Stefan Berger and Dieter Sicker

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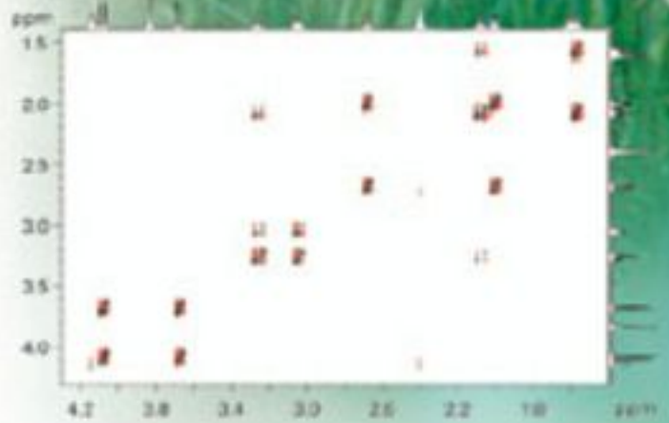
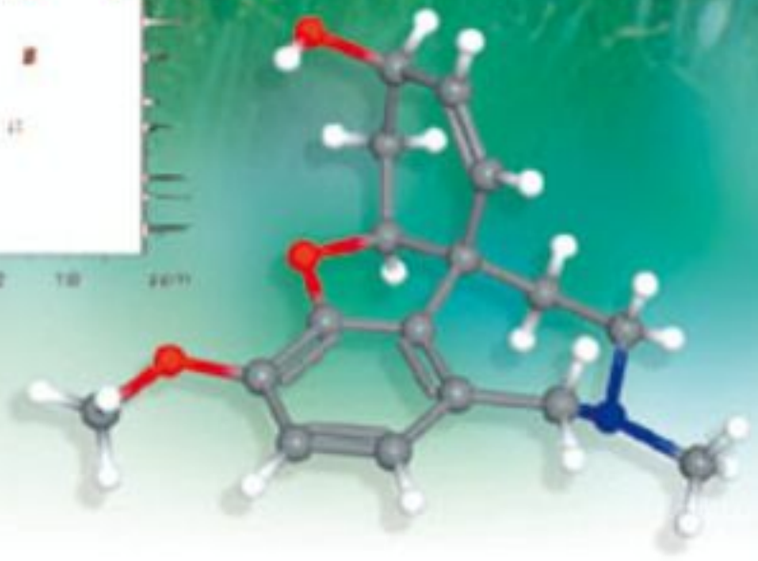
Classics in Spectroscopy

Isolation and Structure Elucidation of Natural Products





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Classics in Spectroscopy

Isolation and Structure Elucidation of Natural Products

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The Authors
Professor Dr. Stefan Berger
Department of Analytical Chemistry
University of Leipzig
Johannisallee 29
04103 Leipzig
Germany

Professor Dr. Dieter Sicker
Department of Organic Chemistry
University of Leipzig
Johannisallee 29
04103 Leipzig
Germany

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Endorsement

The isolation of substances from natural sources ("natural products") is as old as mankind, and the structural identification of these compounds is intrinsically related to the birth of the modern science of chemistry. The discovery that a *vis vitalis* is not required for these substances to be formed was the start of organic synthesis (my love in science). The statement by M. Berthelot (1860), "La chemie crée son objet. Cette faculté créatrice, semblable à celle de l'art lui-même, la distingue essentiellement des sciences naturelle et historiques", actually places synthesis (crée son objet) in the center of chemistry.

On the other hand, synthesis without analysis and identification of the products is fruitless; they are the "objects" of further studies. Thus, chemists have to be trained to master the craftsmanship of isolation, purification, characterization, and identification of substances, no matter whether these come from natural sources or from a synthetic step. In our time of research-oriented laboratory courses there is a danger that both general synthetic and analytical skills become lost; therefore, in bioorganic, biomedical, or nanomaterial laboratories students may experience a very limited education, sometimes becoming familiar with only a single synthetic reaction or a highly specialized isolation procedure and analytical method. This lack of breadth may be one of the reasons why most pharmaceutical companies have closed natural product isolation divisions, which is in contrast to the fact that a large fraction of drugs on the market are natural products or are derived thereof.

There is another aspect of natural products: they are the result of billions of years of evolution of life on our planet. My predecessor *Vlado Prelog* used to state that natural products were the center of his interests because of this fact, and, in his typical "Balkanese" humour, he referred to work on totally artificial target molecules (cf. cubane, tetrahedrane *etc.*) as climbing "Affenfelsen" (artificial rock structures for baboons in zoos). A recent manifesto by *Nicolaou et al.* about the importance of natural product derived antibiotics is worth mentioning here: "to stay ahead of the never ending invasions by our fearful enemies, the superbugs".

The book by *Berger* and *Sicker* shows natural products in their correct light, and it may become an important contribution to preventing the danger of limiting the training of chemists. But it is much more than that, it is absolutely unique! Ori-

ginally, the 30 procedures and supplementary material described in the text were used for a lab course in spectroscopy. The isolation (extraction) of the compounds from natural sources is described in detail. All data and spectra (IR, UV, CD, MS, and especially 2D NMR) were obtained using the isolated ("self made") samples, which include caffeine, nicotine, tetrahydrocannabinol (THC), strychnine, indigo, glucosamine, limonene, and shikimic acid, representatives of most natural product families.

In addition to the molecular *formulae*, three-dimensional models are shown, clarifying the *formulae* which are inadequate (cf. cnicin). In each chapter and for each compound the history, the discovery, and the names (and photographs) of scientists (mostly *Nobel*-prize winners), who made major contributions to the field or to the isolation or structural characterization protocols, are presented. Self made pictures of the plants or their fruits, from which the compounds are isolated, are shown.

To entice the students to go into the lab, there are numerous citations of the nonscientific literature ranging from fiction and poetry to philosophy and songs, from *millennia* of human history and cultures. These citations are given in the original languages and fonts (ancient Greek, Latin, Cyrillic, Tamil, German, French etc.), and the translations into English are presented in an appendix.

Thus, this unusual book is a combination of many: a laboratory-course book, a spectroscopy text, a contribution of the history of natural products, of chemistry, and of plant biology, and even a lab book all in one (there is space left in each procedure for "own observations"). Chemical referencing goes up to 2008, at the end of each section there are demanding questions (with answers in an appendix), and at the beginning of each procedure the level of demand on the experimentalist is indicated as easy, medium or difficult. The book is a scholarly masterpiece, didactically perfect, with beautiful colour pictures and graphic arts. Besides its practical importance for students and young chemical scientists, the book is a pleasure to read and can be used as a reference for the organic and biological chemist, indeed for any person interested in chemistry, life sciences, medicine, sociology, history and belles lettres.

January 2009 *Dieter Seebach*

Preface

Natural products – Discover their challenge and beauty

Natural products have always been among the most fascinating objects of the practicing chemist. In fact, in many definitions of chemistry the isolation, purification and structural elucidation of natural products plays a central role. The history of this art gradually developed from pure chemical means towards physical measurements and finally to the recent spectroscopic techniques. Another driving force of natural products chemistry was and is to make use of their properties, e.g. in medicine. Natural products have "privileged structures" regarding their biological activity. In biological tests they often offer a lead structure for further development, in comparison to a pool of purely synthetic compounds. The reason is that they have already been evaluated for their activity during evolution.

The book in your hands developed from practical courses in our university both in organic and analytical chemistry, where the authors were in charge to teach students the appropriate techniques in their respective disciplines. Some day, the idea emerged to combine the efforts. Hence, we started to produce a collection of self-made isolation procedures; own spectra and their detailed discussions, eventually enriched by background manifold informations on the natural product and its natural source.

The preparative part relies on the collective experience of many years in practical courses, but nevertheless appropriate bachelor and master students have newly prepared each compound described in this book. Not all procedures described are optimized, but all have really been done and led to the desired product, eventually. Explicitly, we appreciate your comments and suggestions for improvements.

Similarly, all spectra have been taken from this freshly prepared material and not from commercial samples. The aim was that this book should offer a very high level of trust: Because the spectra depicted are taken from the compounds obtained as described you will sometimes find some honest impurities. None of the spectra shown has been polished.

Readers for which this book has been written are undergraduate and graduate students who take a course in natural product chemistry or in spectroscopy for the elucidation of organic compounds as well as university teachers on every level in this field. What we want to offer to both parties is the possibility to

deal with certain aspects of chemistry at meaningful examples. An interested reader will soon feel both the challenge and the beauty that is embedded in this subject.

Structural diversity is another aim of this book. 30 natural products have been arranged in six sections, describing seven alkaloids, four aromatic compounds, five dyestuffs, four carbohydrates, eight terpenoids, and a small mixed group. An important selection criterion was that every dedicated reader should have access to the natural source. Each chapter therefore first mentions the raw material, followed by the molecular formula and the factual information like boiling or melting points and the CAS registry number. The chapters are classified as easy, medium or difficult to give you a hint about the practical efforts necessary to reproduce this work. A large photograph on the first page of each chapter shall stimulate you to start with this compound. All photographs have been taken by the authors or their friends, as cited.

1. Background provides you in a journalistic style with a certain amount of cultural history of the specific compound and its natural raw material. Often, really astonishing links between different fields of life manifest. Sometimes, personal experience of the authors has been added. Usually, we lead you from the discovery of the compound to its daily use.

2. Literature can of course not be an extensive or complete survey, since the literature on the compounds described in this book is enormous; sometimes more than 10,000 references exist. We have, if ever possible cited the early, significant papers on the first isolation and structural assignation, and then included some reviews on the importance. Finally, we cite some specialized and recent spectroscopic papers

3. Isolation is divided in three subsections giving first some remarks on the **principle** for isolation of the specific compound. Usually, these ideas are not discussed explicitly in the literature. This is a drawback for students, because they are instructive for those dealing with preparative organic chemistry from many points of view. The **principle** is followed by the **method**, showing how the crude compound will be obtained from its complex natural source. Finally, the subsection **purification** gives advices how to obtain the compound in question sufficiently pure for recording spectra.

A feature should be kept in mind: Never the reader should underestimate the fact, that the trial to reproduce natural product isolation has a particular uncertainty. It consists in the variability of the natural raw material that you buy or collect. Whereas you can purchase a liter of THF with a definite composition, you will not be able to do this with 100 g of chamomile flowers, e.g. . Their constituents will vary from many factors uninfluenceable by the chemist, like the chemical race, the climate, the season, the region, the soil etc. Therefore, despite carefully described procedures you will have to find your own way, from time to time.

4. Spectra and Comments gives you as detailed as space allows the spectral result, always starting with the UV/vis-spectrum and, if appropriate the CD-spectrum. This is followed by the IR-spectrum. The main part form many different NMR-spectra that are discussed in considerable detail and finally also the mass-spectra are commented. The layout is arranged in a manner that always a numbered formula is present close to spectra and the pertinent text. Therefore, never pages have to be turned and it is easy to follow the in part ambitious discussion.

5. Questions are sometimes rather intricate and certainly will demand a fair amount of consideration by the reader. All these questions are answered in detail in the appendix.

6. Own Observations provide some space to scribble in the book your own remarks. This section means that the book in your hand is really a working book.

Red Margins. During the writing of this text the idea emerged to include some citations and pictures at the margin. Whereas the sense of the pictures will be obvious, the citations should not be from chemistry, but from the general literature. As it turned out, these citations really document the global importance of the selected compounds. Authors from all ages, continents and cultural backgrounds have contributed to this. To point to this global aspect of natural products we have left these citations, wherever possible, in their original language and writing. Of course, this may be difficult sometimes – but English translations are provided at the end of each answer section. The city of Leipzig has two excellent libraries, the Bibliotheca Albertina as the University Library and the German National Library. These two institutions were extremely helpful to provide the original texts.

During the progress of this work the idea emerged to provide 3D structures of all compounds involved. This task has been worked out by PD Dr. Stefan Immel, using X-ray structures or closely related material. Dr. Immel also created in addition an own website, where you can inspect these structures, turn them around and measure distances or angles. For those who do not want to build individual mechanical models, this website is extremely helpful:

http://csi.chemie.tu-darmstadt.de/ak/immel/structures-nmr.html

Producing such a book is not only a scientific task. To create a stimulating text in a convincing layout a person was needed who fully commits to this project and this person is our secretary Mrs. Uta Zeller. We are extremely grateful to her many valuable contributions.

This book would not have been realized without the help of many friends and colleagues. We have to thank first Prof. K.-P. Zeller, University Tübingen, for his interpretational help in the mass spectral analysis. Painstakingly, he remarked many of our errors or misconceptions and suggested better solutions. This is also true for Dr. C. Birkemeyer and Dr. D. Hofmann at our university. We thank Mrs. R. Oehme and Mr. G. Reinhardt for recording the mass spectra. The polarimetric values have been recorded by the second author. All NMR spectra have been personally recorded and processed by the first author, and thus he is

the only one to blame for eventual insufficiencies. Mrs. K. Maywald has recorded all the UV/vis and IR spectra, some CD spectra and was an invaluable help for many HPLC runs. Mrs. H. Petzold was an essential and skilful assistance in the lab. Mrs. S. Finsterbusch calibrated the CD instrument and was responsible for the majority of these spectra. We thank Mrs. Mona Knop, IMBIO at University Bonn, for excellent photographs from the Botanic Garden in Bonn. Two daughters of the first author, Prof. Franziska Berger (New York) and Dominika Berger (Berlin) were extremely helpful to provide various translations and photographs.

The 30 compounds described have been prepared by the following students: Sandra Aurich, Madleen Busse, Claudia Ernst, Stefanie Finsterbusch, Madlen Fischer, Michael Göpel, Tillmann Heinisch, Nicole Jahr, Pham Ngo Nghia, Dirk Ortgies, Sebastian Rauch, Tom Rautenberger, Frank Richter, Katja Richter, Franziska Schulze, Fabian Schwarzkopf, Stefanie Till and Lisett Valentin.

We further thank Dr. Torsten Blitzke (Bell Flavors & Fragrances, Miltitz), Dr. Subhash P. Chavan, NCL Pune, India, Dr. Michael Edmonds (Christchurch, New Zealand), Günter Paetzold (Botanic Garden of the University Leipzig), PD Dr. Margot Schulz (IMBIO at University Bonn), Prof. Joachim Sieler (University Leipzig), Prof. Carla Vogt (University Hannover) and Prof. Ludger Wessjohann (IPB Halle) for various help and encouragement. D.S. wants to thank Prof. Athanassios Giannis (University Leipzig) for his sympathy for this endeavour.

Eventually, it is our sincere wish to thank both our wives Dr. Sigrid Berger-Hauff and Dr. Angelika Sicker for their continuous support, understanding and suggestions during the time in which this book was created.

The authors look forward to any comment or criticism and it would be best if you write either to **stberger@rz.uni-leipzig.de** or to **sicker@chemie.uni-leipzig.de**.

Leipzig, September 2008 *Stefan Berger and Dieter Sicker*

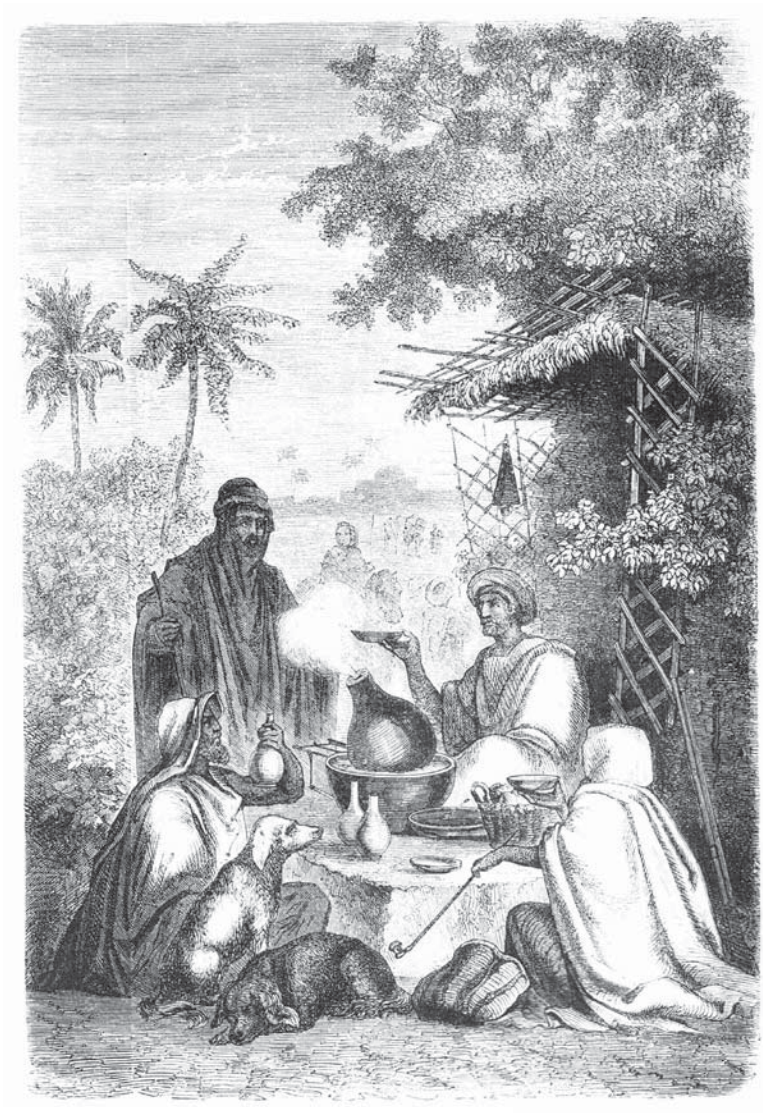
Nil tibi scribo quidem, quod non prius ipse probassem

Heraclius, De coloribus et artibus Romanorum, Prohemium (10th to 12th century)

(I am writing you nothing that I wouldn't have tried out earlier myself)

Contents

Chapter 1 Alkaloids



Coffeeshop in the desert

The field of alkaloids has been investigated by many outstanding chemists. Two Nobel Prize winners should be especially mentioned:

1947

Sir Robert Robinson
(Great Britain, 1886−1975)

Great Britain, Oxford University

“for his investigations on plant products of biological importance, especially the alkaloids”

1965

Robert Burns Woodward
(USA, 1917−1979)

USA, Harvard University, Cambridge, MA

„for his outstanding achievements in the art of organic synthesis“

Index

1.1 Nicotine

3-(*S*)-(1-Methylpyrrolidin-2-yl)pyridine

From tobacco
Nicotiana tabacum L. (Solanaceae)

$C_{10}H_{14}N_2$, MW 162.23

CAS RN 54-11-5, BRN 82109

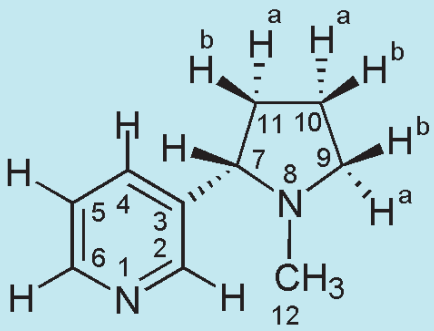
$[\alpha]_D^{24} = -168.5°$ (*c* 0.0465 g/mL, acetone)

Colourless viscous liquid, bp 90−92 °C (500 Pa)

Nicotine is commercially available.

Synonymous names:
3-[(2*S*)-1-Methyl-2-pyrrolidinyl]pyridine,
(–)-Nicotine, (*S*)-(–)-Nicotine

Level: medium



Very strong poison! Warning: Lethal dose for adults: 40-60 mg

Storage under exclusion of air and moisture

Fig. 1.1-1 A young tobacco plant

Ut herbae nicotianae analysis chemica instituatur, principiorum quae inde evadunt et chemica natura et vires quas habent in corpore animali accuratius investigentur, eoque examine, si fieri potest, demonstretur utrum effectus hujus herbae tam acris quam narcotius ab uno eodemque principio pendeat, an a diversis.

Scientific competition,
University of Heidelberg, 1827

1. Background:
Tierra, tierra – the discovery of the big smoke

Tierra, tierra! It was on October 12, 1492 that the three ships of the maritime explorer Christopher Columbus after a long five-week voyage across the ocean ultimately reached an island called Guanahani by the natives, which now belongs to the Bahamas. On October 28, 1492 the expedition landed on what we know as Cuba and came in contact with the chieftain Habaguanex. It was certainly disappointing and in contrast to expectations not to find huge amounts of gold or treasures. However, despite this there were a lot of new and strange discoveries. A fuming material called tabaku by the indigenous people was offered to the navigator and his sailors in form of *zikari*. Indeed, Cuban cigar manufacturers today proudly make tobacco advertisements with the appendix "*since 1492*", a historic year probably known to every school child.

Columbus, concerned about the safety of his men, first tried to prohibit smoking - especially as these natives had not been converted to Christianity. As you may assume: Columbus failed with his proscription. On the other hand, clouds of tobacco smoke were offered by the indigenous people to their own rain god to make a sky full with clouds of water for them – a successful enterprise in a tropical area.

The plant from which zikari or today cigars can be made is named *Nicotiana tabacum* and belongs to the nightshade family. It was indigenous to Middle and South America at the time of its discovery. The leaves are processed to products that can be smoked, sniffed or chewed. The ingredient of interest that causes most of the physiological effect is nicotine. The amount of this classical alkaloid in the tobacco may differ considerably depending on the variety of *Nicotiana tabacum* and is reported to be between only 0.05% up to 7.5% (Russian "Machorka"). Nicotine is biosynthesized in the roots and accumulated in the leaves. The origin of the N-atom in the pyridine ring is L-aspartic acid, the N in the pyrrolidine ring results from ornithine, which in turn is made from L-arginine. It is clear that the high level of the alkaloid made leads to the requirement for a rich soil that has enough ammonium ions to be taken up by the plant. The benefit for the plant is believed to be protection against pests. Indeed, nicotine has strong insecticidal and anthelminthic properties.

Therefore, it has been used in the form of tobacco broth as one of the first means of pest control when this technique arose in the 19th century. This natural product is very effective against aphids and the like and was used at that time in day-to-day life. In households, tobacco broth was made as an application form from cheroots by the housewife herself using an aqueous extraction procedure. The broth showed a powerful effect; however, it had one serious drawback, the terrible stench. Therefore, soon after the development of synthetic pesticides people stopped using tobacco broth. Certainly, you will find it in your great grandmother's book of household management.

Despite the discovery of tobacco plants immediately after the armada reached the new beaches, the first tobacco plants did not arrive in Spain until 1511. It was Jean Nicot de Villemain, a French ambassador in Portugal, who grew tobacco plants himself and brought such seeds to Paris in 1560. This event is regarded as the source of a tobacco boom connected with a lot of dramatic stories, the majority of which we will only be able to mention in passing here. However, it is clear that already at that time tobacco polarized society. Consequently, it was Nicot´s name that was used first to name the plant and later the alkaloid. In the golden age of alkaloid discovery at the beginning of the 19th century, nicotine was first isolated in 1828 by the German chemists Posselt and Reimann at the University of Heidelberg [1]. The correct structure was established between 1891 and 1893 by two other German chemists, Pinner and Wolffenstein [2], whereas it were the French chemists Pictet and Crepieux who succeeded with the first synthesis in 1893. Nicotine as a strongly basic compound fitted at that time very well the very first definition of the term alkaloid given in 1819 by Meissner, who regarded them just as *plant-derived substances that react like alkalis* [3]. Today the definition has been modified by Hesse to: *Alkaloids are nitrogen-containing organic substances of natural origin with a greater or lesser degree of basic character* [4].

Nicotine, once in the bloodstream, is an extremely deadly poison: 40−60 mg can be a lethal dosage for adults. This fact should not be underestimated. It has been reported that death can result if a small child ingests only one cigarette. Also for adults the lethal amount is not much more: it would be in as little as half of a cigar or three cigarettes, if they were to be swallowed. At first glance, it seems therefore impossible to smoke at all because there seems to be an instantaneous risk of passing away. However, this apparent contradiction disappears if one knows that only a small fraction of nicotine contained in a tobacco product is released as such into the smoke. This is a result of the chemical processes taking place during smoke formation from tobacco [5]. The basic reason for this can be found in the physical properties of this alkaloid. Its volatility is high enough to yield a vapour with a flash point of 95 °C, or in non-chemical words: most of the nicotine released by a smouldering cigarette is just burned off. Despite this, enough can be inhaled to provide the known desired effects. Nicotine-rich blood reaches the brain from the lungs within only seven seconds. There, it stimulates the release of chemical messengers such as acetylcholine, dopamine and β-endorphine. These chemicals produce feelings of calmness, alertness, relaxation, enhanced pleasure and decreased anxiety, which can be summarized as a mildly euphoric state. Concentration and memory are enhanced by the increased acetylcholine level. The effects last up to two hours. A receptor was named after nicotine, the nicotinic acetylcholine receptor. It is stimulated by low nicotine concentrations and blocked by high ones, which is the reason for nicotine's toxicity and insecticidal activity. In the liver, nicotine is metabolized to cotinine, the 2-pyrrolidinone derivative. This metabolite remains in the blood for up to four days and can be detected within the blood, urine or saliva by drug tests looking for tobacco smoke exposure. As expected, the toxicology,

December 1st. - We steered for the island of Lemuy. I was anxious to examine a reported coal-mine which turned out to be lignite of little value, in the sandstone (probably of an ancient tertiary epoch) of which these islands are composed. When we reached Lemuy we had much difficulty in finding any place to pitch our tents, for it was spring-tide, and the land was wooded down to the water's edge. In a short time we were surrounded by a large group of the nearly pure Indian inhabitants. They were much surprised at our arrival, and said one to the other, "This is the reason we have seen so many parrots lately; the cheucau (an odd red- breasted little bird, which inhabits the thick forest, and utters very peculiar noises) has not cried 'beware' for nothing." They were soon anxious for barter. Money was scarcely worth anything, but their eagerness for tobacco was something quite extraordinary. After tobacco, indigo came next in value; then capsicum, old clothes, and gunpowder. The latter article was required for a very innocent purpose: each parish has a public musket, and the gunpowder was wanted for making a noise on their saint or feast days.

Charles Darwin (1809−1882)
The Voyage of the Beagle, Chap. 13

Figs. 1.1-2, -3 and -4 Leaves of tobacco plants are divided into five types: capa, ligero, capote, seco and volado. The composition of these types makes the secret of the cigar. Special attention is paid to the wrapper (capa), which is handled in a more humid state than the other leaves in a leather-like condition that makes it ductile

pharmacology and psychoactive effects, and addiction, have been well studied in great detail [6]; also the possible use of nicotine and cotinine in the treatment of Alzheimer's disease and Parkinson's disease is now under investigation.

To be clear: apart from all these more or less scientific considerations, there is today no doubt that long-term tobacco smoking enhances significantly the risk of developing cancers or stroke as well as respiratory and cardiovascular diseases. Statistically, tobacco smoking is associated with shorter life expectancy [7]. Without going into details here, the reason for this is that tobacco smoke represents a complex mixture of more than 1000 volatile chemicals of in part toxic or reactive character that are able to react with the body. If you want to come in contact with a similar complex mixture of hundreds of chemicals without taking a chance – just have a cup of coffee. This experiment has been going on around the world for some hundred years, too, interestingly without comparable harm.

As already mentioned, the use of tobacco, especially by smoking, has always divided society. Looking back, some periods can be detected. At the beginning, it was fashionable among part of the aristocracy. Hence, snuff introduced by Nicot was very popular at the French court. From there the custom spread out into fashionable Paris society, which made Nicot a celebrity. In Prussia, Friedrich Wilhelm I, called the *Soldatenkönig* (Soldiers' King), made the so-called *Tabakskollegium* (tobacco council) into a daily evening institution, i.e. a club open for conversation and amusement whose participants were pipe smokers. At the same time, other noblemen tried to interdict and suppress smoking by the hardest punishments imaginable. Though such stories as reported in [4] and elsewhere are interesting, there is only the space for one: In 1634, Shah Safi I of Persia prescribed the punishment of pouring molten lead into the throat of smokers. When it was obvious that tobacco smoking could not be suppressed easily, another idea arose: the possibility of taxing tobacco goods. This worked very well for the treasury until modern times. Nowadays again, based on reported medical findings, serious efforts are being made both to convince and force people to abstain from tobacco use. Interestingly, just a nicotine patch may be helpful in getting out of the habit of smoking – for nicotine easily penetrates the skin.

Since ancient times murder by poisoning has been a terrible act mainly driven by avarice or imperiousness. Hundreds of novels deal with this subject based on real history: think, for example, of the Medici dynasty and the like. On the other hand, such crimes created an inherent driving force to convict the murderer and to verify the poison used, especially after the Middle Ages. Two milestones in forensic medicine are especially important: first, the development of a test for arsenic in tiny amounts by Marsh in 1836 to discover the use of arsenic trioxide, called the "inheritance powder", and second, after this test existed, the establishment of a verification for nicotine and other toxic alkaloids by the Belgian analytical chemist Stas and the German druggist Otto shortly after a murder by using nicotine in Belgium in 1850.

As with many other goods made on the basis of a natural raw material (for example, think of fabrics made of silk, wool or cotton, coffee, wine, leather, perfumes) during the centuries, mankind has invested a lot of work into the everlasting refinement of these products. It is the same with tobacco. One of the authors, while travelling through Cuba, learned a lot about the cultivation, harvesting and treatment of tobacco on the way to a cigar factory. Unfortunately, taking photographs in such a historic factory was not allowed. However, the principles of how a good cigar is composed and eventually made were shown in the shop of a special cigar smokers' hotel (incredible thought, but true!) in Havana. Here, taking photographs was possible. In every respect, enjoying a fine cigar is different from engulfing a cigarette in hurry. A real ritual was developed around it, beginning with how the end is canonically cut off to get a perfect mouthpiece, followed be the correct manner of lighting it up and crowned by the kind of enjoyment by inhaling the smoke just into the mouth and puffing it away with sobriety. In the margin, you will find a series of photographs that take you from the tobacco field to the air-conditioned cigar shop.

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[8] W. Gattermann, "Die Praxis des organischen Chemikers", Walter de Gruyter, 43rd edition, **1982**, 670f.

[9] D. F. Glenn, W. P. Edwards III, "Synthesis and mass spectrometry of some structurally related nicotinoids" *J. Org. Chem.* **1978**, *43*, 2860−2870.

Figs. 1.1-5 to -9 Cutting a valuable cigar can be done exactly with a cigar cutter. A thin rod of cedar wood is regarded as an appropriate cigar lighter. During this operation no air is sucked through the cigar

[10] J. F. Whidby, W. B. Edwards III, T. P. Pitner, "Isomeric nicotines. Their solution conformation and proton, deuterium, carbon-13, and nitrogen-15 nuclear magnetic resonance" *J. Org. Chem*. **1979**, *44*, 794−798.

3. Isolation

3.1 Principle

There was as many as one loafer leaning up against every awning-post, and he most always had his hands in his britches-pockets, except when he fetched them out to lend a chaw of tobacco or scratch. What a body was hearing amongst them all the time was:

"Gimme a chaw'v tobacker, Hank"

"Cain't; I hain't got but one chaw left. Ask Bill."

Maybe Bill he gives him a chaw; maybe he lies and says he ain't got none. Some of them kinds of loafers never has a cent in the world, nor a chaw of tobacco of their own. They get all their chawing by borrowing; they say to a fellow, "I wisht you'd len' me a chaw, Jack, I jist this minute give Ben Thompson the last chaw I had" -- which is a lie pretty much everytime; it don't fool nobody but a stranger; but Jack ain't no stranger, so he says:

"YOU give him a chaw, did you? So did your sister's cat's grandmother. You pay me back the chaws you've awready borry'd off 'n me, Lafe Buckner, then I'll loan you one or two ton of it, and won't charge you no back intrust, nuther."

Mark Twain (1835−1910)
Huckleberry Finn, Chap. 21

Nicotine is a classical alkaloid the separation of which teaches the main principles of an alkaloid isolation. Consisting of a combination of two tertiary amines of which the pyrrolidine is the stronger basic one, nicotine is protonated in the plant and forms carboxylate salts such as formate, acetate or maleate. Therefore, the first and typical step is to bring the alkaloid into a distinctively strong alkaline environment, such as NaOH solution to cleave the organic salts and release free nicotine into the aqueous solution. Nicotine is readily soluble in water due to its ability to act as an acceptor for hydrogen bonds to water. Another useful property is its volatility with water vapour. This allows steam distillation to be used for a very selective separation of nicotine from many other water-soluble tobacco constituents. In the distillate, the alkaloid is protonated by addition of hydrochloric acid and the nicotinium ions formed are precipitated by addition of sodium picrate solution. The yellow nicotinium picrate formed is pure. To obtain the free alkaloid base, a second alkaline cleavage as with the starting tobacco is necessary with the nicotinium picrate. The free base is extracted from the basic solution with diethyl ether and finally purified by a distillation in vacuo.

3.2 Method

This isolation is based upon a method reported in the literature [8]. Fine cut dark tobacco (80 g − in our example the brand name was Schwarzer Krauser®) was treated in a 1 L beaker with 4 N NaOH solution (650 mL) in a water bath at 50 °C for 2 h with occasional stirring with a glass rod. A dark brown solution forms, which smells intensely of tobacco. The mixture is filtered with a Buchner funnel yielding 390 mL of tobacco broth. The tobacco is subjected to a second extraction with 4 N NaOH solution (400 mL) as described above. Intensive filtration with squeezing of the tobacco mass affords another 590 mL crop of tobacco broth. The combined aqueous extracts are subjected to a steam distillation which is run until 2 L of distillate have passed over. This distillate has a pale yellow colour. In a rotary evaporator (45 °C, 50 to 20 mbar) the solution is concentrated to 200 mL. A strongly basic, cloudy yellow solution remains. Concentrated hydrochloric acid (3 mL) is added to adjust the pH to 3. The solution becomes clear. For the next step, a solution of 2,4,6-trinitrophenol (11.45 g, 50 mmol) and NaOH (2.0 g, 50 mmol) in water (750 mL) is prepared. From this solution, 325 mL can be slowly stirred into the initial nicotinium chloride solution with precipitation of yellow nicotinium picrate at the moment of dropping into the solution. Addition is stopped when this effect ceases. The yellow precipitate is filtered over a small sintered glass filter funnel and shows a broad melting range of 208−220 °C. This crude product is

recrystallized from 1 L of boiling water to yield yellow needles of pure nicotinium picrate which are dried in vacuo (2.2 g, 5.6 mmol) and show mp 215–218 °C. This is in accordance with literature data. To obtain the free base, 2.15 g of the above picrate are stirred with 1 N NaOH (20 mL) for 5 min. A yellow solution forms, which is extracted with diethyl ether (4 × 60 mL). The ethereal extract shows a yellow coloration, is dried over Na_2SO_4, reduced by distillation to a volume of 5 mL and transferred into a micro distillation apparatus. The last ether portion is distilled off, then the nicotine is distilled in vacuo by means of an electronic heat gun as a colourless viscous liquid, which shows only a very weak smell due to its high boiling point. Only a few mg of a dark solid remain in the distillation flask.

Yield: 368 mg (2.3 mmol), which corresponds to only 40% of the starting nicotinium picrate, bp 90–92 °C (500 Pa), *n* 1.5240, optical rotatory power $[\alpha]_D^{24} = -168.5°$ (*c* 0.0465 g/mL, acetone) (both corresponding with literature data).

3.3 Purification

Unfortunately, it is not possible to avoid that in the final ether extraction, together with nicotine a small portion of picrate acid/picrate in water is extracted into the ether which is able to take up a few percent of an aqueous solution. This requires a final distillation to separate pricric acid and nicotine. Though nicotine shows remarkable thermal stability and can in principle be distilled at ambient pressure (bp then 246–248 °C), for a small amount as above a distillation in vacuo is recommended. The refractive index could be measured with a single drop to avoid loss of material. The loss of more than half of the nicotine subjected to the last step is a strong hint at the high solubility of nicotine in water and the small partitioning coefficient with ether.

Василий Андреич между тем, распустив шубу и закрываясь полами ее, теродну серную спичку за другой о стальную коробку, но руки у него дрожали, изагоравшиеся спички одна за другою, то еще не разгоревшись, то в самую ту минуту, как он подносил ее к папиросе, задувались ветром. Наконец одна спичка вся загорелась и осветила на мгновение мех его шубы, его руку с золотым перстнем на загнутом внутрь указательном пальце и засыпанную снегом,выбившуюся из-под веретья овсяную солому, и папироса загорелась. Раза два онжадно потянул, проглотил, выпустил сквозь усы дым, хотел еще затянуться, но табак с огнем сорвало и унесло туда же, куда и солому. Но и эти несколько глотков табачного дыма развеселили Василия Андреича.

Lev Nikolayevich Tolstoy
(1828–1910)
Master and Men

4. Spectra and Comments

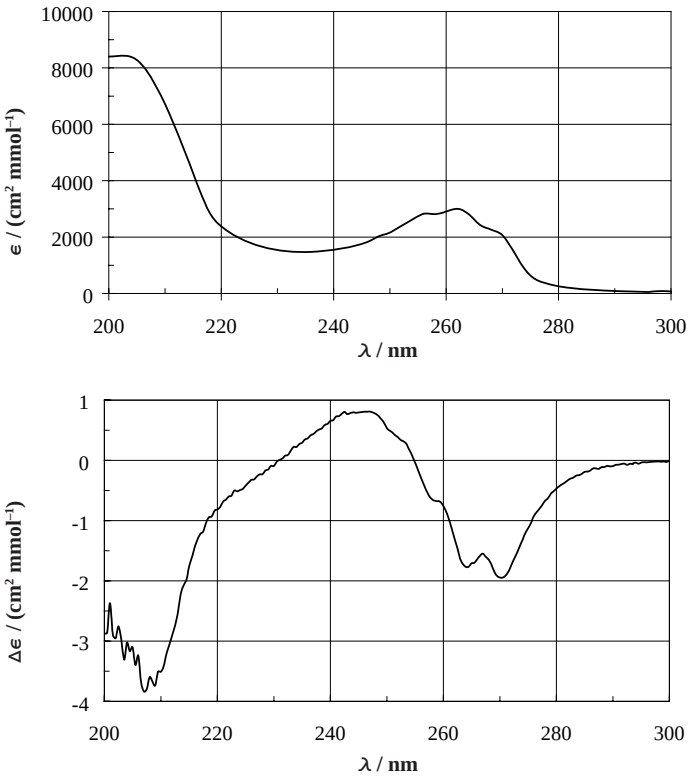
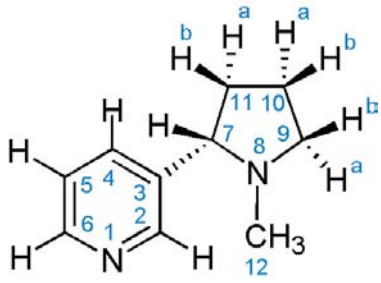


Fig. 1.1-10 UV and CD spectra in ethanol

The UV spectrum is typical for an aromatic compound, since the pyrrolidine part of the molecule gives no additional absorption. The vibrational fine structure is hardly visible due to the lack of rigidity of the molecule. The aromatic chromophore is in the direct vicinity of the chiral centre and therefore a CD spectrum is expected. Both UV absorption bands at 210 and 270 nm show a negative Cotton effect.

Fig. 1.1-11 A mature tobacco plant ready to be harvested. The middle shoot was cut when the plant was young to cause an increased growth of the lower leaves.
Picture from a plantation at San Miguel, Azores



Scheme 1.1-1

Fig. 1.1-12 A cottage made from eucalyptus stakes covered with palm fronds is the typical equipment for processing raw tobacco leaves in Cuba

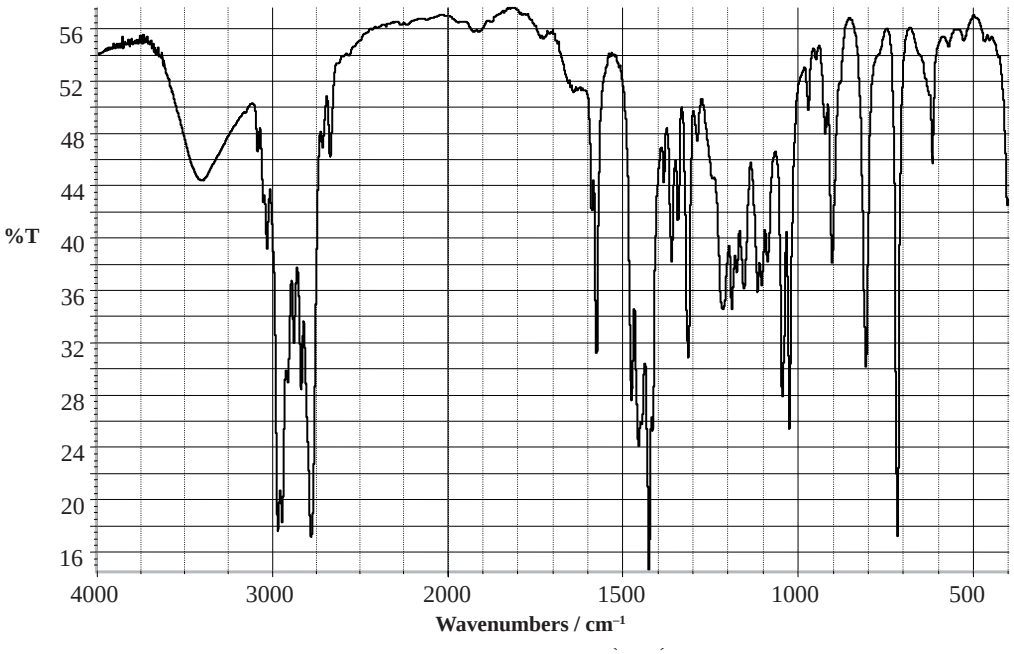
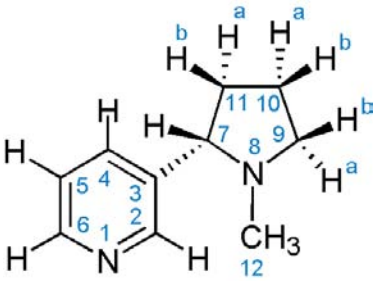


Fig. 1.1-13 IR spectrum as film

The IR spectrum shows frequencies which can also be seen in the spectrum of pyridine, namely the slightly split band for the C=C vibration at 1600 cm^{-1} and the bands at 700 and 800 cm^{-1}. The aliphatic part of the molecule is present in the very strong CH valence vibrations from 3000 to 2800 cm^{-1}.



Scheme 1.1-2

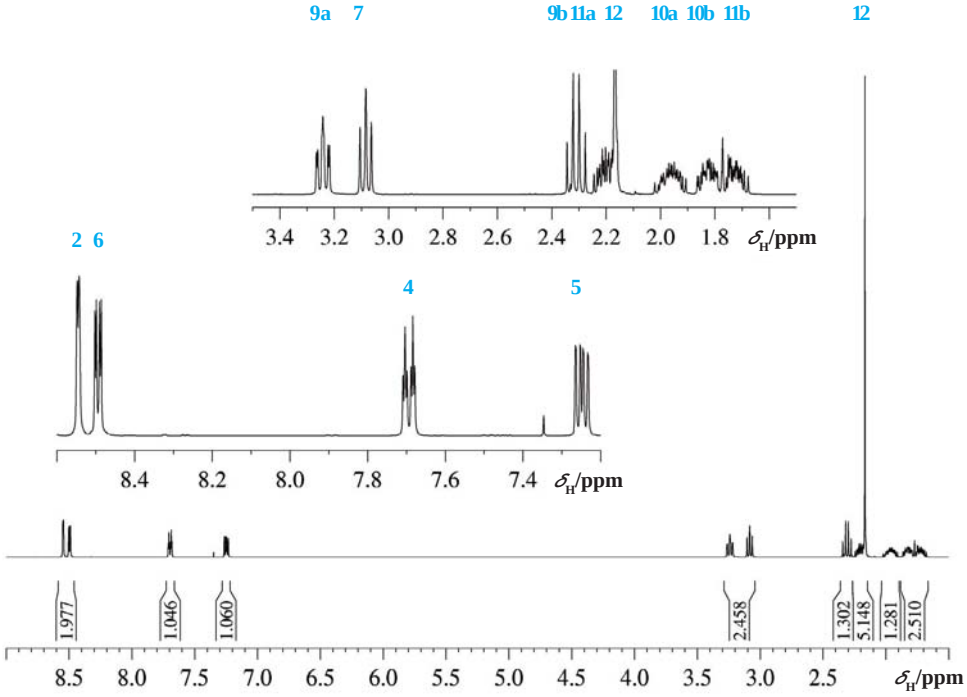


Fig. 1.1-14 ^{1}H NMR spectrum at 400 MHz in $CDCl_3$

The NMR spectra of nicotine are nicely separated into the aliphatic and aromatic parts of the molecule. In the ^{1}H NMR spectrum the four pyridyl protons can be easily assigned, since the two *ortho* protons with respect to the nitrogen are largely deshielded and appear at about 8.5 ppm. H-2 is recognized since it displays only one long-range coupling constant to H-4, whereas H-6 shows a large coupling to its neighbouring proton H-5. The safe distinction between H-5 and H-4 will be recognized in the COSY spectrum. The aliphatic part of the ^{1}H NMR spectrum is much more complicated, since all methylene groups of nicotine are diastereotopic and their safe assignment will be performed with the help of the HSQC spectrum. At this stage it can be assumed, however, that the two signals at about 3.2 ppm should belong to H-9 and H-7.

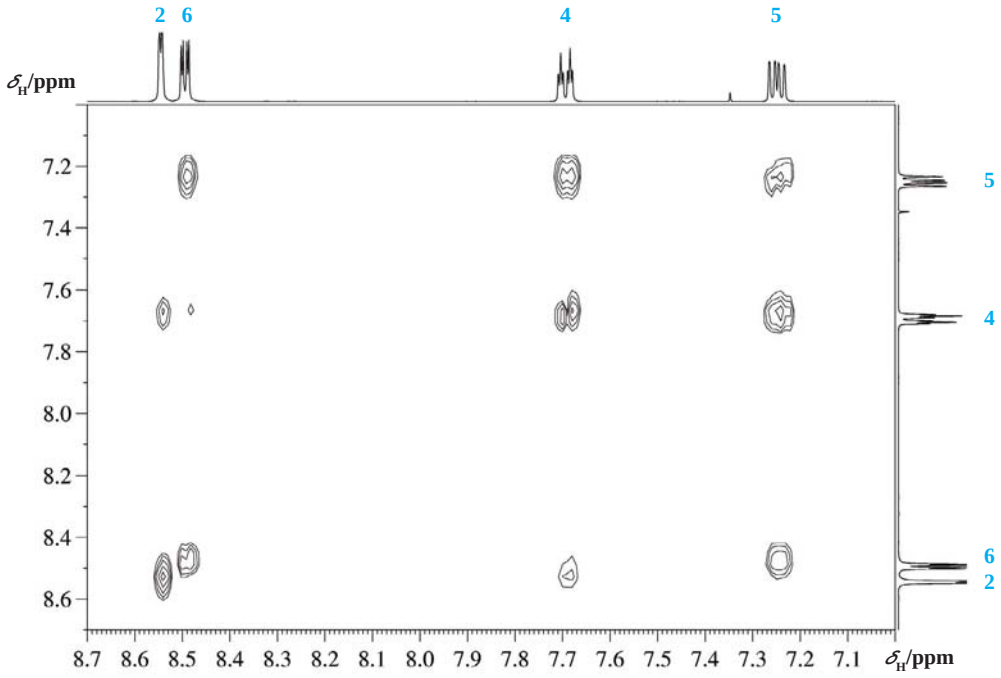


Fig. 1.1-15 Expansion of the COSY spectrum in the aromatic region

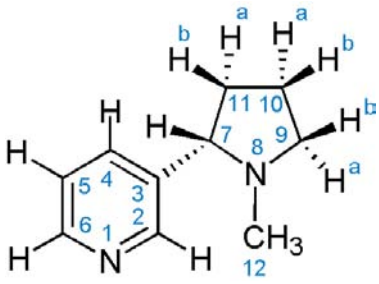
The expansion of the COSY spectrum in the aromatic region is a good example of the assignment of an aromatic four-spin system.The cross peaks starting from H-2 and H-6 give a firm assignment for H-5 and H-5, which, of course, couple to each other in turn.

With his left band he dipped into his side pocket, brought out a loose wheat-straw paper and shifted it to his right hand close by the revolver. Again he dipped, transferring to the paper a pinch of brown, flaky tobacco. Then he proceeded, both hands just over the revolver, to roll the cigarette.
"From the way you hover close to that nasty weapon, you seem to be afraid of me," she challenged.
"Not exactly afraid of you, ma'am, but, under the circumstances, just a mite timid."
"But I've not been afraid of you."
"You've got nothing to lose."
"My life," she retorted.

Jack London (1876–1916)
The Night Born, Chap. 9

Fig. 1.1-16 *Nicotiana rustica* (Farmer's tobacco), a traditional variety of tobacco

Fig. 1.1-17 Tobacco field in Valle Vinales, Cuba



Scheme 11-3

"I'm a heavy grubber, dear boy," he said, as a polite kind of apology when he had made an end of his meal, "but I always was. If it had been in my constitution to be a lighter grubber, I might ha' got into lighter trouble. Similarly, I must have my smoke. When I was first hired out as shepherd t'other side the world, it's my belief I should ha' turned into a molloncolly-mad sheep myself, if I hadn't a had my smoke." As he said so, he got up from table, and putting his hand into the breast of the pea-coat he wore, brought out a short black pipe, and a handful of loose tobacco of the kind that is called Negro-head. Having filled his pipe, he put the surplus tobacco back again, as if his pocket were a drawer. Then, he took a live coal from the fire with the tongs, and lighted his pipe at it, and then turned round on the hearth-rug with his back to the fire, and went through his favourite action of holding out both his hands for mine.

Charles Dickens (1812−1870) *Great Expectations*, Chap. 40

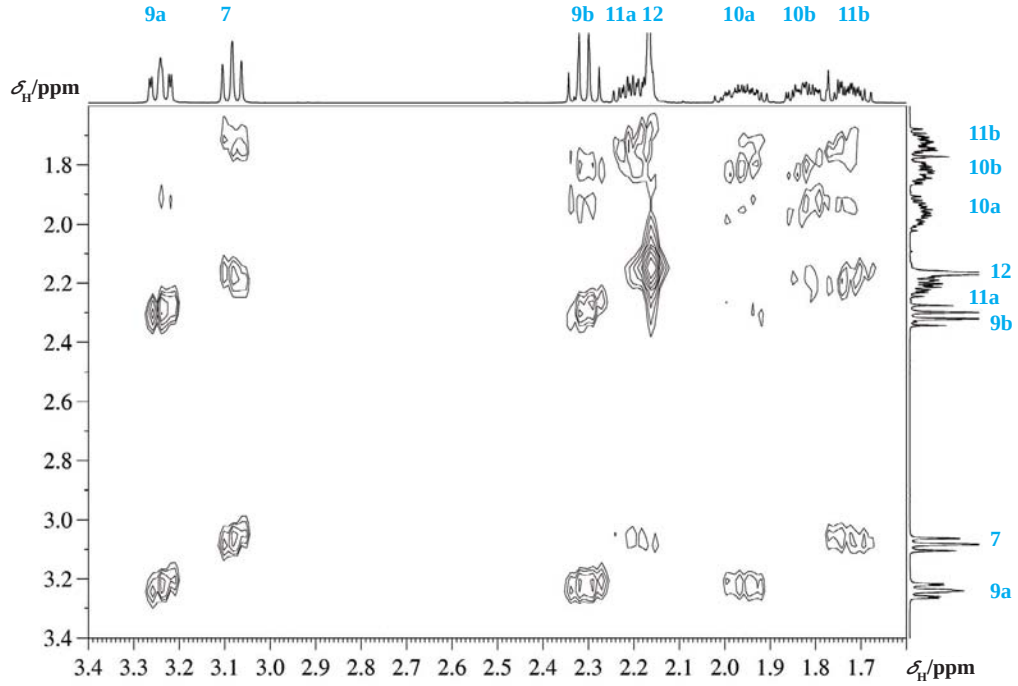


Fig. 1.1-18 Expansion of the COSY spectrum in the aliphatic region

In the aliphatic expansion of the COSY spectrum one observes several diastereotopic methylene group signals which strongly couple to each other. Their safe assignment, however, has to await the discussion of the ^{13}C NMR and of the HSQC spectrum.

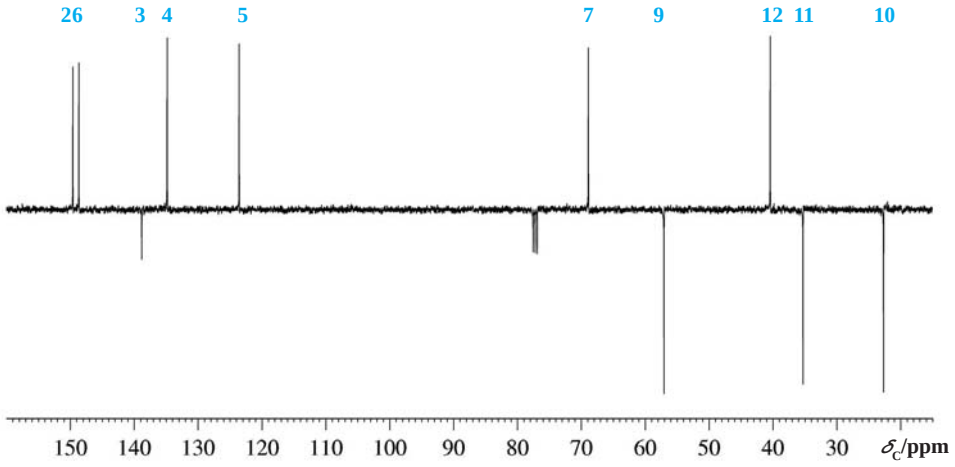


Fig. 1.1-19 APT ^{13}C NMR spectrum at 100 MHz in $CDCl_3$

As with the proton NMR spectrum, the ^{13}C NMR spectrum is nicely divided into an aromatic and an aliphatic part. In the former, the quaternary signal of C-3 at 138.8 ppm is immediatedly recognized, and in the latter, the signals of C-7 at 68.9 ppm and of the methyl group C-12 at 40.4 ppm can also be safely assigned using their sign in the APT spectrum.

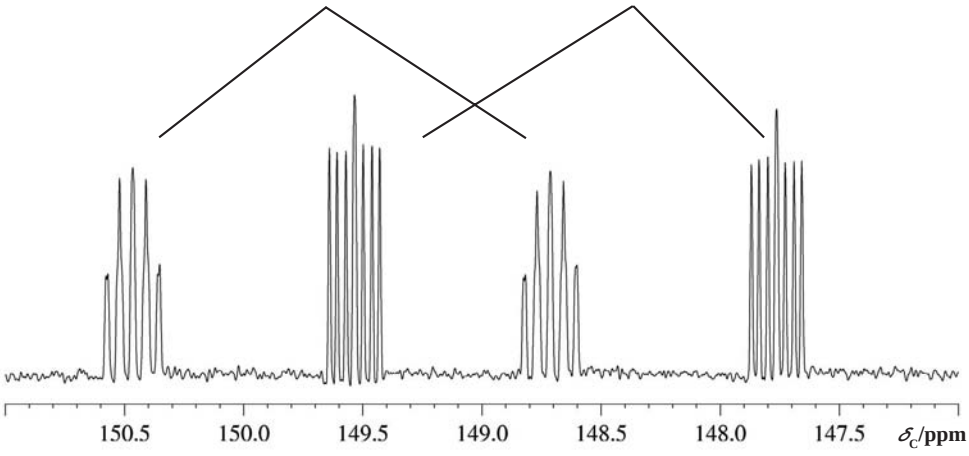


Fig. 1.1-20 Expansion of the gated decoupled ^{13}C NMR spectrum at 100 MHz in $CDCl_3$ in the aromatic region

A particular impressive spectroscopic pattern is revealed by the gated decoupled ^{13}C NMR spectrum in the aromatic part; shown here is the expansion of the signals of C-2 and C-6.

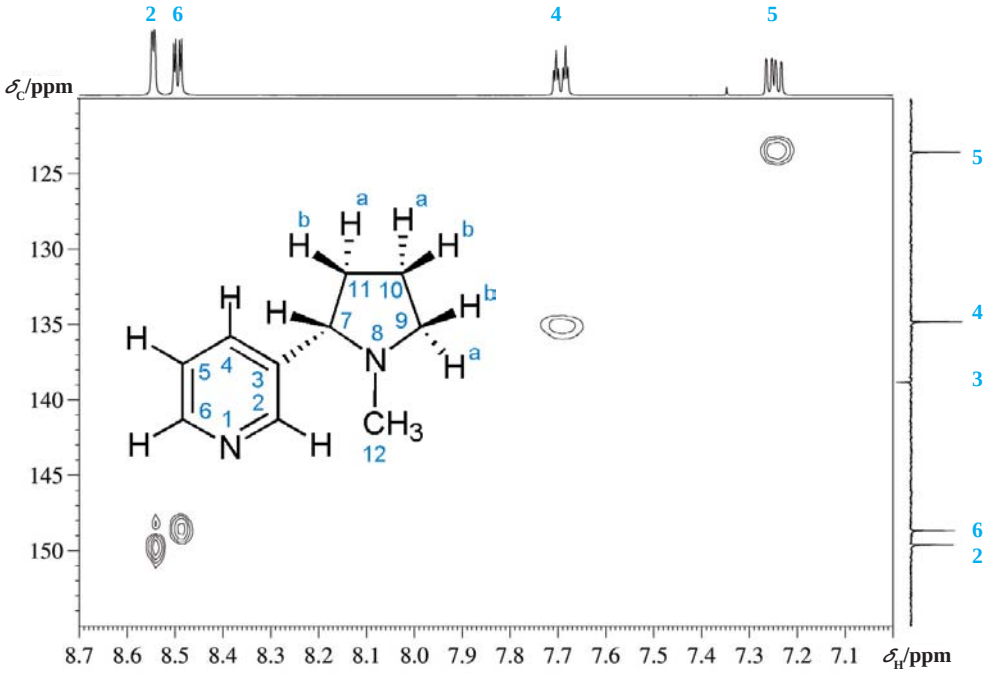


Fig. 1.1-21 Expansion of the HSQC spectrum in the aromatic region

In the HSQC expansion of the aromatic region the ^{13}C NMR signal assignment is straightforward, since we have already assigned the four proton signals using the COSY spectrum.

Cependant, le jour du rendez-vous, le jeune homme, en demi-toilette, avait établi son quartier général dans le petit salon du rez-de-chaussée. Là, sur une table entourée à distance d'un divan large et moelleux, tous les tabacs connus, depuis le tabac jaune de Pétersbourg, jusqu'au tabac noir du Sinaï, en passant par le maryland, le porto-rico et le latakiéh, resplendissaient dans les pots de faïence craquelée qu'adorent les Hollandais. À côté d'eux, dans des cases de bois odorant, étaient rangés, par ordre de taille et de qualité, les puros, les régalias, les havanes et les manilles ; enfin dans une armoire tout ouverte, une collection de pipes allemandes, de chibouques aux bouquins d'ambre, ornées de corail, et de narguilés incrustés d'or, aux longs tuyaux de maroquin roulés comme des serpents, attendaient le caprice ou la sympathie des fumeurs.

Alexandre Dumas (1802−1870)
Le Comte de Monte Cristo, Chap. 39

Fig. 1.1-22 Tobacco leaves are hung in a cottage on eucalyptus slats for a three-month period for drying and fermentation

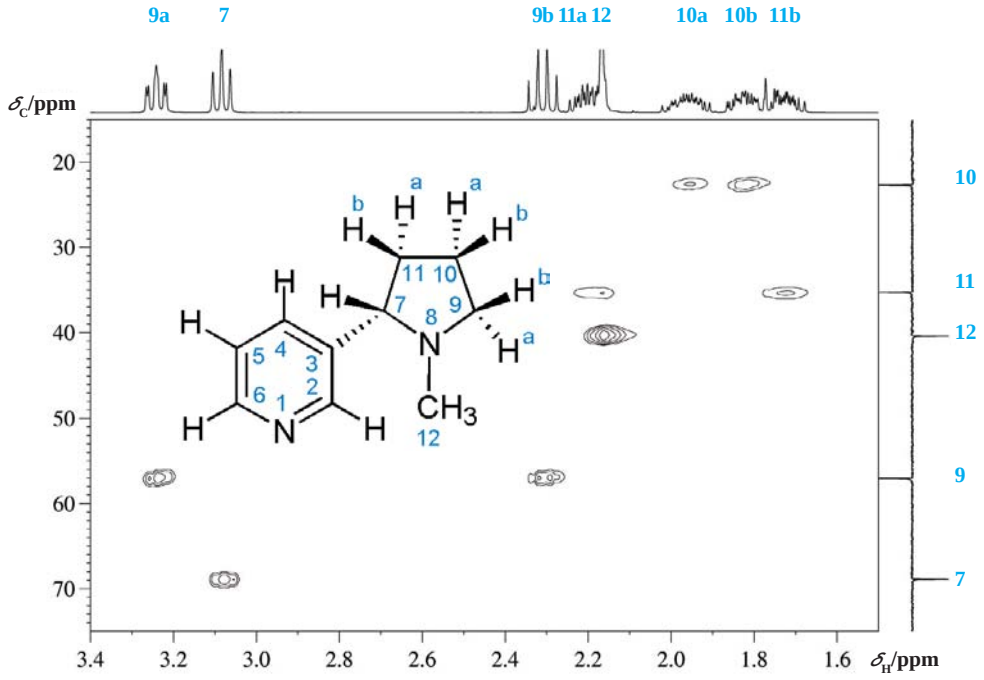


Fig. 1.1-23 Expansion of the HSQC spectrum in the aliphatic region

The aliphatic expansion of the HSQC spectrum demonstrates the assignment power of this technique. Since we already have assigned C-7 and C-12 from the APT ^{13}C NMR spectrum, we can now easily assign H-7 at 3.17 ppm. Furthermore, we find three pairs of diastereotopic protons, where always two proton signals are connected to one carbon atom signal. As the HSQC spectrum reveals, the amount of this diastereotopicity is very different for the three methylene groups. Chemical shift arguments identify the proton at 3.25 ppm as the other proton H-9 in the vicinity of the nitrogen N-8. In the COSY and HSQC spectra one finds the diastereotopic partner proton at 2.9 ppm and the corresponding carbon C-9 at 57.0 ppm. The most shielded carbon signal in this molecule must belong to C-10 at 22.6 ppm and the corresponding protons are at 1.9 and 1.8 ppm. This leaves the residual methylene group signal for C-11 at 35.3 ppm with H-11 at 2.2 and 1.7 ppm. Interestingly, these proton signals “embrace” those of H-10.

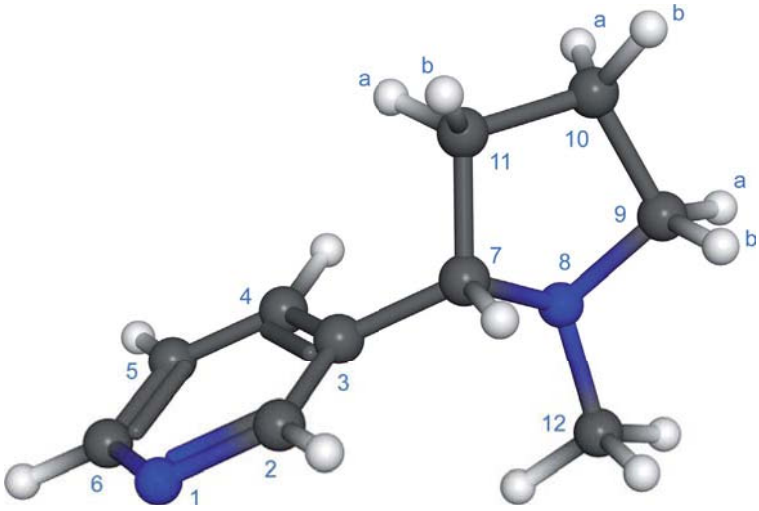


Fig. 1.1-24 Molecular model of nicotine

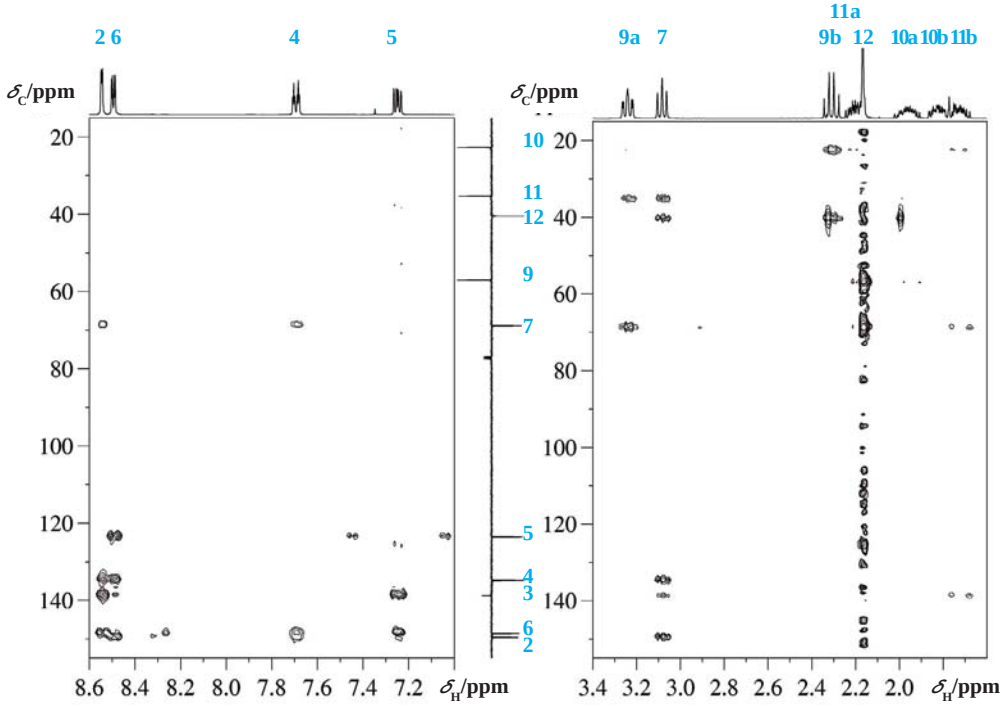


Fig. 1.1-25 HMBC spectra

In the HMBC expansion of the aromatic part, the cross peaks of H-2 and H-4 to C-7 are significant for the structure of the molecule. The other HMBC correlations in the aromatic region confirm the previous assignments. Especially rewarding is the signal of H-7 in the HMBC spectrum, because it reveals five different coupling partners, C-2, C-3 and C-4 in the aromatic part and C-11 and C-12 in the aliphatic part, indicating its central position for the connectivities in this molecule.

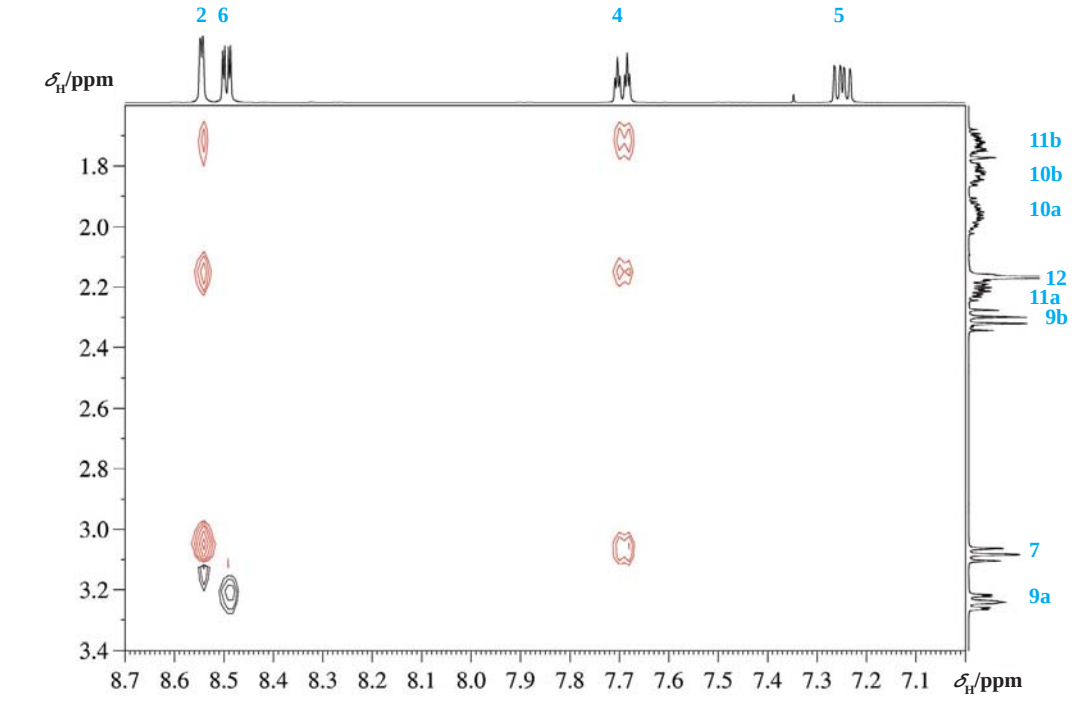


Fig. 1.1-26 NOESY spectrum connecting the aromatic with the aliphatic region

The NOESY spectrum is very interesting and helps to assign the individual protons in the aliphatic part, stereochemically. In the expansion which connects the aliphatic with the aromatic part, one finds NOE contacts from H-2 to H-7 and to one of H-11 as well as to the methyl group protons. Similarly, H-4 displays contacts to H-7, the methyl group and to one of the protons at C-11. This first indicates that the conformation of nicotine usually drawn in the chemical formula is not the only one populated.

Fig. 1.1-27 “Giving up smoking is the easiest thing in the world. I know because I’ve done it thousands of times”

Mark Twain

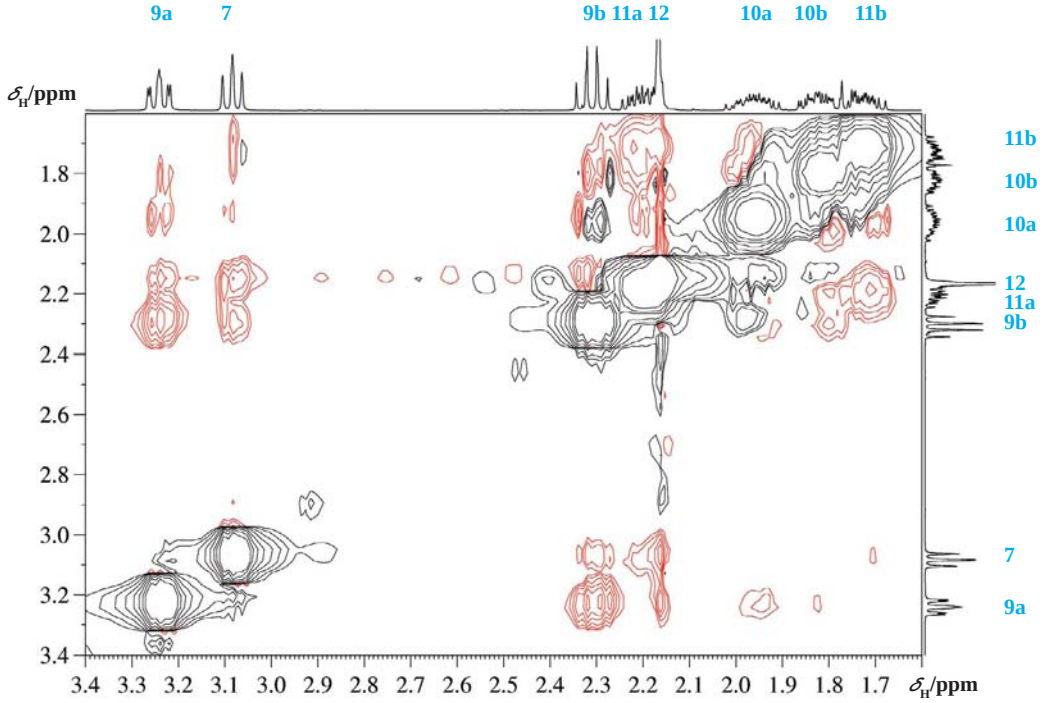


Fig.1.1-28 Expansion of the aliphatic region of the NOESY spectrum

In the aliphatic part H-7 has only one NOE contact to the proton at 2.3 ppm of the methylene group at C-9 and not to the other H-9 at 3.25 ppm. This determines the assignment of H-9b in our formula at 2.3 ppm sitting on the same side of the five-membered ring as H-7. Similarly, H-7 displays an NOE contact to H-11b at 1.7 ppm but not to the other H-11 at 2.2 ppm, and this determines the signal H-11b on the same side of the pyrrolidine ring as H-7. H-9a shows a stronger NOE contact to the signal of H-10a at 1.9 ppm than to H-10b at 1.8 ppm.

Fig. 1.1-29 The Catalan J. Partagás Ravelo established of one of the most famous cigar factories in Havana in 1845. He made *Havanas* a legend by developing a fermentation process which subjected the air-dried leaves from the field to a 60-day fermentation process in wooden barrels under secret conditions. The factory is still working under the original conditions and any visitor is really soaked with different kinds of tobacco flavours on passing through the floors of the building and admiring the sophistication of this handicraft

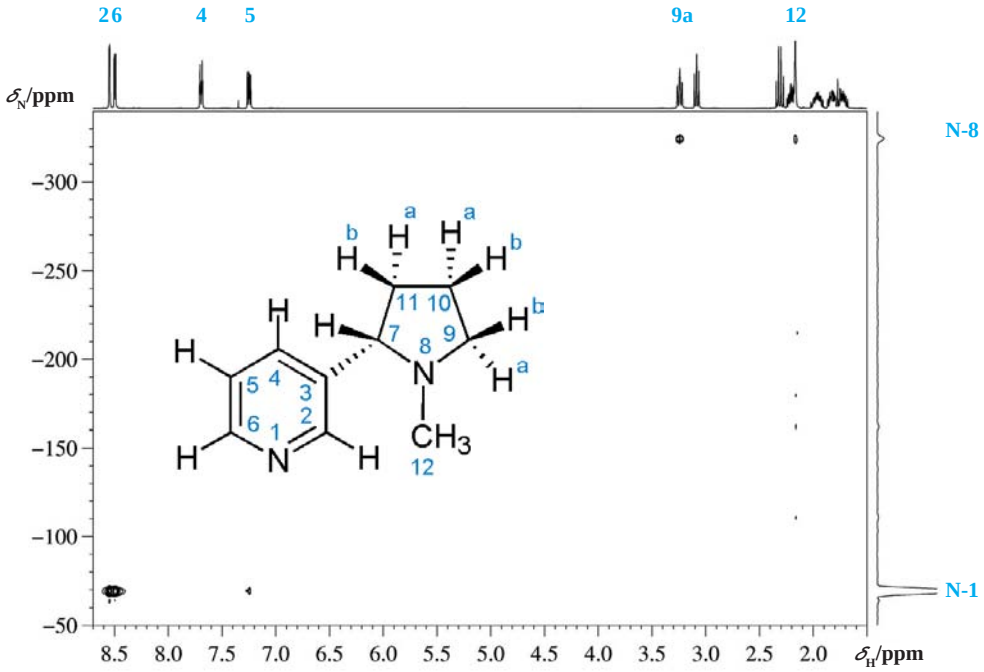


Fig. 1.1-30 $^{1}H^{15}N$ HSQC spectrum

Finally, in the $^{1}H^{15}N$ HSCQ spectrum, the two types of nitrogen atoms with their typical chemical shifts are easily identified by their cross peaks to the aliphatic and aromatic protons. It is of stereochemical interest that for N-8 cross peaks are displayed to H-9a and to the methyl group, but not to H-7 and H-9b.

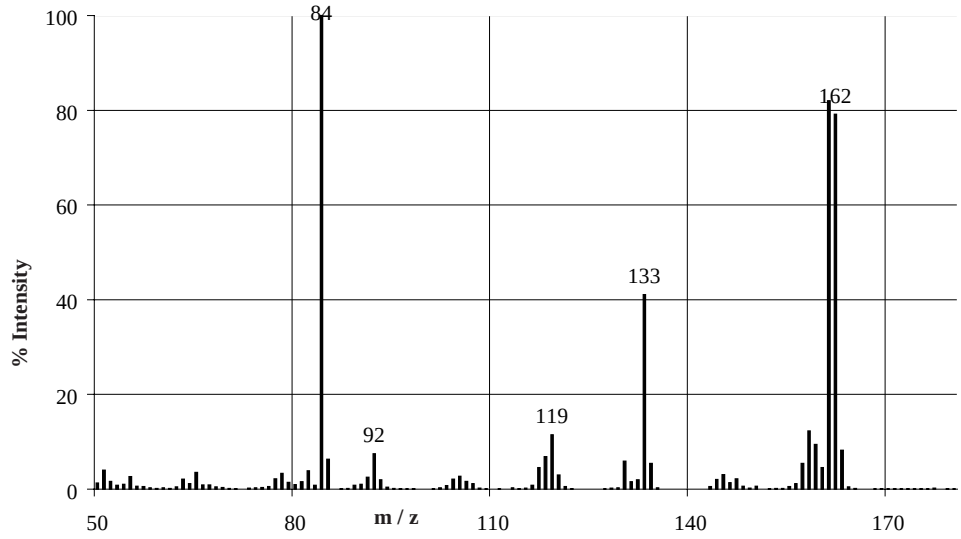
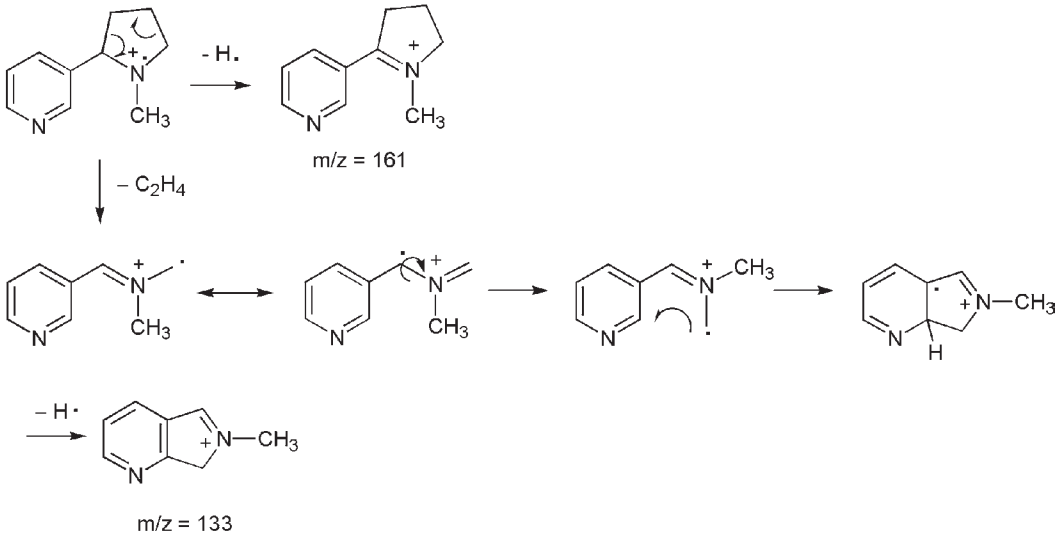


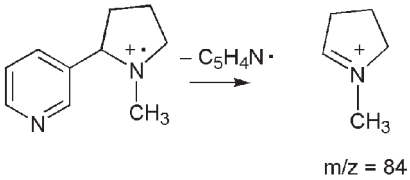
Fig. 1.1-31 Mass spectrum (EI)

In the mass spectrum one observes a very significant M–1 signal which has been shown by the analysis of deuterated derivatives to stem 40% from the hydrogen of C-7 and also from C-9 (35%) and C-10 (10%) after ionization at the pyrrolidine nitrogen and subsequent α-cleavage. The signal at *m*/*z* = 133 has been shown to be created by a two-step process:



Scheme 1.1-4 Fragmentation of nicotine

Ethene is formed from C-10 and C-11 via a process analogous to the retro-Diels–Alder reaction. A subsequent ring closure forms a bicyclic species which loses hydrogen. The base peak of the mass spectrum at m/z = 84 results from the bond cleavage between the two heterocyclic rings.



Scheme 1.1-5 Base peak

^{13}C Signals δ/ ppm	Type of Carbon	Assignment	Proton Signals δ/ ppm, J / Hz
149.6	CH	C-2	8.54, ^4J (H_2, H_4) = 1.9 ^5J (H_2, H_5) = 0.4
148.7	CH	C-6	8.49, ^3J (H_6, H_5) = 4.8 ^4J (H_6, H_5) = 1.8
138.8	C_q	C-3	
134.8	CH	C-4	7.69 ^3J (H_4, H_5) = 7.9
123.6	CH	C-5	7.26
68.9	CH	C-7	3.08
57.0	CH_2	C-9	9a: 3.25, 9b: 2.3
40.4	CH_3	C-12	2.17
35.3	CH_2	C-11	11a: 2.2, 11b: 1.7
22.6	CH_2	C-10	10a: 1.9, 10b: 1.8

Table 1.1-1 NMR data for nicotine

5. Questions

A. Where do you expect the most basic centre in nicotine and why?

B. The prefix “nor” has a general meaning in organic nomenclature. What is it? So, what compound is nornicotine - which is also a natural product? Give its name.

C. What do you expect to be the biological sense of nicotine for the plant itself obtained by the tobacco plant during its evolution?

D. In the 19th century smoking cigars was popular, whereas cigarettes are regarded as an invention of the in general accelerated society of the 20th century. Looking in old books with household hints for the “perfect housewife” you will certainly find advice on how to prepare tobacco juice from tobacco remainders or cheroots. What was the purpose of tobacco juice?

E. What special requirement for the soil will a tobacco plant have in general if you think of its alkaloid content? Some tobacco varieties such as Burley contain up to 4% nicotine, and the Russian Machorka known from the older Russian poetry even up to 7.5%. What do you expect to be necessary from the viewpoint of a tobacco farmer for such plants?

F. What do you expect from a comparison of the UV spectra of pyridine and nicotine?

G. Interpret the multiplet patterns seen in the expansion of the gated decoupled ^{13}C NMR spectrum with the help of a spin simulation program.

H. In the $^{1}H^{15}N$ HSQC spectrum H-9a shows a cross peak to the nitrogen, but not H-7. Explain.

I. Why have the methylene protons of C-9 the largest diastereotopicity?

J. Typically, *N*-methyl groups resonate between 2.5 and 3 ppm. In this molecule the methyl protons have a chemical shift of 2.2 ppm. Explain.

K. Suggest an NMR method to prove the absolute configuration of nicotine.

L. Suggest a structure for the ion with m/z = 119.

6. Own Observations

1.2 Caffeine

1,3,7-Trimethyl-3,7-dihydro-1*H*-purine-2,6-dione

From green tea leaves
Camellia sinensis L. (Theaceae)

or from green coffee beans
Coffea arabica L. (Rubiaceae)

$C_8H_{10}N_4O_2$, MW 194.19

CAS RN 58-08-2, BRN 17705

Colourless needles
mp 233−235 °C (Fischer cuvette)

Caffeine is commercially available.

Synonymous names:
1,3,7-Trimethylxanthine, Theine, Guaranine, Mateine

Level: easy

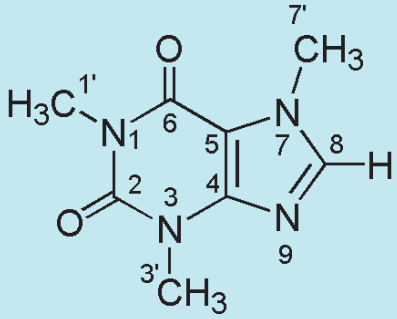


Fig. 1.2-1 Japanese green tea

Fig. 1.2-2 Yerba Mate − this picture shows a package of 500 g of Mate leaves produced in Argentina together with two traditional drinking vessels, a clay mug covered with ox-hide (left), typical for Argentina, and a calabash mounted with brass (right), typical for Chile. In any case, the hot or cold beverage made by infusion with water is sucked in over the *bombilla*, i.e. a tube with a sieve at the end. Drinking mate is common in South America

Fig. 1.2-3 Dried green tea leaves

1. Background: How a poet pushed a chemist

According to an arabic legend, a flock of goats in the region of *Kaffa* attracted attention by their intensive overnight activity. Searching for the reason for this unusual behaviour, people found it in their feeding of leaves and fruits of wild coffee shrubs. Both Abyssinia Christians and Muslims claim to have invented the custom of roasting the beans and producing a drinkable hot extract. Coffee as a beverage influencing mind and mood was not generally accepted from the beginning by some of the sovereigns but eventually any opposition was overcome. It is said that the argument that coffee keeps the faithful awake during religious ceremonies convinced those who were in doubt of the advantages. If not true, this seems at least to be a good story.

From Abyssinia coffee spread inexorably to the North to Mecca (1511), Constantinople (1530), Damascus (1544), Aleppo (1573) and Cairo (1580). The first European botanical description comes from a French physician P. Alpin from Padua as early as 1580. Drinking coffee became popular in Italy around 1645, reached London in 1652, France in 1658, and Germany in 1694.

The first coffee plantations around the world were founded by the Dutch, who brought coffee beans from *Mokka* to Java in 1690. Cultivation was so successful that the Botanical Garden in Amsterdam had a colony of coffee trees in 1710 and a tree with ripe fruits was presented to the French monarch Louis XIV as an exotic gift in 1714. Around 1720 the first coffee plants were shipped to Martinique in the West Indies [1].

Certainly, the rituals developed around making tea and coffee belong to the most sophisticated ones. Think of the Japanese tea ceremony, the British institution of five o'clock tea, the hundreds of ways to make a real coffee (all are correct), or the rich Austrian coffee culture. It developed partly based upon coffee sacks which Turkish troops left behind in their camp when they were forced to stop the siege of Vienna in 1683. Further, think of the challenging preparation of a real Italian espresso. Industries have developed around the latter to be able to supply you with impressive machinery which is able to produce a few millilitres of a complex extract, reliably and absolutely reproducibly.

All of these rituals are aimed at a finely balanced equilibrium of a relaxating and stimulating break in daily life. And it is caffeine which is responsible for the rush part. Occurring both in tea leaves (up to 5%) and coffee beans (up to 1.5%), there is no difference between *theine* from tea and *caffeine* from coffee [2], they are identical. Other sources are mate tea (yerba mate) (ca. 1%), guarana, kola nuts and cocoa beans (0.2%).

Tea plants, growing as evergreen shrubs, have their natural origin in Assam on the slopes of the Himalayas. Coffee trees (*Coffea arabica* and other species) come from the Ethiopian province of Kaffa. It is a separate and exciting story how tea and coffee plants have been distributed over the world and how they have influenced the culture of civilization around the globe [3]. Not by chance, the oldest coffee

house, “Zum Arabischen Coffe Baum” in Germany (founded in 1694), is located in Leipzig (where this book has been written) – it was just a town of fairs, trade and merchants for centuries, and once a day a sack of coffee must have been an amazing novelty.

Within the *alkaloids* (see section 1.1 on nicotine for this term), caffeine belongs to the xanthine family, as do theobromine and theophylline, all related to the purine skeleton. As is typical for any *alkaloid* isolation, caffeine as a weak base has to be set free before extraction from the plant material by a stronger inorganic base, i.e. by an *alkaline* influence.

However, did you know that it was a poet who gave the impetus to deal with the “active principle” of coffee to a chemist? Indeed, the famous German poet J. W. von Goethe, a man with very broad interests also in science (metamorphosis of creatures, light, colour, minerals, geology – to name just a few) encouraged the young chemist F. F. Runge in 1819 to analyse coffee. For that purpose, Goethe gave him a small box with green coffee beans as a present, a very valuable gift at that time. Runge had already proved his extraordinary talent working on the mydriatic effect of belladonna poison on cats’ eyes. Goethe did not exclude that Runge might be able to discover a belladonna antidote in his coffee. Although this did not come true finally, Runge was successful in the isolation of caffeine by sublimation from the green beans as a “coffee-base” in 1820 and this is one of the methods described later in this chapter [4]. Elucidating the structure took a little longer; in 1873 even the opinion on the molecular formula of caffeine was still that it would be $C_{16}H_{10}N_4O_4$ [1].

In exercises, caffeine is isolated from tea leaves and not from real coffee powder, for a simple reason. Coffee powder would be clearly a more complex starting material due to the roasting process, which produces some hundreds of decomposition and transformation products from the green coffee beans as the carriers of the typical coffee taste and scent. Astonishingly, despite the roasting procedure which produces a multitude of products that have not been completely elucidated yet, coffee is not regarded as chemically dangerous to health, in contrast to tobacco or too much alcohol. Obviously, the test running for some hundred years with the public has not given cause for serious concerns in this direction. To avoid the effort of separating caffeine from the complex coffee source, many similar methods describe its isolation from black tea leaves. However, we recommend not doing that, but instead isolating it directly from green tea leaves. If you compare green and black tea, it is obvious that green tea is closer to the plant’s leaves than black tea, which after harvesting is subjected to fermentation with the intention of causing chemical changes. Thereby, new products are formed which we appreciate for their contributions to colour, scent and taste. However, they make the isolation of the alkaloid more difficult: often isolations from black tea leaves include a lead salt precipitation of the tannins. In contrast, green tea is only subjected to steaming, which preserves the original colour. For this reason, the very straightforward isolation from green tea reported by Japanese authors in 1996 [5] has been adopted for this book. Moreover, we thought, why should we

Fig. 1.2-4 Old German household coffee mill

Fig. 1.2-5 Coffee shrub at the end of the dry season in April in Cuba

Fig. 1.2-6 Roasted coffee for espresso

Fig. 1.2-7 Coffee tree in a botanic garden

Conquerebatur enim quidam Camelorum, seu ut alii aiunt, Caprarum Custos, ut communis Orientaliu sert traditio, cum Monachis cuiusda Monasterij, in Ayaman regione, quae est Arabia felix, sua armenta non semel in hebdomana vigilare, imo per totam nostrem, praeter consuetum saltitare; Illius Monasterij Prior curiositate ductus, hoc ex pascuis provenire arbitratus est, & attente considerans una cum eius socio locum ubi Caprae, vel Cameli illa nocte, qua saltitabant pascebantur, invenit ibi queadam arbuscula, quorum fructibus, seu potius baccis vescebantur; huiusce fructus virtutes voluit ipsemet experiri, ideoque illos in aqua ebulliens statim illorum potum noctu vigilantem excitare expertus est.

Faustus Naironius Banesius (1671)
De saluberrima potione Cahve

Fig. 1.2-8 Ripe fruits on a coffee tree

not behave as the young Runge and isolate caffeine by sublimation from green beans? Hence, in our second isolation method we have tried to re-invent Runge's procedure. Pure caffeine is a product of commercial importance, accessible by isolation or synthesis. Caffeine-free coffee is obtained by destraction, a supercritical fluid extraction (SFE) process using supercritical carbon dioxide as a selective solvent for decaffeination [6] under comparatively mild conditions conserving all other constituents. Caffeine, natural or synthetic, is used for pharmaceutical preparations. The main physiological effects are: stimulation of the central nervous system; positive psychotropic effects (buzz or rush expected by consumers); stimulation of heart rate and respiration; diuretic effect. The mode of action is well understood at the molecular level and consists in blocking of adenosine receptors and increasing levels of the hormone adrenaline and the neurotransmitter dopamine. Above a certain blood level caffeine has been put on the doping list of substances.

Overdose and abuse are to be avoided. They may cause intoxication and have even led to death. The lethal dosage LD_{50} is from ca. 10 g up for an average adult. Although continued consumption causes tolerance, one may feel the desire for some caffeine if one has to abstain from it for other reasons. Certainly, this will have been true for Balzac who is reported to have had up to 60 cups of coffee per day; 60 was an important number for Beethoven, too: he used to count exactly 60 beans to make one cup of coffee.

Finally, why do plants make caffeine? To equip an alkaloid with as many as four atoms of N, which is clearly a harder to get element for plant biosynthesis than C, H or O, must have a purpose from the viewpoint of the plant, which cannot waste its nitrogen. Caffeine is regarded as a chemical defence of the plant to paralyse, deter, poison or even kill insecticidal plant pests. But caution! With such a toxic compound, the plant has to take its own precautions: therefore, caffeine is stored as a pre-infectional ready for use weapon in the vacuole, a special cell compartment suitable for the plant-safe storage of aqueous solutions.

2. Literature

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Fig. 1.2-9 Roasted coffee contains considerably less caffeine than green coffee.

3. Isolation

3.1 Principle

For method 1: Caffeine is set free from the plant material by a dilute NaOH solution, extracted with dichloromethane and recrystallized from isopropanol.

For method 2: Caffeine is set free by sublimation from dried and ground green coffee beans on controlled heating at 220 °C. Commercial roasted coffee powder is not an eligible substitute.

Note some chemical peculiarities on the isolation of caffeine:

1. Recrystallization is not an easy task, because it is readily soluble not only in water but also in many organic solvents. A minute amount of isopropanol is sufficient to obtain fine long needles of colourless caffeine.

2. It is impossible to take a correct melting point under ambient conditions because at ca. 175 °C the compound sublimes without forming a liquid phase. Therefore, melting of caffeine crystals can only be brought about by enclosing a few crystals in a tiny flat micro-ampoule (Fischer cuvette) and heating this sealed micro-vessel. Only, in this way, under its own vapour pressure, do caffeine needles melt.

3. To be sublimable is a rather rare property for an organic compound. However, if it exists, it opens the chance to achieve an amazingly simple removal of just a single compound from a complex mixture by solid−gas−solid phase transfer. Caffeine is an ideal example. Sublimation may be done with or without vacuum. In this case, ambient pressure is suitable. The heating procedure used here leads to a kind of roasting, as becomes obvious from the dark discoloration of the coffee powder. The evaporation of some liquid constituents can be observed. In comparison experiments we found that caffeine sublimes only from ground green coffee beans and not from roasted beans. In conclusion, on roasting of coffee a certain amount of the original caffeine is surely lost by sublimation and a certain amount is held tight in the coffee powder; only this part can be subjected to hot water extraction to obtain coffee.

The coffee-maker was almost ready to bubble. I turned the flame low and watched the water rise. It hung a little at the bottom of the glass tube. I turned the flame up just enough to get it over the hump and then turned it low again quickly. I stirred the coffee and covered it. I set my timer for three minutes. Very methodical guy, Marlowe. Nothing must interfere with his coffee technique. Not even a gun in the hand of a desperate character.

Raymond Chandler (1888−1959)
The Long Good-Bye

Fig. 1.2-10 Vietnamese green coffee beans

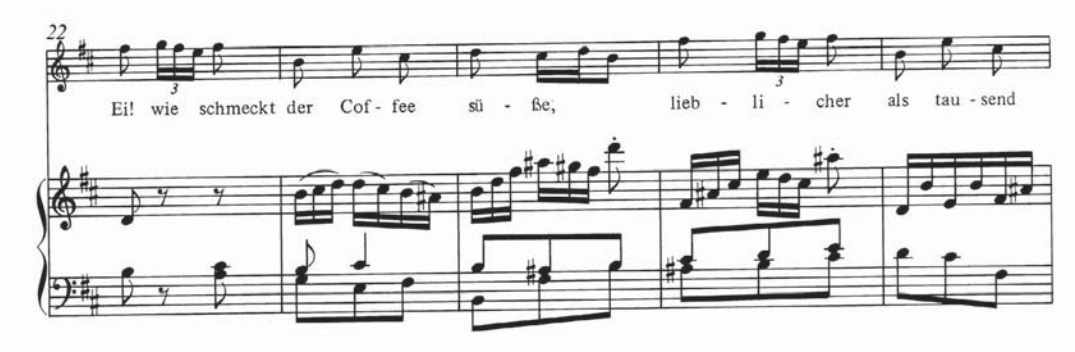


Fig. 1.2-11 The Coffee Cantata

Fig. 1.2-12 J. S. Bach
"Ei! wie schmeckt der Coffee süße,
Lieblicher als tausend Küsse,
Milder als Muskatenwein.
Coffee, Coffee muß ich haben,
Und wenn jemand mich will laben,
Ach, so schenkt mir Coffee ein!"

Johann Sebastian Bach (1685–1750)
The Coffee Cantata BMV 211

Fig. 1.2-13 Oldest German coffee house in Leipzig

3.2 Methods

Method 1

Green tea leaves (4 g) are placed in a 100 mL Erlenmeyer flask together with dichloromethane (30 mL) and 0.2 M NaOH (10 mL). The flask is closed with a stopper and the mixture carefully shaken at a rate excluding formation of an emulsion. Tea leaves are then removed by gravitational filtration and washed with dichloromethane (30 mL) that is added to the filtrate, which consists of a dark aqueous and a nearly colourless organic phase. Filtration without suction is a good means to obtain two separate phases. The organic phase is separated using a separating funnel or a pipette and the solvent is removed in vacuo. The crude caffeine remaining in the flask is carefully washed (3 × 1 mL) with a mixture of petrol ether–diethyl ether (1:1, v/v). The solvent turns green and the caffeine becomes pale grey. Each washing is decanted from the alkaloid. Finally, the caffeine remaining is dissolved in dichloromethane (1 mL), sucked into a Pasteur pipette and squeezed through a tiny pad of cotton wool placed in front of the tip of the pipette into a 5 mL flask. The pad is washed with CH_2Cl_2 (2 × 0.5 mL). The solvent is removed and crude caffeine of green colour is obtained.

Method 2

Green coffee beans (25 g) are ground to a powder and dried in an oven for 5 h at 100 °C to remove as much water as possible. A 15 g amount of the dried powder is then placed in a wide-necked 300 mL Erlenmeyer flask. Use a flask equipped with a large 45 mm top joint instead of the 29 mm standard, preferably, whenever possible. The flask is equipped with a reduction adapter (bottom joint 45 mm, top joint 29 mm) and a water-cooled sublimation head. The apparatus is immersed about 5 cm deep into a silicone oil bath with the temperature controlled at 220 °C for 2 h. On heating, the coffee slowly turns brown and vapours are set free and partly condense at the sublimation finger in the form of colourless droplets. Each 15 min, the sublimation finger is removed carefully and these droplets are wiped off before the finger is reset. Caffeine needles are formed not at this sublimation head unit but at that part of the inner wall of the Erlenmeyer flask which is just above

the surface of the oil bath. Sometimes a chaplet of needles forms at the bottom of the reduction adapter (see Fig. 1.2-14). Such needles are pure enough to obtain NMR spectra without additional signals and also pure according to TLC.

3.3 Purification

Method 1

The crude caffeine is dissolved in 2–3 mL of isopropanol under reflux. On standing, pure caffeine crystallizes in the form of tiny thin needles. Finally, the flask is put into ice-water to complete crystallization. The needles are filtered off and washed with the petrol ether–diethyl ether mixture as above (2 × 1 mL) whereupon the caffeine becomes colourless.

Yield: 20 mg, mp 233–235 °C (Fischer cuvette).

For a TLC check of the purity we used as an eluent $CHCl_3$–MeOH (9:1, v/v) with silica gel 60_{F254} plates. On UV 254 nm detection caffeine is visible as a yellow spot, R_f = 0.5. This method was adapted from the literature [5].

Method 2

Caffeine deposited as colourless needles on the inner vessel wall should be wiped off with a piece of cellulose. Note: a layer of sticky brownish vapour deposit above the caffeine may be formed and should be removed by wiping off with acetone-tinctured cotton before harvesting the caffeine needles in the same manner. Caffeine removed in this way is extracted from the cotton with boiling acetone (15 mL). The acetone extract is concentrated to dryness in vacuo. The residue remaining is recrystallized from 2 mL of isopropanol to yield caffeine needles on standing. Yield: 50–60 mg, mp as above.

4. Spectra and Comments

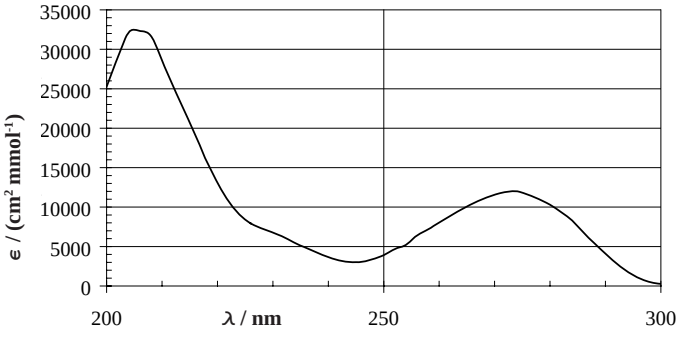


Fig. 1.2-16 UV spectrum in ethanol

The electronic system of caffeine can be regarded approximately as the combination of an imidazole system condensed with one urea unit and an additional C=O group. Therefore the UV spectrum reflects this situation.

"Nachdem Goethe mir seine größte Zufriedenheit ... ausgesprochen, übergab er mir noch eine Schachtel mit Kaffeeebohnen, die ein Grieche ihm als etwas ganz Vorzügliches gesandt. "Auch diese können Sie zu Ihren Untersuchungen brauchen!" sagte Goethe. - Er hatte recht, denn bald darauf entdeckte ich darin das wegen seines großen Stickstoffgehalts so berühmt gewordene "Coffein"."

F. F. Runge
Hauswirthschaftliche Briefe, #36
Mein Besuch bei Goethe im Jahre 1819

Fig. 1.2-14 Caffeine needles after sublimation

Fig. 1.2-15 A crop of 320 mg of caffeine isolated from green tea leaves according to the method described

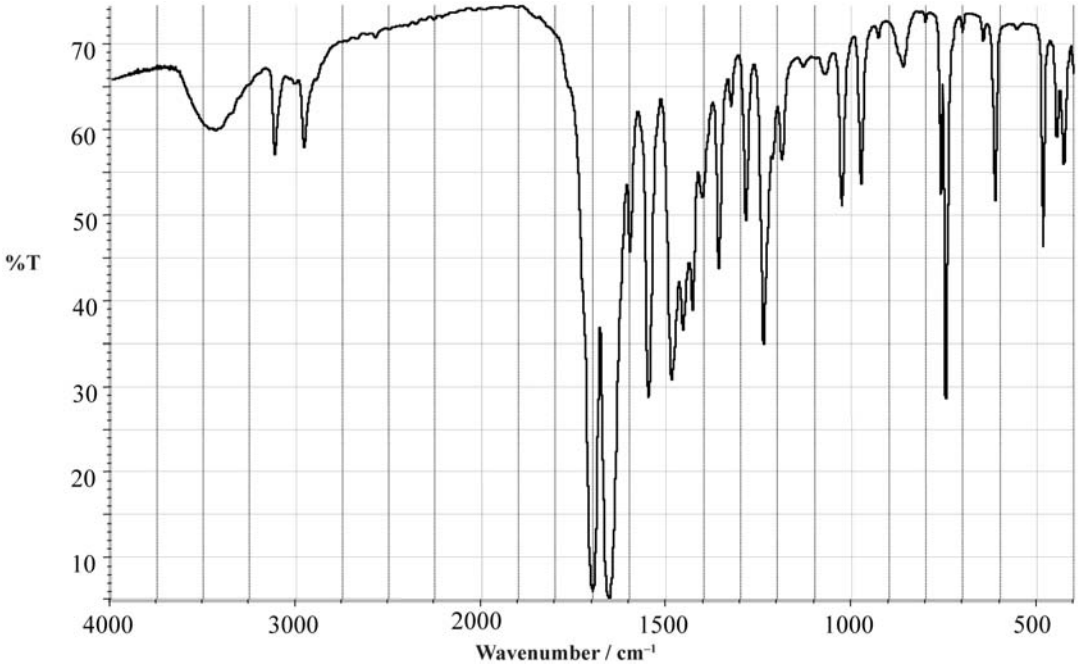


Fig. 1.2-17 IR spectrum in KBr

In the IR spectrum, recorded in KBr, some residual humidity can be detected which is also present in the ^{1}H NMR spectrum. Very clear is the separation of sp^3- and sp^2-CH valence vibrations; also the two C=O valence bands are nicely separated.

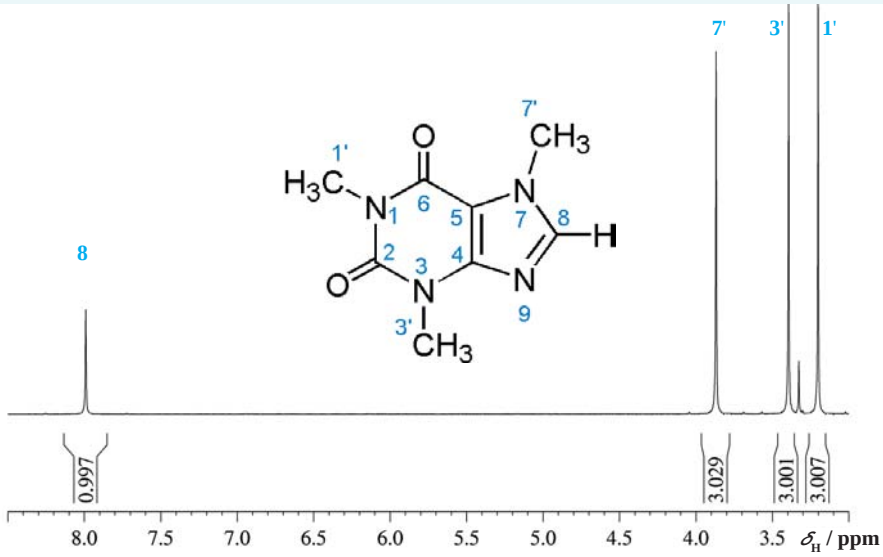


Fig. 1.2-18 ^{1}H NMR spectrum at 400 MHz in DMSO-d_6

Although the ^{1}H NMR spectrum looks very simple, the correct and safe assignment of all the signals is difficult, since no spin splittings can be observed. Of course, the proton signal for the single olefinic H-8 at 8.0 ppm can be directly assigned. A COSY spectrum is not shown, because of the lack of spin-spin couplings.

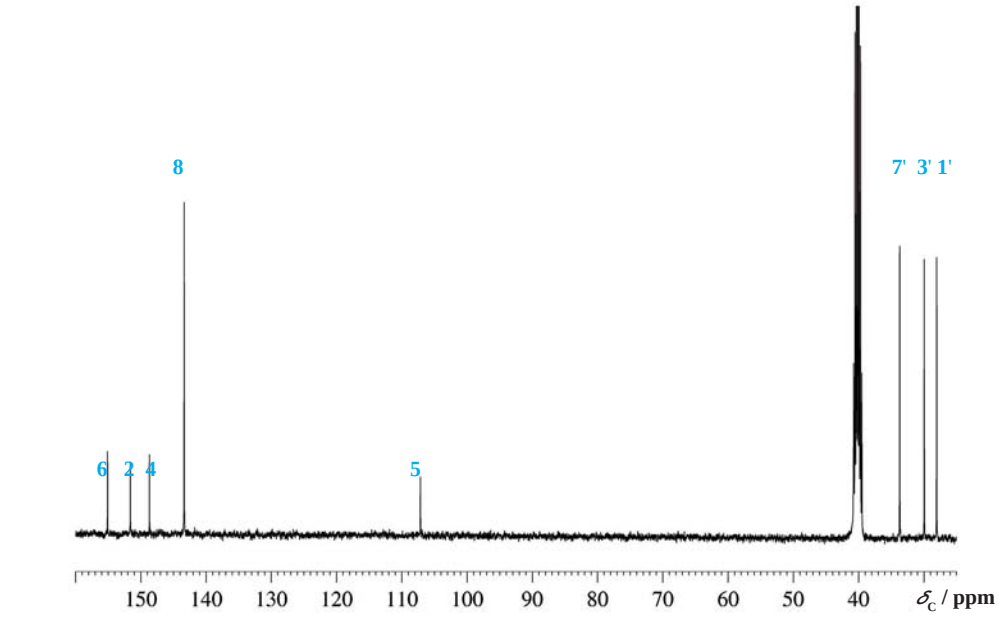


Fig. 1.2-19 ^{13}C NMR spectrum at 100 MHz in DMSO-d_6

Like the proton spectrum, the ^{13}C NMR spectrum looks very simple. However, only C-8 (143 ppm) can be directly assigned due to its intensity.

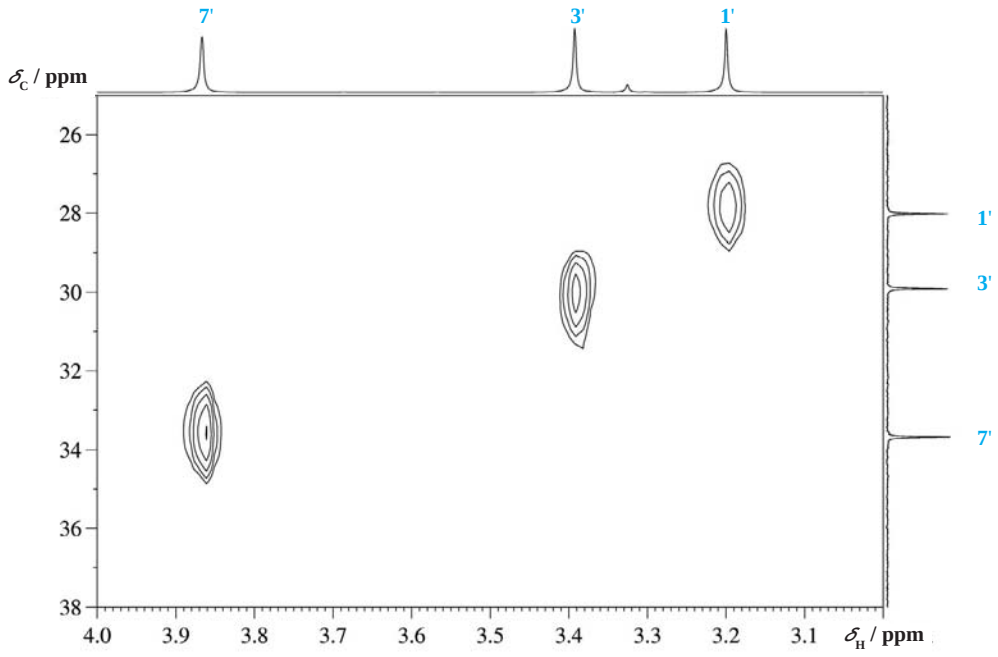


Fig. 1.2-20 Expansion of the HSQC spectrum in the aliphatic region

The expansion of the HSQC spectrum shows that the chemical shift order of the proton signals for the methyl groups in this compound is the same as for ^{13}C.

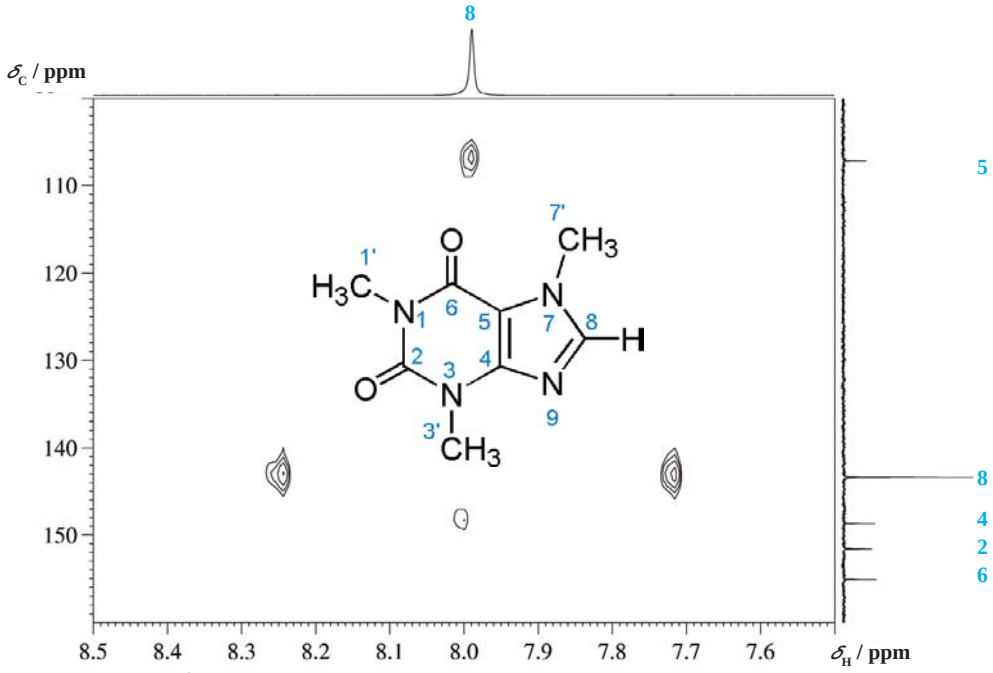


Fig. 1.2-21 Expansion of the HMBC spectrum in the aromatic region

In the first expansion of the HMBC spectrum we see a breakthrough of the ^1J(C,H) of the imidazole proton H-8, which is due to its unusually large value of 210 Hz. Therefore, the low pass filter used is not sufficient to suppress these signals. The key point of the further assignment, however, is the two long-range correlations of this proton seen to the carbon signals at 107.2 and 148.7 ppm. One has to establish which of these two signals belong to C-4 and C-5.

Fig. 1.2-22 Flowering coffee shrub with first green beans

Fig. 1.2-24 A tea shrub on a rainy day

Fig. 1.2-23 Sign found in the Botanic Garden of Brooklyn

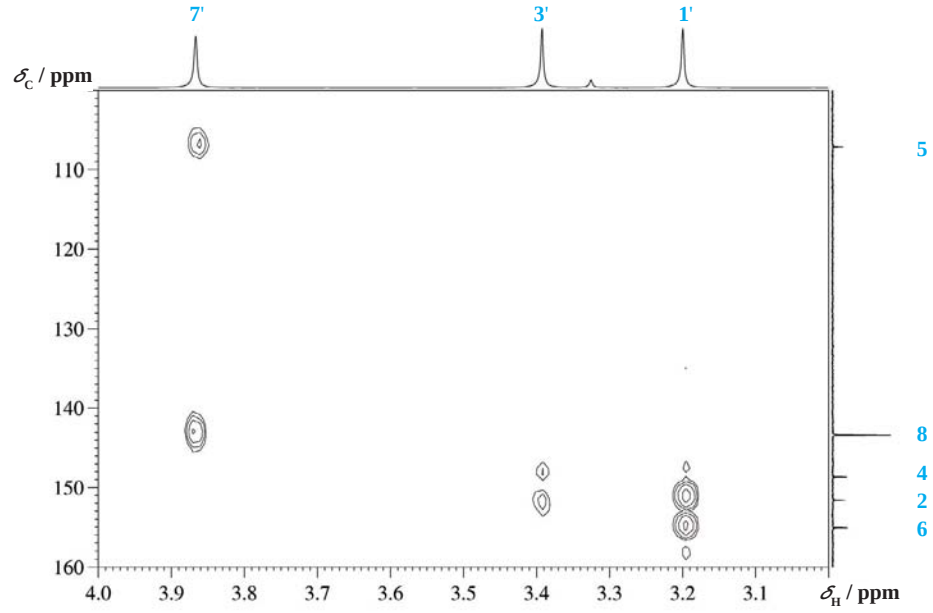


Fig. 1.2-25 Expansion of the HMBC spectrum in the carbonyl region

One observes that the proton signal of the methyl group at 3.4 ppm is connected to the carbon atom at 148.7 ppm and to a C=O carbon at 151 ppm, whereas the methyl group signal at 3.2 ppm is connected with two carbonyl signals. Thus, once again, the HMBC technique proves to be the most important in signal assignment and structural elucidation.

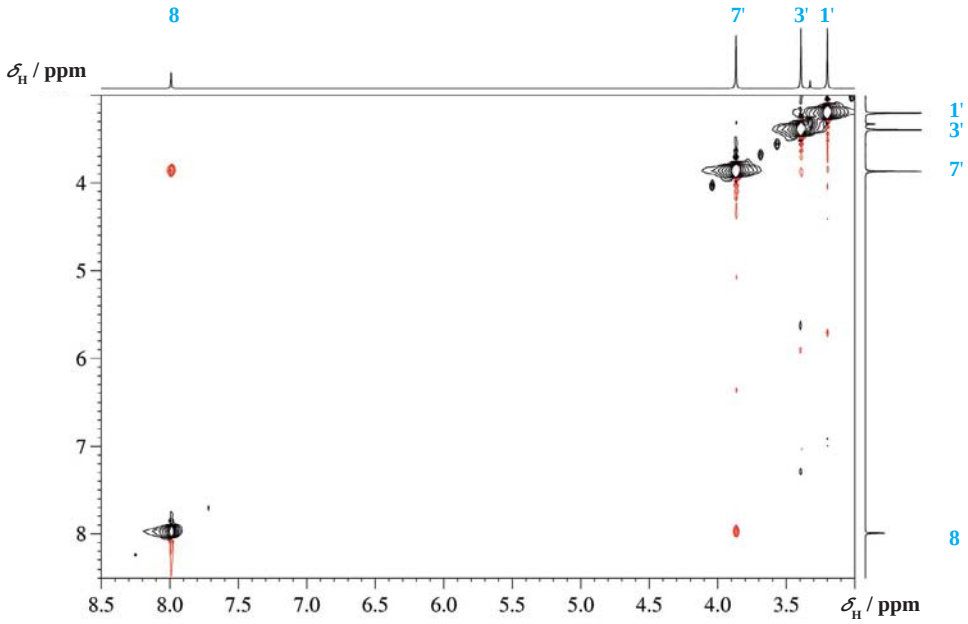


Fig. 1.2-26 NOESY spectrum connecting the aromatic with the aliphatic region

The NOESY spectrum shows clearly which signal belongs to the methyl group C-7' and this was already corroborated by the expansion of the HMBC spectrum.

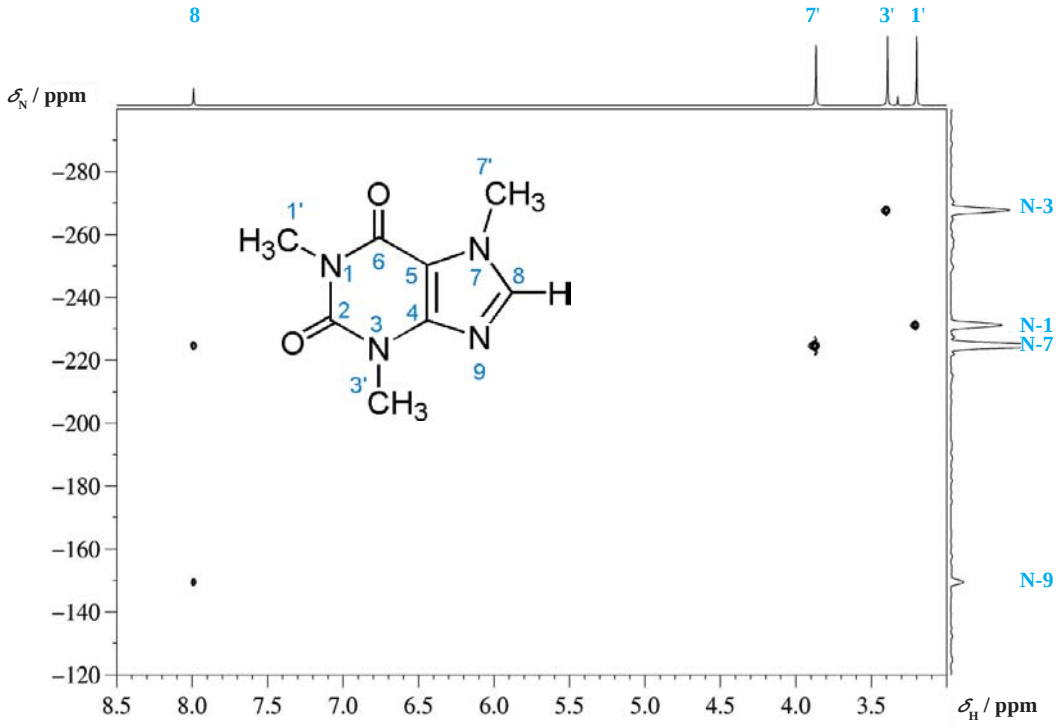


Fig. 1.2-27 ^{1}H ^{15}N HMQC spectrum

Since caffeine contains four quaternary nitrogen atoms, it is a valuable task of current NMR spectroscopy to obtain their chemical shift values. This can be done via the inverse $^{1}H^{15}N$ correlation spectrum shown, which nicely confirms the assignment for the methyl groups given above. However, there is no high-resolution 1D ^{15}N NMR spectrum plotted at the right side of the 2D graph, since this is very difficult to obtain. The chemical shifts of the four nitrogen atoms are given with respect to nitromethane as a reference and clearly reflect their electronic situation.

Bien loin d'être nuisibles, le café et le thé, pris même en abondance, mais pourtant sans excès (eh! quel excès n'est pas nuisible), sont très-salutaires. Au moins les Allemands leur doivent-ils un avantage fort précieux, et qui à lui seul mérite une très-grande reconnoissance. Ces boissons ont tempéré plus efficacement en Allemagne le vice de l'ivrognerie , que les leçons des moralistes et des théologiens, et même que le progrès des lettres et l'instruction.

Honoré Gabriel Riqueti, Comte de Mirabeau (1749–1791)
De la Monarchie Prussienne, sous Frédéric le Grand

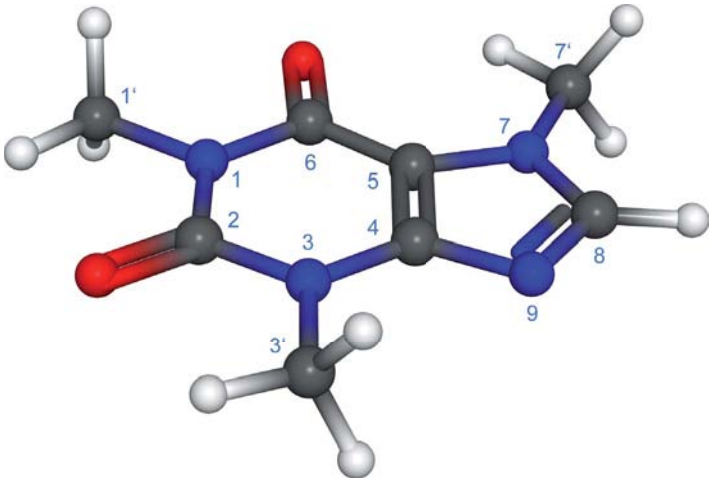


Fig. 1.2-28 Molecular model of caffeine

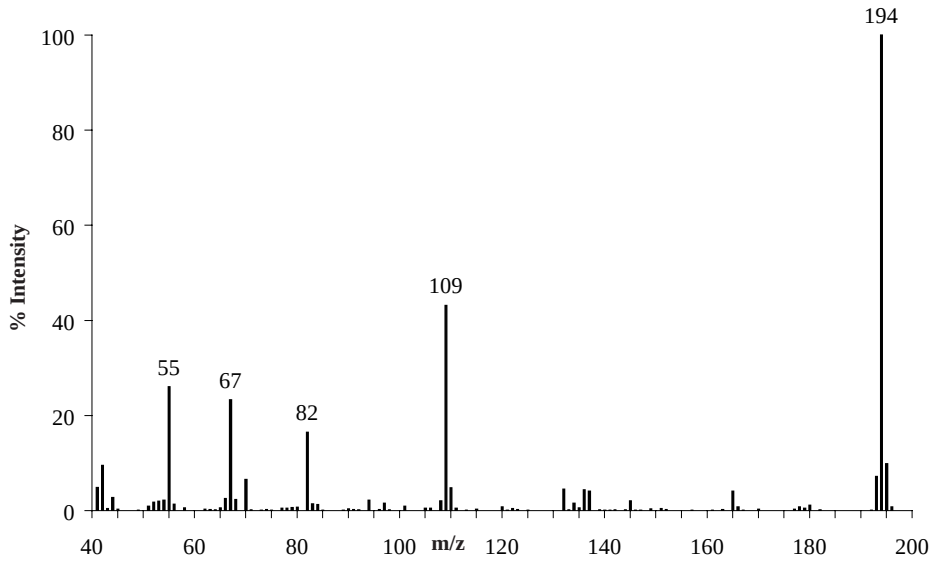
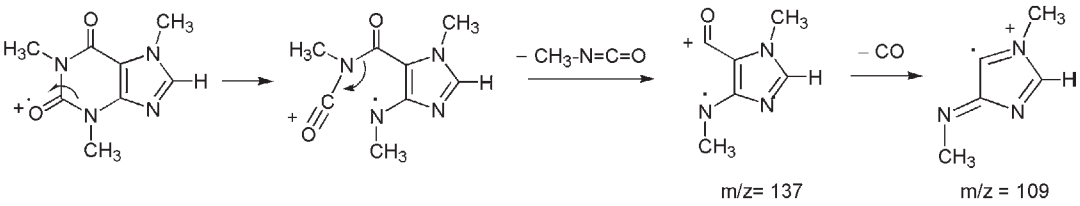


Fig. 1.2-29 Mass spectrum (EI)

In the mass spectrum, the molecular ion peak forms the base signal which is often found for such heterocycles. The spectrum is rather empty and does not show many characteristic fragments. It is very similar to that of theobromine and shows the same fragment ions. The dominant fragment ion with m/z = 109 can be explained by elimination of CH_3NCO and subsequently of CO:



Scheme 1.2-1 Fragmentation of caffeine

^{13}C Signals δ / ppm	Type of Carbon	Assign-ment	Proton Signals δ / ppm, J / Hz	^{15}N Signals δ / ppm
155.1	C_q	C-6		
151.6	C_q	C-2		
148.7	C_q	C-4		
143.3	CH	C-8	8.0	
107.2	C_q	C-5		
33.7	CH_3	C-7'	3.87	N-7 –224.7
29.9	CH_3	C-3'	3.39	N-3 –267.7
28.0	CH_3	C-1'	3.2	N-1 –231,4
				N-9 –149.6

Table 1.2-1 NMR data for caffeine

Fig. 1.2-30 Tea from Europe? Not impossible! Tea plantation at Porto Formosa on the north coast of San Miguel, Azores, a part of Portugal

5. Questions

A. Why is caffeine readily soluble in water (20 g/L at 20 °C)?

B. Draw the structures of the heterocycles xanthine and purine and the structures of the natural products uric acid, adenine, guanine and theophylline.

C. Birds and reptiles excrete the ammonium salt of uric acid in a nearly solid form (main guano constituent!). Make a proposal as to which biochemical function is fulfilled in this way.

D. Compare the UV spectrum with those of imidazole and urea.

E. Assign the two IR bands at 1650 and 1700 cm^{-1} to the two carbonyl groups.

F. According to the ^{13}C NMR spectrum, the C_4=C_5 double bond is highly polarized. Discuss which of the two carbons is the more shielded one and draw resonance structures to explain its chemical shift.

G. Assign all carbon and proton signals using the information given in the HMBC spectrum. Finally assign the four nitrogen signals. Is their relative order in line with what you expect from the estimated electron density at these nitrogen atoms?

H. Suggest why in the mass spectrum there is no significant M-15 peak, although the compound contains three methyl groups.

J. Many people react quite differently to caffeine from a cup of tea or coffee. Suggest an explanation.

6. Own Observations

1.3 Theobromine

3,7-Dihydro-3,7-dimethyl-1*H*-purin-2,6-dione

From cocoa of the cacao tree
Theobroma cacao L. (Sterculiaceae)

$C_7H_8N_4O_2$, MW 180.16

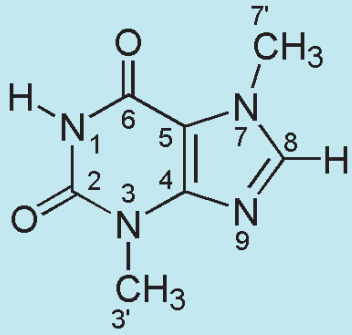
CAS RN 83-67-0, BRN 16464

Colourless crystals, mp 346−347 °C (Fischer cuvette)

Synonymous names:
3,7-Dimethylxanthine, Santheose, Theosalvose, Theostene, Thesal

Theobromine is commercially available.

Level: easy



1. Background: Deliver your tribute in beans

The emperor took no other beverage than the chocolatl, a potation of chocolate, flavoured with vanilla and other spices, and so prepared as to be reduced to a froth of the consistency of honey, which gradually dissolved in the mouth. This beverage, if so it could be called, was served in golden goblets, with spoons of the same metal or of tortoise-shell finely wrought. The emperor was exceedingly fond of it, to judge from the quantity − no less than fifty jars or pitchers − prepared for his own daily consumption. Two thousand more were allowed for that of his household.

William H. Prescott (1796−1859)
History of the Conquest of Mexico,
Book IV, Chapter I

Fig. 1.3-1 Cocoa pods are directly on the stem

Do you think that one can extract a natural product from a currency? If not, read this section. Theobromine is a purine alkaloid occurring in the seeds of the cacao tree, *Theobroma cacao*, growing in tropical America up to about 10 m in height. In Mexico, the tree has been cultivated for centuries and cocoa beans have been highly esteemed by the indigenous rulers, long before their empire was conquered by Cortéz and his troops in 1528. The word cacao comes from the word *kakawa* from the language of the Olmecs people and was already used around 1000 BC. Later, when the Aztecs established their empire in the 14th century, they called a cocoa bean *cacahuatl*. Further, also the Maya culture knew and used *kakaw* as a divine kind of food. The first Europeans who encountered cacao were Columbus and his crew in 1502. They captured a canoe with aborigines off the island of Guanaja in the Caribbean near Honduras. The odd “almonds” they saw in it did not offer them their secret, nor did Columbus acquire the traditional beverage from the Indians. Why should he?

The name *Theobroma* of the plant is derived from the Greek words *theo* for “god” and *broma* for “food”, hence the meaning “food of the gods” arises. This phrase is not an expression of eccentricity in naming plants from exotic countries. It has an unemotional reason in the rich nutritious properties of cocoa – we will report on it under isolation: more than 30% (!) of cocoa powder dissolves in hot water and can be digested. That means that cocoa is tasteful and very nutritious. What seems like a side effect for us was very important in former times. In catholic countries each year a period of fasting had to be observed in the course of the annual religious rituals. However, to be hungry for a long time is unpleasant in any religion. Therefore, substitutes for meat and the like have been sought. Calorie-rich beverages were the solution to this problem. The cultivation of fish in ponds of monasteries was another. Both strong beer and cocoa fulfilled the nutritional demands very well. However, European friars were not the first to discover this property. That cocoa is so substantial already made it a staple food in the pre-Columbian Mesoamerican civilizations. Also, chocolate, as a beverage, was not a European invention: it came from the New World in 1544 together with the Maya noble Kekchi, who was taken to the Spanish court by Dominicans to pay a courtesy visit to Prince Philip. From that time on, chocolate became a popular beverage, but not cheap and not easy accessible. Solid chocolate as a food was still unknown. It was an invention of Swiss and German confectioners at the beginning of the 19th century. In the 17th century, chocolate was still regarded as an aphrodisiac (test it, if you are in doubt) and sold in pharmacies as a tonic.

The name theobromine, for an alkaloid which does not contain a bromine atom at all, was just derived from the plant. This term was coined in 1842 when the alkaloid was first isolated from cocoa beans [1]. Cocoa powder from the present-day supermarket contains between 1 and 3% theobromine. The first synthesis of theobromine was achieved in 1882

from xanthine (3,7-dihydro-1*H*-purin-2,6-dione), the non-methylated stem purine base for caffeine, theophylline and theobromine [2].

The world cocoa harvest is about 3 000 000 tons per year. Cocoa grows in the hot and rainy areas of the tropics (e.g. Ivory Coast, Ghana, Indonesia, Brazil, Nigeria, Cameroon). It is noteworthy that the main growing area has been shifted from Central America to the tropical part of Africa. Three main cultivar groups are under cultivation: cocoa trees of the Criollo Group (10% only, best quality, a Maya-derived cultivar, very aromatic and only slightly bitter), the Forastero Group (80%) and Trinitario (10%), a hybrid of the other two. A cocoa tree shows the strange phenomenon of cauliflory, which means that a cocoa tree flowers and fruits directly from the stem (see Figs. 1.3-1 and 2) and not from new-grown branches or shoots. Ripe cocoa pods have a rugby ball-like shape with a leathery, hard, yellow skin. They are up to 20 cm in length and 500 g in weight. Inside are around 50 pale seeds lying within a white, slimy, sweet pulp. If you see for the first time a cocoa bean cut off it looks really strange and different from all other fruits. The beans inside are still far away from a cup of cocoa or a piece of chocolate. In ancient times, these seeds were a treasure and were treated as such. They were at the same time a sacrificial offering, a major currency system and an ingredient for a beverage. It is known that Spanish conquistadores under Cortéz on subduing the Aztecs found 25 000 hundredweight of cocoa beans in the treasury house of the last emperor Montezuma II. Loads with millions of cocoa beans belonged to the yearly tribute that had to be paid to him. It is of interest to compare this huge amount with the equivalent value of a slave: 100 cocoa beans only, which is just the content of two cocoa pods. A new cloth mantle was of the same price. It is handed down that also prostitutes in the Aztec empire were paid with cocoa beans.

However, their beverage *xocólatl*, which gave the name to our chocolate, was a mixture which we possibly would find not sweet enough. It consisted of water, cocoa, maize, vanilla and hot pepper. Indeed, *xocólatl* means “bitter water”. In a certain respect, with cocoa beans the conquistadores had conquered “brown gold”, accidentally. We will completely abstain from writing anything about the making of chocolate. Although interesting, this is too far away and others have done it, impressively [3,4]. However, the steps from crude cocoa beans to a cocoa mass suitable for further processing will be summarized because they are the link that converts a botanical subject into a real treat.

The raw cocoa beans taste too bitter, and not at all like chocolate. They undergo a long process, reminding the author of the journey that a tobacco leaf undergoes from the plantation to a cigar. Fermentation is the first step. Ripe cocoa pods are plucked by hand and opened, and the beans together with the slimy pulp are then allowed to ferment for about 10 days. During this process they acquire their brown colour and characteristic taste. The heat of fermentation by microorganisms which are left behind by insects such as flies warms the mixture to 50 °C and separates the beans from the pulp. However, the unavoidable

Fig. 1.3-2 Flowers of the cocoa tree

“Mais vous ne vous portez point bien, vous n’avez point dormi: le chocolat vous remettra”, puis quelques mois plus tard: “Je veux vous dire, ma chère enfant, que le chocolat n’est plus avec moi comme il l’était; la mode m’a entraînée, comme elle le fait toujours: tous ceux qui m'en disaient du bien, m’en disent du mal. On le maudit, on l’accuse de tous les maux qu’on a, il est la source des vapeurs et des palpitations; il vous flatte pour un temps et puis allume tout d’un coup une fièvre continue qui vous conduit à la mort.”

Marie de Rabutin-Chantal,
Marquise de Sevigné (1626−1696)
Correspondance avec sa fille

"pollution" of the cocoa beans with a mixture of microorganisms has to be taken into account – nobody wants to find them in cocoa powder or chocolate. Drying is the second step. The beans are air-dried by the sun and lose half of their mass in the form of water. At the end, their water content is as low as ca. 7% and the fat content is about 50% in the form of cocoa butter.

The process continues with the fabrication of cocoa paste. The content of about 10 cocoa pods is required for 1 kg of this paste, which is required for both cocoa powder and chocolate. Cleaning and roasting of the beans at temperatures up to 160 °C (i.e. clearly not as hot as applied with coffee beans) for ca. 30 minutes bring about the typical cocoa flavour and dry the beans. Then, a so-called debacterizing step follows (an autoclaving procedure with overheated steam related in principle to that known from clinical sterilization of medical equipment) which makes the crude cocoa microbially safe for human use. These beans are then peeled and broken. Peel and core particles are separated. The broken cores are called *nibs*. Finally, they are finely ground to a viscous mass, the so-called cocoa paste. This paste can be subjected to a pressing procedure, leading to a separation of very soft cocoa butter from the pressing cake. Cocoa powder (think of the information "strongly defatted" on the box) is made from the pressing cake. Cocoa butter is used for making several chocolate products. Cocoa paste itself is used as an ingredient for making dark chocolate. Today, a lot of effort in the advertisement of chocolate is directed at conveying to a possible purchaser the feeling of buying an individual cocoa product, the origin of which can be followed back to a special tropical area of the world. This is a distinct difference from former times when chocolate was just thrown on the market as a more or less uniform and cheap staple article. If one thinks of the effort required to make it, that is really not a desirable destiny.

Zu meinem Namenstag hat er mir eine große Schachtel Schokolade geschickt, es war sehr lieb und aufmerksam. Ich hatte vergessen, es Euch damals zu schreiben, erst jetzt, da Ihr mich fragt, erinnere ich mich daran. Schokolade, müßt ihr wissen, verschwindet nämlich in der Pension sofort, kaum ist man zum Bewußtsein dessen gekommen, daß man mit Schokolade beschenkt worden ist, ist sie auch schon weg.

Franz Kafka (1883−1924)
Der Prozess, Chap. VI

Fig. 1.3-3 Germinating cocoa beans as a whole and when cut in half

Finally, the physiological effect of theobromine will be described. Theobromine is a stimulant, but in a different manner to caffeine, which acts with a strong, immediate effect and causes increased awareness. Instead, theobromine acts as a mild and lasting stimulant. It has a mood-brightening effect which is generally associated with the consumption of chocolate, or in other words which is expected to come about from it. Theobromine has a bitter taste but it is not the only bitter ingredient of cocoa. Another question is, in what amounts does chocolate contain theobromine at all? Dark chocolate has about 10 g/kg and mild chocolate between 1 and 5 g/kg. These amounts are no risk for humans, even if one eats chocolate in very large quantities – which occasionally has happened. Medically, theobromine is used as a diuretic, myocardial stimulant and vasodilator. It is helpful in treating asthma. It relaxes the smooth muscles, and hence those of the bronchi. Antitussive effects superior to those of codeine are currently under investigation [5,6]. A too high dose would result in sleeplessness, restlessness and tremors, with increased production of urine, being very similar to caffeine in this respect. However, enzymatic metabolization is rapid in the human body – quite different to that in the body of a dog or cat, which for example, have different enzymatic equipment that can easily and of

course unintentionally expose them to jeopardy. Thus, theobromine poisoning may occur with as small an amount as 50 g of chocolate for a small and 400 g for a larger dog. If recognized early, the animal can be treated, but prevention is better.

2. Literature

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[9] B. Goeber, R. Kraft, "Zum massenspektrometrischen Abbau von Theophyllidin und Coffeidin sowie der Methylxanthine Coffein, Theophyllin und Theobromin" [Mass spectrometric analysis of theophyllidine, caffeidine, caffeine, theophylline and theobromine] *Pharmazie* **1978**, *33*, 717−720.

3. Isolation

3.1 Principle

Our own isolation method was stimulated by the circumstance that the isolation of theobromine was described using trichloroethylene, a rather nonpolar and lipophilic solvent formerly used as a degreasing agent in dry-cleaning. This seemed extraordinary.

If one thinks of the solubility of theobromine in hot water that ensures it will be taken up in a beverage such as cocoa, the same property should be utilizable to isolate this alkaloid. The water solubility is based on the physicochemical property of theobromine to be able to form hydrogen bonds to water molecules both as a donor (by an N−H unit) and an acceptor (by C=O and –N= units) molecule.

Fig. 1.3-4 Chocolate leaves

La duchesse, au désespoir, hasarda d'aller dans le salon où se tenait le marquis Crescenzi, de service ce jour-là. Au retour de la duchesse à Parme, il l'avait remerciée avec effusion de la place de chevalier d'honneur à laquelle, sans elle, il n'eût jamais pu prétendre. Les protestations de dévouement sans bornes n'avaient pas manqué de sa part. La duchesse l'aborda par ces mots: – Rassi va faire empoisonner Fabrice qui est à la citadelle.
Prenez dans votre poche du chocolat et une bouteille d'eau que je vais vous donner. Montez à la citadelle, et donnez-moi la vie en disant au général Fabio Conti que vous rompez avec sa fille s'il ne vous permet pas de remettre vous-même à Fabrice cette eau et ce chocolat.

Stendhal (1783−1842)
La Chartreuse de Parme, XXV

Fig. 1.3-5 Chocolate truffles

Unter gemahlnen Kaffee wird Zichorie oder anderes wohlfeiles Zeug gemischt, ja sogar unter ungemahlnen, wobei die Mischung in die Form von Kaffeebohnen gebracht wird. Kakao wird sehr häufig mit feiner brauner Erde versetzt, die mit Hammelfett gerieben ist und sich dann mit dem echten Kakao leichter vermischt. Tee wird mit Schlehenblättern und anderem Unrat vermischt, oder ausgebrauchte Teeblätter werden getrocknet, auf kupfernen heißen Platten geröstet, damit sie wieder Farbe bekommen, und so für frisch verkauft. Pfeffer wird mit Staub von Hülsen usw. verfälscht; Portwein wird geradezu fabriziert (aus Farbstoffen, Alkohol usw.), da es notorisch ist, daß in England allein mehr davon getrunken wird, als in ganz Portugal wächst, und Tabak wird mit ekelhaften Stoffen aller Art vermischt in allen möglichen Formen, die diesem Artikel gegeben werden.

Friedrich Engels (1820−1895)
Die Lage der arbeitenden Klasse in England

Fig. 1.3-6 Bensdorp cacao

Therefore, in the first step, theobromine, together with all other water-soluble compounds, especially carbohydrates, is extracted from strongly defatted cocoa powder with hot water. One may be astonished at the fact that nearly one-third of the cocoa powder is water soluble; however, the nutritious effect mentioned at the beginning in the background information comes just from this carbohydrate portion of the cocoa powder. After separation of the water phase, the second step uses the circumstance that theobromine is not as hydrophilic as carbohydrates.

Therefore, it is possible to extract theobromine selectively with methanol as an organic solvent of distinctly lower polarity than water from the residue remaining after drying the aqueous extract. Disaccharides and polysaccharides will not dissolve in methanol at all, and monosaccharides, such as glucose, if present at all, only to a very small extent – which has not been found to be disturbing during the extraction.

After crystallization and recrystallization from methanol, a portion of theobromine was eventually further purified by sublimation in vacuo. The distinguishing property to sublime under heat is common to both theobromine and caffeine due to their closely related structures. In contrast, if present in the crude product at all, a pure carbohydrate would not be able to sublime. A feature is that melting points from compounds which are sublimable under standard conditions can only be taken when a minute amount of the compound is inserted in a small sealed glass vial (in Germany called a Fischer cuvette). Doing so allows that, on heating in the tiny closed ampoule, a vapour pressure of theobromine is built up which corresponds to different conditions in the phase diagram of theobromine under which a liquid phase exists and can be observed.

3.2 Method

Strongly defatted cocoa powder (100 g, of the brand Demeter®, containing 11% cocoa butter) and water (1 L) are placed in a 2 L round-bottomed flask, stirred mechanically and heated in a water bath at 90 °C for 30 min. The suspension obtained is cooled to room temperature and centrifuged in portions at 3000 rpm for 5 min. The brown and cloudy aqueous phases are separated and extracted with methyl *tert*-butyl ether (3 × 100 mL) to remove any lipids. The brown ethereal phases are discarded. The aqueous phase is dried in two steps. First, water is distilled off in a rotary evaporator at 45 °C and 16 mbar. A slurry is obtained from which, second, the remaining water is removed by means of an oil pump at 0.1 mbar and 45 °C. A pale brown solid remains (31.8 g). To this solid methanol (400 mL) is added and heated under reflux for 15 min. The suspension is filtered to yield a brown extract which is concentrated in vacuo to 15 mL. On standing overnight, colourless crystals precipitate. These are filtered off and washed with 3 mL of ice-cold methanol. This crude theobromine has a mass of 700 mg. The crude material is dissolved in hot methanol (250 mL) and allowed to stand in an open beaker overnight, leading to recrystallization of pale grey theobromine (300 mg when dried using an oil pump), mp 349−351 °C (taken in a sealed Fischer cuvette as described above). Standard

workup of the mother liquor yields another crop of 280 mg of the same quality.

3.3 Purification

A 100 mg amount of this material is subjected to sublimation in a vacuum sublimator which is heated in the air stream (320 °C) of an electronic heat gun under a membrane pump vacuum (down to 40 mbar) for 15 min until sublimation ceases. Colourless sublimed theobromine (50 mg) can be obtained, mp 346–347 °C (Fischer cuvette). This melting point is in full accordance with that of an authentic sample of commercial colourless theobromine. In this case, the slight lowering of the melting point did not come along with a poorer purity, just the opposite was true on looking at the NMR data for crude and sublimed theobromine samples. The melting point is always also an expression of the time and conditions available for making up crystals. Obviously, sublimation delivered a clean sample here, but without an ideal inner crystal shape.

4. Spectra and Comments

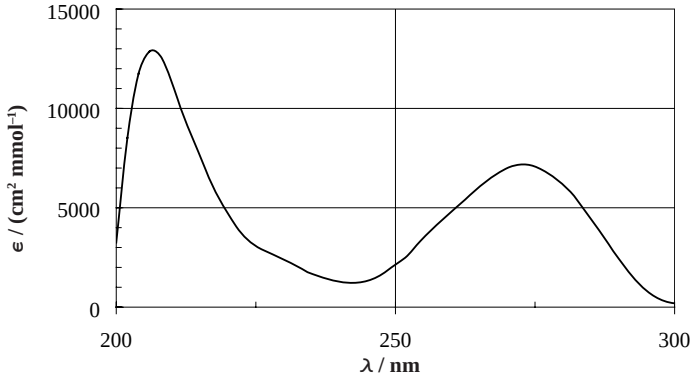
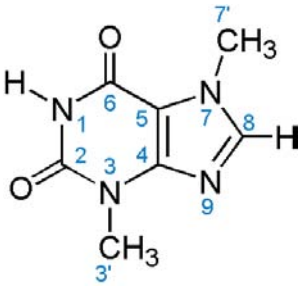


Fig. 1.3-7 UV spectrum in ethanol

The UV spectrum of theobromine is very similar to that of caffeine, as expected, since both compounds differ only in one methyl group, which will not affect the electronic system much.



Scheme 1.3-1

Des Morgens stehen wir (ich und mein Herr nämlich) nicht zu früh, aber auch nicht zu spät auf; das heißt, auf den Schlag elf Uhr. – Ich muß dabei bemerken, daß mein breites weiches Lager unfern dem Bette des Barons aufgeschlagen ist, und daß wir viel zu harmonisch schnarchen, um beim plötzlichen Erwachen zu wissen, wer geschnarcht hat. – Der Baron zieht an der Glocke, und sogleich erscheint der Kammerdiener, der dem Baron einen Becher rauchender Schokolade, mir aber einen Porzellannapf voll des schönsten süßen Kaffees mit Sahne bringt, den ich mit demselben Appetit leere wie der Baron seinen Becher. Nach dem Frühstück spielen wir ein halbes Stündchen miteinander, welche Leibesbewegung nicht allein unserer Gesundheit zuträglich ist, sondern auch unsern Geist erheitert.

E. T. A. Hoffmann (1776–1822)
Die Lebensansichten des Katers Murr

Fig. 1.3-8 Defatted cocoa powder

Fig. 1.3-9 Ripe cocoa fruits

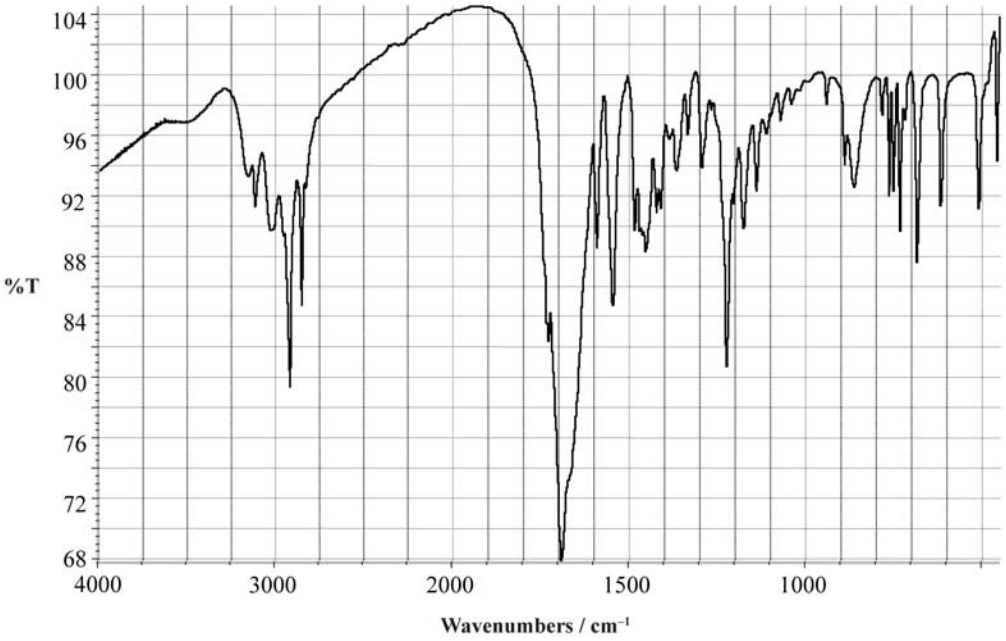
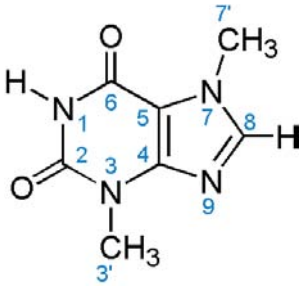


Fig. 1.3-10 IR spectrum in KBr

In contrast to the UV spectrum, the IR spectrum of theobromine looks remarkably different to that of caffeine. Of course, the bands of the functional groups are at similar places, but here the sp^2- and sp^3-CH valence band region at 3100–2900 cm^{-1} is much more detailed than in caffeine, whereas the carbonyl band is not separated into two absorptions as in caffeine. The C=C double bond vibration at 1600 cm^{-1} is identical in its intensity in both compounds, but the fingerprint region again looks very different.



Scheme 1.3-2

Fig. 1.3-11 Cocoa pod with germinating beans. The size is ca. 60% of the original

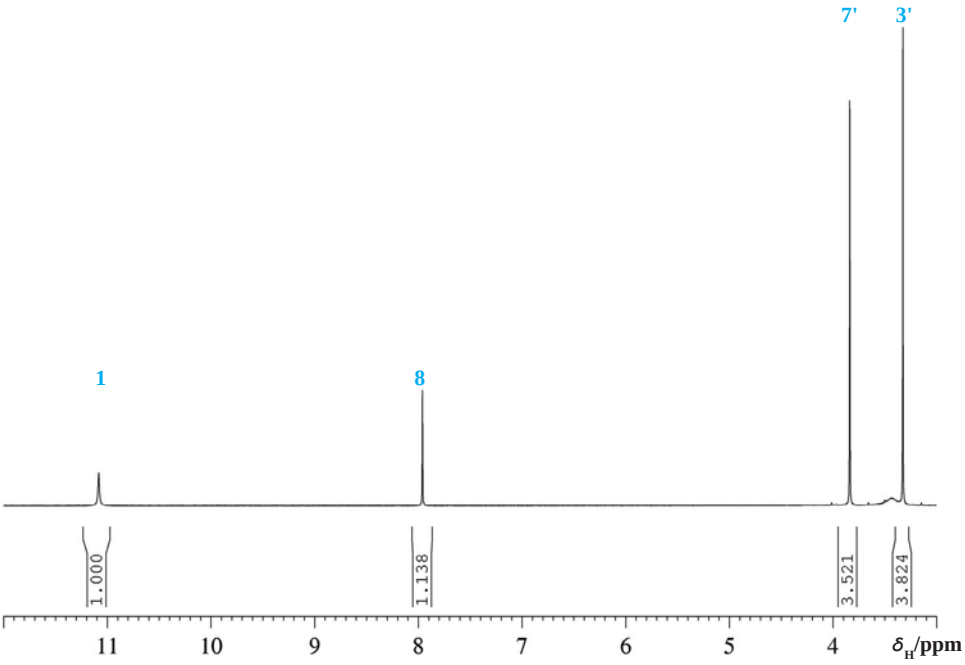


Fig. 1.3-12 ^{1}H NMR spectrum at 400 MHz in DMSO-d_6

The very simple ^{1}H NMR spectrum displays four singulets, two of which can obviously be directly assigned, namely the NH proton H-1 at 11.08 ppm and H-8 at 7.96 ppm due to their typical chemical shift ranges. The individual assignment of the two methyl groups has to await the analysis of the NOESY spectrum.

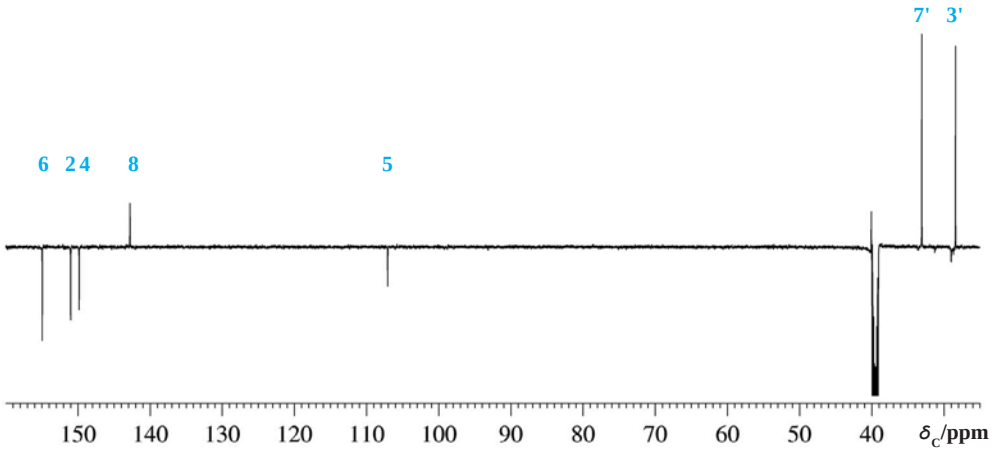


Fig. 1.3-13 APT ^{13}C NMR spectrum in DMSO-d_6

The very simple-looking APT ^{13}C NMR spectrum displays seven signals, of which only the positive signal at 142.7 ppm can be assigned with safety to C-8, and in analogy with caffeine the signal of the quaternary carbon at 107 ppm to C-5. The safe assignment of the other signals has to await the results from HMBC and NOESY spectra.

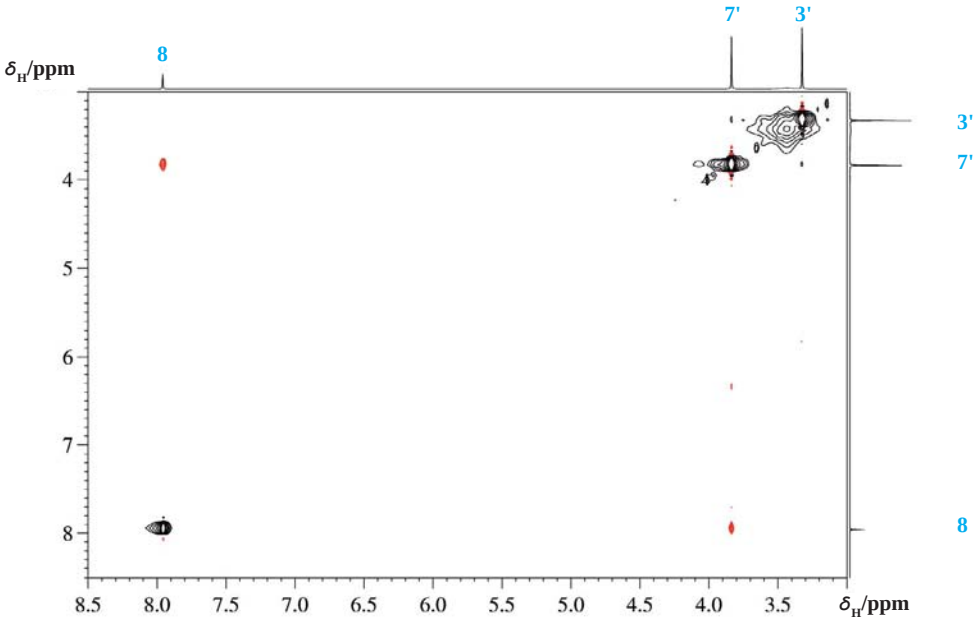


Fig. 1.3-14 NOESY spectrum connecting the aromatic with the aliphatic region

The NOESY spectrum immediately clarifies the assignment for the methyl group signals, since only the more deshielded one shows an NOE effect to H-8, hence this signal must stem from H-7'. A COSY spectrum is not shown, since no spin couplings are to be evaluated.

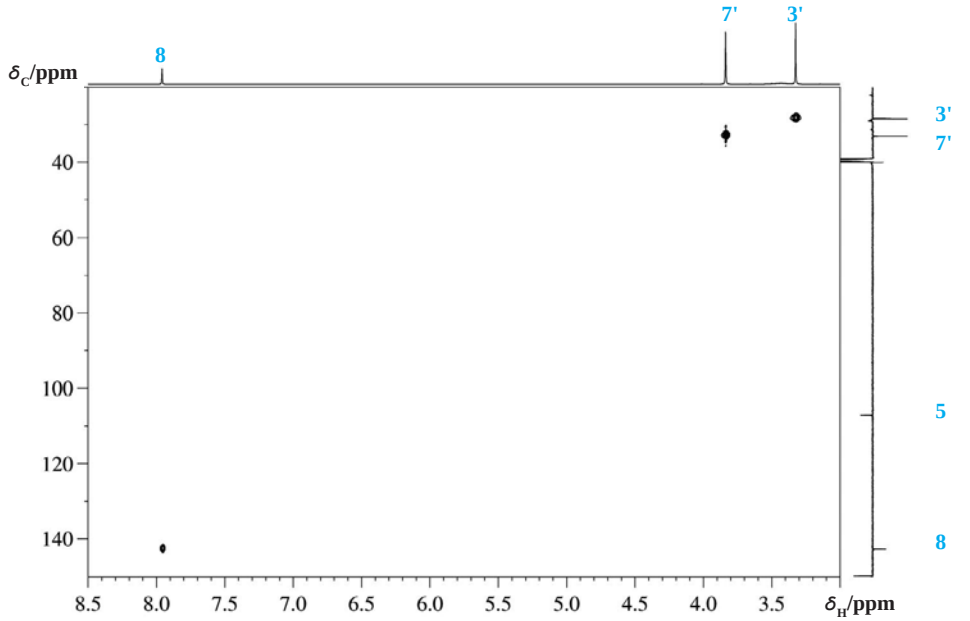


Fig. 1.3-15 HSQC spectrum

With the assignment information obtained from the NOESY spectrum, now the carbon signals for the methyl groups can be safely assigned due to their direct connectivity with the corresponding protons. The chemical shift values are nearly exactly the same as in caffeine.

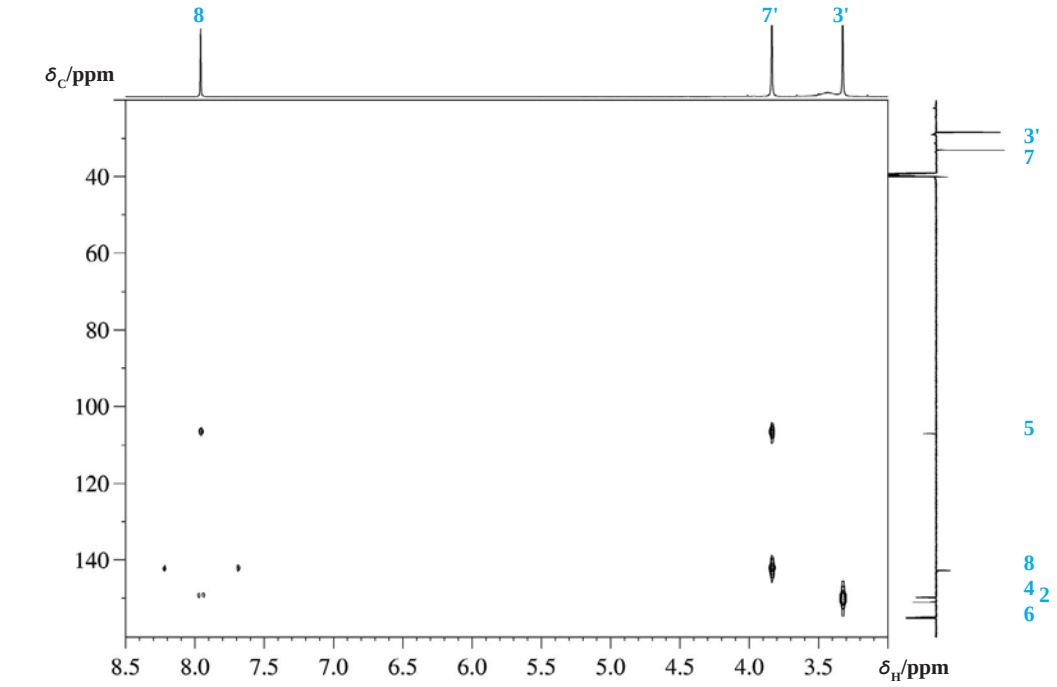


Fig. 1.3-16 HMBC spectrum

The HMBC spectrum clarifies the remaining open questions. H-8 displays cross peaks with C-5 at 107.1 ppm and to C-4 at 149.8 ppm, both via three bonds. H-7' finds C-5 and also C-8, which have already been assigned. H-3' shows HMBC connections via three bonds both to C-4 at 149.8 ppm and to C-2 at 151 ppm. This leaves the signal at 154.9 ppm for C-6, which shows no HMBC correlation at all, and this is in accordance with the ^{13}C chemical shift assignment in caffeine.

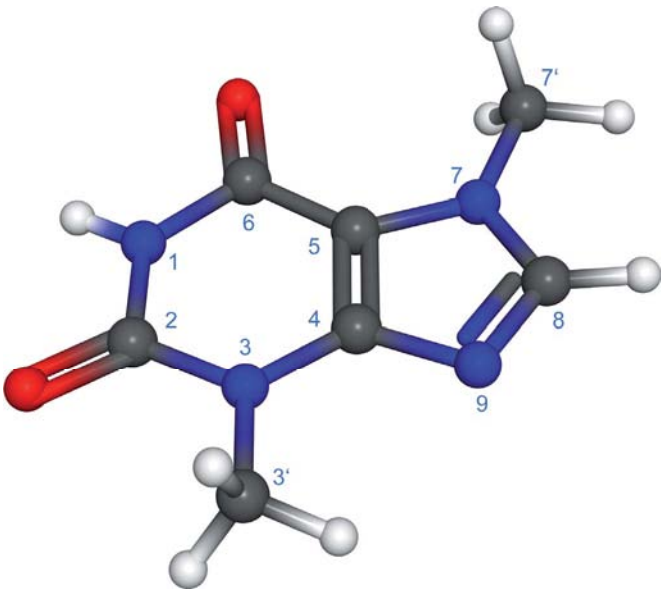


Fig. 1.3-17 Molecular model of theobromine

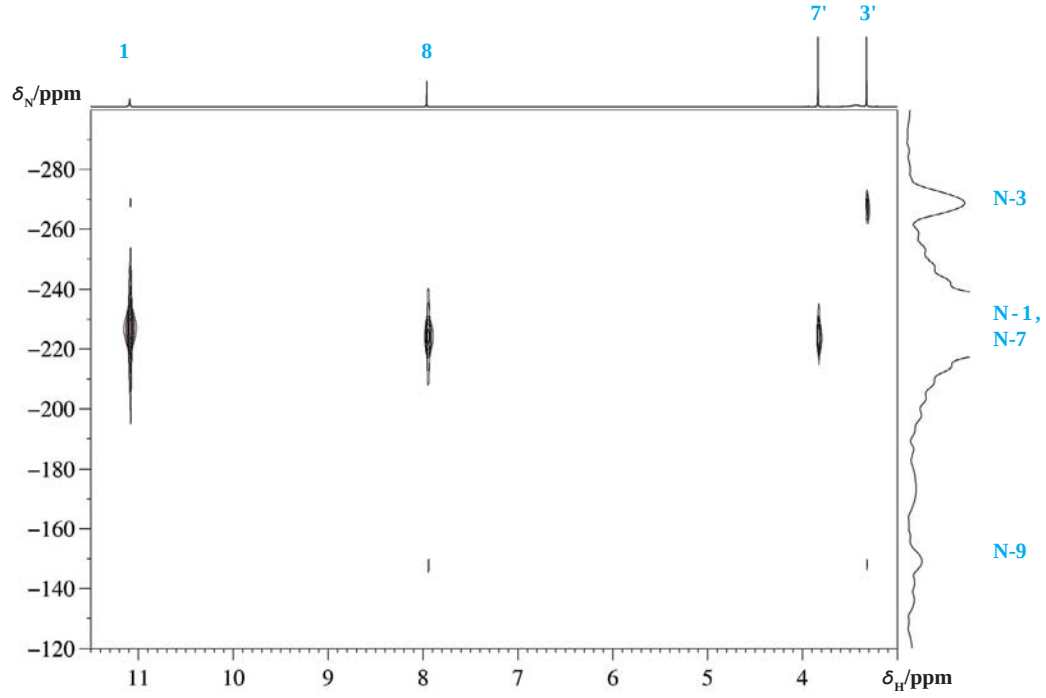
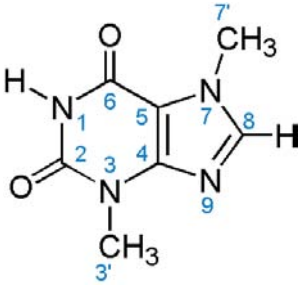


Fig. 1.3-18 ^{1}H ^{15}N HMQC spectrum

Due to the rather limited solubility of theobromine in comparison with caffeine the recording of a ^{1}H,^{15}N HMQC spectrum is much more difficult than with caffeine and, furthermore, one has to compromise between one signal of an NH moiety and three quaternary nitrogen atoms. The nitrogen chemical shift values, however, are very similar to those in caffeine, as expected. Their relative assignment is obvious from the correlation with the corresponding proton signals.



Scheme 1.3-3

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz	^{15}N Signals δ / ppm
154.9	C_q	C-6		
151.0	C_q	C-2		
149.8	C_q	C-4		
142.7	CH	C-8	7.96	
107.1	C_q	C-5		
33.0	CH_3	C-7'	3.84	N-7 –224.0
28.4	CH_3	C-3'	3.32	N-3 –267.4
			NH: 11.08	N-1 –227.0
				N-9 –147.8

Table 1.3-1 NMR data for theobromine

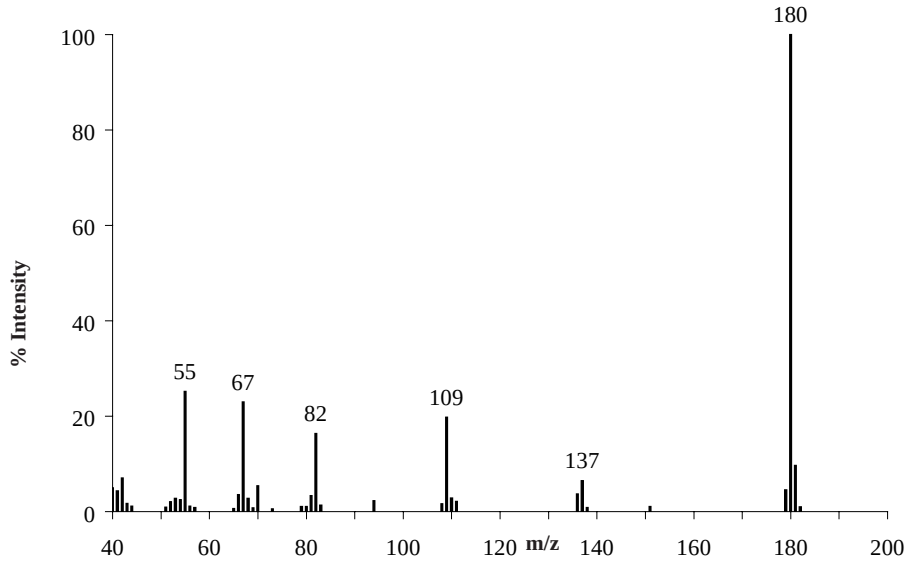
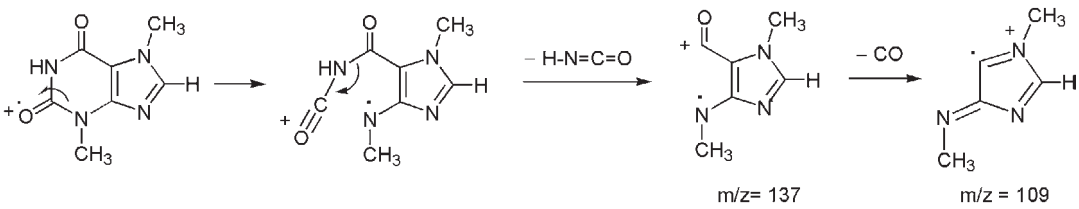


Fig. 1.3-19 Mass spectrum (EI)

The electron impact mass spectra of caffeine and theobromine look remarkably similar. In both compounds the molecular ion also forms the base peak, which is, due to the lack of a methyl group in theobromine, 14 mass units lower than in caffeine. Nearly all other fragment peaks, however, are identical for both compounds, suggesting a common fragmentation pathway. The first significant fragment ion of m/z = 137 can therefore be postulated as being formed by loss of HNCO from the molecular ion, which subsequently loses CO to form the ion with m/z = 109:



Scheme 1.3-4 Fragmentation of theobromine

Fig. 1.3-20 Flowering cocoa tree

5. Questions

A. Methylation reactions belong to the biochemical finishing of many natural products, with theobromine, theophylline and caffeine being examples. A typical reactant is *S*-adenosylmethionine, a reactive sulfonium salt which is used by many methyl transferases to transfer a methyl group to an O or N atom. Although transgenic organisms are controversial, make a suggestion as to what may be the driving forces for the efforts to construct coffee plants that are unable to do methylations and – just the opposite – to build up an opium poppy species with elevated capability for methylation. In what kind of economically useful plants would this end?

B. The annual demand for caffeine is about 20 000 tons, mainly used for beverages, only in part for pharmaceuticals. About 5000 tons are gathered by destraction of coffee, the remaining amount has to be synthesized. Still, the principle of the first successful synthesis of Traube (1900) is used with some improvements. Write a formula scheme for the following steps:

(1) reaction of *N*,*N* '-dimethylurea and cyanoacetic acid;
(2) nitrosation of the heterocycle obtained in (1) to form the product as oxime tautomer;
(3) catalytic hydrogenation of the oxime to form a diamine;
(4) cyclization of the diamine with formic acid to yield theophylline;
(5) full methylation with dimethyl sulfate to yield caffeine.

C. The HMBC cross peak of H-8 to C-4 shows a significant splitting of 13 Hz, whereas that to C-5 seems to be a singulet at the given resolution. Comment.

D. The ^{15}N NMR signal of N-1 in caffeine appears at –231.4 ppm, whereas the signal of N-1 in theobromine has a chemical shift value of –227 ppm. Compare this methylation effect with the chemical shift changes introduced by a methyl group in ^{13}C NMR.

E. Suggest an explanation for the ion with m/z = 82, found in the mass spectra of both caffeine and theobromine.

6. Own Observations

1.4 Piperine

(2*E*,4*E*)-5-(1,3-Benzodioxol-5-yl)-1-(1-piperidinyl)-2,4-pentadien-1-one

From black pepper
Piper nigrum L. (Piperaceae)

$C_{17}H_{19}NO_3$, MW 285.34

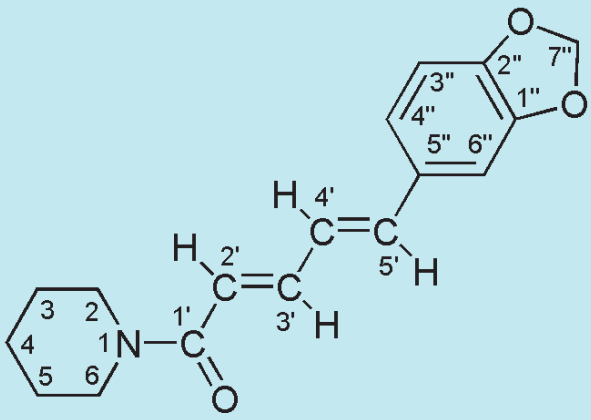
CAS RN 94-62-2, BRN 90741

Colourless crystals, mp 128−129 °C

Piperine is commercially available.

Synonymous names:
(*E*,*E*)-1-Piperoylpiperidine,
Bioperine, Piperin

Level: difficult



1. Background: From Malabar to any table

Fig. 1.4-1 Pliny the Elder (23−79)

Pepper is surely the top spice. Nowadays, a salt cellar and a pepper pot appear on every dining room table around the globe. In ancient times, pepper was incredibly expensive due to the risks and dangers during land transportation with caravans from India and the Orient. Clearly, not only the desire for gold but also for exotic goods such as pepper pushed the global sea expeditions of the 15th and 16th centuries. At that time the profits made from trading of spices from the Moluccan Islands, for example, were fabulous, whether ships were lost or not. Common parlance in Germany called rich Dutch merchants “Pfeffersaecke” (English: pepper sacks), combining in this word the origin of the opulence, together with admiration, envy and contempt.

Peppercorns are the fruits of the Asian vine *Piper nigrum* L. originating from the Malabar coast in southwest India and are available as black, white and green types. To obtain black pepper the unripe fruits are dipped into hot water and air dried. White pepper is gained by fermentation of ripe red pepper fruits, involving loss of the pulp. Mild green pepper with a much shorter tradition in the kitchen is an invention of the 1970s and is made by pickling unripe green peppercorns in brine.

Pepper contains 10−15% of an alkaloidal fraction. Among these ca. 15 alkaloids, piperine constitutes 90% and is the main alkaloid, responsible for the hot taste. The aromatic part of the taste is not due to piperine but to a small fraction of terpenoid ethereal oils in the peppercorns.

Passim vero quae piper gignunt iunipiris nostris similes, quamquam in fronte Caucasi solibus opposita gigni tantum eas aliqui tradidere. Semina a iunipiro distant parvulis siliquis, quales in phasiolis videmus. Hae prius quam dehiscant decerptae tostaeque sole faciunt quod vocatur piper longum, paulatim vero dehiscentes maturitate ostendunt candidum piper, quod deinde tostum solibus colore rugisque mutatur.

Plinius Maior
Naturalis Historia Liber XII, 26

Piperine is the main alkaloid in fruits of black pepper. Its Latin botanical name provided the root for the trivial name of the alkaloid and also for the nitrogen-containing heterocycle piperidine, well known as a base in the laboratory. However, piperine reacts neutral and not basic because the N atom is part of an amide unit. Biogenetically, the ε-amino group of an L-lysine is the precursor of the N atom. Piperine belongs to the alkaloids, which were isolated in the first blossoming of alkaloid chemistry at the beginning of the 19th century. It was first isolated by the Danish scientist H. C. Oersted [1].

Fig. 1.4-2 Peppercorns

Piperine is only very sparingly soluble in water (4 mg/L). The pepperiness can still be felt after dilution to 1:200 000. Piperine has an antimicrobial effect, which helps the seed in which it is collected to withstand the attack of pests in the tropical climate. Pepper is traditionally used in ethnomedicine, e.g. in the Indian ayurveda formulation “Trikatu” [2]. Physiological effects of piperine are increased salivation, enhanced secretion of gastric juice and gall which lead to better digestion and bioavailability of nutrition constituents [3]. A well-known phenomenon is sweating after having a hot meal due to the general stimulation of the metabolism. Similarly to capsicum preparations, pepper preparations can be used in dermal applications to cause a heat sensation and a local anaesthetic effect. However, such treatment can only be applied for a short time due to simultaneous irritation of the skin. Pepper can due to its physiological activity be regarded as a spice and a drug.

Piperine as a chemical is classified as hazardous material if swallowed (classification Xn Harmful) and is commercially available.

2. Literature

[1] H. C. Oersted, "Ueber das Piperin, ein neues Pflanzenalkaloid", [On piperin, a new plant alkaloid] *Schweiggers J. Chem. Phys*. **1820**, *29*, 80−82.

[2] R. K. Johri, U. Zutshi, "An Ayurvedic formulation "Trikatu" and its constituents" *J. Ethnopharmacol.* **1992**, *37*, 85−91.

[3] M. Watanabe, "Bioperine: effect on natural acceleration of nutrient absorption" *New Food Ind.* **2004**, *46*, 31−36.

[4] E. Stahl, W. Schild, In: "Isolierung und Charakterisierung von Naturstoffen", G. Fischer Verlag, Stuttgart, **1986**, 143−145.

[5] U. Braumann, H. Haendel, K. Albert, R. Ecker and M. Spraul "Online monitoring of the supercritical fluid extraction process with proton nuclear magnetic resonance spectroscopy" *Analytical Chemistry*, **1995**, *67*, 930−935.

[6] A. Banerji, M. Sarkar, T. Ghosal, S. C. Pal "Carbon-13 NMR spectra of Piper alkamides and related compounds" *Org. Magn. Reson*. **1984,** *22*, 734−736 .

3. Isolation

3.1 Principle

Powdered black pepper is extracted with chloroform in a Soxhlet apparatus. The extract contains all lipophilic constituents with low polarity. In the concentrated extract, triglycerides present are cleaved by saponification with aqueous ethanolic KOH solution, whereas crude piperine crystallizes on standing in the cold.

3.2 Method

Powdered black pepper (25 g) is placed in the thimble of a Soxhlet apparatus and extracted with chloroform for 2 h to obtain the piperine. At the end of this operation, the extract obtained is colourless. All of the solvent is removed in vacuo and a brown oil remains; 20 mL of a 10% KOH solution in 50% aqueous ethanol are added with stirring.

The mixture is filtered. The filtrate is allowed to stand overnight in a refrigerator at 4 °C. Crystals of crude piperine formed are removed by suction filtration over a sintered disc filter funnel and washed with 2 mL of cold water to remove the adhering base. The crystals are air-dried and recrystallized from cyclohexane−toluene (4:1, v/v). Use 10 mL of this solvent for each 200 mg of crude piperine (recovery ca. 60%). Piperine crystallizes on standing in a beaker as shiny, pale yellow crystals which are filtered off and washed with a few mL of cyclohexane.

Yield: 200−500 mg depending on the pepper, mp 130−131 °C.

Fig. 1.4-3 Gourmets like their pepper freshly ground

I will now take the lecher; he is at my house; he cannot 'scape me; 'tis impossible he should; he cannot creep into a halfpenny purse, nor into a pepperbox: but, lest the devil that guides him should aid him, I will search impossible places.

William Shakespeare
The Merry Wives of Windsor, III, 5

"There's certainly too much pepper in that soup!" Alice said to herself, as well as she could for sneezing.
There was certainly too much of it in the air. Even the Duchess sneezed occasionally; and as for the baby, it was sneezing and howling alternately without a moment's pause. The only things in the kitchen that did not sneeze, were the cook, and a large cat which was sitting on the hearth and grinning from ear to ear.

Lewis Caroll
Alice's Adventures in Wonderland

3.3 Purification

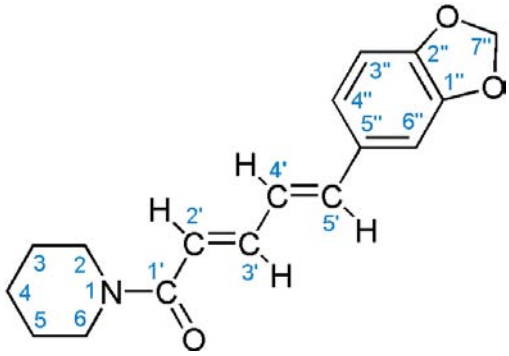
Fig. 1.4-4 Green pepper

Contrary to the nice appearance of this material, TLC [TLC alumina foils, Kieselgel 60_{F254} toluene–ethyl acetate (1:1)] indicates, however, that it is not pure at all. Besides the desired main compound ($R_f = 0.53$), a second stereoisomer ($R_f = 0.26$) of higher polarity is present. This finding is supported by the ^1H NMR spectrum, also showing signals of this side component. If NMR tubes with a piperine preparation in $CDCl_3$ are kept standing in daylight, at least three of the four possible stereoisomers around the two double bonds can be detected. To obtain pure piperine, 150 mg of the crystals were subjected to column chromatography [silica gel 60, 0.063–0.200 mm, eluent toluene–ethyl acetate (2:1)] to afford 135 mg of piperine after workup of the corresponding fractions as colourless crystals; mp 127–129 °C.

Piperine shows fluorescence quenching using TLC alumina foils of silica gel 60_{F254} on illuminating with 254 nm UV light and blue fluorescence on excitation with 366 nm UV light.

The method described here has been adapted from [4].

Fig. 1.4-6 This amount would have been invaluable in ancient times



Scheme 1.4-1

4. Spectra and Comments

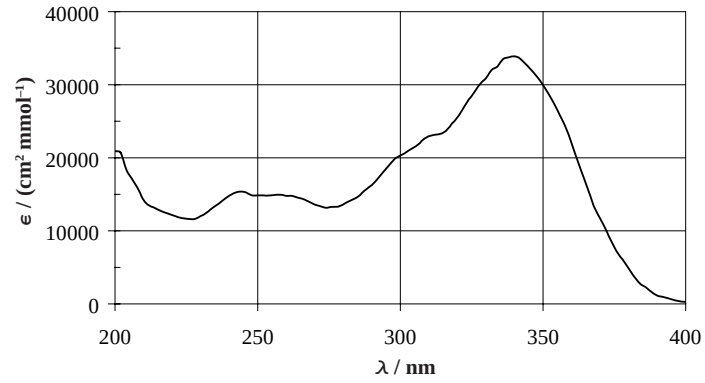


Fig. 1.4-7 UV spectrum in ethanol

Fig. 14-8 Crystals of piperine

As for most compounds in this book, the UV spectrum does not show a vibrational fine structure due to a lack of molecular rigidity. Piperine provides an aromatic 6π electron system in conjugation with a *trans*-butadiene unit and a carbonyl function which serves here as an auxochromic group. Therefore, λ_{max} is at 341 nm.

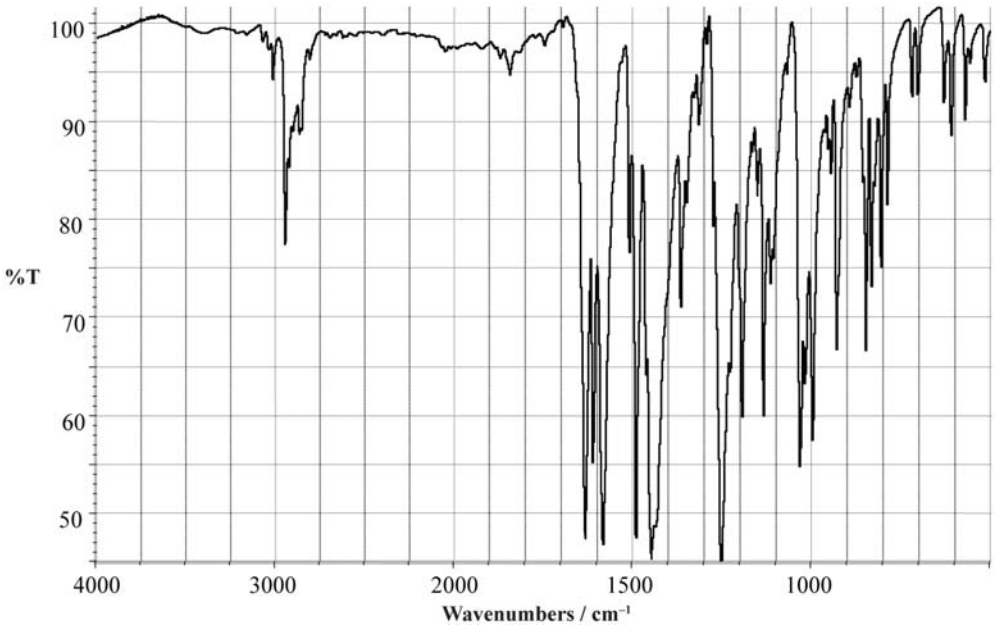


Fig. 1.4-8 IR spectrum in KBr

The IR spectrum reveals CH valence bands for sp^3- and sp^2-type carbon atoms. In the double bond region the CO band is nicely separated from different C=C bands. The strong absorption at 1250 cm^{-1} is likely due to the C–O–C vibration within the dioxolane ring.

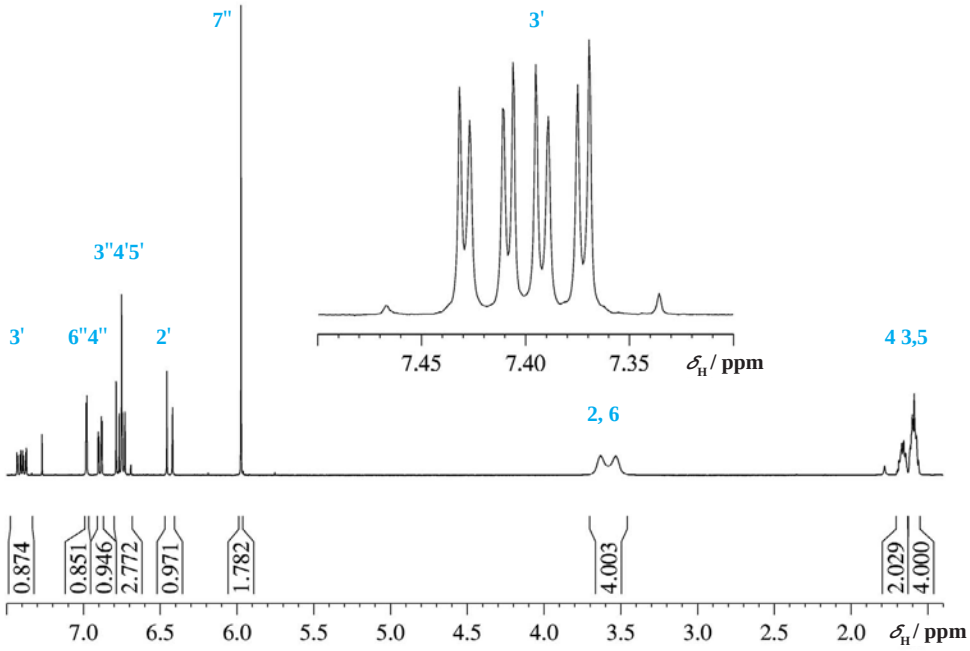
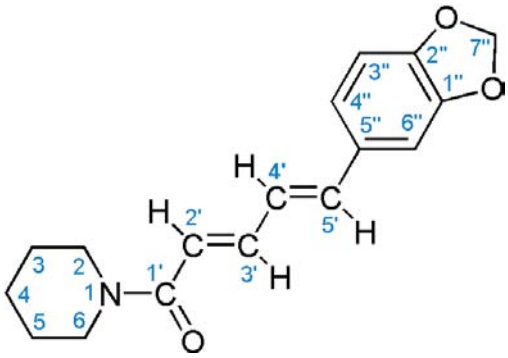


Fig. 1.4-9 ^{1}H NMR spectrum at 400 MHz in $CDCl_3$

The correct analysis of the proton NMR spectrum is only possible with the help of spin simulation, since the resonance positions of the two protons 4' and 5' are very close together, which leads to higher order effects. The most deshielded signal at 7.4 ppm is assigned to H-3', as its chemical shift is typical for a β-proton in α/β-unsaturated carbonyl compounds. It reveals a pattern, which, however, must not be interpreted in terms of a long-range coupling, since no other signals show this fine splitting. Note that the two small lines shown in the expanded inset for this signal also belong to the pattern for this proton.

Fig. 1.4-10 Unripe pepper fruits



Scheme 1.4-2

Fig. 1.4-11 Black peppercorns in close-up

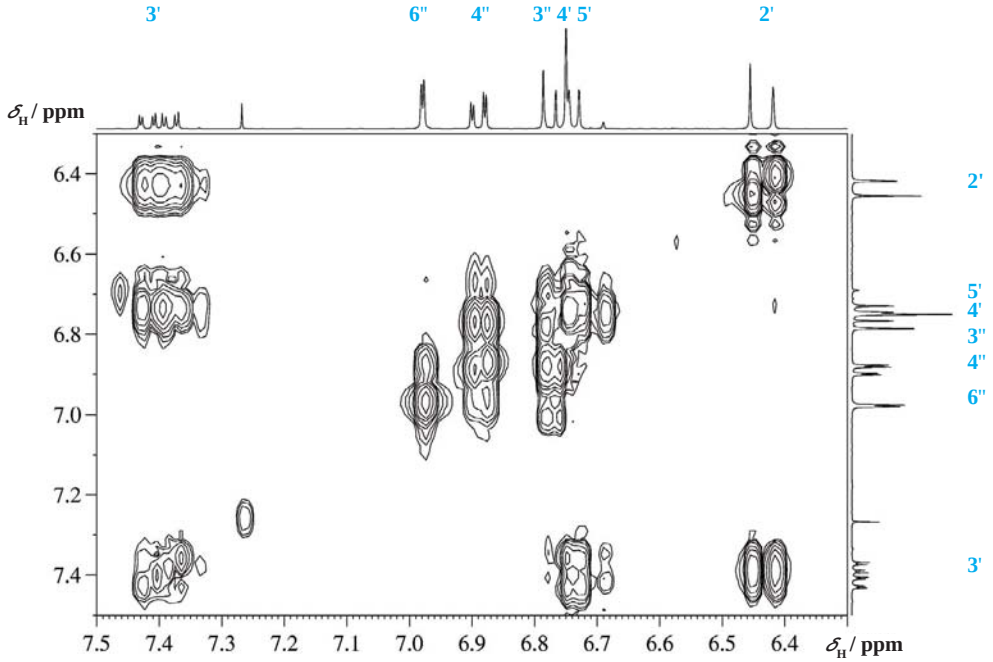


Fig. 1.4-12 Expansion of the COSY spectrum in the olefinic/aromatic region

The COSY spectrum shows that the proton at 7.4 ppm is connected to two other protons, one at 6.73 ppm and the other at 6.44 ppm. The latter shows only a sharp doublet with a spin coupling of 14.9 Hz, hence this proton can be safely assigned to H-2'. Proton H-4' resonates very close to H-5'. The three proton signals at 6.98, 6.89 and 6.78 ppm constitute the very typical pattern of a 1,2,4-trisubstituted aromatic compound. The assignment of these protons is self-evident due to the aromatic *meta spin* coupling over four bonds.

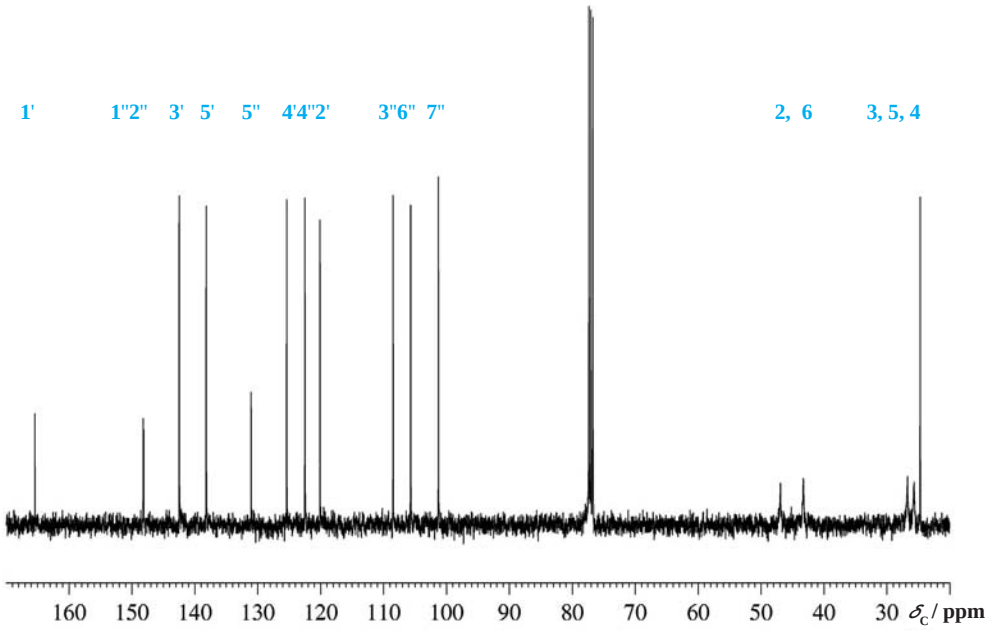


Fig. 1.4-13 ^{13}C NMR spectrum at 100 MHz in $CDCl_3$

Three signals of the ^{13}C NMR spectrum can be immediately assigned due to their significant chemical shift: the amide carbonyl atom at 165.4 ppm, carbon 7" in the dioxolane ring at 101.3 ppm and C-4 of the piperidine ring at 24.7 ppm. The assignment of the other carbon atoms needs the help of the HSQC and HMBC spectra. Note the broadening of the signals for C-2/C-6 and C-3/C-5.

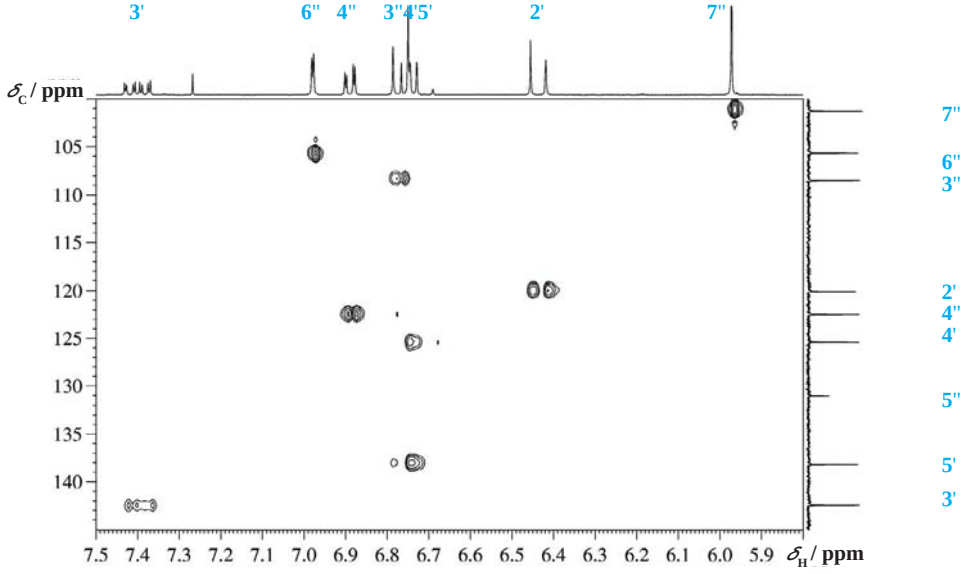


Fig. 1.4-14 Expansion of the HSQC spectrum in the olefinic and aromatic region

The HSQC spectrum helps nicely to disentangle the three overlapping proton resonances at 6.7 ppm. In fact, distinguishing the proton signals at 6.73 and 6.75 ppm can only be achieved with chemical shift arguments from the ^{13}C and the HMBC spectra.

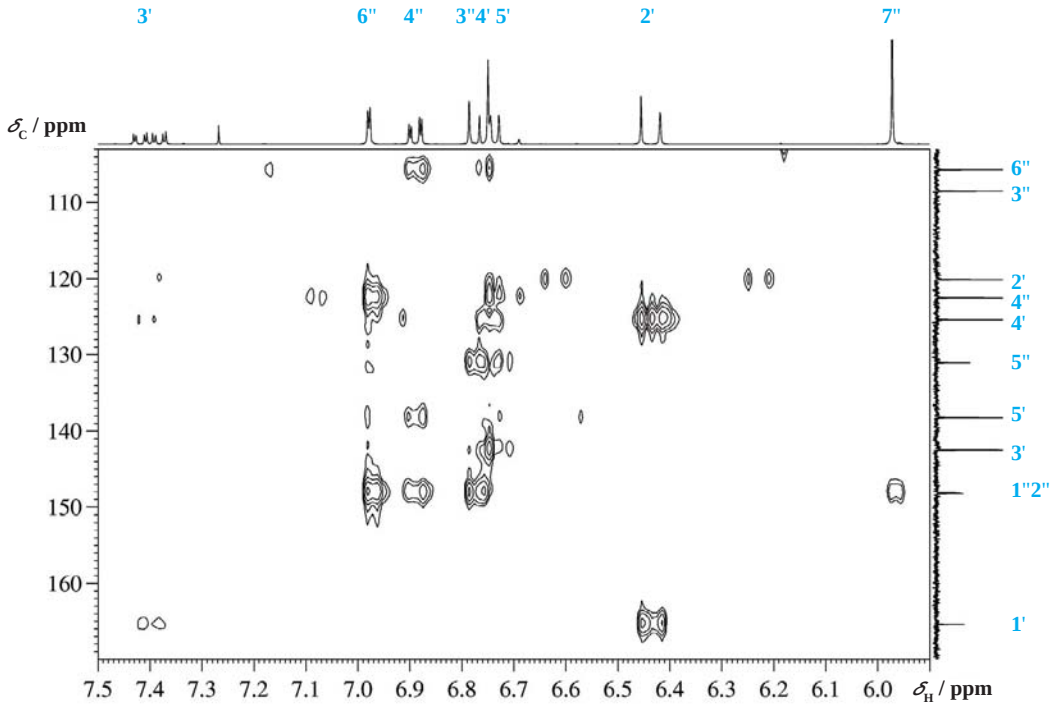
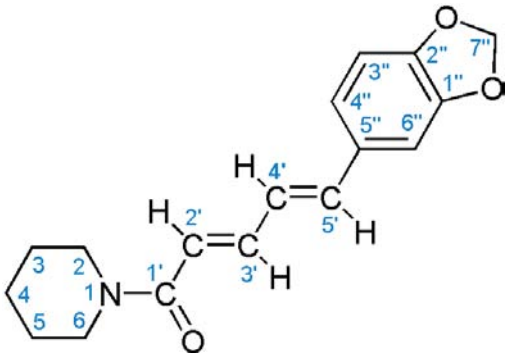


Fig. 1.4-15 Expansion of the HMBC spectrum in the aromatic region

The HMBC spectrum confirms the assignments mentioned above and further helps to assign the quaternary carbon signal at 131.0 ppm to C-5", since cross peaks both from H-4' and H-3" can be seen. However, the individual distinction between the very closely resonating carbon signals of C-1" and C-2" at 148.2 and 148.1 ppm requires more advanced methods if needed at all. Very important is the cross peak of H-2' to the ^{13}C signal at 125.4 ppm, which secures this to C-4'. The carbonyl atom is seen from H-2' and H-3'.



Scheme 1.4-3

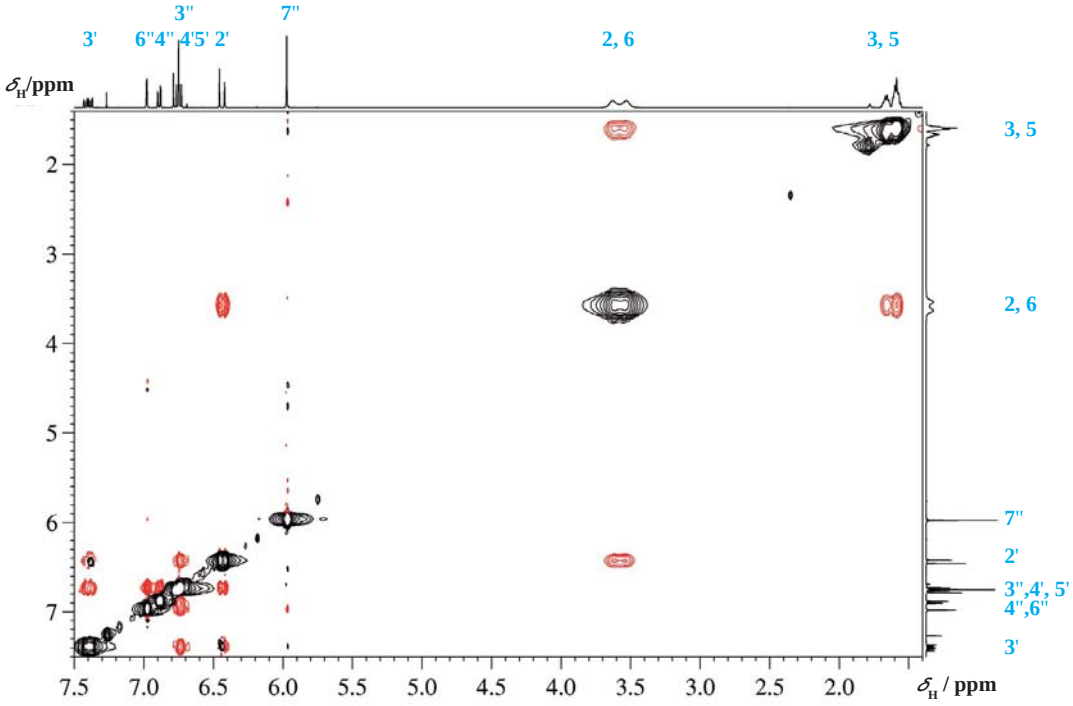


Fig. 1.4-16 NOESY spectrum

The NOESY spectrum confirms the attachment of the piperidine ring, due to the strong cross signal between H-2/6 and H-2'. It also shows the close overlap of H-4' and H-5' with their respective cross signals to H-2' and H-3'.

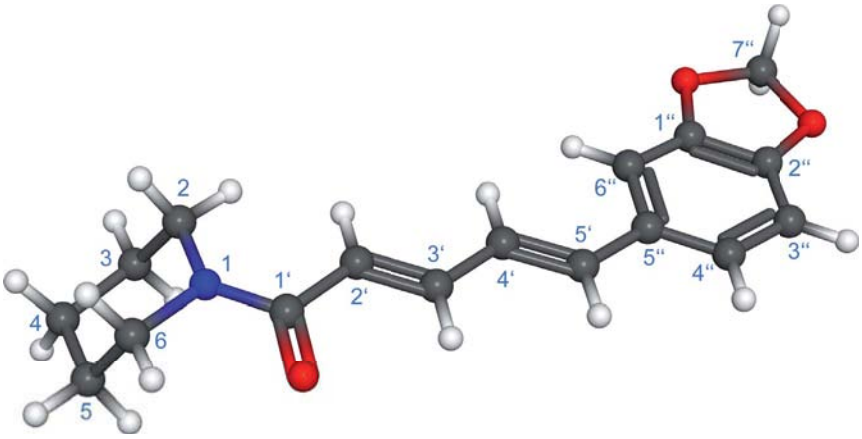


Fig. 1.4-17 Molecular model of piperine

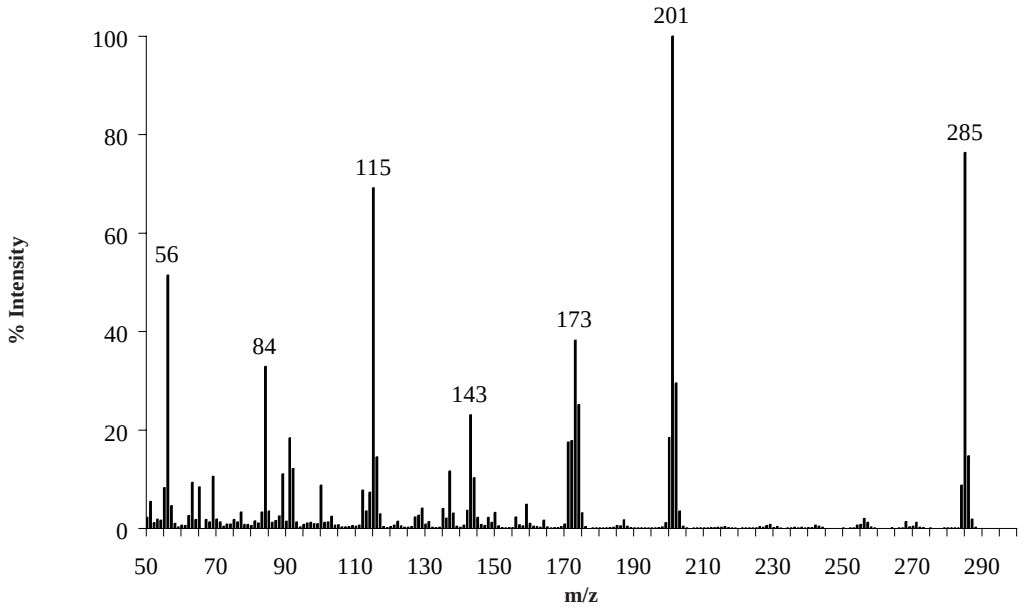


Fig. 1.4-18 Mass spectrum (EI)

In the mass spectrum, the basic peak at m/z = 201 is formed after the apparent loss of the piperidyl ring by an α-elimination, assuming ionization at the carbonyl group. Note that also the ion at m/z = 84 is observed, which is formed by cleavage of the same bond, but with the ionic and radical fragments interchanged.

^{13}C Signals δ / ppm	Type of Carbon	Assignment	Proton signals δ / ppm, J / Hz
122.5	CH	C-4"	6.89, $J_{4",6"}$= 1.8
120.1	CH	C-2'	6.44 $J_{2',3'}$= 14.9
108.5	CH	C-3"	6.78 $J_{3",4"}$= 8.0
105.7	CH	C-6"	6.98, $J_{4",6"}$= 1.8
101.3	CH	C-7"	5.97
46.9	CH_2	C-2/6	3.58
43.3	CH_2	C-2/6	3.58
26.7	CH_2	C-3/5	1.59
25.7	CH_2	C-3/5	1.59
24.7	CH_2	C-4	1.67

Table 1.4-1 NMR data for piperine

Well it was 20 years ago today,
Sergent Pepper taught a band to play,
They've been going in and out of style,
But they're guaranteed to raise a smile,
So may I introduce to you,
The act you've known for all these years,
Sergent Pepper's lonely hearts club band!

The Beatles (1967)

Fig. 1.4-19 Black pepper

5. Questions

A. Which products result from the alkaline cleavage of piperine?

B. Piperine undergoes isomerization to form three stereoisomers under the influence of light: isopiperine (*Z*,*E*)-configuration (CAS 30511-76-3), chavicine (*Z*,*Z*)-configuration (CAS 495-91-0), and isochavicine (*E*,*Z*)-configuration (CAS 30511-77-4). Draw the structures of these diastereomers.

C. Although more and more biological processes are understood at the molecular level, phenomena such as smell and taste are not well comprehended at this stage, because it is very hard to obtain a molecular picture of the receptors responsible. However, by comparison of structure and activity, some empirical rules can be established. Compare the structures of capsaicine and piperine and make an intuitive proposal as to which structural features may be required for interaction with the hot taste receptor.

D. The longer the conjugated system the more sensitive it is towards double bond isomerizations. Retinal (retinaldehyde) with five double bonds is such a natural example from another sensation: perception of light in the photoreceptors of the retina in the eye. The fundamental reaction for the transduction of light into an electrical signal for the brain is a chemical one: isomerization of (11*Z*)-retinal into (11*E*)-retinal [= (all-*E*)-retinal]. The energy for this process is taken from the impinging photons. The strong structural change caused is transferred into a conformational change of a protein coupled to (11*Z*)-retinal and finally transformed into a neural signal. Recycling of the *E*- to *Z*-isomer occurs chemically, without the need for light. Draw the scheme for the light-induced isomerization of (11*Z*)-retinal into (11*E*)-retinal.

E. At first glance, it is astonishing that H-2' is much more shielded than H-3', although H-2' is much closer to the electron-withdrawing carbonyl group. Explain.

F. In the ^{13}C NMR spectrum it is seen that the signals of the carbon atoms C-2/6 and C-3/5 are rather broad, whereas all other carbon signals give sharp signals. The proton signal at δ = 3.6 ppm is also broadened. What is the reason and how could one obtain sharp signals?

G. The carbon atom C-7'' within the dioxolane ring gives a signal at δ = 101.3, which is normally very typical for an anomeric carbon atom of a sugar residue. Not knowing the structure, with which experiment would you distinguish between these two situations?

H. In the HMBC spectrum there are four small signals at the carbon chemical shift of 120 ppm, although no corresponding proton signals can be seen. Explain.

I. The relative assignment of the carbon atoms C-4' and C-5' may be explained using the same arguments as for the pair H-2' and H-3'. Show by analysis of the HMBC spectrum how the assignment can be secured.

J. Spin simulation of the proton NMR spectrum gave $^3J_{2',3'}$ = 14.9 Hz, $^3J_{3',4'}$ = 12.0 Hz and $^3J_{4',5'}$ = 15.4 Hz . What would you expect for the known (*E*,*Z*) stereoisomer?

K. Explain the mass spectrum fragments at *m*/*z* = 173, 143 and 115.

6. Short Own Observations

1.5 Cytisine

(1*R*,5*S*)-1,2,3,4,5,6-Hexahydro-1,5-methano-8*H*-pyrido[1,2-*a*][1,5]diazocin-8-one

From the seeds of the golden chain tree
Common laburnum, *Laburnum anagyroides* Fabr. (Fabaceae)

$C_{11}H_{14}N_2O$, MW 190.24

CAS RN 485-35-8, BRN 83882

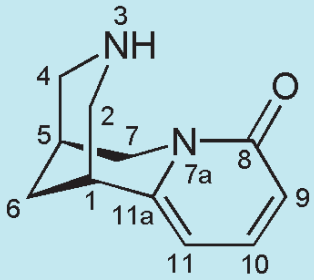
$[\alpha]_D^{22}$ –108.1° (*c* 0.0198 g/mL, ethanol)

Colourless crystals, mp 154–155 °C
Cytisine is commercially available.

Synonymous names:
(−)-Cytisine, Baptitoxine, Laburnin, Sophorine, Tabex, Ulexine

Level: easy

Caution: Cytisine is a strong poison!



1. Background: Hands off and keep your horse away!

When I was a little boy I would roam around with my friends through the park. From time to time my mother warned me urgently against eating the fruits of two trees: the yew (dark red seed cones) and the golden chain (black seeds in beans). The warning was so especially impressive for it was definite and short: That's very poisonous! On looking back, for many children such an interdiction will have been the first real contact with an abstract term: poison. And we learned a poison to be a hidden danger that Nature may mask with appealing wrapping. More generally: obviously, not all things are only what they seem to be.

"But you have not seen it yet," said she, rising; "come to the window and take a better view." I followed her; she opened the sash, and leaning out I saw in full the enclosed demesne which had hitherto been to me an unknown region. It was a long, not very broad strip of cultured ground, with an alley bordered by enormous old fruit trees down the middle; there was a sort of lawn, a parterre of rose-trees, some flower-borders, and, on the far side, a thickly planted copse of lilacs, laburnums, and acacias. It looked pleasant, to me – very pleasant, so long a time had elapsed since I had seen a garden of any sort. But it was not only on Mdlle. Reuter's garden that my eyes dwelt; when I had taken a view of her well-trimmed beds and budding shrubberies, I allowed my glance to come back to herself, nor did I hastily withdraw it.

Charlotte Brontë (1816–1855)
The Professor

The golden chain is a shrub native in Europe from France to the Balkan Peninsula and belongs to the pea family Fabaceae. The pea family? – you may think now of the symbiosis of soil bacteria (rhizobia) in root nodules of the legumes (Fabaceae) able to split nitrogen from the air and fix it as ammonia usable by the plant. The biological outcome of such a symbiosis for the plant host is that it is able to make protein-rich fruits, such as peas, beans, lentils (compare section 5.5 on onocerin) and alkaloids. Indeed, each cytisine has two N-atoms and the seeds are rich in the alkaloid. What about the plant's name, *Laburnum anagyroides*? Pliny the Elder in his encyclopaedia *Naturalis Historia* used the closely related name alburnum already to describe the white (*alba*) sapwood of the golden chain that is hard and esteemed by woodturners and carpenters. The species name anagyroides is due to the similarity of this shrub with the stinking bush *Anagyroides foetida*. The name cytisine for the alkaloid is related to an older synonymous name for this plant (*Cytisus laburnum*) that is borrowed from the Greek island Kythisos.

The poetic name golden chain tree for laburnum descends from the yellow flowers that adorn the blossoming shrub in spring by their pendulous racemes that are about 20 cm long. No wonder that laburnum is a very popular garden tree. In fact, the seeds used in the isolation described below and the photographs stem from a beautiful tree in the garden of a neighbour of one of the authors. All parts of laburnum are poisonous. If consumed in excess there is really a lethal danger. Of course, this may be true for many plants. However, in this case, the insidious danger consists in the circumstance that young children feel attracted by the seeds, which they mistake for peas and want to test. But 3 or 4 fruits containing 15–20 seeds may be lethal for a child. All plant parts contain alkaloids, the leaves (ca. 0.4%), the flowers (ca. 0.9%) and the pericarp, i.e. the fruit-making body around the seed (ca. 0.1%), but most of all it is contained in the ripe seed (1.5–3%). Two structural classes are present, quinolizidine and pyrrolizidine alkaloids. Cytisine as the main alkaloid belongs with its *N*-methyl derivative to the former class, whereas laburnamine and laburnine belong to the latter. Other well-known plants containing cytisine are the common broom (*Cytisus scoparius* L.), mescalbean species (*Sophoreae*) and the common gorse (*Ulex europaeus* L.).

Cytisine is a nicotinic acetylcholine receptor agonist. Both nicotine and cytisine interact with the same receptors in the brain, a fact that

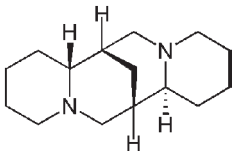
seems understandable when comparing the two structures. This leads to the pharmacological effect of cross-tolerance between the compounds. Cytisine intoxication is similar to nicotine poisoning. The central nervous system is first stimulated and then paralysed. The symptoms, which set in rapidly within an hour, include salivation, stinging in the mouth, thirst, feeling of sickness, nausea, prolonged and sometimes bloody vomiting, sweating, papillary dilatation, whirl, convulsions, headache, coma and heart pain. Larger doses may cause death via respiratory failure. Intoxications with golden chain poison are not an academic curiosity. The Berlin medical centre for the treatment of intoxications recorded 550 cases within 15 years, mainly with children aged up to 10 years. That means they are ranked among the top positions of plant-caused intoxications. It can be regarded as a kind of blessing in disguise that one effect caused by cytisine is in favour of the poisoned, namely the vomiting that evacuates the stomach. Therefore, the mortality in cases of such intoxication is about 2%; of course, this is still a tragedy. An efficient first aid step is to initiate vomiting. The sensitivity of other mammals to cytisine is absolutely different. Whereas sheep and goats seem to be less sensitive, horses are very strongly affected (lethal dose 0.5 g/kg body weight) with intensive sweating as an alarm signal.

As in many other cases, the high physiological activity of cytisine has led to the considerations of whether it could not also be a beneficial compound in any manner. An interesting field is the use of cytisine for smoking cessation. Cytisine has been used and studied as a nicotine replacement since the 1960s, mainly in Eastern Europe. Recently, a review concluded that trials with cytisine as a substitute are of poor quality [1]. Furthermore, in recent times a pharmaceutical preparation for the treatment of nicotinism has become available that contains the active agent varenicline (approval 2006 for Pfizer) as a smoking cessation drug. This compound (7,8,9,10-tetrahydro-6,10-methano-6*H*-pyrazino[2,3-*h*][3]benzazepine; CAS RN 249296-44-4) has a structural resemblance to cytisine. Its tartrate is sold in the USA as Chantix® and in the EU as Champix®. It is the first approved partial agonist of the nicotinic receptor. In its pharmacokinetics it is different from nicotinic antagonists and also from nicotine replacement with nicotine patches or nicotine gum. It can help people to quit smoking because it reduces the craving for it and lowers the pleasurable effects of tobacco products.

As with many natural compounds, the history of the structural elucidation and total synthesis of cytisine covers many decades. Cytisine was first isolated by Husemann and Marmé [2]. The complex determination of the constitution took more than eight decades and was brought about by the work of many chemists with main contributions of the Austrian chemist Galinowsky [3]. A first total synthesis was reported by Bohlmann *et al.* for racemic cytisine, containing a classical cleavage of the racemate via the formation of diastereomeric salts with (+)-camphorsulfonic acid [4]. Although (−)-cytisine identical with an authentic natural sample was obtained, even this success did not mean that the absolute configuration was known. Eventually, the stereochemistry was assigned in 1962, almost a century after the first isolation, by unequivocally showing a link to the known absolute configuration of the related alkaloid (−)-anagyrine

– Adieu, adieu, adieu! dit-elle sans que l'âme communiquât une seule inflexion sensible à ce mot.
C'était l'impassibilité de l'oiseau sifflant son air.
– Elle ne me reconnaît pas, s'écria le colonel au désespoir. Stéphanie! c'est Philippe, ton Philippe, Philippe.
Et le pauvre militaire s'avança vers l'ébénier; mais quand il fut à trois pas de l'arbre, la comtesse le regarda, comme pour le défier, quoiqu'une sorte d'expression craintive passât dans son oeil ; puis, d'un seul bond, elle se sauva de l'ébénier sur un acacia, et, de là, sur un sapin du Nord, où elle se balança de branche en branche avec une légèreté inouïe.

Honoré de Balzac (1799−1850)
Adieu



Scheme 1.5-1 (–)-Sparteine

[5]. Recently, the synthetic strategies leading to cytisine in the form of both enantiomers or as the racemate have been compared in a review [6]. The biosynthesis was studied by incorporation of labelled [1,5-^{14}C] cadaverine and [2-^{14}C] lysine into cytisine and *N*-methylcytisine. The conclusion was that it is likely to assume that cytisine is formed by oxidative degradation from (−)-sparteine as its natural precursor [7]. The structural similarity between the cytisine and sparteine skeletons became of interest some 30 years later from the viewpoint of asymmetric synthesis. The drawback of many natural products of the chiral pool is that they are not available in the form of both enantiomers on the same scale. This means a restriction of their application because "the other half" is out of reach. Whereas (−)-sparteine, a chiral ligand for asymmetric synthesis, as a lupin alkaloid is readily accessible, its (+)-enantiomer is not. Therefore, successful attempts have been made to synthesize (+)-sparteine surrogates that are structurally derived from (−)-cytisine. The deciding structural difference is that they lack the D-ring of sparteine, which does not mean, however, that their performance as chiral ligands imitating (+)-sparteine is reduced [8]. This is an example where a chiral pool member has not been used as a precursor for the synthesis of another natural product but as a precursor for a useful imitation to overcome a fault.

Fig. 1.5-1 Golden chain blossoms

What improvements might be subsequently introduced?
A rabbitry and fowlrun, a dovecote, a botanical conservatory, 2 hammocks (lady's and gentleman's), a sundial shaded and sheltered by laburnum or lilac trees, an exotically harmonically accorded Japanese tinkle gatebell affixed to left lateral gatepost, a capacious waterbutt, a lawnmower with side delivery and grassbox, a lawnsprinkler with hydraulic hose.

James Joyce (1882−1941)
Ulysses

2. Literature

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[11] T. Brukwicki, W. Wysocka, "Geometry of tricyclic quinolizidine-piperidine alkaloids in solution by NMR spectroscopy" *J. Mol. Struct.* **1999**, *474*, 215−222.

3. Isolation

3.1 Principle

This is an example for a classical alkaloid extraction process. The alkaloid cytisine is released from the seeds by the basicity of concentrated ammonia (any alkaloid salts would be cleaved thereby, if present) and released into a two-phase system in which an organic extracting agent (CH_2Cl_2) takes up the alkaloid. Extracted plant material and ammonia are then separated and discarded. The next step consists in the selective transfer of the alkaloid from CH_2Cl_2 into the aqueous phase by formation of water-soluble cytisine hydrochloride. This is ensured by reaction with hydrochloric acid. Any other neutral organic compounds that may have also been extracted at the beginning into the organic solvent cannot form such a salt and will remain in the CH_2Cl_2. Therefore, this step is crucial for the selectivity of the isolation. Now, the acidic aqueous phase is separated and the organic phase can be discarded. To obtain the alkaloid in hand it has now to be brought back in its neutral form. This is achieved by alkalizing with concentrated ammonia and re-extraction into CH_2Cl_2. It is astonishing that a single recrystallization step from toluene is sufficient to obtain pure cytisine. Toluene is a suitable solvent for several reasons: it is nonpolar, whereas the compound is polar due to its lactam unit; it has a certain temperature solubility gradient as its bp 111 °C is distinctly above RT; it has a certain structural similarity to cytisine in its structure, the aromatic ring, therefore it is a better solvent than e.g. cyclohexane would be; finally, it is always favourable if the bp of the solvent is below the mp of the solid, because then a supersaturation effect can be excluded. This last feature becomes more difficult to fulfil the lower the mp is.

3.2 Method

This is based on the method described in [9].

Five portions (10 g each) of dried pods of the golden chain are finely ground in a kitchen grinder for 10 s. The powder obtained is placed in a 500 mL three-necked flask charged with dichloromethane (175 mL), methanol (50 mL) and 25% aqueous ammonia solution (25 mL). The resulting mixture is stirred vigorously with an overhead mechanical stirrer at RT for 3 h, allowed to stand overnight and then stirred for another 4 h. The mixture is filtered by suction through a Buchner funnel.

Fig. 1.5-2 Seeds of the golden chain tree

Une bise froide et aiguë sifflait à travers les branches dépouillées, et cependant la sueur ruisselait sur mon front. Je me rappelai que j'avais reçu le coup de poignard au moment où je piétinais la terre pour recouvrir la fosse; en piétinant cette terre, je m'appuyais à un faux ébénier; derrière moi était un rocher artificiel destiné à servir de banc aux promeneurs; car en tombant, ma main, qui venait de quitter l'ébénier, avait senti la fraîcheur de cette pierre. À ma droite était le faux ébénier, derrière moi était le rocher, je tombai en me plaçant de même, je me relevai et me mis à creuser et à élargir le trou: rien! toujours rien! le coffret n'y était pas.

Alexandre Dumas (1802−1870)
Le Comte de Monte-Cristo,
Chapter 67

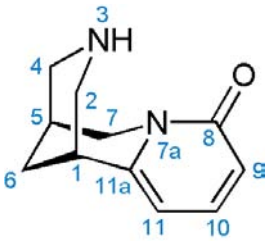
The filter cake is washed with dichloromethane until a colourless filtrate is obtained. The filtrate is transferred into a separating funnel and shaken with 3.3 M HCl (ca. 140 mL). The contents are left in the funnel for 2 h and shaken repeatedly to ensure good mixing and passing over of the alkaloid as hydrochloride into the aqueous phase. The aqueous phase shows a pH of 1–2 when tested with pH paper. After phase separation of the two layers, the aqueous phase is transferred into an Erlenmeyer flask and 25% aqueous ammonia solution (ca. 40 mL) is added dropwise within 1 h under magnetic stirring with external cooling using a cold water bath until pH 9–10 is reached (tested with pH paper). Stirring is continued for 2 h. The alkaloid is then extracted from the aqueous phase with dichloromethane (10 × 20 mL). The extracts are combined, dried over $MgSO_4$, filtered and the solvent is completely removed in vacuo to leave crude cytisine as a yellow-brownish solid.

3.3 Purification

The crude solid cytisine is recrystallized from toluene (5 mL) to yield 277 mg of pure cytisine as a yellow crystalline solid; mp 154–155 °C.

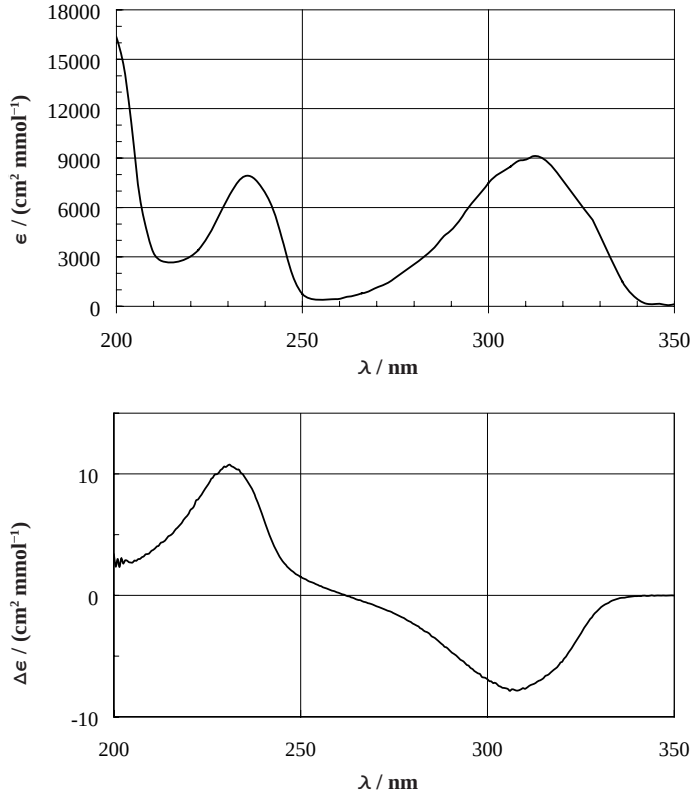
$[\alpha]_D^{22}$ –108.1° (*c* 0.0198 g/mL, ethanol).

Fig. 1.5-3 Laburnum tree in the street of one of the authors



Scheme 1.5-2

4. Spectra and Comments

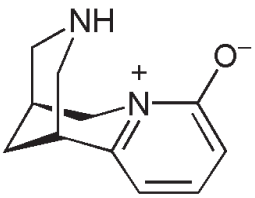


In einem Garten
unter dunklen Bäumen
erwarten wir die Frühlingsnacht.
Noch glänzt kein Stern.
Aus einem Fenster,
schwellend,
die Töne einer Geige. . . .
Der Goldregen blinkt,
der Flieder duftet,
in unsern Herzen geht der Mond auf!

Arno Holz (1863−1929)
Phantasus

Fig. 1.5-4 UV and CD spectra in ethanol

The UV spectrum shows two distinct maxima, one at 234 nm with ε = 8000 cm^2 $mmol^{-1}$ and the second at 312 nm with ε = 9000 cm^2 $mmol^{-1}$. Whereas the the first absorption typically resembles the π → π * part of the chromophore, it can be assumed that the surprisingly strong band at 312 nm involves structures where the free electron pair of the nitrogen is part of the extended mesomeric system:



It is very interesting that the CD spectrum of cytisine shows two rather strong bands at 234 and 312 nm with opposite polarity, whereas the first UV absorption band at 202 nm has no Cotton effect.

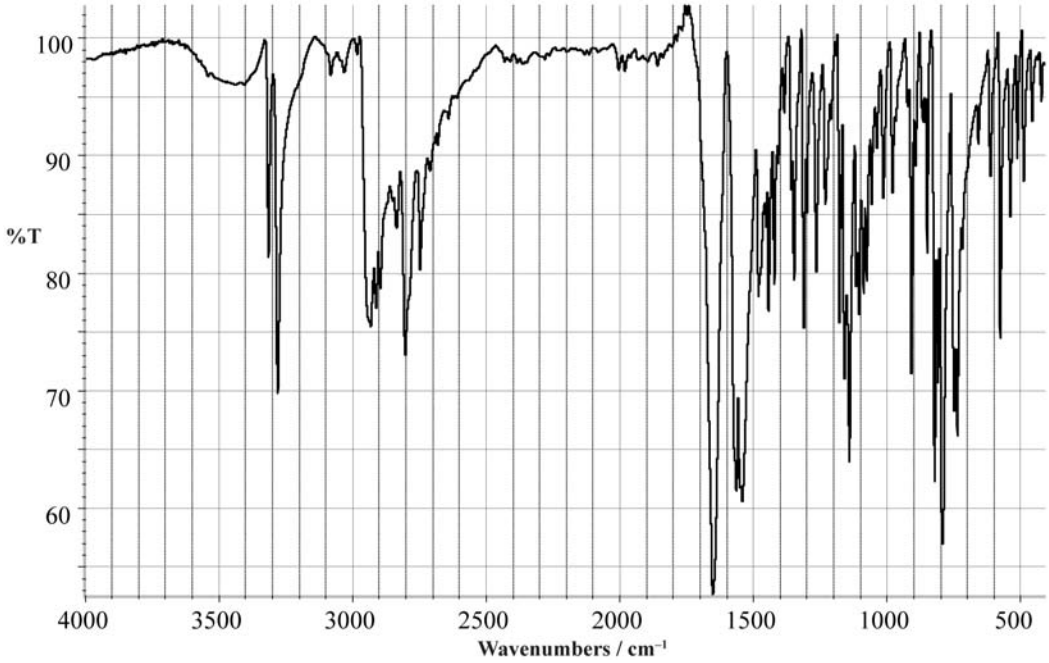


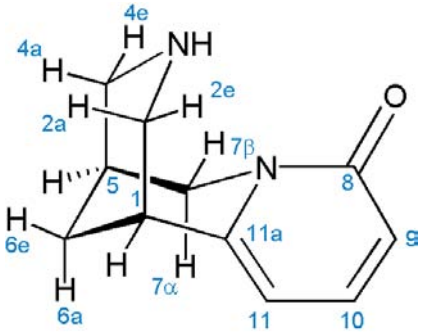
Fig. 1.5-5 IR spectrum in KBr

The IR spectrum displays a sharp and split NH valence vibration at 3300 cm^{-1}. The sp^2 CH valence vibrations between 3100 and 3000 cm^{-1} are remarkably weak and they are followed by many aliphatic CH valence bond vibrations from 2950 to 2750 cm^{-1}. Typical for the amide-type structure is the lowered frequency of the carbonyl C=O vibrations at 1650 and 1570 cm^{-1} and these resemble very well the amide I and amide II IR absorptions for this class of compounds.

Fig. 1.5-6 Seeds of common laburnum

It was when looking down from that suburban eyrie over the whole confounding labyrinth of London that he was filled with that great irresponsible benevolence which is the best of the joys of youth, and conceived the idea of a perfectly irresponsible benevolence in the first plan of Pippa Passes. At the end of his father's garden was a laburnum "heavy with its weight of gold," and in the tree two nightingales were in the habit of singing against each other, a form of competition which, I imagine, has since become less common in Camberwell.

G. K. Chesterton (1874−1936)
Robert Browning



Scheme 1.5-3

A bush of May flowers with the bees about them;
Ah, sure no tasteful nook would be without them;
And let a lush laburnum oversweep them,
And let long grass grow round the roots to keep them
Moist, cool and green; and shade the violets,
That they may bind the moss in leafy nets.

John Keats (1795–1821)
Poems

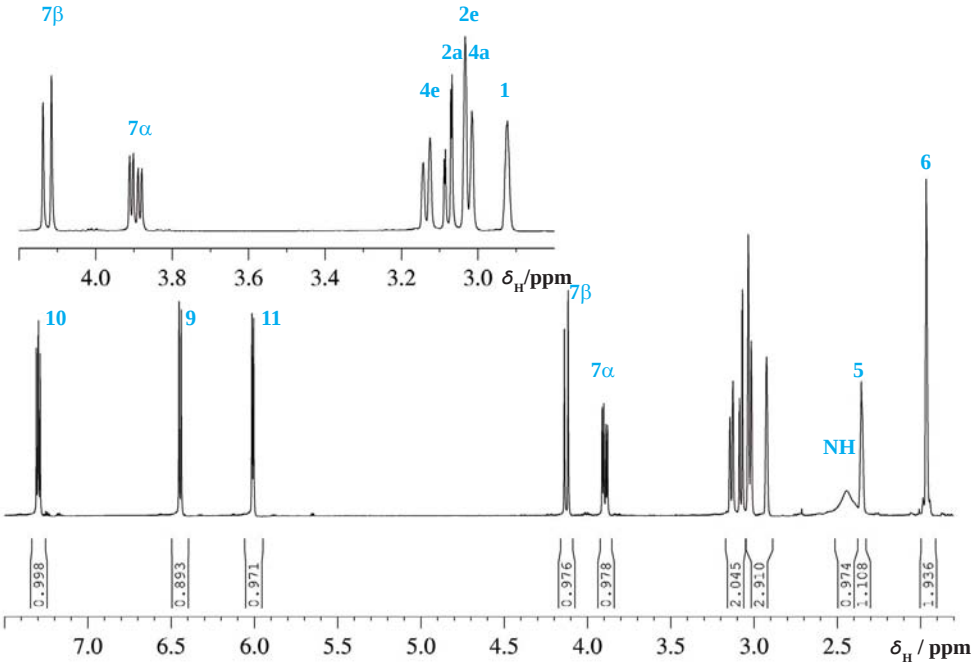


Fig. 1.5-7 ^{1}H NMR spectrum at 700 MHz in $CDCl_3$

The ^{1}H NMR spectrum consists of 12 absorptions well dispersed over the entire chemical shift range. In the olefinic/aromatic regions we find two doublets at 6.45 and 6.0 ppm and a doublet of doublets at 7.30 ppm. The last clearly belongs to H-10 due to its β-position with respect to the carbonyl group and the spin couplings to both H-9 and H-11. A safe assignment of which of the two signals at 6.5 and 6.0 ppm belongs to H-9 or H-11 cannot be decided at this stage of the analysis. There is a diastereotopic methylene group with signals at 4.13 and 3.89 ppm, indicative of an NCH_2 group, where one of these signals is further coupled to another proton. This observation leads to the assignment for H-7α and H-7β, where the pseudo-axial proton at 3.89 ppm displays the additional spin coupling to H-5. For the pseudo-equatorial proton a Karplus angle close to 90° can be seen from a model. In a flattened ring system the terms axial and equatorial are no longer valid and the designations α and β, which are defined for protons below and above the mean ring plane, are better suited. The signal group of five protons at about 3 ppm will be disentangled using the COSY spectrum, but clearly the most shielded signal at 1.96 ppm belongs to the two protons at C-6.

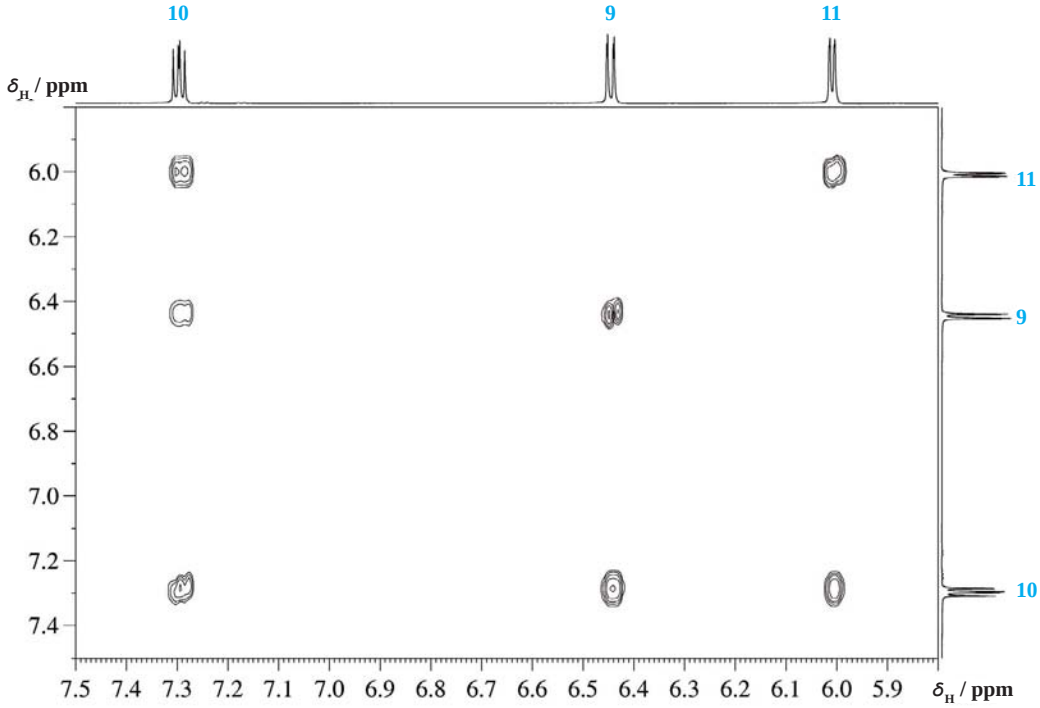


Fig. 1.5-8 Expansion of the COSY spectrum in the aromatic region

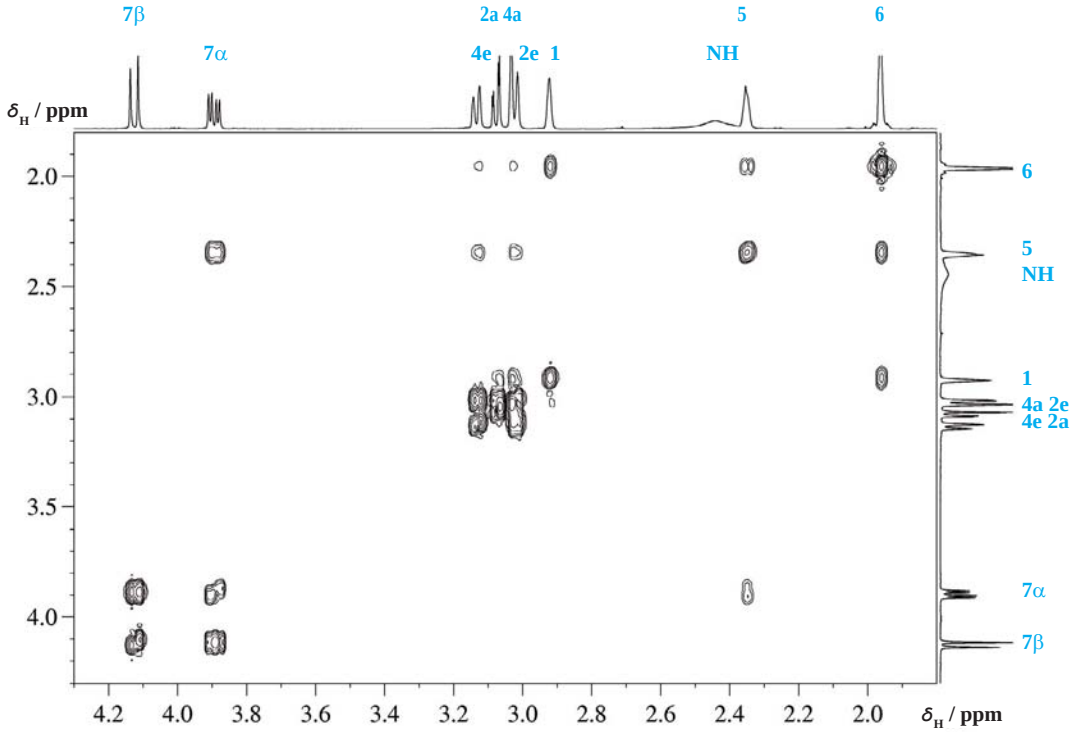
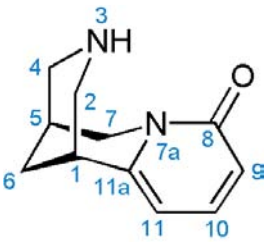


Fig. 1.5-9 Expansion of the COSY spectrum in the aliphatic region

Since the ^{1}H NMR spectrum is not too crowded, in contrast to many other cases in this book, the COSY spectrum will this time be extremely helpful or even already decisive for the signal assignment. The aromatic expansion is a scholarly example of a simple ABC spin system. In the aliphatic expansion we start with the NCH_2 group of H-7 and find a cross peak leading from H-7α to H-5 at 2.35 ppm. In turn, we find from H-5 a cross peak leading to the most aliphatic signal of H-6 at 1.96 ppm, which appears as a broadened singlet. The protons H-6 are also coupled to H-1, as indicated by the corresponding cross peak to this signal at 2.92 ppm. The signals of the remaining two diastereotopic methylene groups are secured by the corresponding cross peaks from the bridgehead protons H-5 and H-1. Thus H-4 is found at 3.13 and 3.02 ppm whereas H-2 is present at 3.08 and 3.02 ppm.



Scheme 1.5-4

Der Rosenschein

Lautlos über den spiegelnden Plan
ziehen die Schwäne silberne Bahn.
Goldregen in schimmerndem Schweigen
Rinnt von den zitternden Zweigen,
Nachtigall fleht im Syringenbaum,
Auf lauen Schwingen ein Schattentraum
Weht über die blauenden Matten.

Max Dauthendey (1867−1918)
Dornröschen

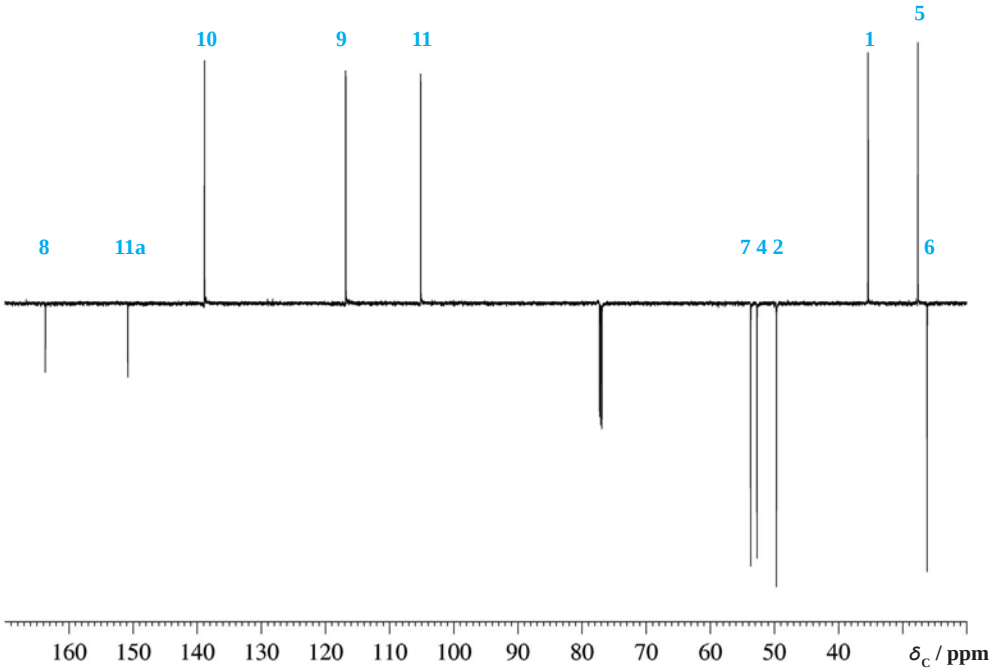


Fig. 1.5-10 APT ^{13}C NMR spectrum

The edited ^{13}C NMR spectrum displays the correct number of signals with five CH moieties, four methylene groups and two quaternary carbon atom signals. The assignment is straightforward for the amide carbonyl at 163.7 ppm and for C-11a at 150.8 ppm. In the aliphatic region only the signal of C-6 can at present be assigned with safety at 26.2 ppm.

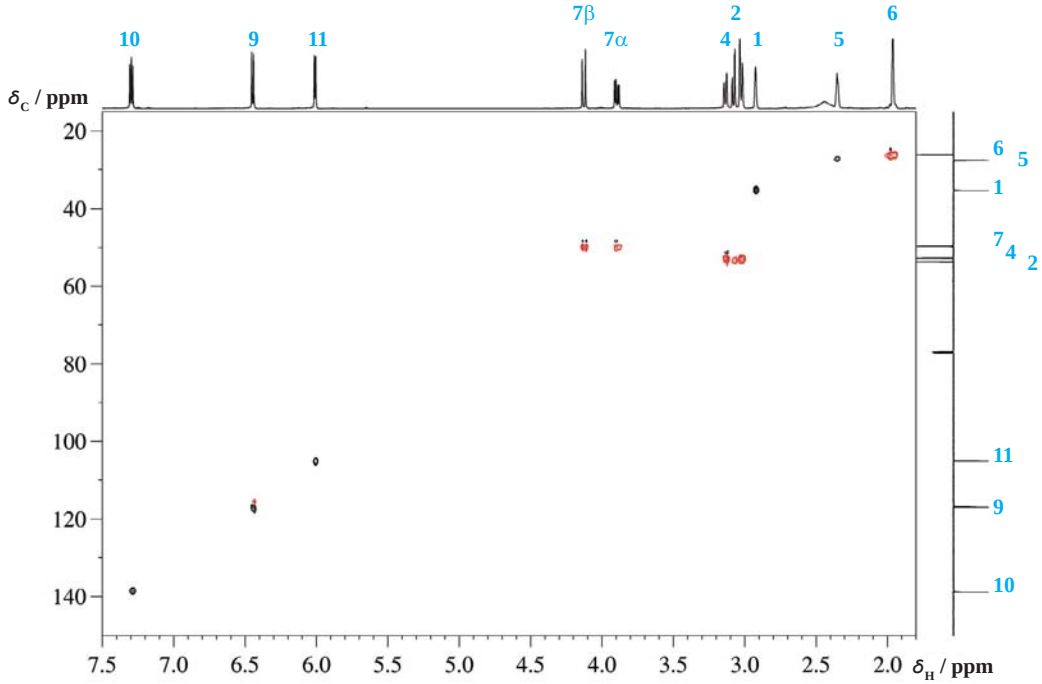
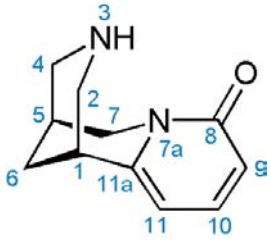


Fig. 1.5-11 HSQC spectrum

Since we have completely assigned the proton spectrum of the molecule, we use the HSQC spectrum for the residual assignments of the carbon spectrum. We observe that in the olefinic region the relative sequence for both carbon and protons is identical. We can identify the signal of C-5 at 27.6 ppm and that of C-1 at 35.4 ppm and we can differentiate between the two closely resonating methylene groups C-4 and C-2.



Scheme 1.5-5

The expansion of the HMBC spectrum in the aromatic region displays very clearly the correlation signals of the three proton signals. H-10 is connected via three bonds to C-11a at 150.8 ppm and to the carbonyl signal at 163.7 ppm. The signal of the proton at 6.45 ppm is connected to the carbon signal of C-11 at 105.1 ppm and to the signal of the carbonyl atom. The spectrum was recorded in a manner such that also the ^{1}J(CH) connectivities can be seen in the aromatic region and these help with the relative assignment. The proton signal at 6 ppm is connected to C-11a at 150.8 ppm and to C-9 at 116.8 ppm, and most significantly also to an aliphatic carbon atom which must therefore be C-1 at 35.4 ppm.
In the aliphatic region H-7 is connected to C-11a and to the carbonyl C-atom, and in addition to C-6 and C-5. The connectivity to C-11a is decisive for the assignment of H-2. Due to their central position, the proton signals of H-6 display the highest number of CH correlations over two and three bonds.

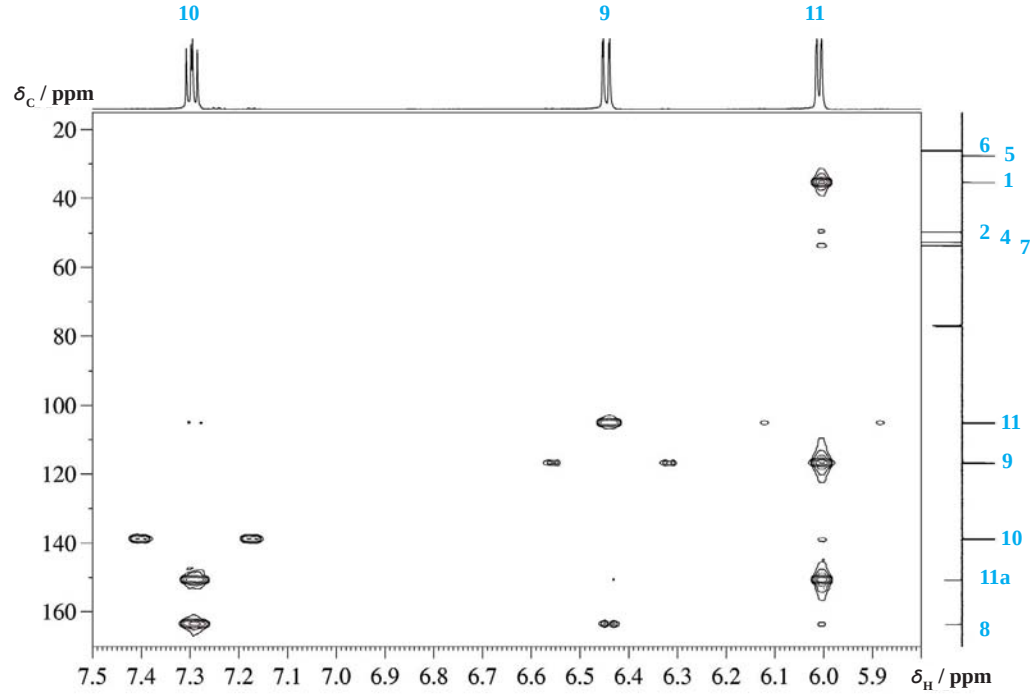


Fig. 1.5-12 Expansion of the HMBC spectrum in the aliphatic region

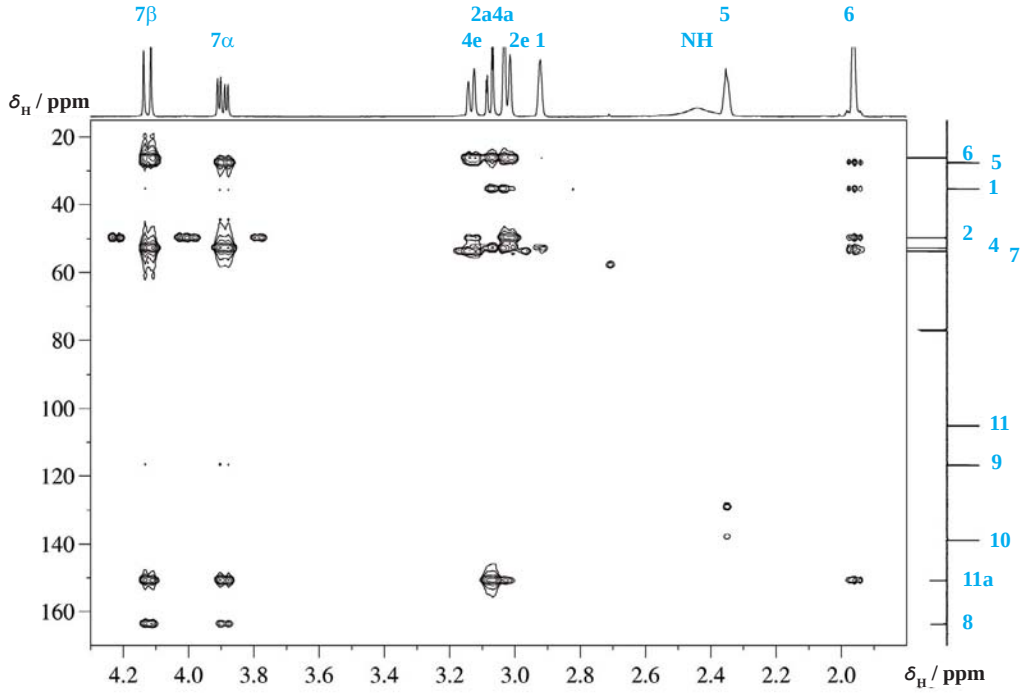


Fig. 1.5-13 Expansion of the HMBC spectrum in the aromatic region

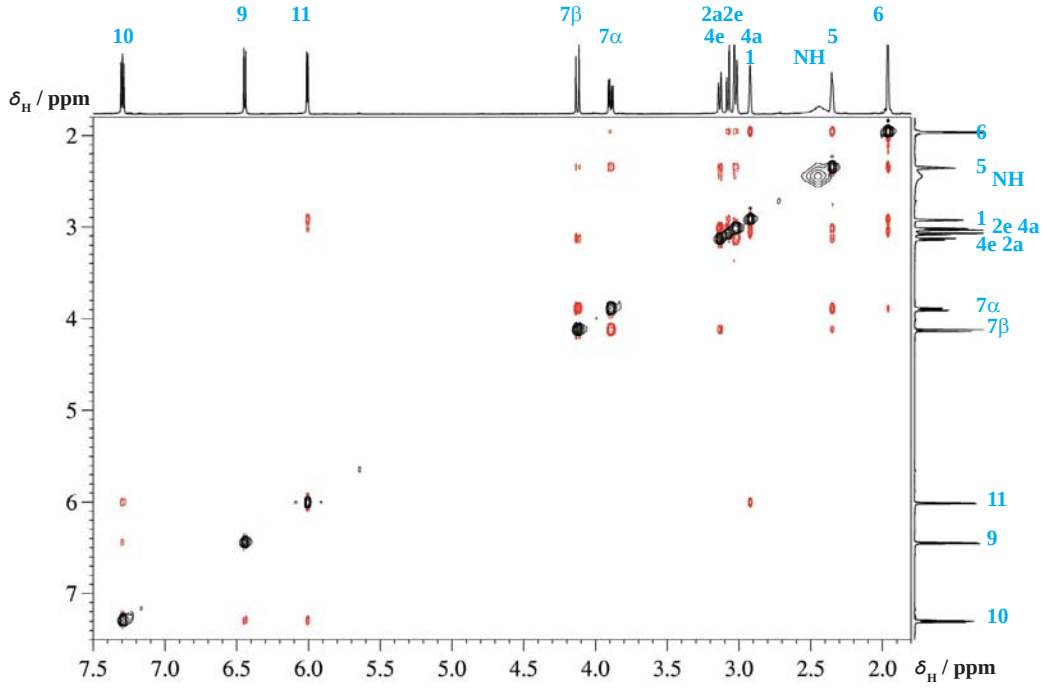


Fig. 1.5-14 NOESY spectrum

After having safely assigned all proton and carbon signals in this molecule, we use the NOESY spectrum only to determine the stereochemistry of the individual CH_2 groups. Looking at H-7β at 4.13 ppm we find an NOE contact to H-4e, which is not displayed by H-7α; similarly, H-7α shows an NOE contact to H-6 which is not displayed by H-7β. Both H-7 show an NOE contact to H-5. Finally, both H-4a and H-2a show NOE contacts to H-6, hence they can be assigned relative to their equatorial counterparts.

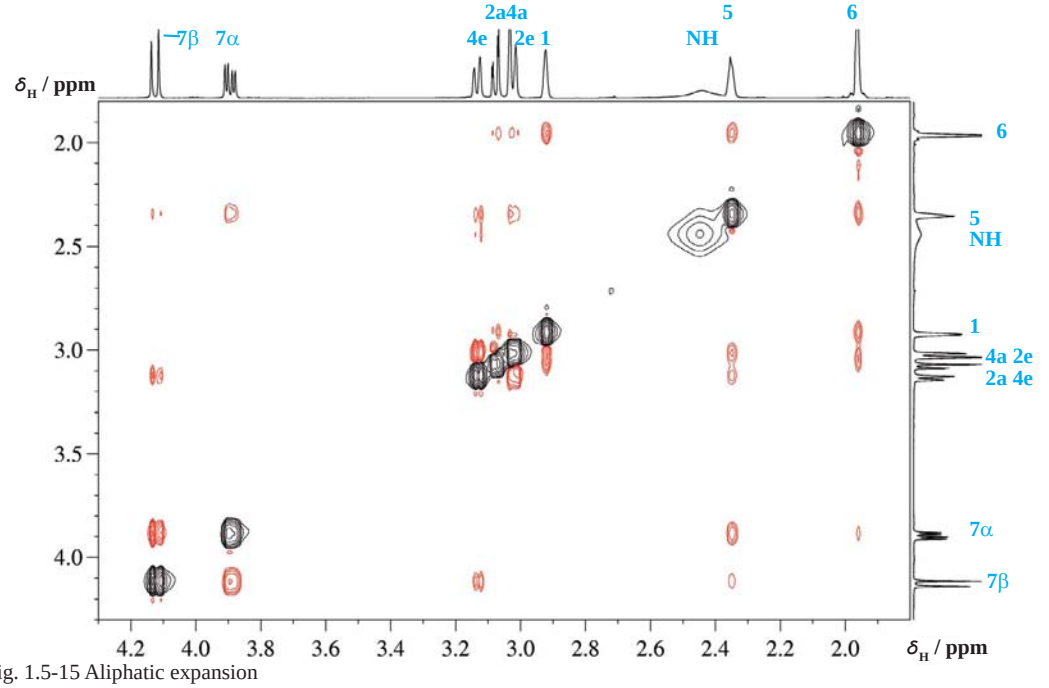
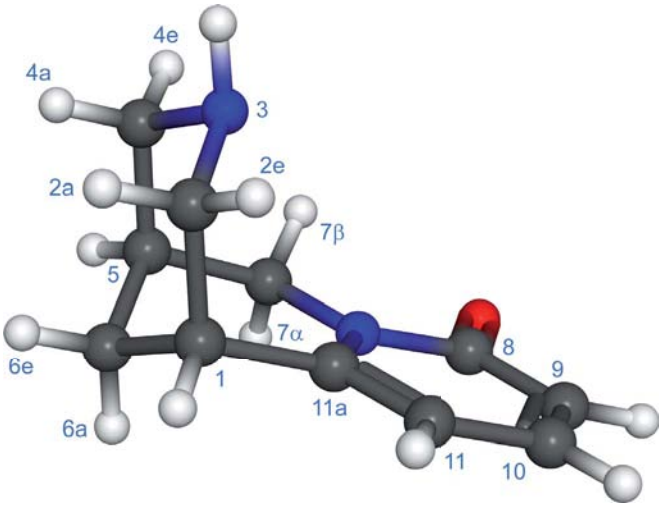


Fig. 1.5-15 Aliphatic expansion



Und so setzte denn, als eben Goldregen und Syringen im Garten des Vetters sich zum Blühen anschickten, ein braunes, rosiges Mädchen zum erstenmal den Fuß über die Schwelle seines Hauses; und der Vetter konnte nicht begreifen, weshalb auch drinnen die alten Wände plötzlich zu leuchten begannen. Erst später meinte er bei sich selber, es sei der Strahl von Güte, der aus diesen jungen Augen gehe. Die Großtante freilich schüttelte etwas den Kopf über diese gar so jugendliche Haushälterin, und womit die alte Caroline geschüttelt, das hat der Vetter niemals offenbaren wollen.

Theodor Storm (1817−1888)
Beim Vetter Christian

Fig. 1.5-16 Molecular model of cytisine

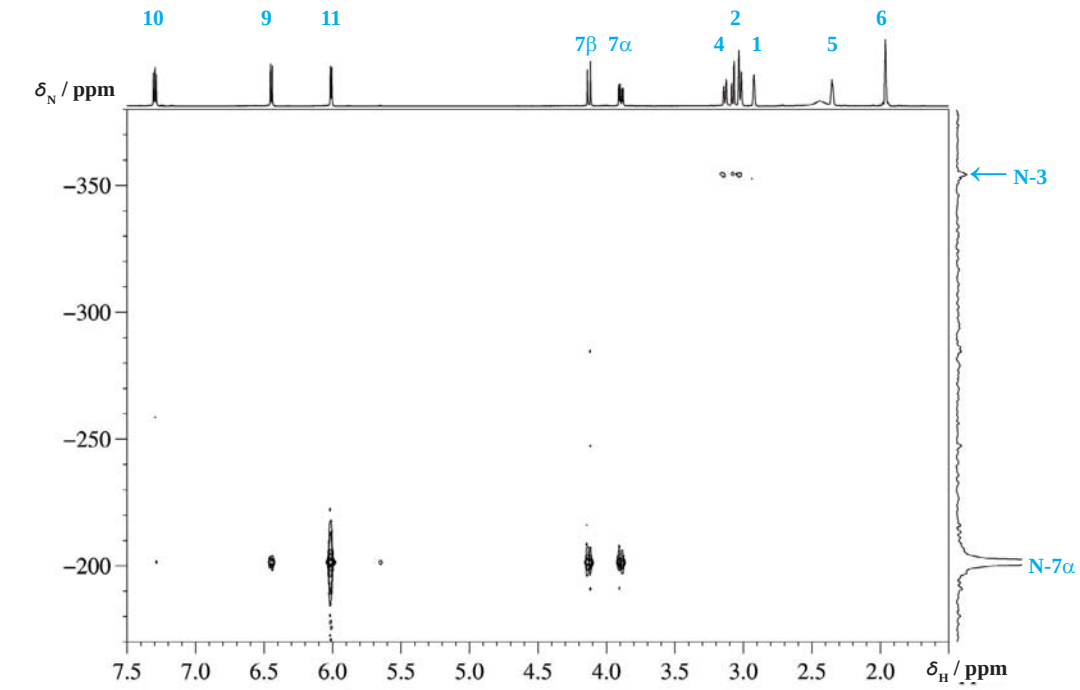
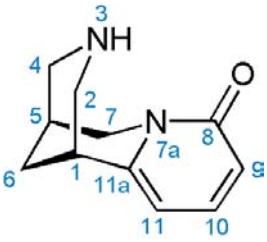


Fig. 1.5-17 $^{1}H^{15}N$ HMQC spectrum

Since cytisine is an alkaloid with two nitrogen atoms, it was worthwhile to record a $^{1}H^{15}N$ HMQC spectrum to reveal the ^{15}N information. The spectrum is very similar to that of strychnine, since both compounds contain an amide nitrogen and a secondary aliphatic amine group. The amide nitrogen N-7a appears at –201.7 ppm (referenced versus nitromethane) and is detected by H-11, H-9 and H-7. The amine nitrogen N-3 is detected by H-2 and H-4, at –354.8 ppm.

"Ja, liebe Johanna, das ist alles ganz gut, aber was sollen wir damit? Wir haben ja den Weg gesehen. Oder wollen Sie den Kirchhof..."
"Freilich will ich. Ich habe da so meine Gefühle, besonders an solchem Tage wie heute. Und es ist immer gut, sich zu erinnern, daß man sterben muß. Und wenn dann der Flieder so blüht..."
"Aber, Johanna, der Flieder blüht ja gar nicht mehr, höchstens noch der Goldregen, und der hat eigentlich auch schon Schoten. Du meine Güte, wenn Sie so partout für Kirchhöfe sind, so können Sie sich ja den in der Oranienstraße jeden Tag ansehen. Aber ich weiß schon, mit Ihnen ist nicht zu reden. Zeuthen und Kirchhof, alles Unsinn. Da bleiben wir doch lieber hier und sehen gar nichts. Kommen Sie, Kleine, geben Sie mir Ihren Arm wieder."

Theodor Fontane (1819−1898)
Irrungen Wirrungen, Chap. 13



Scheme 1.5-6

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz	^{15}N Signals δ / ppm
163.7	C_q	C-8		
150.8	C_q	C-11a		
138.8	CH	C-10	7.30, $J_{10,11}$ = 6.85, $J_{10,9}$ = 9.0	
116.8	CH	C-9	6.45, $J_{9,11}$ = 1.49, $J_{9,10}$ = 9.0	
105.1	CH	C-11	6.00, $J_{11,10}$ = 6.85, $J_{11,9}$ = 1.0	
53.7	CH_2	C-7	a: 4.13, e: 3.89, $J_{7\alpha,\beta}$ = −15.4, $J_{7\alpha,5}$ = 6.60	
52.7	CH_2	C-4	3.13, 3.02, $J_{4e,4a}$ = −12.6	
49.7	CH_2	C-2	3.08, 3.02, $J_{2e,2a}$ = −12.0, $J_{2,1}$ = 2.3	
35.4	CH	C-1	2.92	
27.6	CH	C-5	2.35	
26.2	CH_2	C-6	1.96	
		N-3	NH: 2.44	−354.8
		N-7a		−201.7

Table 1.5-1 NMR data for cytisine

Fig. 1.5-18 Laburnum tree in autumn

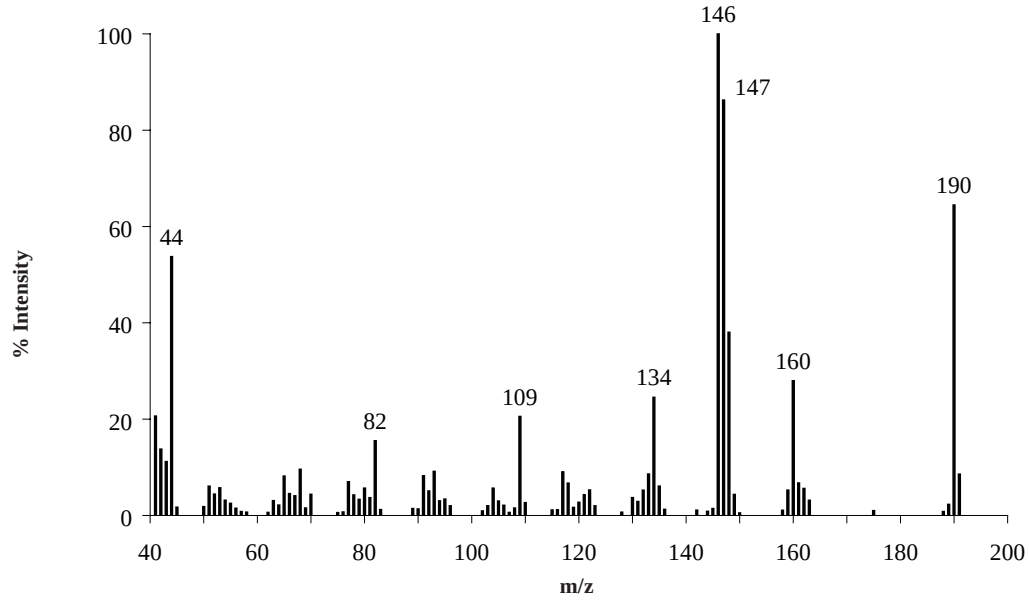
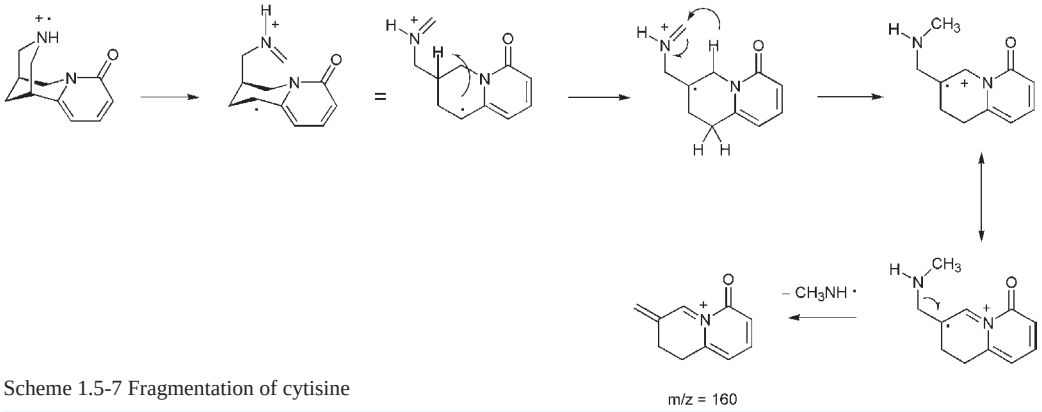


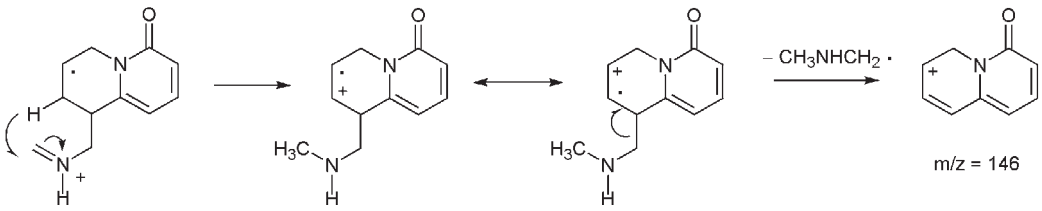
Fig. 1.5-19 Mass spectrum

The EI mass spectrum displays the molecular ion signal at m/z = 190 and indicates a loss of 30 Da to form the ion with m/z =160. This is explained by assuming the first ionization at the secondary amine group, subsequent hydrogen transfer and elimination of a methylamine radical according to:



Scheme 1.5-7 Fragmentation of cytisine

There is a cluster of signals from m/z = 149 to 146 involving the base peak at m/z =146. The latter can be understood by a very similar mechanism involving the loss of a dimethylamine radical:



Scheme 1.5-8 Further fragmentation

5. Questions

A. Reflect on the structural reasons for the physicochemical difference in forming a liquid or a solid at ambient temperature within the following pairs of compounds:

(a) nicotine (liquid at ambient temperature) and cytisine (solid);

(b) squalene (liquid) and lanosterol (solid);

(c) *n*-hexane (liquid) and cyclohexane (but solid already at 6 °C).

B. What is the meaning of the term *chiral pool*?

C. Do all alkaloids contain N or are there exceptions?

D. Why is the active principle varenicline mentioned in the text administered in its pharmaceutical formulations Chantix® (USA) and Champix® (EU) as tartrate?

E. CH vibrations at 2850 and 2750 cm^{-1} are seldom in the IR spectra in this book. Which structural element is responsible?

F. Give at least two arguments from the spectra for the relative assignment of H-11 and H-9, with respect to their corresponding carbon atoms.

G. The HMBC spectrum of H-7α and H-7β reveals the interesting fact that one, H-7β, is coupled to C-6 at 25 ppm, whereas the other, H-7α, is connected to C-5 at a slightly different chemical shift of 26 ppm. Explain.

H. A standard method to elucidate the absolute stereochemistry of chiral compounds is the formation of derivatives with both enantiomers of α-methoxy-α-trifluoromethylphenylacetyl chloride (Mosher's acid chloride). In this case, the derivatives formed of the *R* and *S* acid chloride each show two sets of proton spectra. Explain.

I. If one obtains a mass spectrum of an alkaloid with an even mass for the molecular ion, as is true for cytisine, what is the direct structural conclusion?

J. Give a mechanism for the formation of the ion at m/z = 147.

6. Own Observations

1.6 Galanthamine

(4a*S*,6*R*,8a*S*)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6*H*-benzofuro-[3a,3,2-*ef*][2]benzazepin-6-ol

From the bulbs of daffodils
Narcissus pseudonarcissus L.
subspecies *pseudonarcissus* Carlton (Amaryllidaceae)

$C_{17}H_{21}NO_3$, MW 287.35

CAS RN 357-70-0, BRN 93736

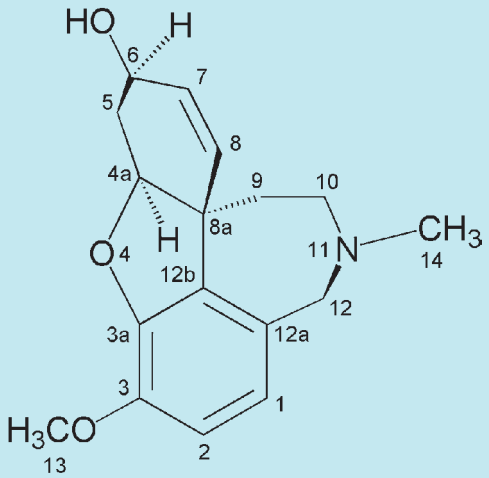
Colourless crystals, mp 128 °C (database value)

$[\alpha]_D^{22}$ –125° (*c* 0.0063 g/mL, ethanol)

Galanthamine is commercially available.

Synonymous names:
(–)-Galanthamine, Galantamin, (–)-Galantamine, Jilkon, Lycoremine

Level: difficult



1. Background: All inclusive: pretty, poisonous and useful!

This is a chapter about a pretty, poisonous and useful plant, the daffodil. The alkaloid galanthamine that we isolate from special daffodil bulbs was, however, discovered in the bulb of another spring flower, the Caucasian snowdrop (*Galanthus woronowii*), a relative of the common snowdrop (*Galanthus nivalis* L.).

Fig. 1.6-1 Common Snowdrops in spring

In contrast to other alkaloids described here, the discovery of galanthamine is not that old (around 1950). It has a kind of origin that we have not discussed here yet. It was found by becoming aware of its ethnopharmacological use. This means that laymen without medical education but with traditional knowledge on the physiological effects of plant ingredients use plant preparations to cure people. By the way, the most widespread story in this context is that the knowledge about the effect of cardiac glycosides from the common foxglove (*Digitalis purpurea* L.) in the treatment of cardiac failure was taken over at the end of the 18th century by W. Withering, an English medical doctor, from the experience of a healer woman from the country. With galanthamine the story is quite similar. Around 1950, Russian pharmacologists noticed that villagers from the Ural mountains used the wild Caucasian snowdrop to treat poliomyelitis in children. From this plant galanthamine was then isolated as an active principle in 1952 [1]. The name galanthamine is an artificial combination of the Greek words γάλα for milk, ἄνθος for flower (this makes the genus name galanthus = "milky flower") and the word amine highlighting the basic nature of the alkaloid. Thorough investigation of this special snowdrop, the well-known common snowdrop and other species belonging to the family Amaryllidaceae soon proved that they all were full of alkaloids. Interestingly, in this case none of the often cited antique or mediaeval texts mentions a medical application of such plants. In the margin of the next page the main patterns of such alkaloids are shown. The skeletons of the main Amaryllidaceae alkaloid types besides galanthamine are represented by the following compounds: norbelladine (nonheterocyclic), crinine, haemanthamine, homolycorine, lycorine, montanine, narciclasine and tazettine.

Fig. 1.6-2 Schnee-Tropfen, the old German name for the common snowdrop

The assignment of the galanthamine structure was much faster than depicted elsewhere for some of the "historical" alkaloids isolated in the early 19th century. Whereas the first Russian researchers mentioned in [1] assigned several structural subunits of the molecule, the absolute configuration was discovered by means of an X-ray structure [2] in 1964, i.e. only a dozen years after the isolation of the compound.

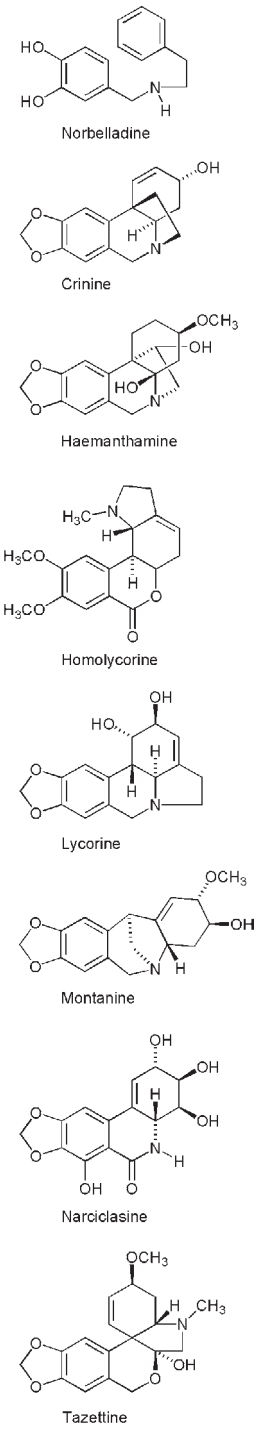
Early, its acetylcholinesterase-inhibiting property was recognized and the ability to act as an antagonist of curare's action. In fact, galanthamine enhances the cholinergic function by increasing the level of the neurotransmitter acetylcholine in the brain. Furthermore, galanthamine is also capable of influencing the nicotinic cholinergic receptors in a second pathway and so again increasing the release of acetylcholine. Initially, the compound was therefore used to reverse the relaxation of muscles caused by curare used during surgery. Additionally, the alkaloid

was used in cases of pathological muscle weakness. These early uses of galanthamine were developed during the time of the Cold War in Bulgaria and the USSR behind the Iron Curtain.

None of the ethnopharmacological uses mentioned above gave a hint that another use of the alkaloid would also be possible. The crucial point to open the door to this new research direction was the consideration that galanthamine is able to penetrate the blood–brain barrier and to augment the central cholinergic function, specifically. This led to studies on the use of galanthamine as a symptomatic treatment, not a cure, for patients suffering from Alzheimer's disease. Dementia, when defined as an illness in 1907, was a relatively rare event. Today, it has become a common illness of elderly people that will become even more important with increasing life expectancy. A feature of this neurodegenerative disease is the progressive decline in memory in combination with damage to one or more cognitive functions. At the end of the 1970s, it was discovered that Alzheimer patients had brains deficient in the neurotransmitter acetylcholine, necessary to pay attention and facilitate learning. This finding and the fact that galanthamine could pass the barrier to the brain led in the 1980s in Western Europe to investigations on the use of the alkaloid for enhancing the cholinergic transmission. Galanthamine preparations have become a therapeutic option to decelerate neurological degeneration. Galanthamine has a twofold effect on the cholinergic system. It acts as an inhibitor of acetylcholinesterase and thus slows the cleavage of acetylcholine to a certain extent, and it allosterically modulates the nicotinic acetylcholine receptor, which eventually also ends in an increase in the desired acetylcholine. The peculiar story of this alkaloid's pharmaceutical development is described in recent reviews [3,4].

However, galanthamine used today for medical purposes is no longer implicitly gained by plant extraction. Synthetic procedures have been developed and patented and are in use. The first company that obtained a patent on a synthetic access was the Austrian Sanochemia Pharmazeutika in 1997, which later began a cooperation with Janssen Pharmaceutica (Belgium). The drug is now on the market under trade names such as Reminyl, Razadyne and Nivalin and is indicated for mild to moderate cases of the disease. Since that time, about 20 patents have been taken out, which illustrates the interest in such a medication. The synthetic approaches have been discussed for processes leading both to racemic galanthamine and to the enantiomerically pure form. The two key reaction steps are an oxidative phenol coupling reaction and an intramolecular Heck reaction [5]. In addition, some further extractive methods from plants have also been claimed.

Regarding the biological purpose for the producing plant, it can be assumed that such alkaloids are an advantage in the struggle for survival. If one considers that the mechanically only weakly protected bulb (without bark, as existing on other roots) has to stay all the time in the soil full of microorganisms, worms and the like, it is understandable that such a bulb has got weapons against such parasites: poisons! This is comparable to a soft coral in the ocean. About 150 Amaryllidaceae



Scheme 1.6-1 Further Amaryllidaceae alkaloid types besides galanthamine shown at typical individual representatives; note that norbelladine is a non-heterocyclic secondary amine.

Fig. 1.6-3 The natural wild daffodil (*Narcissus pseudonarcissus* L.) as found in the Botanical Garden of the University of Leipzig

alkaloids are known, differing in the kind of ring systems formed. The biogenesis, which cannot be discussed here, starts in any case at L-tyrosine and L-phenylalanine, which are transformed into derivatives of the open chain *N*-benzyl-*N*-β-phenylethylamine. Oxidative coupling is then the main means to form a variety of cyclic structures. For a deeper insight, a recent book can be consulted [6].

The natural source used here is bulbs of a special variety of the species *Narcissus pseudonarcissus*, a creation closely related to the natural wild daffodil (see photographs of this wild form and of the species used here in the margin and compare). The botanic name *Narcissus* stands for a genus of mainly hardy plants growing from bulbs, that all belong to the Amaryllis family, native to Europe, North Africa and Asia. Most of their species are spring-flowering. A common feature of all species is the central corona that may have a trumpet-, bowl- or disc-like shape and is surrounded by six floral leaves, three sepals and three petals. The coloration of floral leaves and corona may be the same (e.g. yellow) or different and extends from white for the former to orange for the latter.

It is most likely that the name narcissus was derived from the narcotic properties of the plants ingredients, i.e. from the Greek word ναρκειν for to numb, which is closely related to narcosis, which means to bring somebody to fall asleep. Another, literary story tells us that it has its origin in Νάρκισσος, the "self-admirer", a figure in Greek mythology, who was renowned for his extraordinary beauty. Doubtless, the legend may be highly poetic, but the naming according to a given physiological property seems more convincing.

Fig. 1.6-4 Picture taken in a garden showing the flowering cultivar Carlton, an economically important variety that belongs to the group of large-cupped daffodils

Indeed, the bulbs of daffodils are poisonous. Intoxications have happened occasionally by confusing them with ordinary onions for the kitchen, to which they are akin (compare Figs. 1.6-5, 1.6-6 and 1.6-7). Consumption of narcissus bulbs may lead to a sick feeling, vomiting, diarrhoea, sweating, somnolence, collapse and paralysis. Large amounts are life-threatening. It has been reported that a patient, being feverish and hence thirsty unthinkingly drank the water from a vase containing daffodils and died from it. Therefore, such flowers are not to be recommended when visiting a sick person. If daffodil bulbs have been swallowed accidentally, an emergency doctor should be consulted immediately; activated charcoal tablets are considered helpful to absorb the toxins.

Florists may suffer from local skin irritation. This is a kind of contact dermatitis called "daffodil itch", caused by calcium oxalate together with other ingredients of the plants.

Narcissus species are native around the coasts of the Mediterranean Sea and its islands, i.e. southwestern Europe, Corsica, Sardinia and northwestern Africa. They occur to the East as far at the coasts of the Black Sea. More than 50 natural species are known, but of course they cannot be discussed in detail here. Curiosities exist, such as the species *N. elegans* that blossoms in autumn. The coloration can be rather different, e.g. *N. tazetta* (Chinese sacred lily) has white outer flowers with a dark orange cup-like inner crown. This species is widespread

from the Middle East via Iran to Kashmir. In addition to the huge number of hybrids created by gardeners, natural ones also exist at the overlaps of their ranges of distribution.

Daffodils gained admission to occidental horticulture in the so-called Oriental Phase from about 1560 to 1620. However, the main flower of this era was not the narcissus but the tulip, which was an admired symbol of the Orient in Europe. Surely, you will have read stories about the incredible amount of money that was paid, e.g. in The Netherlands at the height of this hype during Rembrandt's times, to purchase just one special tulip bulb. Hyacinths and daffodils have been the motivation to satisfy this yearning for distance.

Today, more than 24 000 narcissus cultivars are known that have been bred over the centuries. They are registered in the International Daffodil Register and Classified List. During late winter, daffodils belong to the main business of flower shops. Together with other bulb flowers, they represent an important branch of trade for countries with a suitable climate such as The Netherlands, Ireland and the UK. Just to give a rough idea of the business: about 10% of Dutch bulb production is represented by daffodils (ca. 1800 hectares). The annual harvest of daffodil bulbs is about 250 million. There are traditional cultivars that make up for the main business, such as "Carlton", "Ice-Follies" (white) and "Golden Harvest". The cultivar Carlton, the bulbs of which are used here, is really a classic, and received its approval in 1927. Although easily affordable by everyone, daffodils symbolize the hope for an end of winter darkness and the onset of spring. They have a peculiar meaning in some countries. Thus, the daffodil is Wales' national flower and daffodils are a standard decoration during the Chinese New Year or so-called spring festival that is the most important Chinese holiday.

Hic puer, et studio venandi lassus et aestu,
procubuit faciemque loci fontemque secutus.
dumque sitim sedare cupit, sitis altera crevit.
Dumque bibit, visae correptus imagine formae
spem sine corpore amat: corpus putat esse, quod unda est
adstupet ipse sibi, vultuque inmotus eodem
haeret, ut e Pario formatum marmore signum.
Spectat humi positus geminum, sua lumina, sidus
et dignos Baccho, dignos et Apolline crines
impubesque genas et eburnea colla decusque
oris et in niveo mixtum candore ruborem,
cunctaque miratur, quibus est mirabilis ipse.
Se cupit imprudens et qui probat, ipse probatur,
dumque petit, petitur, pariterque accendit et ardet.

P. Ovidius Naso (43 BC–17 AD)
Metamorphoses III, 413−426
(Narcissus)

2. Literature

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Fig. 1.6-5 Bulbs of Carlton daffodils

Fig. 1.6-6 Peeled bulbs of Carlton daffodils

Fig. 1.6-7 Diced daffodil bulbs

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3. Isolation

3.1 Principle

Important note: all work reported here is mainly inspired by the findings reported by Kreh in the 1990s [7]. The advantage of his investigations is that they are directed at the optimization of many factors, e.g. what is the most suitable Narcissus species and what is its best extractive separation under appropriate pH conditions with a most suitable solvent, and so on. All this was studied very carefully and is just used here following its principles (see section 3.2). Despite this isolation in the laboratory is still tricky, when considered technologically.

The isolation of galanthamine follows the classical principle. The alkaloid is first set free from the salt form – in which it is contained in the cells – by reaction with a strong base. Then the alkaloid is extracted with an organic solvent. Finally, it is purified. This seems to be rather simple. However, in practice, it has two crucial points.

The first problem is that this alkaloid is not contained in all of the many Narcissus species. The difficulty is that, of course, a gardener cannot tell you if a special daffodil variety actually contains the alkaloid or not. In case of need, it has to be confirmed at the beginning by checking the literature whether a certain variety is known to contain galanthamine or not. Also, the content differs considerably within the species making galanthamine. A special dealer of bulbs should be contacted and, at the correct time of the year (early summer), a definite purchase order for a pure cultivar should be placed to obtain the daffodil bulbs in autumn. We want to dissuade readers from buying cheap mixed narcissus collections from a do-it-yourself store.

The second problem is a technological one. The transfer from the biological matrix of the bulb into an organic solvent is very difficult to accomplish. It is not easy to crush the rather tough bulbs together with a

suitable solid base such as soda ash in a manner that brings about close contact, leading to release of the free alkaloid from the cells. Furthermore, if this seems to have been achieved, it is tricky to extract the alkaloid into an organic solvent. When using an anhydrous base such as solid soda ash, the usual laboratory stirrers are incapable of bringing about really intense mixing of the gummy bulb–soda mass with the solvent. When using an aqueous base (trials were made with aqueous sodium carbonate), intense mixing of the crushed bulbs, the basic solution and the solvent is necessary. Attempts to do so probably end in an emulsion that on standing does not separate well in a reasonable time. Hence centrifugation on a large scale of volume is necessary, and so on. The point is that the technical equipment of the standard organic laboratory probably does not fulfil the needs that are caused by the separation problem. One always knows what would be a helpful unit (for stirring, mixing, centrifugation, etc.), but it is never at hand: a laboratory is not a factory.

Fig. 1.6-8 The tough meat mincer used

Therefore, when starting with this interesting challenge, the most powerful tool will be improvization. The crucial operation is the transfer of enough alkaloids from the alkalinized crushed bulb mass into heptane. Make sure by a check of the mass of the crude alkaloids extracted that there is really enough raw material to end up with, after all chromatographic steps, an amount still sufficient for the spectroscopic analyses.

Fig. 16-9 Centrifuge vessel with acidic phase below containing alkaloids as salts

It will be found that the chromatographic separations are rather difficult. In this respect, our procedure is inspired by the method described in [7] but it is no repeat. The separation there is done at a much larger scale with a different equipment not described in detail. Therefore, every successor is encouraged to find a better way. In particular, we want to emphasize that the chromatographic runs described below are not optimized. Readers should feel encouraged to look for their own alternatives once a crop of crude galanthamine is available for the final purification.

3.2 Method

Twenty-two bulbs of *Narcissus pseudonarcissus* L. subspecies *pseudonarcissus* Carlton (mass 2 kg) are peeled and the root rudiments are cut off. The mass is then 1.75 kg (see photograph). The bulbs are then diced like onions in the kitchen and thoroughly mixed with solid sodium carbonate (170 g). The mixture is twice passed through a meat mincer (see photograph), a procedure that requires a lot of power and time. In our case, 90 min were necessary to accomplish the operation. A sticky, beige mass is obtained. It is divided into 10 equal portions of 180–190 g. A single portion is placed in a 2 L beaker together with *n*-heptane (400 mL) and stirred as vigorously as possible with a kitchen mixer normally used to make a purée (in our laboratory a "Zauberstab M100" mixer from ESGE Switzerland was used; see photograph) for 3 min. The pale yellow heptane phase is removed from the gummy plant material by filtration. This is done with any of the 10 portions in the same manner. The heptane phases are combined, dried over $MgSO_4$ and the heptane is completely removed in a rotary evaporator. An oily yellow

Fig. 1.6-10 Mixer "Zauberstab M100"

He stopped before a bed of narcissus, gathered one of the while, starry flowers, and inhaled its perfume until he felt the blood hammering in his temples. He had never examined this flower minutely.
But during the last term they had read Ovid's story of Narcissus. He had not discovered a deeper meaning in the legend. What did it mean, this story of a youth who, from unrequited love, turned his ardour upon himself and was consumed by the flame when he fell in love with his own likeness seen in a well? As he stood, examining the white, cup-shaped petals, pale as the cheeks of an invalid with fine red lines such as one may see in the faces of consumptives when a pitiless cough forces the blood into the extremest and tiniest blood-vessels, he thought of a school-fellow, a young aristocrat, who was a midshipman now; he looked like that.

August Strindberg (1849−1912)
Married

liquid remains (2.44 g). It is dissolved in heptane (350 mL) and extracted with 2% aqueous H_2SO_4 (5 × 100 mL) to transfer galanthamine as salt into the aqueous phase. Each of the extraction steps leads to an emulsion with a very low tendency to separate just on standing in a separation funnel. Therefore, each of the emulsions obtained is separated by centrifugation (10 min at 3200 rpm – or faster if possible). All aqueous phases containing galanthamine and other alkaloids in protonated form are collected. All heptane phases are discarded. The combined aqueous phases are then adjusted to pH 5 by careful addition of concentrated ammonia solution (ca. 14 mL are necessary). To remove colouring impurities, this pale yellow aqueous solution is extracted with diethyl ether until the ether is colourless (2 × 100 mL portions). The ethereal extracts are discarded. Then, the colourless aqueous phase is adjusted to pH 9 by dropwise addition of concentrated ammonia solution (ca. 2 mL). The free alkaloid bases are extracted into diethyl ether (5 × 100 mL). The ethereal phases are combined, dried over $MgSO_4$ and the ether is completely distilled off in vacuo to leave a viscous, colourless liquid (465 mg) containing crude galanthamine. Several TLC tests were undertaken to find a suitable solvent for a column chromatographic separation. Pure ethanol was found to show $R_f = 0.20$ for galanthamine and $R_f = 0.36$ for the main accompanying compound.

3.3 Purification

Conditions for the first column chromatography: column, 30 × 3 cm; stationary phase, silica gel 60 (0.040–0.063 mm); eluent, ethanol. The above crude product is dissolved in 20 mL of ethanol and placed on the top of the column with a pipette. During the chromatography, 160 fractions (of 6.5 mL each) are taken. A TLC check shows that fractions 60–150 contain galanthamine. These fractions are combined, the solvent is removed in vacuo and 309 mg of a colourless oil remain. However, the separation from the main impurity is not yet satisfactory. Again, TLC tests were made to find a more suitable solvent.

Conditions for the second column chromatography: column, 45 × 1 cm; stationary phase, silica gel 60 (0.040–0.063 mm); eluent, THF. The material from above is dissolved in 3 mL of THF, placed on the top of the column and 140 fractions (of 3 mL each) are taken in this run. A TLC check shows that fractions 58–83 contain enriched galanthamine whereas earlier and later fractions contain impurities and can therefore be discarded. Fractions 58–83 are combined and the solvent is removed in a rotary evaporator to leave 78 mg of a colourless paste, with a high galanthamine content according to NMR – but not pure.

At this point, column chromatography did not seem to be a suitable means to remove the last impurities. Hence preparative thin-layer chromatography (TLC) was used for the final separation step. Merck silica gel 60_{F254} glass plates for preparative TLC, size 20 × 20 cm with concentrating zone 2.5 × 20 cm were used. The 78 mg of crude product are dissolved in 4 mL of methanol and divided into four portions of 1 mL each. Every portion is placed in a line in the concentrating zone of a preparative TLC plate. All four plates are then placed in a TLC chamber

and developed with methanol as eluent. This takes about 2 h. The plates are removed and allowed to dry, then a second run is performed under the same conditions; this takes again 2 h. After final drying in air, the plates are illuminated with UV light of 254 and 366 nm. The following result can be observed: at R_f = 0.05–0.1, a compound with azure fluorescence under 366 nm UV irradiation appears. At R_f = 0.25, a compound is observed that quenches the fluorescence of the fluorescence indicator in 254 nm UV light. Neither compound is galanthamine. The alkaloid is found at R_f = 0.45 and also quenches the indicator in 254 nm UV light. A final test was made with Dragendorff's reagent for alkaloids. (Preparation of the reagent: 1.7 g of basic bismuth nitrate and 20 g of a tartaric acid are dissolved in 40 mL of water, added to a mixture of 16 g of potassium iodide in 40 mL water, stirred for 1 h and filtered. Stored cool in a dark bottle, the reagent can be used for serveral weeks. Dilute with a triple amount of water prior to use.)

To carry out the test, 4 cm of a plate is cut off with a glasscutter and this small plate is sprayed with the above reagent. Brown zones as a feature of alkaloids are visible at the two upper spots. The zones around R_f = 0.45 are scratched off, collected and extracted with hot chloroform to yield 14 mg of a colourless amorphous solid after removal of the solvent.

All spectra and the measurement of the optical rotatory power, $[\alpha]_D^{22}$ –125° (*c* 0.0063 g/mL, ethanol), show this material to be pure galanthamine.

An exact melting point determination was impossible because the rapid removal of the solvent did not allow for the formation of large crystals that show the required melting behaviour. However, if more material is available, it should be noted that the literature [7] recommends crystallization from isopropanol, but the procedure is mentioned as being rather tricky.

I wandered lonely as a cloud
That floats on high o'er vales and hills,
When all at once I saw a crowd,
A host, of golden daffodils;
Beside the lake, beneath the trees,
Fluttering and dancing in the breeze.

Continuous as the stars that shine
And twinkle on the milky way,
They stretched in never-ending line
Along the margin of a bay:
Ten thousand saw I at a glance,
Tossing their heads in sprightly dance.

The waves behind them danced; but they
Out-did the sparkling waves in glee:
A poet could not but be gay,
In such a jocund company;
I gazed – and gazed – but little thought
What wealth the show to me had brought:

For oft, when on my couch I lie
In vacant or in pensive mood,
They flash upon that inward eye
Which is the bliss of solitude:
And then my heart with pleasure fills,
And dances with the daffodils.

William Wordsworth (1770−1850)

Fig. 1.6-11 Spring is more than daffodils! Note the tulips developing in the upper right corner

4. Spectra and Comments

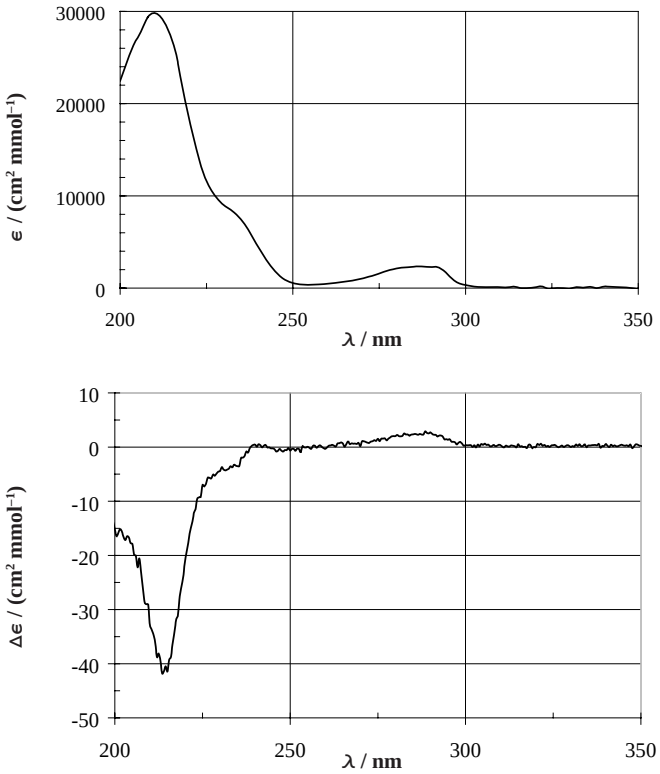
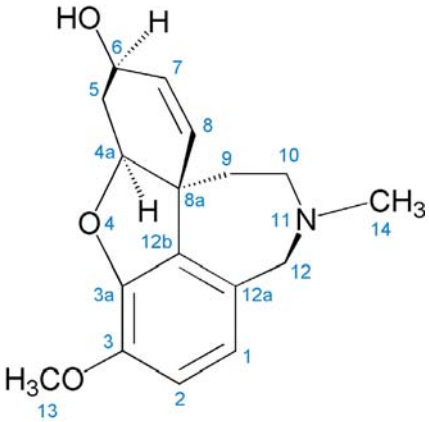


Fig. 1.6-12 UV and CD spectra in ethanol

Due to the aromatic ring with two auxochromic oxygen groups attached and the additional double bond, the compound has a very strong π–π* transition at 212 nm with a shoulder at 240 nm. As seen for instance in the spectrum of eugenol, we observe a weaker band at 280 nm. Since the compound is a chiral alkaloid, we expect a CD spectrum. As in other cases, e.g. cytisine, we find both polarities for the Cotton effect; the strong band at 212 nm shows a strong and negative Δε of – 40 cm^2 $mmol^{-1}$, whereas the band at 280 nm shows only a very weak but positive Δε.



Scheme 1.6-2

Fig. 1.6-13 Garden daffodils

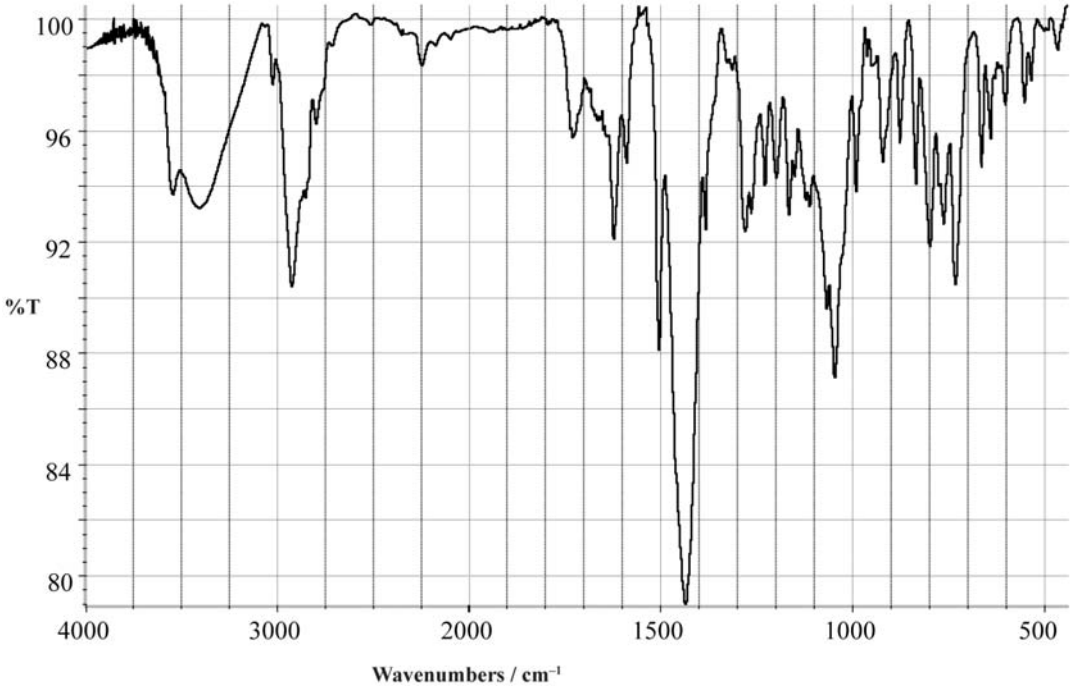


Fig. 1.6-14 IR spectrum as film

The IR spectrum was obtained directly from a film of the substance. The spectrum reveals the OH vibration and rather broad CH valence band from 3050 to 2800 cm^{-1}. In the double bond region, we find two absorptions near 1600 cm^{-1}. The strong band at 1430 cm^{-1}, most likely a CH deformation vibration, is predominant.

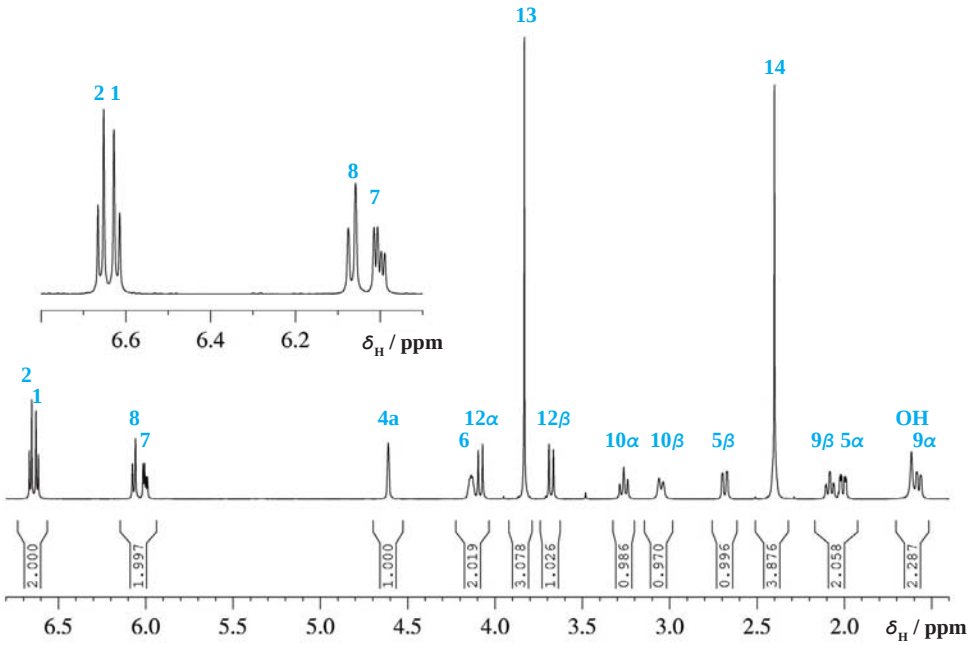


Fig. 1.6-15 ^{1}H NMR spectrum at 600 MHz in $CDCl_3$

As found in many alkaloids, the signals of the 21 hydrogen atoms are very well dispersed over the entire proton chemical shift range. At the left side of the spectrum we see an aromatic AB system at 6.65 ppm and we therefore assign this to H-2 and H-1, without being able at this stage to assign these two protons individually. Another AB spin system follows at 6.03 ppm, where the right part is further split by an additional spin coupling. Hence these two protons are assigned to H-8 and H-7. The signal of H-7 is further split by H-6. Next are two broadened singlets at 4.61 and 4.14 ppm. As this is the CHO region, they must stem from H-4a and H-6; again, an individual assignment is doubtful at this stage. An isolated AX spin system at 4.08 and 3.68 ppm resonates clearly in the CHN region and can be safely assigned to the protons H-12. The two large singlets at 3.83 and 2.40 ppm can easily be assigned to the methoxy group and the NCH_3 group. There are seven more proton signals between 3.3 and 1.5 ppm which are best discussed with the help of the other spectra given below.

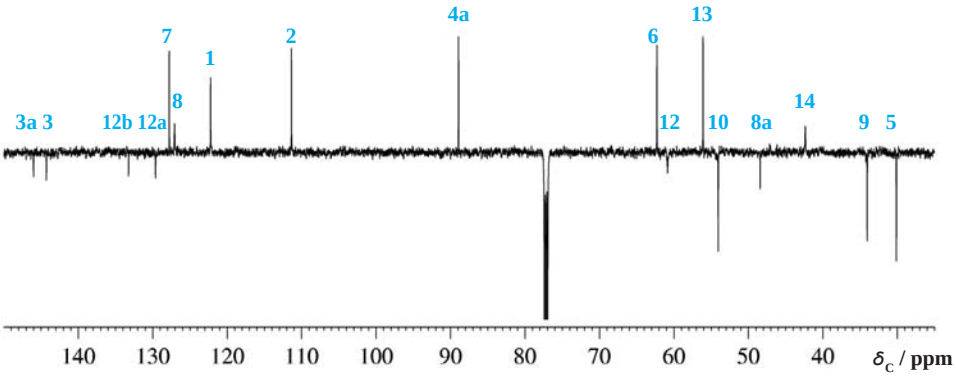
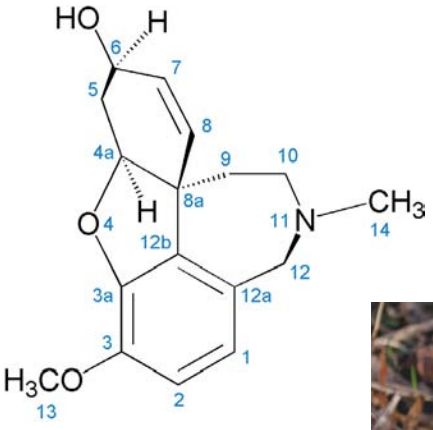


Fig. 1.6-16 APT ^{13}C NMR spectrum at 150 MHz in $CDCl_3$

As required, we find in the olefinic/aromatic region of the spectrum the signals of four quaternary carbon atoms and four signals of CH groups. The two most deshielded signals at about 150 ppm clearly belong to the two oxygen-substituted carbon atoms C-3 and C-3a, which leaves the other two signals at about 130 ppm as C-12b and C-12a. The assignment of the CH groups will easily follow from the HSQC spectrum. In the CHO region we find three signals at 88.9, 62.3 and 60.9 ppm. The first two must be from C-4a and C-6, but cannot yet assigned individually. The signal of the NCH_3 group C-14 with a typical chemical shift value of 42.4 ppm is very small, as are some other signals from the NCH_2 groups, and the reason for this will be addressed in the Questions section.



Scheme 1.6-3

Fig. 1.6-17 The common snowdrops *Galanthus nivalis* L.

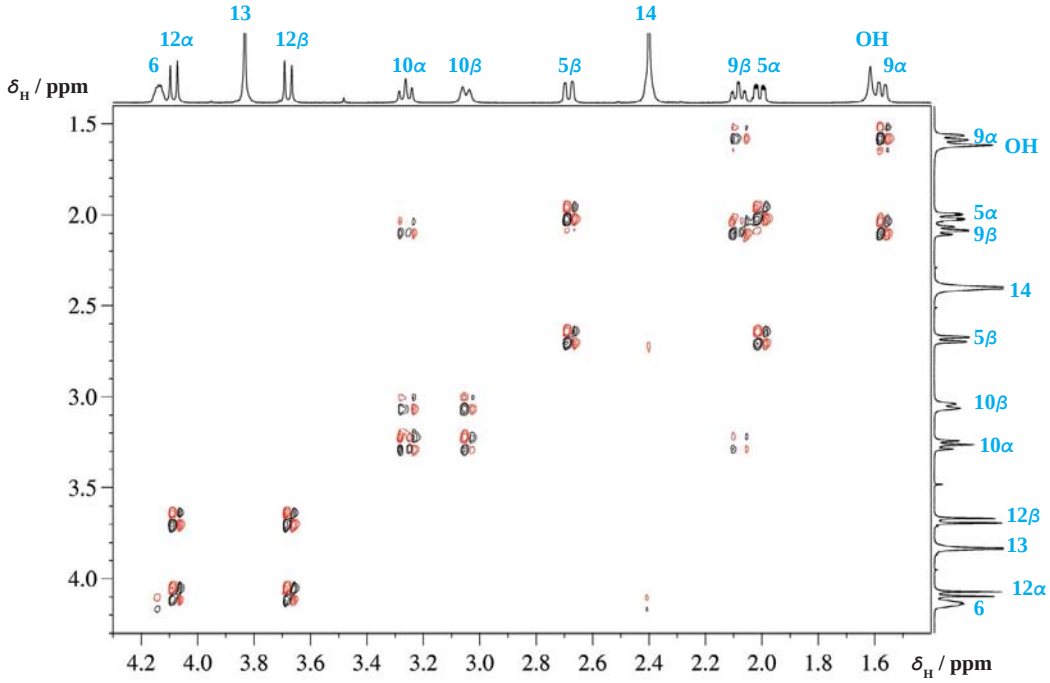


Fig. 1.6-18 Expansion of the DQF-COSY spectrum in the aliphatic region

In the COSY spectrum we see four typical squared patterns for diasterotopic methylene protons. As already discussed for the proton spectrum, we assign the most deshielded AX spin system to H-12. The most shielded squared pattern at 2.08 and 1.57 ppm can be attributed to the most aliphatic protons H-9 of the molecule. One signal is interconnected by a cross peak to the signal at 3.26 ppm, hence these two squared patterns form a CH_2–CH_2 moiety, where the signals at 3.05 and 3.26 ppm can be safely assigned to H-10. Hence the remaining diasterotopic protons at 2.69/2.01 ppm must be from H-5.

"It is of no use asking the flowers; they only know their own old rhymes, and can tell me nothing." And she tucked up her frock, to enable her to run quicker; but the Narcissus gave her a knock on the leg, just as she was going to jump over it. So she stood still, looked at the long yellow flower, and asked, "You perhaps know something?" and she bent down to the Narcissus. And what did it say? "I can see myself–I can see myself! Oh, how odorous I am! Up in the little garret there stands, half-dressed, a little dancer. She stands now on one leg, now on both; she despises the whole world; yet she lives only in imagination. She pours water out of the teapot over a piece of stuff which she holds in her hand; it is the bodice; cleanliness is a fine thing. The white dress is hanging on the hook; it was washed in the teapot, and dried on the roof. She puts it on, ties a saffron-colored kerchief round her neck, and then the gown looks whiter. I can see myself–I can see myself!"
"That's nothing to me," said little Gerda. "That does not concern me." And then off she ran to the further end of the garden.

Hans Christian Andersen (1805–1875)
The Snow Queen

Fig. 1.6-19 Native exemplar of a wild daffodil

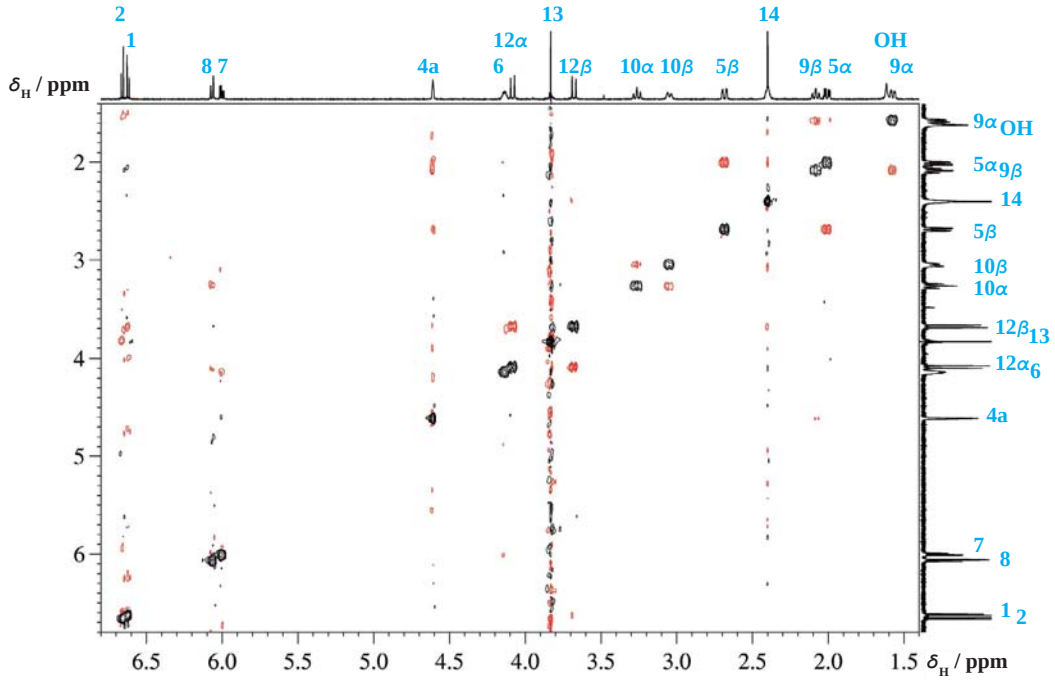
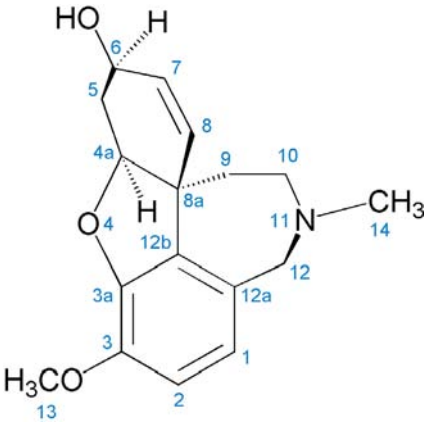


Fig. 1.6-20 NOESY spectrum

From the NOESY spectrum we can first assign the protons H-2 and H-1, individually. The most deshielded signal at 6.7 ppm displays a cross peak to the methoxy group and hence belongs to H-2, whereas the other signal of the aromatic AB system has a cross peak to the more shielded of the H-12 protons at 3.68 ppm. This assigns the proton at 6.62 ppm to H-1 and the proton at 3.68 to H-12β. Similarly, the proton H-8 at 6.07 ppm displays a NOESY cross peak to the more deshielded signal of the pair H-10 at 3.26 ppm. We can therefore assign this signal to H-10α. The signal at 4.61 ppm, which we had left unassigned in the discussion above, displays three NOESY cross peaks: two to the signals of H-5, therefore the signal at 4.61 ppm stems from H-4a. The NOESY cross peak from H-4a to the more deshielded part of the signal pair of H-9 identifies the signal at 2.08 ppm as H-9β. A faint NOE cross peak from H-9α at 1.57 ppm can be seen on the computer screen to the H-5 proton at 2.69 ppm and therefore this will be assigned to H-5β. Again, it is mandatory to build a molecular model of this compound in order to verify the stereochemical relationships.



Scheme 1.6-4

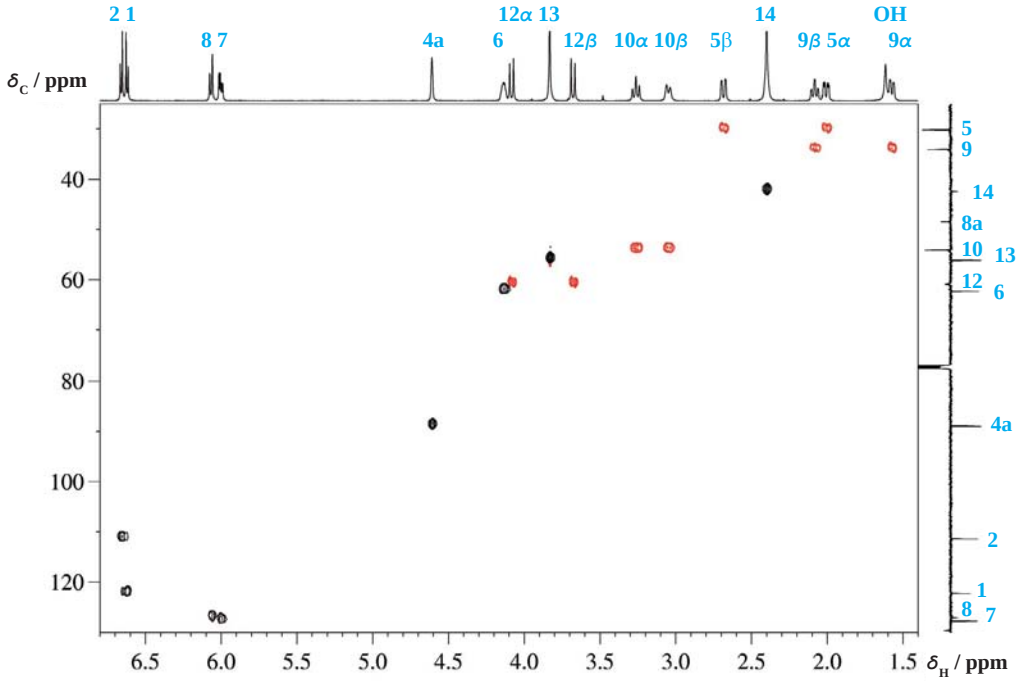


Fig. 1.6-21 HSQC spectrum

Since we have assigned all proton signals, we use the HSQC spectrum only to assign the signals of the protonated carbon atoms. C-2 is very typically much more shielded than C-1 due to its β-position with respect to the oxygen. The four pairs of signals in red indicate the methylene groups and C-6 is clearly identified from the corresponding proton signal. The HSQC spectrum shows that the proton singlet at 1.61 ppm is not connected to a carbon atom and hence this stems from the OH group.

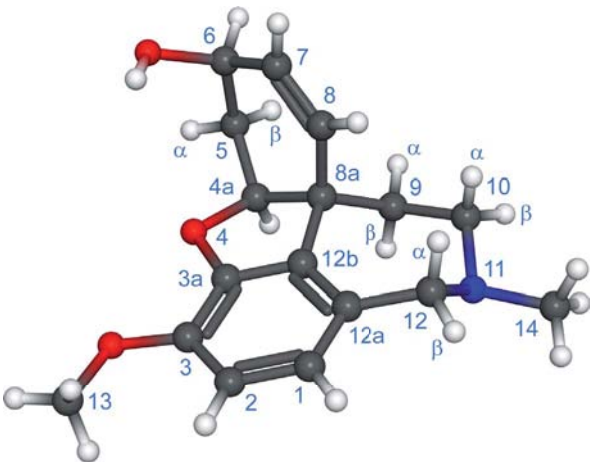


Fig. 1.6-22 Molecular model of galanthamine

Jene wenigen, welche gelegentlich die Einfalt des Abtes etwas belächelten, waren desto mehr von Narziß bezaubert, dem Wunderknaben, dem schönen Jüngling mit dem eleganten Griechisch, mit dem ritterlich tadellosen Benehmen, mit dem stillen, eindringlichen Denkerblick und den schmalen, schön und streng gezeichneten Lippen. Daß er wunderbar Griechisch konnte, liebten die Gelehrten an ihm. Daß er so edel und fein war, liebten beinahe alle an ihm, viele waren in ihn verliebt. Daß er so still und beherrscht war und so höfische Manieren hatte, nahmen manche ihm übel.

Hermann Hesse (1877−1962)
Narziß und Goldmund

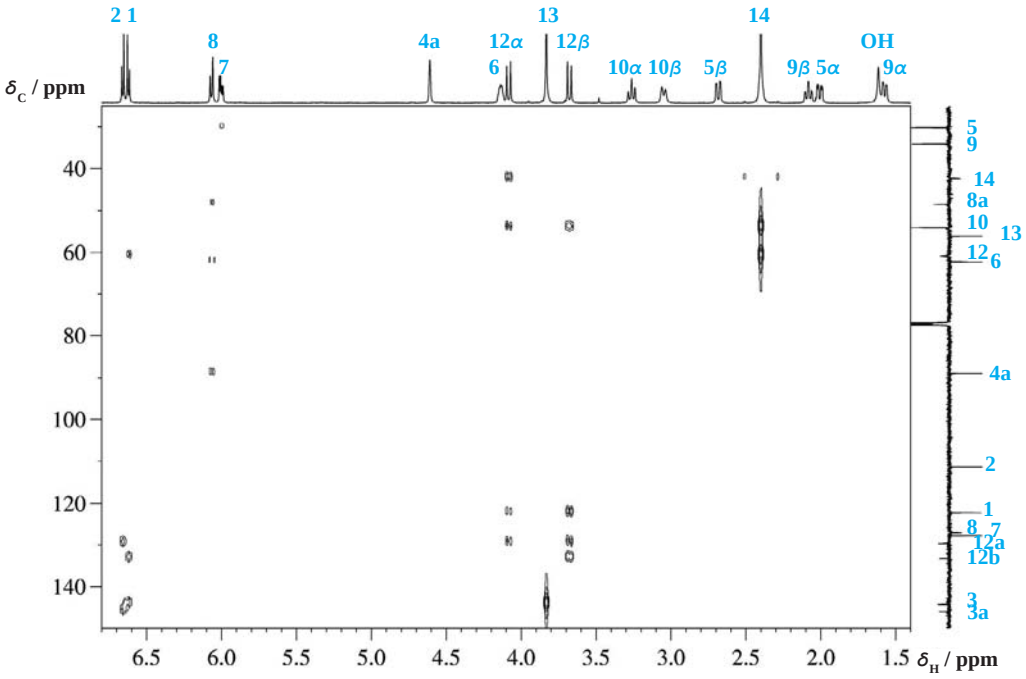


Fig. 1.6-23 HMBC spectrum

The HMBC spectrum will be used to confirm the assignments from the analysis given above. We will use the Questions section for the reader to go through the entire HMBC spectrum and verify the given assignments.

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz
146.0	C_q	C-3a	
144.3	C_q	C-3	
133.2	C_q	C-12b	
129.6	C_q	C-12a	
127.8	CH	C-7	6.00, $J_{7,8}$ = 10.48, $J_{7,6}$ = 4.96
127.0	CH	C-8	6.07, $J_{8,7}$ = 10.48
122.2	CH	C-1	6.62, $J_{1,2}$ = 8.18
111.4	CH	C-2	6.66, $J_{2,1}$ = 8.18
88.9	CH	C-4a	4.61
62.3	CH	C-6	4.14
60.9	CH_2	C-12	H-12α: 4.08, H-12β: 3.68, $J_{12\alpha,12\beta}$ = –14.9
56.1	CH_3	C-13	3.83
54.0	CH_2	C-10	H-10α: 3.26, H-10β: 3.05, $J_{10\beta,9\beta}$ = –14.1, $J_{10\alpha,9\beta}$ = 13.4
48.4	C_q	C-8a	
42.4	CH_3	C-14	2.40
34.0	CH_2	C-9	H-9β: 2.08, H-9α: 1.57, $J_{9\beta,10\alpha}$ = –13.4
30.1	CH_2	C-5	H-5β: 2.69, H-5α: 2.01, $J_{5\alpha,5\beta}$ = –15.7, $J_{5\alpha,4a}$ = 2.23, $J_{5\alpha,6}$ = 4.83
		OH	1.61

Table 1.6-1 NMR data for galanthamine

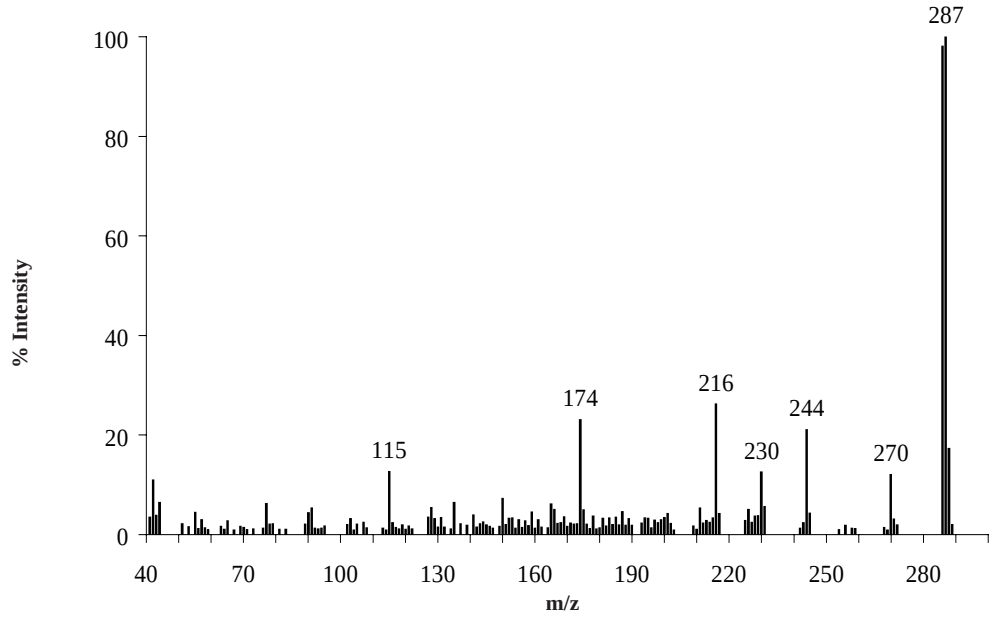
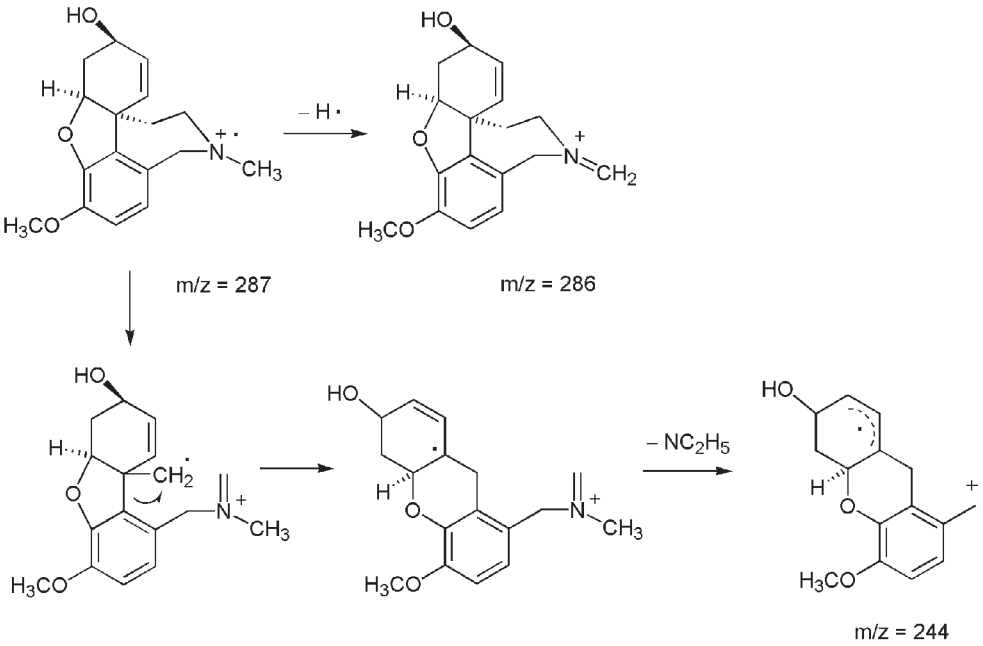
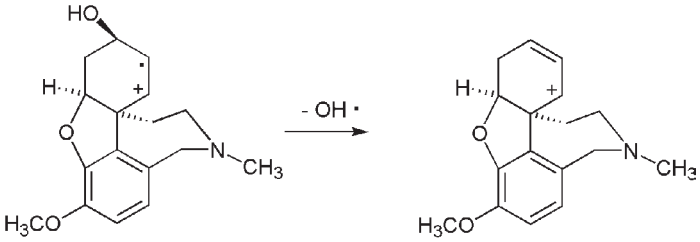


Fig. 1.6-24 Mass spectrum (EI)

The EI mass spectrum of galanthamine displays a very strong M–1 peak, which can easily be explained by ionization at the nitrogen atom and subsequent α-cleavage of a hydrogen radical as indicated. Similarly, the ion at m/z = 244 can be explained starting from the ionization at the nitrogen atom, whereas the ion with m/z = 270 may be explained be loss of a hydroxyl radical after ionization at the double bond of the cyclohexene ring.



Scheme 1.6-5 Fragmentation of galanthamine



m/z = 287 m/z = 270

Scheme 1.6-6 Further fragmentation

5. Questions

A. What are the common features in the structures of galanthamine and strychnine?

B. What are the typical variations within the class of galanthamine alkaloids?

C. The ^{13}C NMR signals of C-12 and C-14 show a surprisingly low intensity. Why?

D. What is the correct nomenclature for the spin system of the methylene groups H-10 and H-9?

E. Galanthamine has three stereogenic centres. Suggest an NMR method to determine its absolute stereochemistry.

F. Discuss in detail all the signals visible in the HMBC spectrum for all proton signals between 6.8 and 3.5 ppm.

G. Explain why in the HMBC spectrum H-12α but not H-12β gives a correlation signal to C-14, and H-12β but not H-12α gives a correlation signal to C-12b.

H. Propose a mechanism for the ions with m/z = 230, 216 and 179.

6. Own Observations

1.7 Strychnine

From the seeds of the strychnine tree
Strychnos nux-vomica L. (Loganiaceae)

$C_{21}H_{22}N_2O_2$, MW 334.42

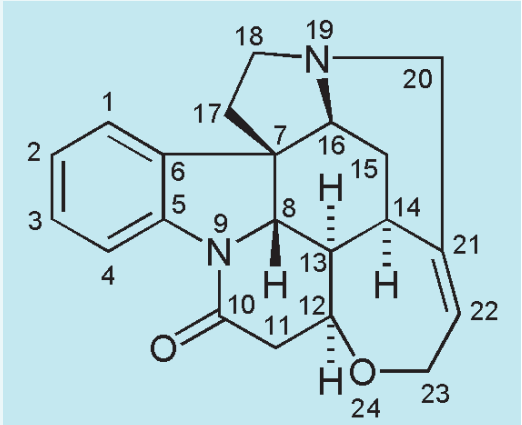
CAS RN 57-24-9, BRN 52979

$[\alpha]_D^{21}$ –139° (*c* 0.0164 g/mL, $CHCl_3$)

Colourless crystals, mp 270–273 °C
Strychnine is commercially available.

Synonymous names:
(–)-Strychnine, Strychnin, Strychnidin-10-one

Level: medium



Note concerning the nomenclature: for very complex structures, such as that of strychnine, the IUPAC name would be over one line in length, and very complicated. Therefore, the trivial name "strychnine" is always used.

Caution! Strychnine is a very strong and dangerous poison even in small amounts. Inform yourself about its toxicological properties prior to beginning and organize your laboratory work during isolation in a manner that does not endanger you or your colleagues. The LD_{50} for an adult is at about 1 mg/kg body weight; however, it may be even lower due to the different individual sensitivities towards the poison. Fatal doses have been reported to be as small as 5–10 mg! Amounts of more than 0.75 g of the seeds are a lethal danger. That means even one seed is very dangerous.

Hence *Strychnos nux-vomica* seeds should be handled with care and kept under lock and key.

1. Background: Don't play with fire – About a bitter poison that did not remain a drug

Fig. 1.7-1 A young strychnine tree plant

The first challenge here is to avoid writing a book about just this one compound! There is a recent one that describes the unique nature of this alkaloid with more facets that can be mentioned here [1]. The strychnine tree is an evergreen deciduous tree native to Southeast Asia (from India, Sri Lanka to North Australia) of up to 25 m in height. It belongs to the Loganiaceae family. The seeds of this tree are knob-like flat slices with a dented velvety surface. They are grey or pale brown in colour and of about 2−3 cm in diameter (see photograph). The German name *Krähenaugen* (= crows' eyes) expresses their eye-like appearance. The husks of the seeds are very hard. These seeds occur naturally inside the pulp of a green-to-orange-coloured fruit of ping-pong ball size and are removed and dried before sale. The seeds have various names such as *semen strychni*, *nux vomica*, *nux metella* and *semen nucis vomica*. However, the association that the Latin "vomica" for vomit means that they cause disgorging is rather a mistake. This is an exception! The seeds have no smell but are reported to have a bitter, hot and evil taste. The next mistake is that the Latin "nux" is suggestive of a nut; however, this fruit is a berry, botanically. The seeds contain 1.5–5% strychnine, accompanied by brucine; both are bound to chlorogenic acid. Blossoms and bark of the tree also contain the poisonous alkaloids.

All Ding' sind Gift und nichts ohn' Gift; allein die Dosis macht, dass ein Ding kein Gift ist.

Philippus Theophrastus Aureolus Bombastus von Hohenheim, named Paracelsus (1493−1541)

Strychnine is best known as a poison, even though small doses were used in former times in medications as a stimulant, a laxative and against other ailments; 75 years ago it was regarded as a valuable drug. However, taking strychnine always was playing with fire, as an episode from the 1904 Olympic Games tells us. The winner of the marathon collapsed before the finishing line. He drank brandy that contained strychnine as a stimulant to help him win the gold medal. Obviously, the dosage had been too high. Nowadays, due to this analeptic, i.e. stimulant effect strychnine is still on the doping list of forbidden performance-enhancing drugs. No recent cases of strychnine doping are known; however, other substances took its place, unfortunately. Eventually, because of its high toxicity and tendency to cause convulsions, medicine abandoned the prescription of strychnine. Nowadays, some safer alternative medications are available.

Fig. 1.7-2 Homeopathic strychnine pills

Nevertheless, it is also possible today to purchase a homeopathic drug called Nux vomica D2 in a German pharmacy. The pills contain saccharose and strychnine in the D2 dilution.

Like morphine and caffeine, strychnine belongs to the very first alkaloids isolated at beginning of the 19th century. Its discovery was reported in 1818 by the French chemists and pharmacists Pelletier and Caventou [2]. They isolated strychnine first from the so-called Saint Ignatius beans, fruits of the *Strychnos ignatia* tree, a Loganiaceae species native to the Philippines. The alkaloid got the name strychnine at the end of an involuted story. Pelletier's father, an apothecary, was an associate of the French nobleman and great scientist Lavoisier. In 1794, at the height of the French revolution, Lavoisier, despite his achievements as a

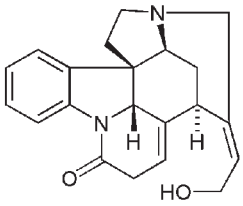
scientist, was beheaded for his leading position in the pre-revolutionary government as administrator of the *Ferme Générale* − a hated private tax collection company. Therefore, Pelletier's father had to look for a new possibility to get his son professionally educated. He sent him as an associate to a young scientist named Vauquelin, who had earned merit by the discovery of the elements chromium and beryllium. But he was also interested in pharmacy and supported natural product isolations, and is regarded as the first to have noticed an "alkali organique" (i.e. an alkaloid). Some 20 years later, Pelletier and Caventou wanted to express their gratitude and proposed to name the new alkaloid just discovered in honour of their patron "Vauqueline". However, knowing the high toxicity of the new alkaloid, the French Academy of Sciences rejected this proposal with the remark that an esteemed name of a scientist should not be applied to a harmful compound. Therefore, strychnine was named after the plant and only brucine in honour of a man, the French explorer Bruce. And brucine is less toxic.

Although known as a pure compound for a long time, its structure was a mystery for more than a century. The search for the structure is connected to the names of two brilliant chemists who worked for four decades from the beginning of the 20th century on with chemical means (oxidation, hydrogenation, alkaline degradation, etc.): Hermann Leuchs (many papers from 1908 to 1944) and Robert Robinson, who with astuteness summarized the findings of both in 1946 and proposed the correct constitution as one of the most complex small compounds known by then, with seven annelated rings [3]. Sir Robert Robinson won the 1947 Nobel Prize in Chemistry for his investigations on plant products, especially the alkaloids and their biological meaning. The remaining problem of the absolute configuration of strychnine was also one of the milestones in the development of the X-ray diffraction method [4] emerging in the 1950s that is most famous for its contributions to the development of the double helix structure of DNA. Strychnine has the most complex structure among the molecules in this book. It has only 24 atoms that form the skeleton, but they form seven rings that include six chiral centres. The absolute configuration of this indole alkaloid is (7*R*,8*S*,12*S*,13*R*,14*R*,16*S*).

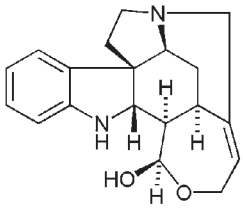
No wonder that strychnine, as soon as its structure was known, became a synthesis target of the best chemists of the world. The first to complete the total synthesis was Woodward, who received the Nobel Prize in Chemistry in 1965 for this and other outstanding achievements. He reported a synthesis of racemic strychnine already in 1954 [5], that went over to isostrychnine in the final step. At the end of the 20th century, strychnine was among the challenging targets for asymmetric syntheses. It was Overman and his group who reported a most impressive first asymmetric synthesis of the natural strychnine in 1995. It is different by proceeding through the so-called Wieland−Gumlich aldehyde, which as isostrychnine is one of the two main degradation products known from the era of structural determination [6]. Finally, a recent synthesis by Shibasaki et al. should be cited: by using the asymmetric Michael reaction, it paves the way in a general form that one day will

We now proceeded with preparations for the launch of the "Lady Nyassa." Ground was levelled on the bank at Shupanga, for the purpose of arranging the compartments in order: she was placed on palm-trees which were brought from a place lower down the river for ways, and the engineer and his assistants were soon busily engaged; about a fortnight after they were all brought from Kongoné, the sections were screwed together. The blacks are more addicted to stealing where slavery exists than elsewhere. We were annoyed by thieves who carried off the iron screw-bolts, but were gratified to find that strychnine saved us from the man-thief as well as the hyena-thief. A hyena was killed by it, and after the natives saw the dead animal and knew how we had destroyed it, they concluded that it was not safe to steal from men who possessed a medicine so powerful. The half-caste, who kept Shupanga-house, said he wished to have some to give to the Zulus, of whom he was mortally afraid, and to whom he had to pay an unwilling tribute.

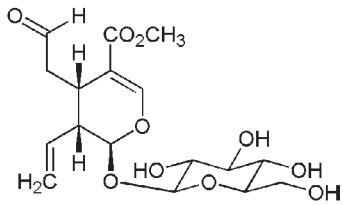
David Livingstone (1813−1873)
A Popular Account of Dr. Livingstone's Expedition to the Zambesi and its Tributaries and the Discovery of Lakes Shirwa and Nyassa



Isostrychnine



Wieland-Gumlich aldehyde



Secologanin

Scheme 1.7-1

"Poirot," I said, "what was in this particular little bottle?"
Poirot looked out of the window.
"Hydro-chloride of strychnine," he said, over his shoulder,
continuing to hum.
"Good heavens!" I said it quite quietly. I was not surprised. I
had expected that answer.
"They use the pure hydro-chloride of strychnine very little –
only occasionally for pills. It is the official solution, Liq.
Strychnine Hydro-chlor. that is used in most medicines. That is
why the finger-marks have remained undisturbed since then."

Agatha Christie (1890–1976)
The Mysterious Affair at Styles

allow access to the series of other strychnos alkaloids [7]. This is not a book dealing with synthetic details in depth. Readers interested in concise descriptions of all the syntheses mentioned that allow useful comparisons are referred to a recent book on natural products synthesis [8]. As chiral bases, both strychnine and brucine can be used for the resolution of racemic acids via diastereomeric salts. However, due to its distinctly lower toxicity, brucine (only 2% of that of strychnine) is usually used for this purpose.

The biogenesis of strychnine starts from tryptophan, which in a series of reactions is condensed with the monoterpene glycoside secologanine, the other key unit for indole alkaloid synthesis, and finally an acetate unit from acetyl coenzyme A [9]. Yet another point is worth mentioning. The understanding of the relationships within the close or remote members of the alkaloid biosynthesis tree is a useful tool for chemotaxonomic analysis. Such a chemotaxonomic appraisal can serve as a tool for botanical classification of plants based upon their composition of alkaloids as secondary metabolites. In this respect, strychnine and its derivatives are specifics peculiar to the family Loganiaceae. How a huge amount of such material has to be organized to find a useful conclusion was shown by Hesse, a grandmaster of alkaloid chemistry [10].

To understand what can be done against strychnine poisoning, which is life threatening, requires a knowledge of how the poison acts. Strychnine is a competitive antagonist of the neurotransmitter glycine at the inhibitory strychnine-sensitive glycine receptor of the central nervous system. This receptor is a ligand-gated chloride channel in the spinal cord and in the brain. The task of such receptors in the brainstem is to slow signals coming into and out of the brain. Normally, that is done by glycine. Strychnine as an antagonist causes the opposite because it paralyses the inhibitory neurons. Hence the signalling becomes more rapid and stimuli are sent without discrimination. This causes overstimulation, leading to severe muscle convulsions. These seizures take place under full consciousness. Also, higher centres, such as those for circulation and respiration, become more excitable under the influence of strychnine. The alkaloid can be ingested into the body by swallowing, inhalation or absorption through the mouth or eyes. The spasms provoked by the poison belong to the most painful and dramatic symptoms of any known intoxication. Therefore, strychnine poisoning has often been used in thrillers to express suspense and fright.

The poison is rapidly absorbed in the gastro-intestinal tract. The main symptoms that set in ca. 15 min after exposure are involuntary convulsions, starting with the head and neck, then spreading out to every muscle. Difficulty in breathing is another feature of the poisoning. Cramps attacks last for some minutes and are triggered by the slightest outer stimulus. Eventually, the backbone forms a continual arch. In this respect the symptoms are similar to those of tetanus, the so-called lockjaw caused by the neurotoxin tetanospasmin, produced by the bacterium *Clostridium tetani*. Death results from asphyxiation, caused by paralysis of the breath control system. Death may also result from exhaustion caused by the continuous convulsions. It is reported that

this happens after 2–3 h after uptake of the poison. To complete the image of horror, at the moment of death the body freezes within the convulsion into instantaneous rigor mortis.

A specific antidote is unknown. Therefore, countermeasures are taken that are directed at the competitive absorption of poison not yet taken up by the gastro-intestinal tract. Thus, activated charcoal can be administered orally to absorb the poison. Vomiting if possible is helpful. The poisoning is better treatable the earlier the patient is presented after exposure. Anticonvulsants such as diazepam are useful to control convulsions. Muscle relaxants can help against muscle rigidity. A patient needs absolute silence to prevent outside stimuli causing further convulsions. Those who survive the first 24 h will probably recover. Strychnine when taken up is metabolized by liver microsomes and the urine excretes about 20% in nonmetabolized form.

It is at first glance strange that a natural product of such high physiological activity has not found some kind of reliable application in medical treatment. This is especially astonishing if one thinks of even more toxic compounds such as botulinum toxin ("botox"), a neurotoxin produced by the bacterium *Clostridium botulinum* that has – in minute amounts – found medical application to treat painful muscle spasms and cosmetic application as an injection against frown lines. In the case of strychnine, just the opposite is true. Earlier attempts to use it as stimulant, tonic or laxative adopted from oriental medicine have been abandoned. Also, its mediaeval use as a cure for pestilence is forgotten. Today, nobody numbs fish with it or poisons a fox, a cat or a crow. The only use that has remained is its occasional application as a rodenticide.

Never in this book are we driven by any intention to prompt the reader to carry out their own physiological experiments with natural products that have been isolated. This is especially true for strychnine, even in minute amounts. We do not believe that it is a good idea to find out if some of the psychotropic effects of the nux vomica seeds reported from followers of the drug scene are true for the reader or not. To find a list here will be sufficient: enhanced perceptual experience in the form of "higher awareness"; enhanced contrast in perception of colours and brightness; enhanced field of view and sense of touch. Finally, an effect as an aphrodisiac has been reported. Our comment is: do not play with fire! Abstain from experiments with nux vomica seeds or even pure strychnine. Let a bitter poison be a bitter and not a deadly one.

As one would expect, a paragraph on murder by strychnine is included. Let us look at this point with the eyes of a toxicologist or a specialist in forensic medicine. A priori, the high toxicity expressed in terms of a low LD_{50} value is in favour of a poison attack. However, various other properties are not. How likely is it that a victim will swallow one of the bitterest substances without noticing? How likely is it that efforts to mask the bitter taste can be successful? Of course, the likelihood is remote in both cases. Furthermore, the symptoms of intoxication are very distinctive. Also, this is a difference from the behaviour of many other poisons. The victim is able to take note of what happens with it,

She read those atrocious lines, without any visible disturbance of the dreadful composure that possessed her. Her mind made no effort to discover the person who had listened and betrayed her. To all ordinary curiosities, to all ordinary emotions, she was morally dead ready.
The one thought in her was a thought that might have occurred to a man.
"If I only had my hands on his throat, how I could wring the life out of him! As it is –" Instead of pursuing the reflection, she threw the letter into the fire, and rang the bell.
"Take this at once to the nearest chemist's," she said, giving the strychnine prescription to the servant; "and wait, please, and bring it back with you."
She opened her desk, when she was alone, and tore up the letters and papers in it. This done, she took her pen, and wrote a letter. It was addressed to Amelius.
When the servant entered the room again, bringing with her the prescription made up, the clock downstairs struck eleven.

Wilkie Collins (1824–1889)
The Fallen Leaves

because he or she is not unconscious. The death struggle is not a short one and not a silent one; on the contrary, there is a lot of turmoil. The ability to verify the poison even after years in a dead body is high due to the extraordinary chemical stability of strychnine. Strychnine is one of the bitterest substances known: at a very low dilution of 1:130 000 its taste is still detectable. This property is against its use as the perfect poison for murder. Did you know that? Did writers who include it in their thrillers consider it? Not all have described it in an adequate manner. Despite all these facts, strychnine has been often used for murder. This really seems incredible. The problem with murder using strychnine is extensively discussed in ref. [1] and elsewhere.

2. Literature

[1] J. Buckingham, "Bitter Nemesis − The Intimate History of Strychnine", CRC Press, Taylor & Francis Group, Boca Raton, FL, **2008**.

[2] P. J. Pelletier, J. B. Caventou, "Note sur un novel alcali" [Note on a new alkaloid] *Annales de Chimie et de Physique* **1818**, 323−336.

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−Est-ce au magistrat ou à l'ami que vous parlez? demanda Villefort.
−À l'ami, à l'ami seul en ce moment; les rapports entre les symptômes du tétanos et les symptômes de l'empoisonnement par les substances végétales sont tellement identiques, que s'il me fallait signer ce que je dis là, je vous déclare que j'hésiterais. Aussi, je vous le répète, ce n'est point au magistrat que je m'adresse, c'est à l'ami. Eh bien, à l'ami je dis: Pendant les trois quarts d'heure qu'elle a duré, j'ai étudié l'agonie, les convulsions, la mort de Mme de Saint-Méran; eh bien, dans ma conviction, non seulement Mme de Saint-Méran est morte empoisonnée, mais encore je dirais, oui, je dirais quel poison l'a tuée.
−Monsieur! monsieur!
−Tout y est, voyez-vous: somnolence interrompue par des crises nerveuses, surexcitation du cerveau, torpeur des centres. Mme de Saint-Méran a succombé à une dose violente de brucine ou de strychnine, que par hasard sans doute, que par erreur peut-être, on lui a administrée.
Villefort saisit la main du docteur.
Oh! c'est impossible! dit-il, je rêve, mon Dieu! je rêve! C'est effroyable d'entendre dire des choses pareilles à un homme comme vous! Au nom du Ciel, je vous en supplie, cher docteur, dites-moi que vous pouvez vous tromper!
−Sans doute, je le puis, mais....
−Mais?...
−Mais, je ne le crois pas.

Alexandre Dumas (1802−1870)
Le Comte de Monte-Cristo, Chap. 73

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3. Isolation

3.1 Principle

The isolation of strychnine is a classic example of alkaloid isolation. The *nux vomica* seeds are crushed and first all lipophilic content is removed by extraction with low-boiling petroleum ether. The seeds do not contain many compounds that dissolve in this step. The *nux vomica* powder is then treated with a strongly basic suspension of $Ca(OH)_2$ to release the free alkaloids strychnine and its companion brucine from salts in which the may be present in the seeds. After removal of the aqueous phase and drying, the alkaloids are extracted with dichloromethane. After removal of the solvent, a solid remains that according to TLC consists mainly of strychnine accompanied by some brucine (the ratio may differ depending on the chemical composition of the individual *nux vomica* plant!), and some other side constituents. All alkaloids are extracted via their soluble sulfates and set free by NaOH solution. At this point two classical pathways for the further processing have been followed. The first consisted in repeated recrystallization from chloroform and the second included a selective precipitation of strychnine as sparingly soluble sulfate. Both pathways afford high-grade strychnine (see below). A final purification of an analytical sample is possible by means of preparative TLC.

Finally, a few words on a question that some of our readers will regard as problematic: how does one obtain such poisonous seeds of the strychnine tree? Indeed, the difficulties of gaining access may differ depending on the country in which you live. In Germany, these seeds were accessible by order from a pharmacy. They were sold as a means of rodent control via an autograph signature after the author had identified himself by his identity card. It may be that such seeds are easier to obtain in oriental countries that produce them such as India, but this may still not answer your entire silent question: possibly, you ask, who is usually a client for such *nux vomica* seeds? It is the farmer who can use them as a rodenticide. It is a matter of course that any commerce has to be done with full responsibility, and that applies to everyone in the laboratory also.

"Before they come," said Holmes, "just put your hand here on this poor fellow's arm, and here on his leg. What do you feel?"
"The muscles are as hard as a board," I answered. "Quite so. They are in a state of extreme contraction, far exceeding the usual rigor mortis. Coupled with this distortion of the face, this Hippocratic smile, or 'risus sardonicus,' as the old writers called it, what conclusion would it suggest to your mind?"
"Death from some powerful vegetable alkaloid," I answered, "some strychnine-like substance which would produce tetanus."

Arthur Conan Doyle (1859−1990)
Sign of the Four

3.2 Method

The method is based on the procedure described in [11]. Seeds of *Strychnos nux vomica* (100 g, ca. 70–90 seeds are required) are ground in a kitchen grinder. The seeds are rather hard and an inhomogeneous mixture containing a fluffy brown powder and parts of the husks is obtained. The mixture is placed in the thimble of a Soxhlet apparatus and extracted for 7 h with ligroin (bp 30–80 °C) for defatting. The ligroin extract is discarded.

The mixture from the thimble is air-dried and has a mass of 92 g. An alkaline suspension of 20 g [$Ca(OH)_2$ in 200 mL of water (pH 11)] is placed in a large mortar, the defatted *nux vomica* powder from above is added and the olive-green suspension obtained is stirred occasionally with a pestle for 2 h. The paste remaining is stretched in thin layers in several glass bowls standing in the hood and allowed to air-dry over 3 days. The dry grey-brown crumbly mass (111 g) is allocated to the thimbles of two Soxhlet apparatuses and extracted with dichloromethane for 5 h. The colourless extracts obtained are combined and the solvent is removed completely in vacuo. A colourless crystalline residue remains (mass 1.174 mg). ^{1}H NMR spectroscopy shows a strychnine:brucine ratio of 92:8 (this may differ depending on the individual sample of *nux vomica* seeds).

The residue is shaken in a flask with chloroform (200 mL) to yield a pale yellow suspension. It is filtered and the colourless solid that separates (106 mg) is discarded. The clear filtrate is extracted with 5% H_2SO_4 (4 × 25 mL). The phase separation is not complete; therefore, emulsion parts are centrifuged at 3600 rpm for 10 min. The combined aqueous phases are neutralized with stirring in a beaker with 10% NaOH solution (ca. 75 mL), and then a further 15 mL of this base are added until pH 11 is reached.

On neutralization, a colourless cloudy precipitation is formed that is stirred for 1 h in an ice-bath for crystallization. The crystals formed are filtered by suction, dried in vacuo and have a mass of 432 mg. ^{1}H NMR spectroscopy shows a strychnine:brucine ratio of 96:4. The two alkaloids are still accompanied by small quantities of a third compound, less polar than strychnine.

Notes on TLC and NMR: the composition of the sample can be followed qualitatively by TLC throughout the procedure. Use standard silica gel plates with an integrated $UV_{254\ nm}$ fluorescence indicator and methanol–25% aqueous ammonia solution (19:1, v/v) as eluent. Both alkaloids quench the fluorescence. The R_f values are 0.60 for strychnine and 0.45 for brucine. The third compound elutes slightly faster than strychnine (R_f = 0.80).

Another alkaloid-selective detection is also possible by means of Dragendorff's reagent for alkaloids, which forms brown spots on dipping the TLC plate in it. Preparation of the reagent: 1.7 g of basic bismuth nitrate and 20 g of a tartaric acid are dissolved in 40 mL of water, added to a mixture of 16 g of potassium iodide in 40 mL of water,

Da sagt der Baron zum Diener – es soll so 'n neuer und 'n Windikus mit einem Arm gewesen sein, den er von der Straß' aufgelesen hat, aber horchen tat er dem Baron wie ein Hund –: "Bringen Sie mir doch gleich aus meiner Schlafstub' die Rhabarbertinktur mit! Sie steht auf der Kommod'! Es ist nur eine Flasche – und Sie können sich gar nicht irren..." Und die Marjell trinkt ihr Selterwasser, und der Baron trinkt seine Medizin. Und wie er fertig ist, da sagt er: "Donnerwetter, schmeckt das Zeug aber bitter! Das ist ja zum Katzen und Hunde vergiften! ..." Und da kommt auch schon der andre, der alte Diener gelaufen, den sein Onkel selig noch gehabt haben soll, und schreit: "Um Gottes willen, Herr Baron, das war ja das Strychnin für die Füchse!" – Und da lacht der Kreth, der Baron, nur und fragt: "Für wieviel Füchse?" – "Ich glaub' für hundert!" – Und da meint der Baron wieder, aber ohne mit der Wimper zu zucken: "Na, da wird's wohl hoffentlich auch für mich langen!" ... Da haben sie ihm denn nachher noch Milch eingegeben und nach 'm Arzt geschickt. Der Arzt kam auch, aber wie gewöhnlich so die Ärzte kommen – 'ne Stund' zu spät ... Dagegen munkelt man, daß die Baronin aus Bussardshof noch im Morgengrauen gekommen wär' und ihm die Augen zugedrückt hätt'. Verlangt haben nach ihr soll er aber nicht! ...

Johannes Richard zur Megede
(1864–1906)
Modeste

stirred for 1 h and filtered. Stored cool in a dark bottle, the reagent can be used for some weeks. Dilute with a triple amount of water prior to use.

Integrals of the following peaks (in $CDCl_3$) can be used to determine quantitively the ratio of the two alkaloids: strychnine: δ= 8.08 ppm (d) and brucine: δ= 7.79 ppm (s).

3.3 Purification

Method 1

A 360 mg amount of the above crude strychnine is dissolved in a small glass bottle in hot chloroform (4.5 mL) and allowed to stand open in the hood overnight for crystallization. The pale yellow mother liquor is removed by pipetting and the crystals are washed rapidly with ice-cold chloroform and dried in vacuo. Colourless crystals remain (150 mg). NMR spectroscopy shows a strychnine:brucine ratio of 97.3:2.7. The procedure is repeated; 81 mg of strychnine are obtained (mp 270−277 °C). TLC and NMR spectroscopy show this now to be free of brucine. However, it contains about 3% of the less polar companion. Integration of the following peak (in $CDCl_3$) can be used to determine its amount quantitively: δ = 7.87 ppm (d). To remove this impurity, part of the sample (13 mg) is subjected to preparative TLC with the above-mentioned solvent using Merck 20 × 20 cm silica gel plates with a concentrating zone for preparative TLC and methanol−25% aqueous ammonia solution (19:1, v/v) as eluent. The small amount of impurity (<1 mg) is between R_f = 0.61 and 0.75. The zone from R_f = 0.20 to 0.47 is worked up and yields strychnine (9 mg). ^{1}H NMR spectroscopy shows this sample to of 99+% purity.

Method 2

The mother liquor from the first recrystallization is reduced to dryness (210 mg). This solid is heated in boiling distilled water (3 mL) in a small glass bottle and 16% H_2SO_4 (320 mg) is added until complete dissolution. A 50 mg amount of powdered charcoal is added and the mixture is filtered by suction. The filtrate is allowed to stand at 4 °C overnight for crystallization. The precipitation obtained is increased by partial removal of water in vacuo without heating. Filtration yields 92 mg of strychninium sulfate. This is dissolved in hot water (3 mL) and 10% Na_2CO_3 solution is added with stirring. A colourless precipitate of crystalline strychnine is formed in the basic solution and stirred for 1 h. It is filtered by suction, washed with ice-cold water and dried in vacuo (mass 68 mg), mp 270−273 °C, $[\alpha]_D^{21}$ –139° (*c* 0.0164 g/mL, $CHCl_3$) both values are in accordance with reference data. ^{1}H NMR spectroscopy shows this sample to consist of 95.5% strychnine, 0.5% brucine and 4.0% of the unknown compound mentioned above.

Preparation of strychninium chloride crystals

Chloroform is saturated with HCl by shalling with 3 M hydrochloric acid and sepration of the organic phase. A few mg of strychnine are dissolved in 2 mL of such chloroform and allowed to crystallize in a small open flask.

Von Apotheker Stannebein in Meißen erzählt: Bei de Indianer

"Änne gans eegendiemliche Geschichte is mir da bei de Indianer bassiert. Eenes Dages nämlich, wie unsere Exbedizjohn so ä wildes Felsendahl durchstreeft, un mir drei Forscher, de Gebrieder Humbold un ich, g'rade unsern Soldaten ä Stickchen vorneweg geeilt sinn un gans arglos aus ä Hohlwege treten – heernse, da komm Sie zwee Drubbs Indianer uff eemal in sausender Karrjere 'rangesprengt – links ä Drubb Sioux un rechts ä Drubb Irokesen – denn ich kannte die Brieder an den Federbischeln – ä Hagel von Feilen saust off uns ein un – hastenichgesähn! stecken m'r ooch schon zwee von den verdammten Dingern in der linken Seite. Nu is es immer gut, wenn der Mensch Kenntnisse un de Oogen offen hat. De Feile waren von links gekomm' un links standen de Sioux, un daß die ihre Feile mit Strychnin vergiften, das wußt'ch schon von der Ferschtenschule her. Die Dinger 'rausreißen war eens. Awer was nu gegen de Werkung von dän Strychnin duhn? Unsre Reiseabodeke war bei d'n Soldaten zerickgebliem. Heernse, da fiel m'r zum Glick ein, daß ja de Irokesen – die von rechts schossen – bei ihren Feilen Kurarin verwenden, was de das Gegengift von Strychnin is! Wie m'r das dorch de Gedanken schoß, war ich ooch schon nach rechts vorgesprungen. Awer in dän Oogenblicke erschienen unsere Soldaten, gingen mit ä dreimaligen Hurra vor un de Indianer kratzten aus. Ich, in der Angst, daß es ze spät fer mich wär'n gennte, renne den eenen Irokesen nach un schreie in eene fort – uff irokesisch nadierlich –: Schießen Se nur noch ä eenz'gen Feil uff mich! Nur ä allereenz'gen! Heernse, sein Se doch so gut! Un das Luderchen muß es endlich ooch begriffen hamm. Denn uff eemal dreht er sich um un huck! sitz m'r ooch schon ä Fitschefeil in Bauche. Ich war gerettet – awer's war ooch de heechste Zeit, un drei Dage haw ich noch von wegen dän Schräcken krank gelegen!"

Georg Bötticher (1849−1918)

The figure below shows a molecule of strychninium chloride prepared as described above. Although this book only reports spectra for all the other compounds, we thought it appropriate to record and present an X-ray structure of this alkaloid of outstanding complexity.

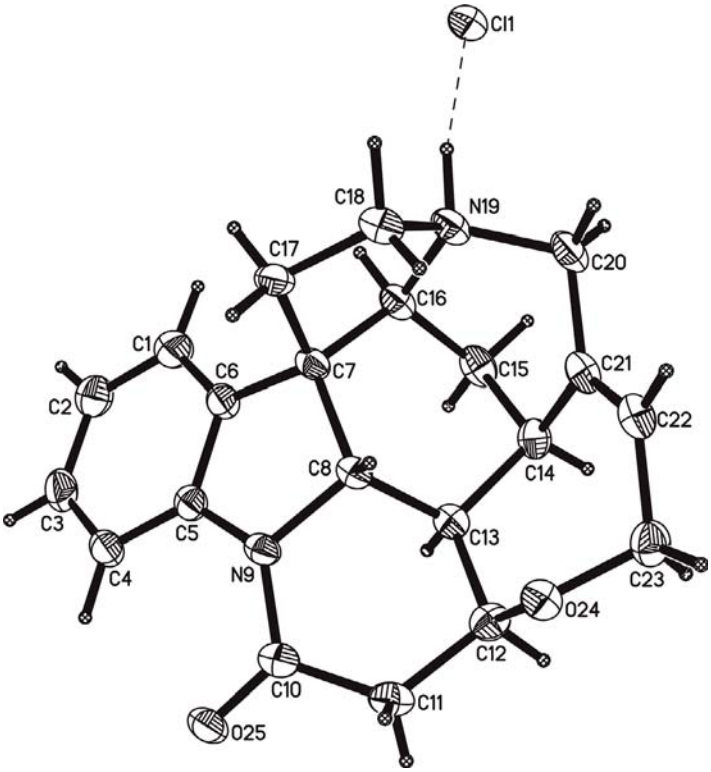
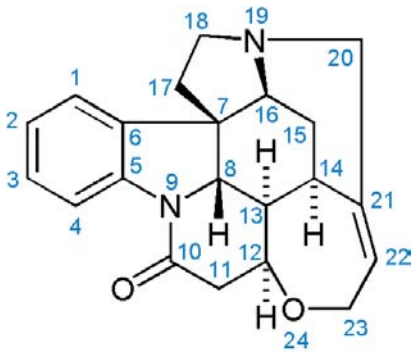


Fig. 1.7-3 X-ray structure
Details of the crystallographic data can be obtained from the Cambridge Crystallographic Data Centre, No. 697488



Scheme 1.7-2

4. Spectra and Comments

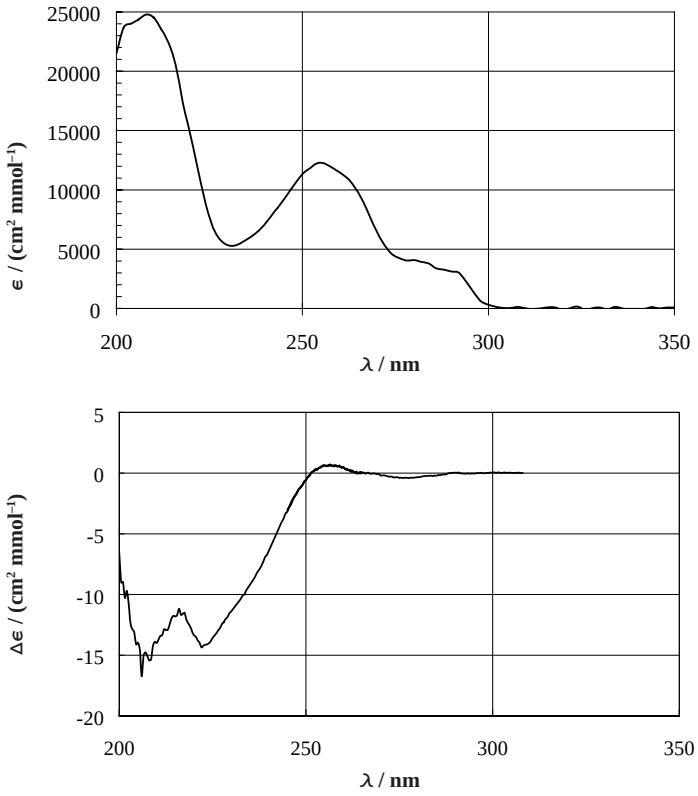


Fig. 1.7-4 UV and CD spectra in ethanol

As chromophores, strychnine has a benzene ring with an N–CO group attached and in addition an isolated double bond. Accordingly, we find in the UV spectrum the typical benzenoid absorptions at 212 and 254 nm. In addition, we see an n–π* transition with a vibrational fine structure at 280 nm. The intensities of these transitions are in the expected range. The compound is chiral and therefore we obtain a CD spectrum. Only the main aromatic π–π* absorption reveals a Cotton effect which is rather strong and negative.

Fig. 1.7-5 A branch of *Strychnos decussata* growing in the Botanic Garden of Cape Town, South Africa

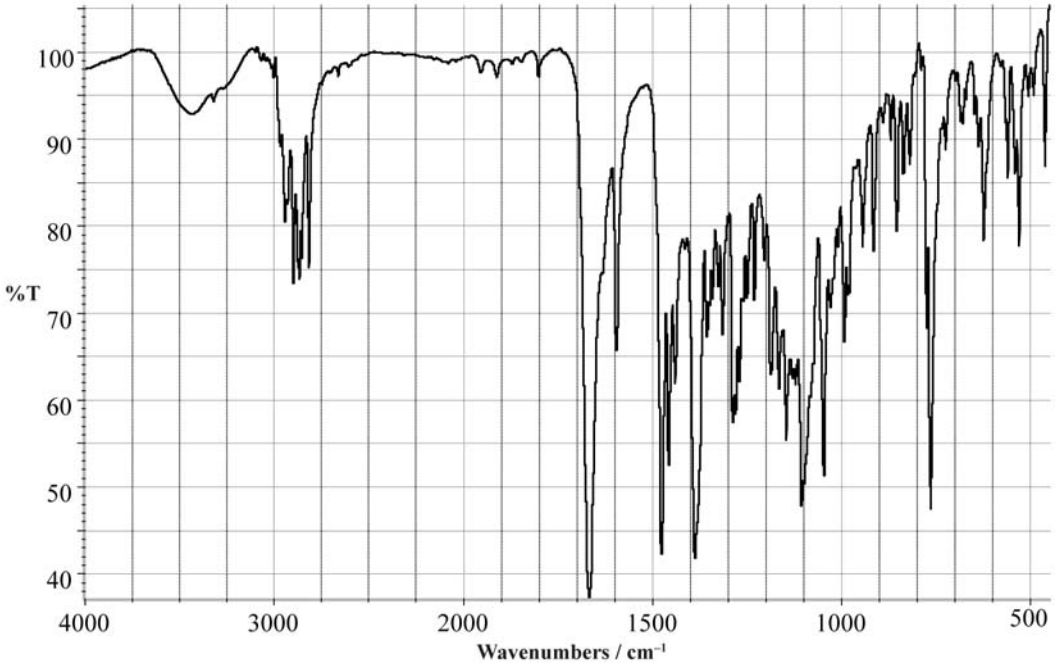
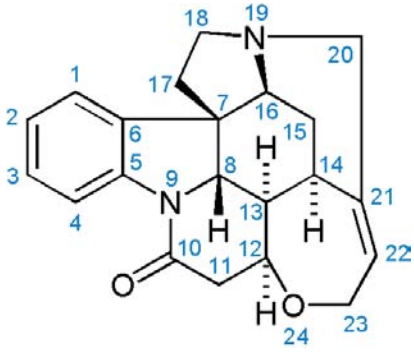


Fig. 1.7-6 IR spectrum in KBr

Interestingly, the CH valence vibrations of sp^2-hybridized CH moieties can hardly be detected although the compound has five such groups. In the CH region, the spectrum is dominated by valence vibrations below 3000 cm^{-1} originating from the many sp^3-hybridized CH groups. The benzene ring can be identified from the IR spectrum by the overtone vibrations between 1800 and 2000 cm^{-1} and these are followed by a typical amide band at 1670 cm^{-1} and a C=C band at exactly 1600 cm^{-1}.

Fig. 1.7-7 A young plant of *Strychnos nux-vomica*



Scheme 1.7-3

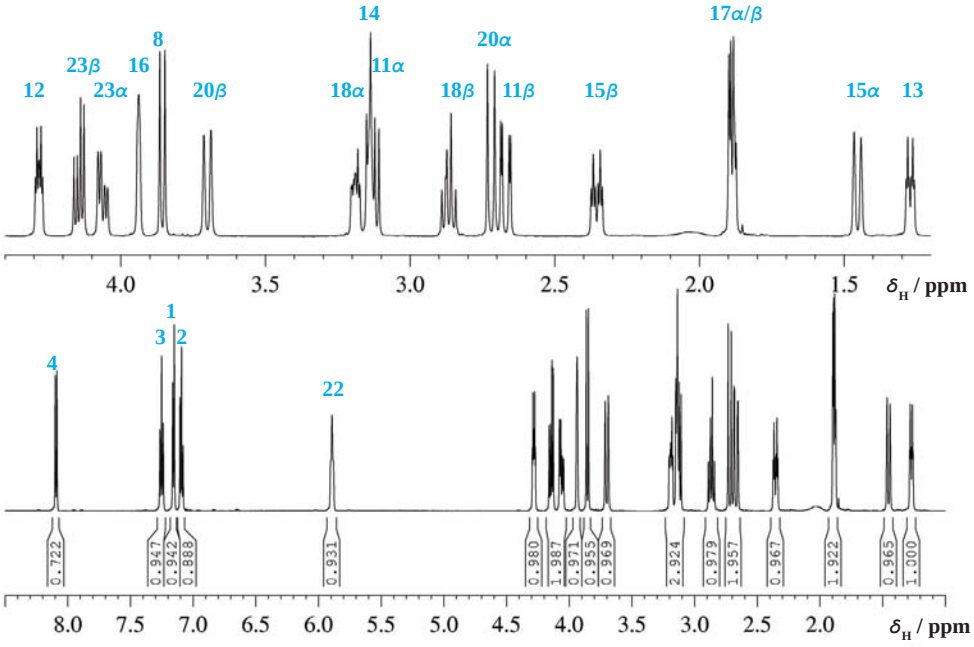


Fig. 1.7-8 ^{1}H NMR spectrum at 600 MHz in $CDCl_3$

Strychnine has developed to become a standard reference compound for NMR spectroscopy in organic chemistry. The reason is that in this compound many typical structural features are present which cause the spectrum to spread over the entire chemical shift range. Nearly every new method or pulse technique introduced in recent years was first demonstrated using strychnine as an example and therefore the NMR spectra are all very well understood and documented. In an earlier book by one of the authors [12] a large variety of strychnine NMR spectra are documented.

Looking at the ^{1}H NMR spectrum we find in the aromatic region four signals, two appearing as doublets and two as triplets, typical for an *ortho*-disubstituted benzene ring. The assignment of the most deshielded signal at 8.2 ppm to H-4 is evident due to the electron-withdrawing power of the amide group. The relative assignment of the two triplets will follow from the COSY spectrum. At 6 ppm we find a broadened signal which must be from the only olefinic hydrogen in this compound, H-22. In the CHO region at about 4 ppm we find a signal from a single proton at 4.288 ppm, which we assign to H-12, being located directly beside the ether oxygen. Close by is an AB pattern with one further spin splitting and this is therefore assigned to the two H-23 protons at 4.148 and 4.066 ppm. At this stage of the analysis it is to early to make a firm assignment of the remaining protons. We only assume that the signals between 4 and 3 ppm are probably from protons close to the nitrogen atoms and that the most shielded signal at 1.276 ppm arises from H-13 having no heteroatom in its direct vicinity.

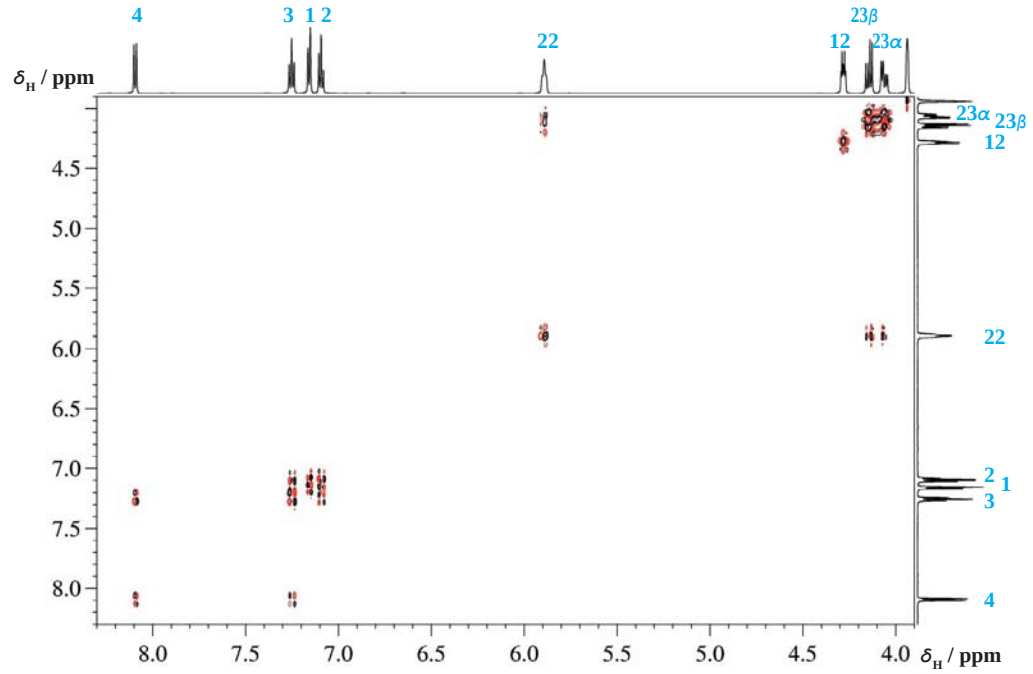


Fig. 1.7-9 Expansion of the DQF-COSY spectrum in the olefinic aromatic region

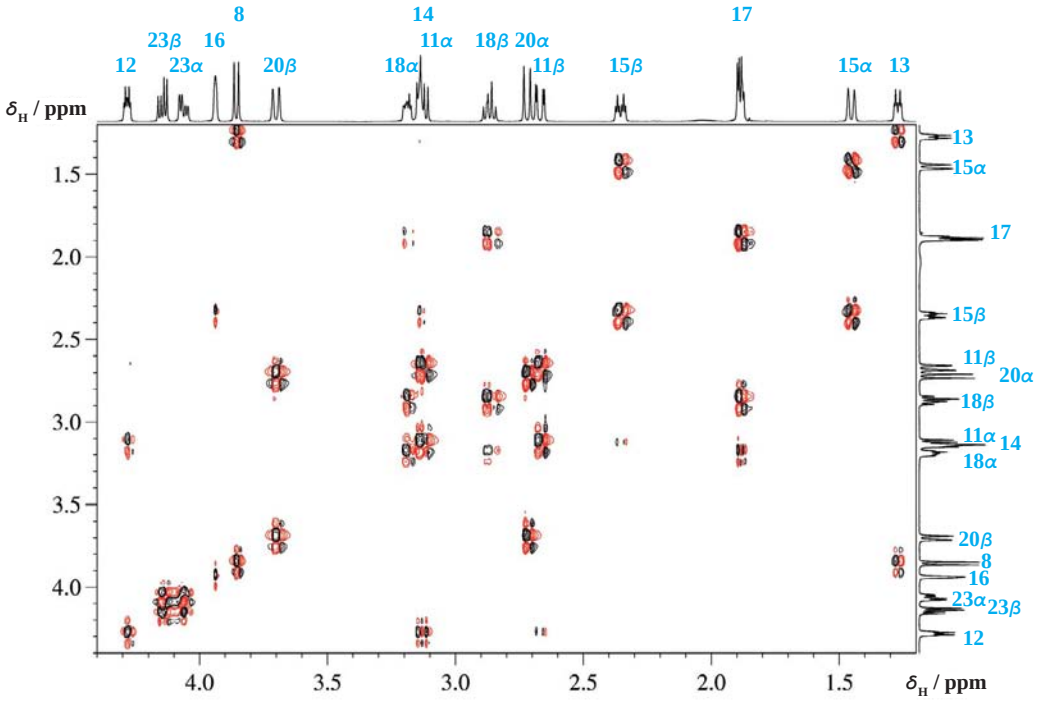
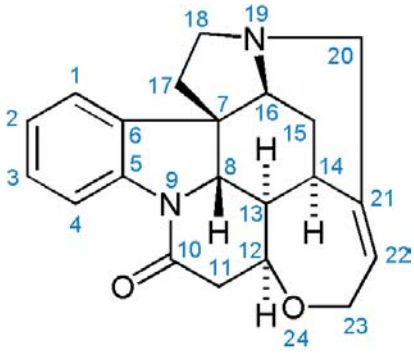


Fig. 1.7-10 Expansion of the DQF-COSY spectrum in the aliphatic region



Scheme 1.7-4

The first expansion of the COSY spectrum in the aromatic/olefinic region confirms the assignment within the aromatic ring with H-3 at 7.255 ppm and also the assignment for both H-23. The second COSY expansion displays three connectivities for H-12, one of which to H-13, however, is only detected on the computer screen. The assignment for H-13 has already been discussed and the two cross peaks to the signals at 3.132 and 2.670 ppm point to the diastereotopic protons H-11. The broadened signal at 3.963 ppm has a COSY cross peak to one multiplet at 2.360 ppm, which in turn is strongly coupled to another multiplett at 1.462 ppm. We assign these two multipletts to both H-15 protons and the signal at 3.963 ppm therefore to H-16. The sharp doublet at 3.860 ppm is connected with H-13 and therefore firmly assigned to H-8. Next, we find an AX pattern at 3.716 and 2.745 ppm and this is assigned to the isolated protons H-20 which have no other direct spin coupling partner. The COSY spectrum further reveals four protons which are strongly coupled to each other. The first two protons of this pattern at 3.219 and 2.878 ppm are most likely CHN protons and assigned to H-18 and these are connected with both H-17 which resonate at 1.89 ppm on top of each other. The only signal not yet discussed is the broad singlet at 3.150 ppm displaying a cross peak to H-13, which identifies it as H-14. In summary, due to the well spread spectrum, the COSY technique is in principle sufficient to assign all protons of strychnine with safety.

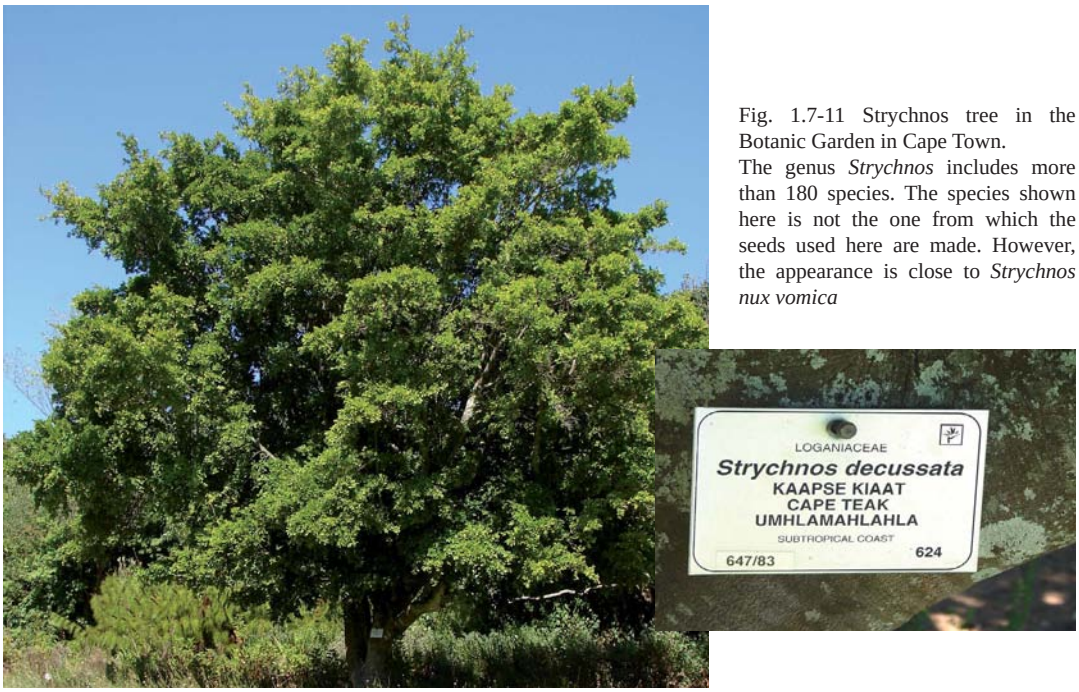


Fig. 1.7-11 Strychnos tree in the Botanic Garden in Cape Town.
The genus *Strychnos* includes more than 180 species. The species shown here is not the one from which the seeds used here are made. However, the appearance is close to *Strychnos nux vomica*

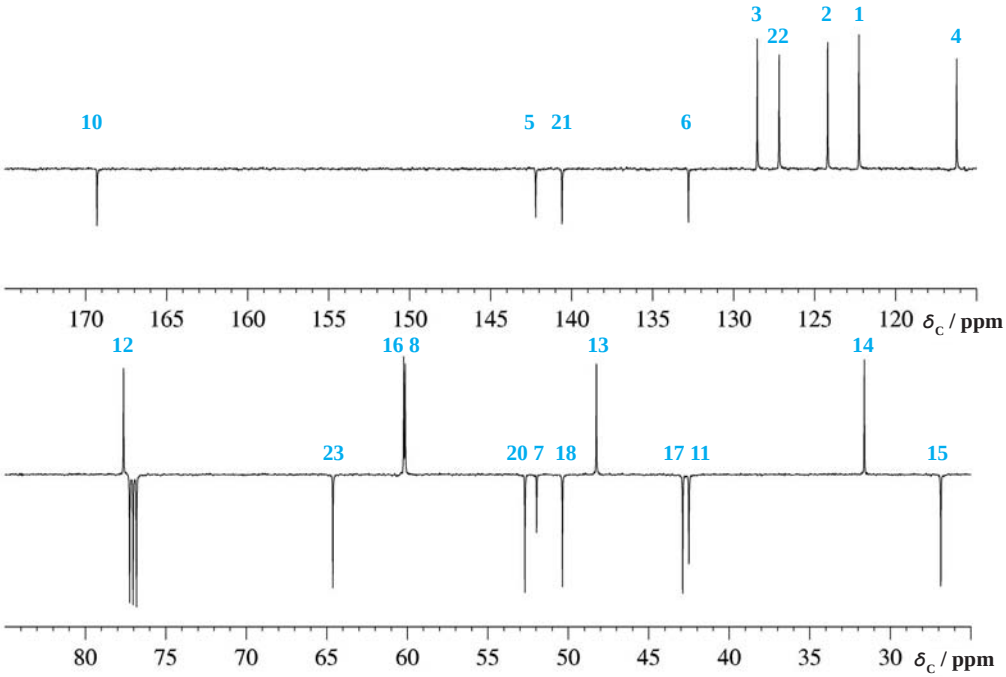
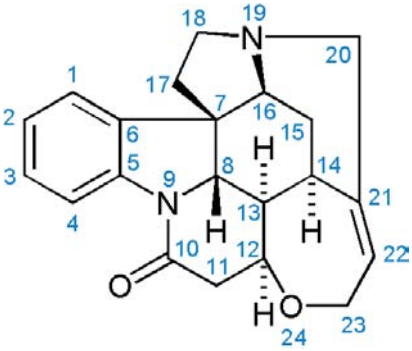


Fig. 1.7-12 APT ^{13}C NMR spectrum at 150 MHz in $CDCl_3$

The only obvious assignment is that for the amide carbonyl at 169.3 ppm. The remaining quaternary carbon atoms will be assigned with the help of the HMBC spectrum. Similarly, since we know all proton assignments, we will use the HSQC spectrum to identify the signals of the CH and CH_2 moieties. The assignment of C-12 close to the chloroform signal, however, is also very clear. C-7, the only aliphatic quaternary carbon atom, may be picked out already here due to its reduced intensity.



Scheme 1.7-5

Fig. 1.7-13 Seeds of *Strychnos nux vomica*

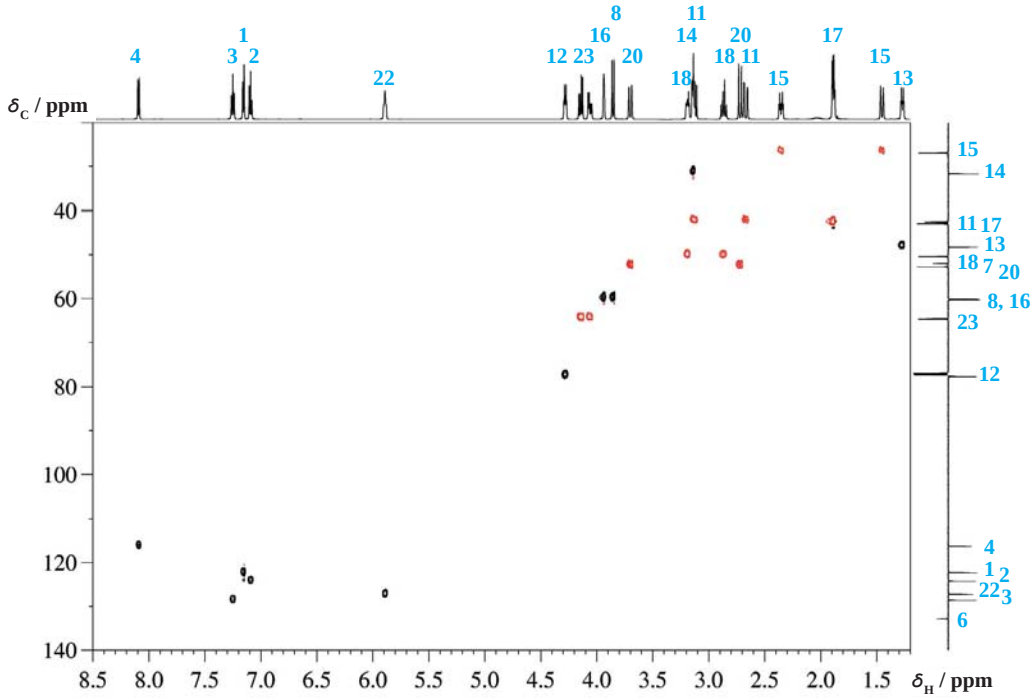


Fig. 1.7-14 HSQC spectrum

The CH edited HSQC spectrum displays in red the signal of all methylene groups. The diasterotopic protons of these groups of course correlate with the same carbon signal and can thus easily be identified. Note that the most shielded proton signal stems from H-13, whereas the most shielded ^{13}C signal stems from C-15. It is also interesting to observe that the signal of C-4 is the most shielded aromatic carbon signal whereas its proton H-4 is the most deshielded proton signal.

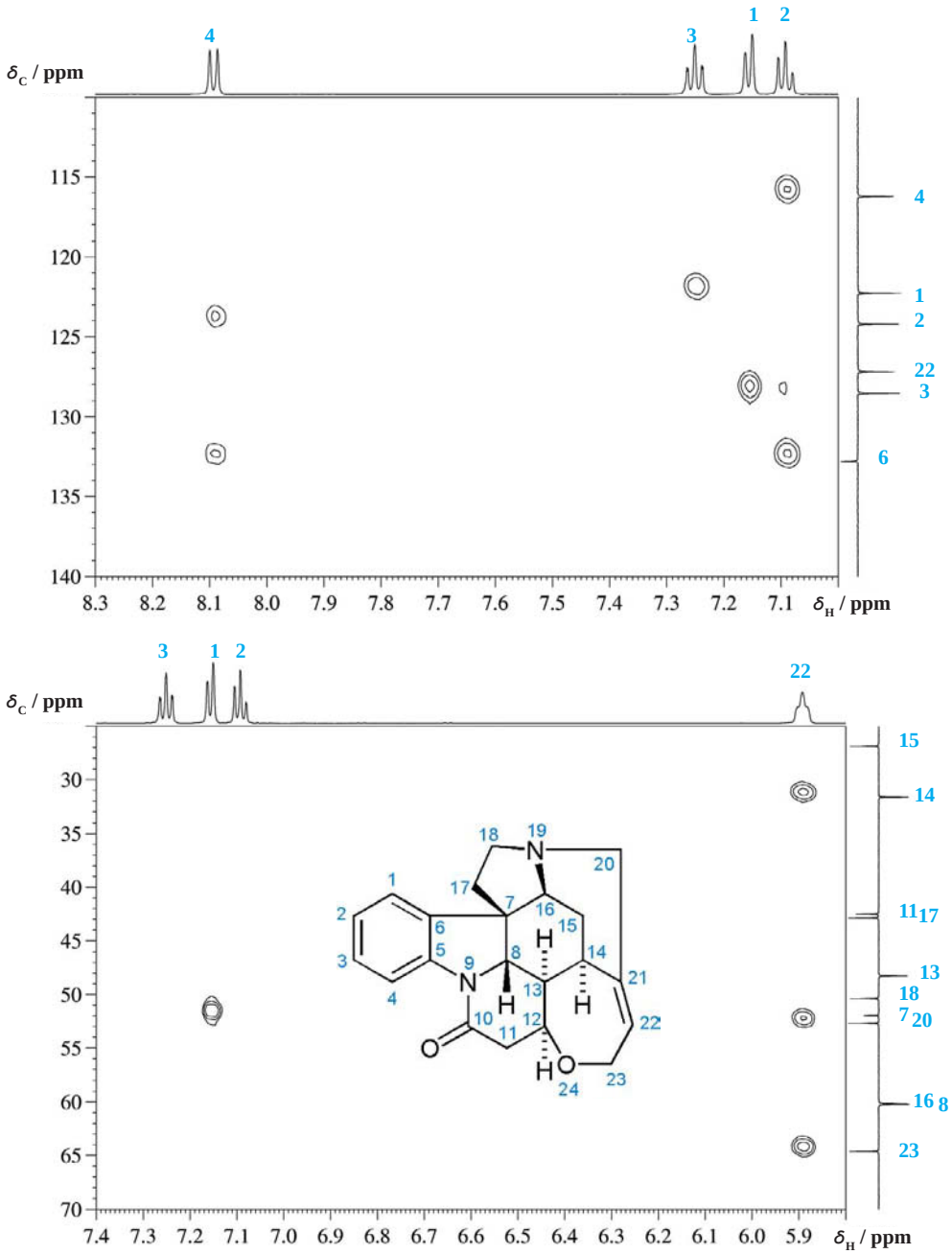


Fig. 1.7-15 Expansions of the HMBC spectra

The first expansion of the HMBC spectrum in the aromatic region is again a textbook example of the power of this method. H-4 shows two connectivities via three bonds to the signals of C-2 and C-6, H-3 identifies C-1 and vice versa H-1 displays a cross peak to C-3. Finally, H-2 is connected to both C-4 and C-6. In the second expansion, devoted to the connections between the olefinic and aliphatic regions, we find a cross peak between H-1 and C-7 via three bonds and three cross signals for H-22. These identify C-14, C-20 and C-23.

We will not discuss in detail the third expansion, which displays a multitude of information. However the reader is asked to go through this diagram and confirm the various assignments. In the final expansion, the most important feature is the verification of the protons H-11 due to their cross peaks to the carbonyl C-atom.

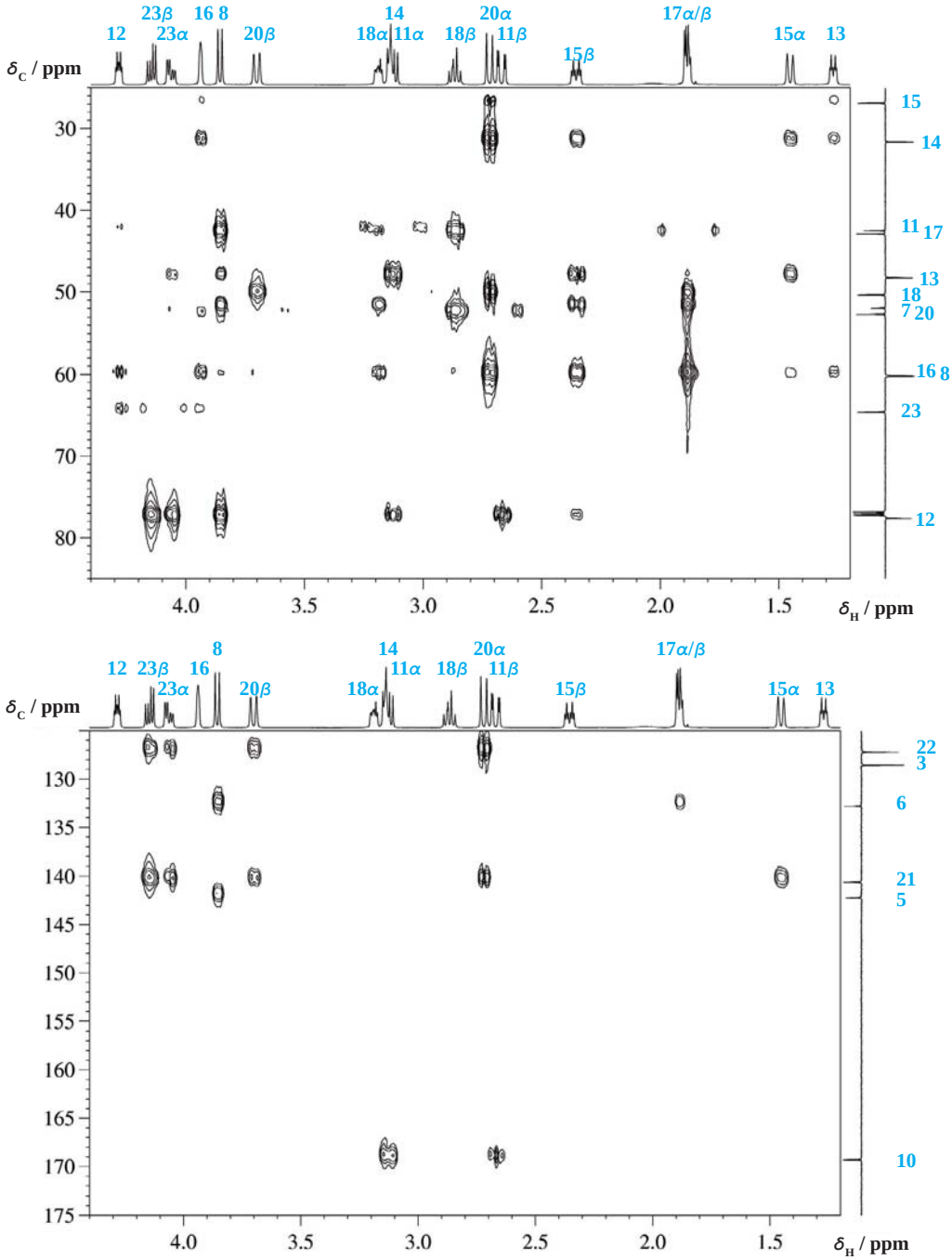


Fig. 1.7-16 Further expansions of the HMBC spectra

Since all assignments have been verified by the spectra discussed above, we use the NOESY information only for the stereochemical assignment of the diastereotopic protons. For this, these protons are labelled α and β in the 3D structure shown below.

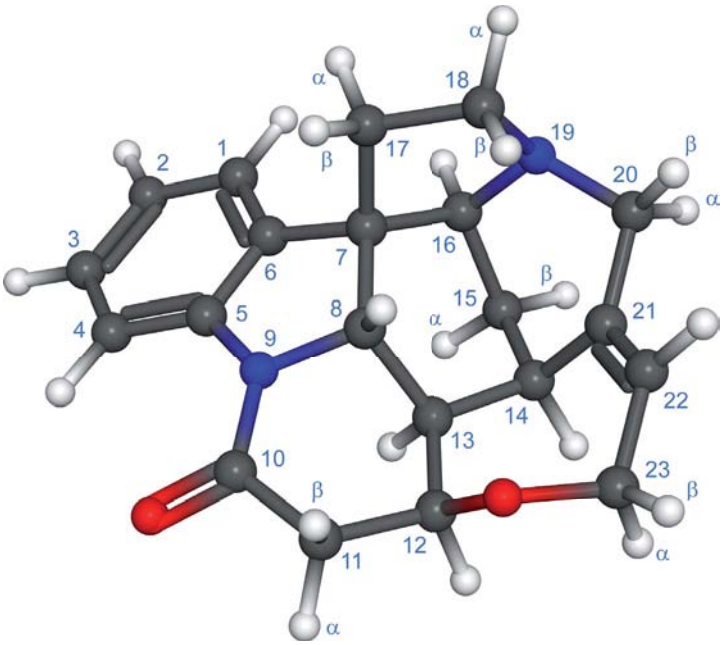


Fig. 1.7-17 Molecular model of strychnine

We start with the protons H-11, which of course show a strong NOE cross peak between each other. Only the H-11 proton at 3.132 ppm displays an NOE cross peak to H-12 and therefore it is assumed to be on the same side of the molecule and hence assigned to H-11α. Exactly the same reasoning is valid for H-23 and therefore the signal at 4.066 ppm is assigned to H-23 α . The distinction between the protons H-15 can be obtained by the cross peak of H-15α to H-13. Only the H-20 at 3.716 ppm shows a cross peak to one of the H-15 protons and this is therefore assigned to H-20β . Finally, the signal of H-18 at 2.878 ppm shows a cross peak to H-8 and therefore it is assigned to H-18β. The remaining diastereotopic protons H-17 are too close together and cannot be differentiated.

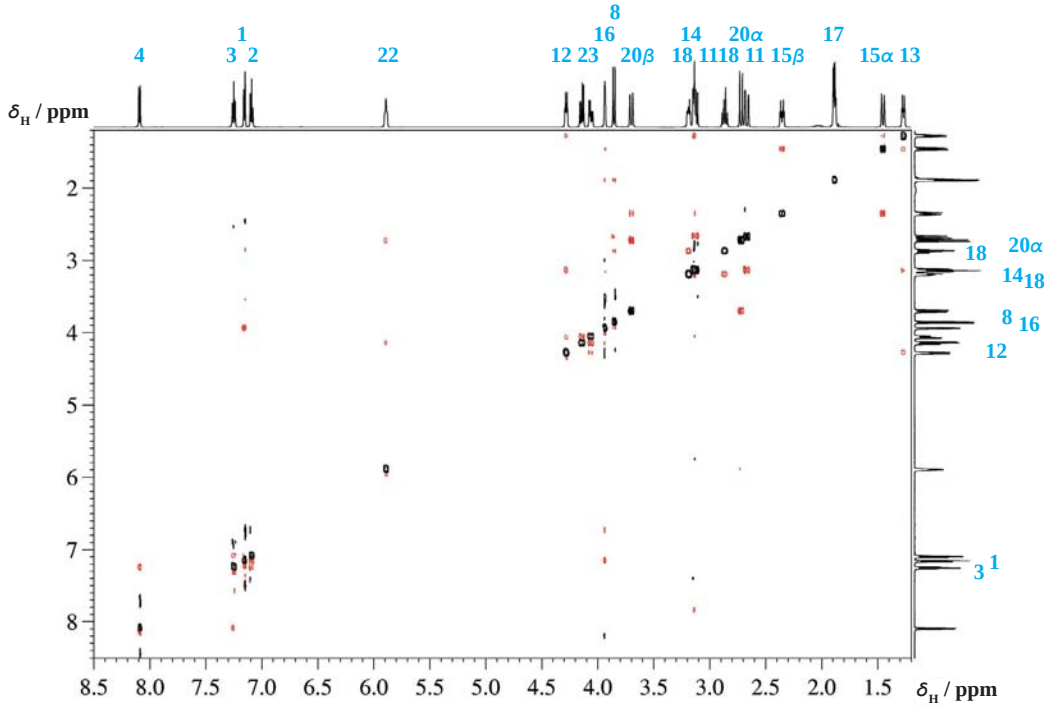


Fig. 1.7-18 NOESY spectrum

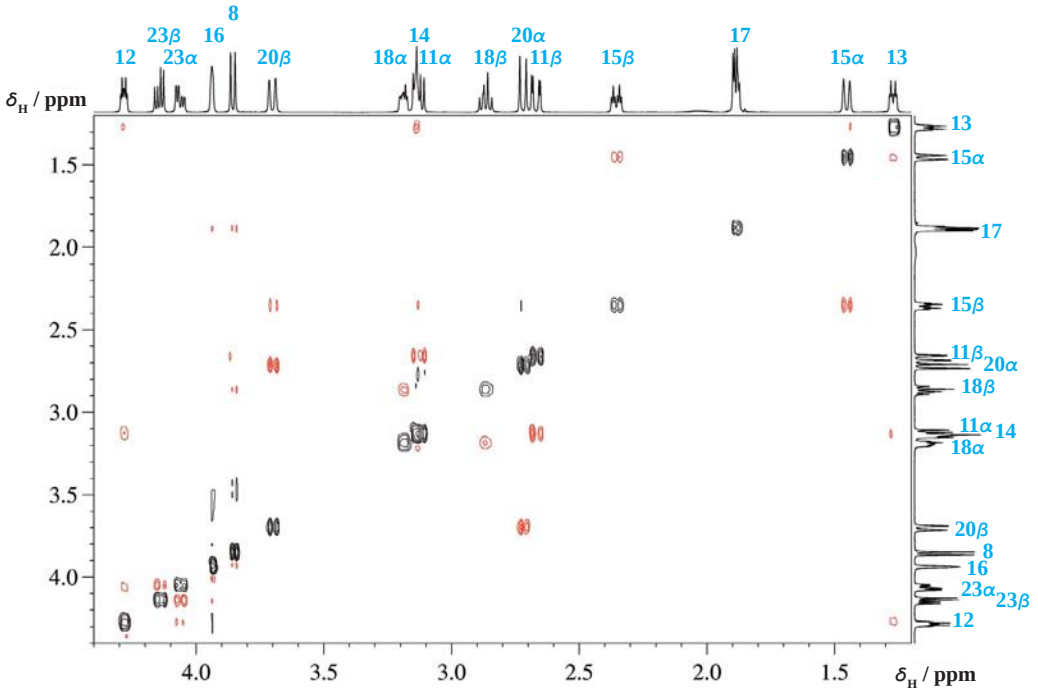


Fig. 1.7-19 Aliphatic expansion

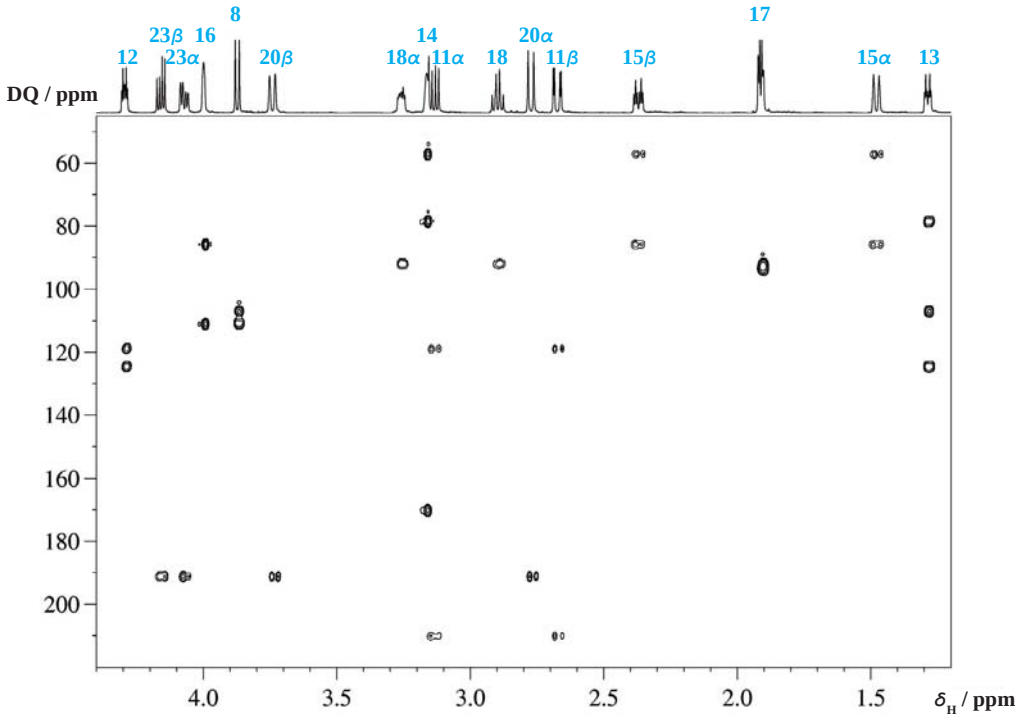
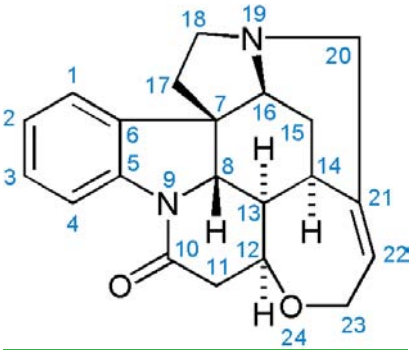


Fig. 1.7-20 ADEQUATE spectrum at 175 MHz

Although not really needed in this case, we display the ADEQUATE spectrum which finally corroborates all of the above assignments. As shown in other cases in this book, one expects from a methine carbon atom connected to three other carbon atoms three correlation signals at the corresponding double quantum frequencies which are the sum of the ^{13}C chemical shifts in question. A methine carbon atom connected to oxygen will display only two correlation signals. The figure shows an expansion of the aliphatic region of the 1,1-ADEQUATE spectrum obtained on an Avance 700 spectrometer using a cryoprobe head. We start the interpretation of the figure at the left-hand side. Proton H-12 has its signal at δ_H = 4.28; two correlation signals at the double-quantum frequencies δ_C = 119.3 and 125.1 can be seen. This corresponds to $\delta_{C\text{-}12}$ = 76.85 + $\delta_{C\text{-}11}$ = 42.48 and $\delta_{C\text{-}12}$ = 76.85 + $\delta_{C\text{-}13}$ = 48.22 and confirms the binding situation for C-12. The protons H-23 (δ_H = 4.0 to 4.2) are situated on a carbon with only one carbon atom neighbour. Therefore, their correlation signals are found at $\delta_{C\text{-}23}$ = 64.60 + $\delta_{C\text{-}22}$ = 127.34 giving 191.9. Going to the right-hand side of the figure, we find H-13 at δ_H = 1.27. C-13 at δ_C = 48.22 is connected to three other carbon atoms, C-8, C-12 and C-14. Therefore, we find the three correlation signals at the corresponding double-quantum frequencies 108.2, 125.1 (as seen before in the signal of H-12) and 79.8. Similarly, all the other correlation signals can be assigned using the table of chemical shift data.



Scheme 1.7-6

Fig. 1.7-21 Small *Strychnos* plant

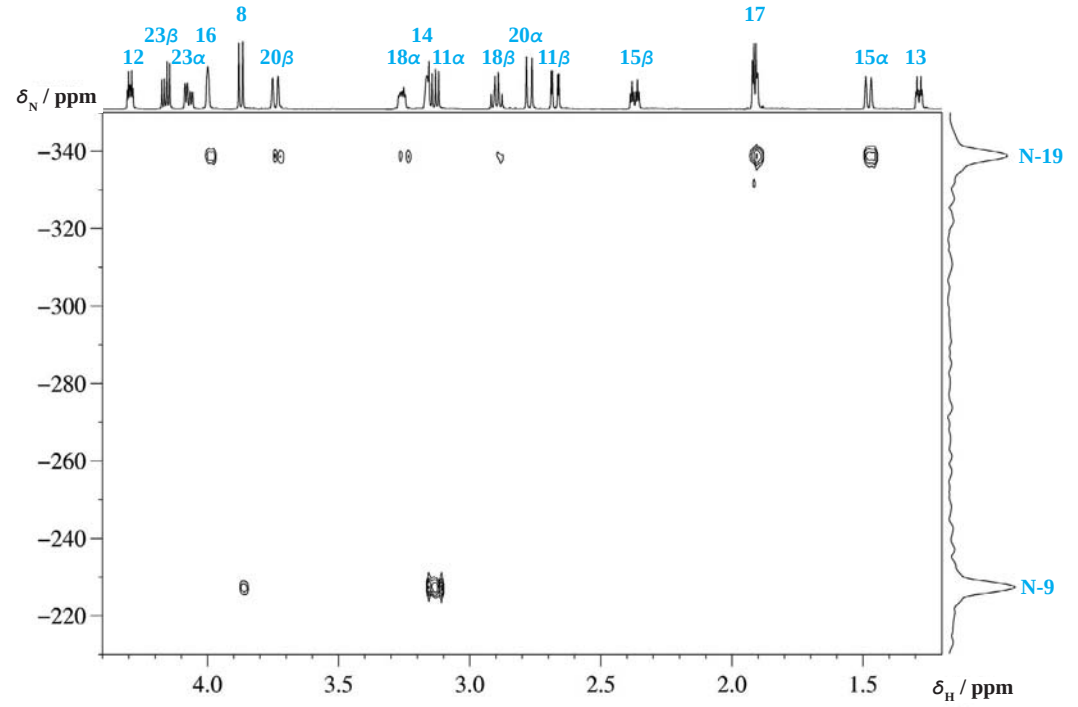


Fig. 1.7-23 ^{1}H ^{15}N HMQC spectrum

Finally, the HN correlation spectrum is shown, nicely revealing the two nitrogen atoms and their different bonding situations. Whereas the aliphatic tertiary amine nitrogen N-19 at –340 ppm is seen by a multitude of its neighbouring protons, the amide nitrogen N-9 at –230 ppm is only detected by H-8 and one of the H-11 protons.

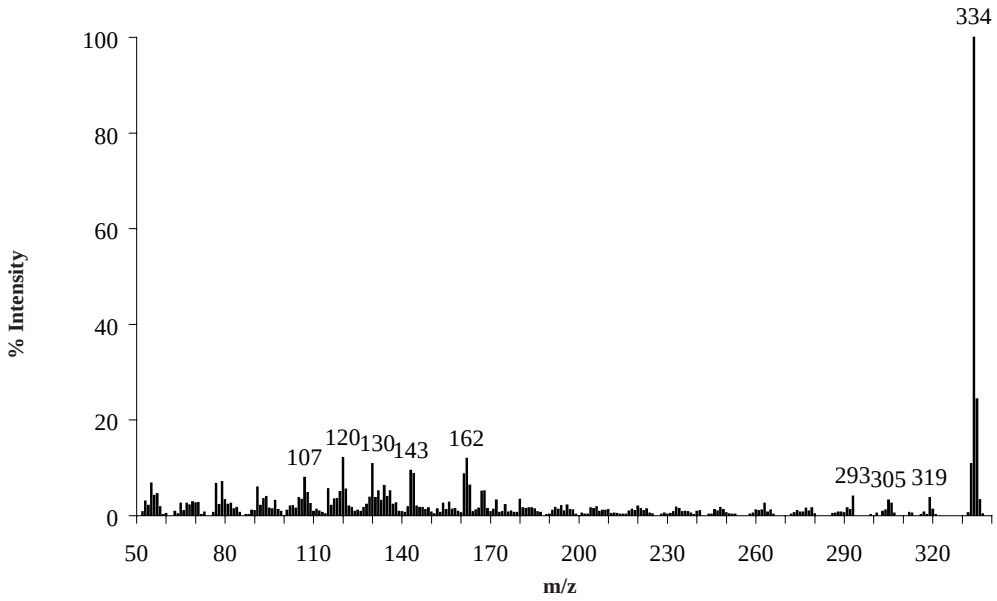
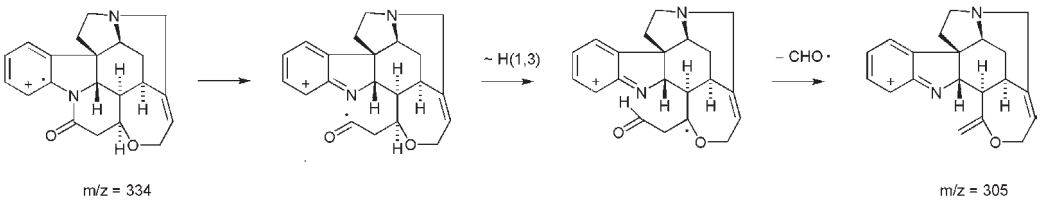


Fig. 1.7-23 Mass spectrum (EI)

As shown for other alkaloids in this book, strychnine is apparently a very stable molecule and does not fragment easily even under electron impact ionization. Accordingly, the mass spectrum displays the molecular ion with m/z =334 as the base peak. Due to the 21 carbon atoms of the molecule, we find an M^{+1} peak with about 20% of the intensity of the $M^{+\bullet}$ signal. A possible mechanism for the loss of 29 mass units is given in the scheme below.



Scheme 1.7-7 Fragmentation of strychnine

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz	$^{1}J_{C,H}$ / Hz
169.28	C_q	10		
142.23	C_q	5		
140.45	C_q	21		
132.72	C_q	6		
128.56	CH	3	7.255, $^{3}J_{3,4}$ 7.90	159.6
127.34	CH	22	5.915,$^{3}J_{22,23\alpha}$ 7.0, $^{3}J_{22,23\beta}$ 6.1	157.7
124.20	CH	2	7.098, $^{3}J_{2,3}$ 7.44, $^{4}J_{2,4}$ 0.98	160.9
122.26	CH	1	7.167,$^{3}J_{1,2}$ 7.49, $^{4}J_{1,3}$ 1.08, $^{5}J_{1,4}$ 0.23	159.0
116.23	CH	4	8.092	168.0
76.85	CH	12	4.288, $^{3}J_{12,13}$ 3.30	145.4
64.60	CH_2	23β	4.148, $^{2}J_{23\alpha,23\beta}$ –13.8	144.3
		23α	4.066	137.2
60.28	CH	16	3.963	146.2
60.10	CH	8	3.860, $^{3}J_{8,13}$ 10.41	145.4
52.68	CH_2	20β	3.716, $^{2}J_{20\alpha,20\beta}$ –14.8. $^{4}J_{20\alpha,22}$ 1.79	141.0
		20α	2.745	141.0
51.96	C_q	7		
50.35	CH_2	18α	3.219, $^{2}J_{18\alpha,18\beta}$ –13.9	136.8
		18β	2.878	
48.22	CH	13	1.276, $^{3}J_{13,14}$ 3.29	124.4
42.85	CH_2	17α	1.89[b], $^{2}J_{17\alpha,17\beta}$ –13.9, $^{3}J_{17\alpha,18\alpha}$ 5.5, $^{3}J_{17\alpha,18\beta}$ 7.2	133.4
		17β	1.89[b], $^{3}J_{17\beta,\,18\alpha}$ 3.2, $^{3}J_{17\beta,\,18\beta}$ 10.7	
42.48	CH_2	11α	3.132, $^{2}J_{11\alpha,\,11\beta}$ –17.34, $^{3}J_{11\alpha,12}$ 3.34	126.3
		11β	2.670, $^{3}J_{11\beta,12}$ 8.47	135.9
31.60	CH	14	3.150, $^{3}J_{14,15\alpha}$ 4.11, $^{3}J_{14,15\beta}$ 1.96, $^{4}J_{14,22}$ 0.47, $^{4}J_{14,20\alpha}$ 1.61	130.1
26.84	CH_2	15β	2.360, $^{2}J_{15\alpha,15\beta}$ –14.35, $^{3}J_{15\alpha,16}$ 4.33	131.4
		15α	1.462, $^{3}J_{15\beta,16}$ 2.42	131.4
^{15}N Signals δ / ppm	Type of Nitrogen	Assignment		
–338,5	N_q	N-19		
–227.3	N_q	N-9		

Table 1.7-1 NMR data for strychnine

5. Questions

A. At present, more than 10 000 alkaloids are known. Make a comment on their classification.

B. What is the chemical reason why a poison such as strychnine is stable for a long time in a cadaver, so that specialists in forensic medicine have a good prospect of identifying this poison even after years?

C. Most alkaloids are N-heterocycles, which means that the N-atom (or the N-atoms) is (are) part of ring(s). Give examples of alkaloids with nonheterocyclic nature, i.e. with N outside cyclic structures.

D. Whereas Pelletier and Caventou did not succeed in naming what now is called *Strychnine* as *Vauqueline* in honour of Vauquelin, another alkaloid was named *Pelletierine* in honour of Pelletier. Give its structure and the group to which it belongs and search for the plant in which this alkaloid occurs. Which well-known alkaloid is structurally closely related to *Pelletierine*?

E. It is obvious that most alkaloids occur in the vegetable kingdom and only a minority in the animal kingdom: think of salamander alkaloids, for example. Suggest a reason for this phenomenon.

F. Suggest a reason why the UV spectrum displays some vibrational fine structure.

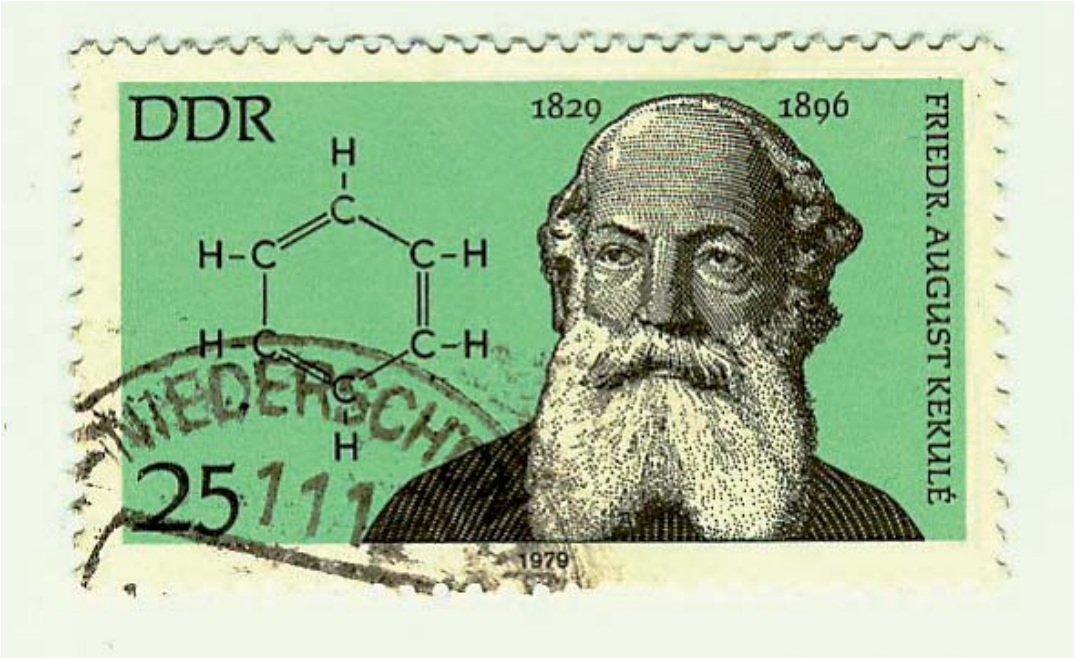
G. Can one deduce from the IR spectrum that an *ortho*-disubstituted aromatic ring is present?

H. Analyse the third expansion of the HMBC spectrum between 2.5 and 1.3 ppm.

I. Can one detect diastereotopic protons in an ADEQUATE spectrum?

6. Own Observations

Chapter 2 Aromatic Compounds



GDR stamp for F. A. Kekulé, inventor of the benzene structure

In the field of aromatic compounds, one of the most engaged persons was the Nobel Prize winner Adolf von Baeyer.

1905

Johann Friedrich Wilhelm Adolf von Baeyer
(Germany, 1835−1917)

Germany, Munich University

“in recognition of his services in the advancement of organic chemistry and the chemical industry, through his work on organic dyes and hydroaromatic compounds”

2.1 Anethole

1-Methoxy-4-[(1*E*)-propenyl]benzene

From anise
Pimpinella anisum L. (Apiaceae)
contained as extract in the spirit Ouzo

$C_{10}H_{12}O$, MW 148.20

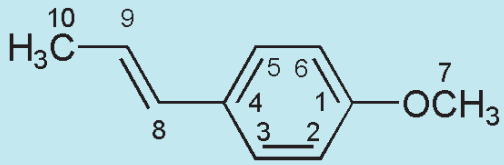
CAS RN 4180-23-8, BRN 636190

Colourless crystals, mp 22 °C
Above mp: colourless liquid, bp 234 °C

trans-Anethole is commercially available.

Synonymous names:
(*E*)-1-methoxy-4-(1*E*)-1-propen-1-ylbenzene,
(*E*)-anethole,
1-methoxy-4-[(1*E*)-1-propenyl]benzene,
1-methoxy-4-*trans*-propenyl-benzene

Level: easy



1. Background: Just filter your ice-cold Ouzo!

Οὐαὶ ὑμῖν, γραμματεῖς καὶ Φαρισαῖοι ὑποκριταί, ὅτι ἀποδεκατοῦτε τὸ ἡδύοσμον καὶ τὸ ἄνηθον καὶ τὸ κύμινον, καὶ ἀφήκατε τὰ βαρύτερα τοῦ νόμου, τὴν κρίσιν καὶ τὸ ἔλεος καὶ τὴν πίστιν: ταῦτα [δὲ] ἔδει ποιῆσαι κἀκεῖνα μὴ ἀφιέναι.

New Testament, Matthew, 23,23

Fig. 2.1-1 Anise seed

Anethole (exact: *trans*-anethole) is an unsatured aromatic ether with a very aromatic taste occurring in several plants such as anise, star anise and fennel. The correct constitution of anethole was described as early as 1877 [1]. However, the very similar taste of licorice, appreciated by many people, is not due to anethole but to another natural sweetener, glycyrrhizic acid, a triterpenoid saponine glycoside, which is 50 times sweeter than sugar. Compounds such as anethole, due to their aromatic flavour, are responsible for the denomination of a class of certain carbocycles as *aromatic compounds* (compare Section 2.2, Eugenol). Anethole is not only aromatic in its flavour but has a distinctly sweet taste in addition. Dilution experiments showed it to be 13 times sweeter than sugar. For these two reasons, the perception of anethole is a pleasant one on the tongue [2]. Only in large quantities, which are clearly above those taken up by food or beverages, is it slightly toxic (spasmolytic) and irritant.

Anise (or aniseed) is an annual herbaceous plant which grows up to 1 m tall. The small, white flowers are arranged in white umbels and finally produce 3–5 mm long seeds. They are often used in south and east Asian cooking. The British confectionary *aniseed balls* contain it as an ingredient, and also the Italian cookies *pizelles*. In India, chewing aniseeds is a standard behaviour after a meal, regarded as acting as a mouth freshener and digestive. Aniseed essential oil can be used in aromatherapy against colds and influenza. In former times, the essential oil was even used to treat scabies and lice.

Mulier ergo, quae obstrusa menstrua patitur et inde dolet, accipiat anesum et febrefugiam aequali pondere et wullenam aliquantum plus, quam unius istorum sit, et eas coquat aperto et fluento flumine, quod et sole et aere temperatur, et tunc etiam sumat lateres et eos in ignem ponat et assum balneum cum praedicta aqua et predictis herbis faciat. Cumque hoc balneum intraverit, super scabellam herbas istas calidas ponat et desuper sedeat atque easdem herbas genitali membro et ita sursum ad umbilicum et toto umbilico calidas circumponat. Etsi interim infrigidatae fuerint, eas iterum in praedicta aqua calefactas in eisdem locis sibi circumponat et hoc faciat, quamdiu in bagno illo sedeat, ut per humores herbarum istarum cutis et caro illius exterius et matrix interius mollificetur et ut venae eius, quae clausae sunt, aperiantur. Nam calor anesi humores commovet; calor autem febrefugiae sanat et calor wullene fluxum parat.

Hildegard Bingensis (1098–1178)
Causae et Curae, Lib IV

A tasteful compound like this was, of course, not ignored by liqueur manufacturers. Accordingly, their list is long, including e.g. Absinthe (see Section 5.3, Thujones), Pastis, Sambuca, Raki, Arak, Mastika and *Ouzo*. Dealing with the last product leads us closer to our experiment. In October 2006, Greece, as a member of the European Union, won the contest for the exclusive right to label Ouzo as a Greek product. This anise-flavoured liqueur is not only widely consumed in Greece, but is also known around the world as a welcome aperitif, which can be drunk straight or diluted with water. The latter initiates the formation of an oil-in-water emulsion which is opalescent due to the Tyndall effect of the colloid particles (called the louche effect in France; compare Absinthe in Section 5.3). If the Ouzo is actually served from a deep freezer, you will find that it contains tiny colourless crystals – these are composed of pure (*E*)-anethole.

However, it is not this book's intention to inaugurate the reader into the art of making liqueurs and spirits. Ouzo making is based on ancient recipes from Byzantine times. After Greek independence, Ouzo distillation increased in the 19th century at a centre on the island of Lesbos. After the banning of Absinthe, Ouzo was a closely related product, but without containing any wormwood ingredients. The basic principle of making Ouzo is a distillation of 96% ethanol in copper stills together with anise and other flavourings. The distillate is then diluted and sugar may be added.

The name Ouzo originates from Greek exports of a liqueur called "tsipouro" in the 19th century, whose name was substituted by Ouzo as the following legend tells. Thessaly exported a number of bottles with "tsipouro" to Marseille and the desire to label the product in a distinguishing manner arose. It was decided to stamp the crates with "uso Massalia", paradoxically the Italian translation of the intended message "to be used by Marseilles". Because the product pleased the short Italian, "uso" became the root of the novel name Ouzo.

Finally, it is of interest that anethole belongs to the groups of chemopreventive agents, i.e. to phytochemicals derived from fruits and vegetables (including also curcumin, eugenol and limonene), which have the ability to suppress the formation of cancer by interfering with several cell-signalling pathways – a matter which is under detailed investigation [3,4].

Fig. 2.1-2 Ouzo at room temperature

2. Literature

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3. Isolation

Based on an idea which arose in one of the authors' minds in a Greek restaurant with a glass of deeply cooled Ouzo in hand.

3.1 Principle

Anethole is a rather nonpolar compound. The solubility of the anethole contained in the Greek liqueur Ouzo is in a range in which the compound crystallizes out in the deep cold whereas it is still soluble in the beverage at room temperature. This effect can easily be observed in a Greek restaurant. When Ouzo is served as a well-chilled aperitif it appears cloudy due to precipitated anethole crystals. On standing and warming, the cloudiness disappears by dissolution of anethole in the aqueous ethanol. There is no other flavour component in Ouzo which

Et anesum adversus scorpiones ex vino habetur, pythagorae inter pauca laudatum sive crudum sive decoctum, item viride aridumve omnibus, quae condiuntur quaeque intinguntur, desideratum, panis etiam crustis inferioribus subditum. saccis quoque additum cum amaris nucibus vina commendat. quin ipsum oris halitum iucundiorem facit faetoremque tollit manducatum matutinis cum zmyrnio et melle exiguo, mox vino collutum. vultum iuniorem praestat. insomnia levat suspensum in pulvino, ut dormientes olefaciant. adpetentiam ciborum praestat, quando id quoque inter artificia deliciae fecere, ex quo labor desiit cibos poscere. ob has causas quidam anicetum id vocavere.

Pliny the Elder (23–79)
Naturalis Historia Liber XX, 73

Fig. 2.1-3 Frozen Ouzo

undergoes this change. Therefore, it can be used for a simple selective separation of anethole just by filtration of the cold liqueur. However, it should be kept in mind that anethole has a rather low melting point of 22 °C. This has to be taken into consideration during any separation operations. All equipment used for filtration has to be precooled to avoid loss of anethole by liquefaction.

It is not to recommend to cool the liqueur still more than described here because then water ice also begins to crystallize. If you cool several brands of Ouzo you will find that the degree of crystallization of anethole is different, which gives a hint about its varying content in the liqueur.

3.2 Method

A 500 mL volume of Greek liqueur Ouzo (brand: PILAOS; ethanol content 37.5%) is allowed to cool in a deep freezer at –20 °C overnight. The viscosity of the solution increases. Anethole crystallizes in the form of colourless leaflets. A sintered glass filter funnel is precooled in the same freezer and used for the filtration operation. The Ouzo is filtered by suction, which requires 30 min because the glass filter easily tends to become blocked by the anethole crystals. To avoid this, it is to recommended to scrape off the material from the filter surface occasionally by means of a pre-cooled spatula. During filtration, the temperature at the funnel should not rise above –12 °C. Finally, a colourless crystalline mass (300 mg) is scraped out of the sintered glass filter funnel, put into a glass vial and immediately evacuated with an oil pump at 20 Pa and 15 °C to remove traces of water and ethanol. Colourless crystals of pure anethole (150 mg) remain in the vial, which, depending on the storage temperature, can be kept as a solid or a liquid.

3.3 Purification

Further purification is not necessary.

4. Spectra and Comments

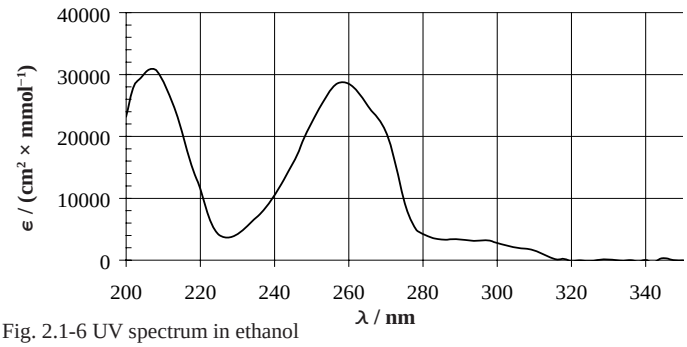


Fig. 2.1-6 UV spectrum in ethanol

The UV spectrum reveals a typical pattern of an aromatic compound with a main π–π* transition at 260 nm and a shoulder due to the auxochromic methoxy group reaching to 320 nm. Due to the flexibility of the side chains, there is no vibrational fine structure to be seen.

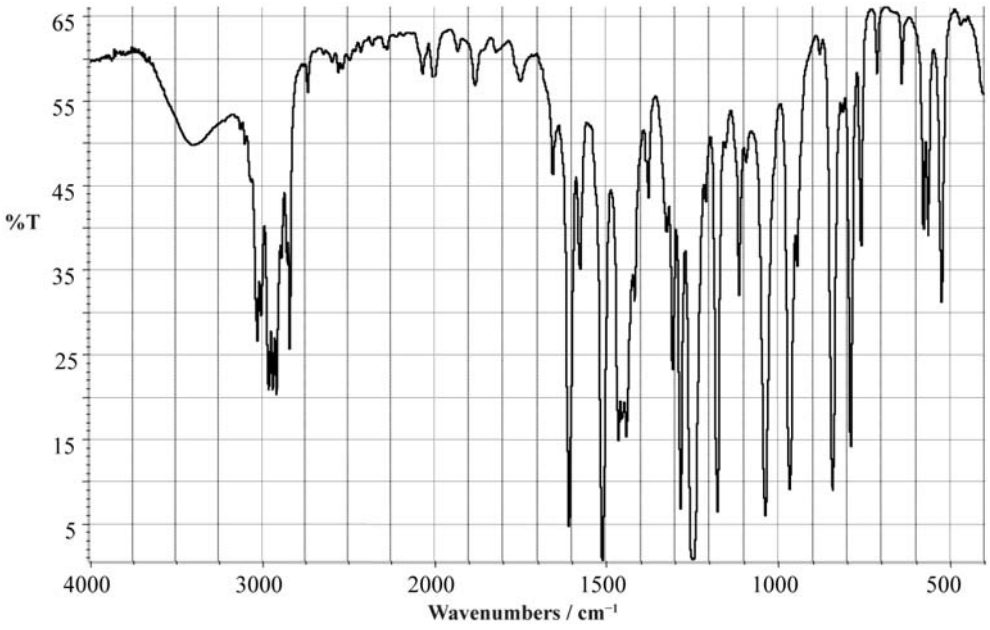
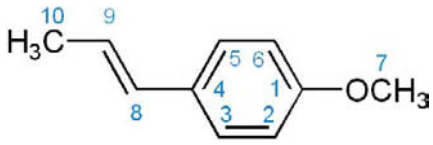


Fig. 2.1-7 IR spectrum in KBr

The IR spectrum shows CH valence bands for both sp^3 and sp^2 units. The aromatic ring is revealed by the overtone vibrations between 2100 and 1700 cm^{-1}. The sharp C=C vibration at 1600 cm^{-1} and the strong band at 840 cm^{-1} indicate the *para*-substituted benzene ring. In the fingerprint region, one finds at 1250 cm^{-1} the C−O−C vibration of an aromatic ether.



Scheme 2.1-1

Fig. 2.1-6 Young anise plants (*Pimpinella anisum* L.)

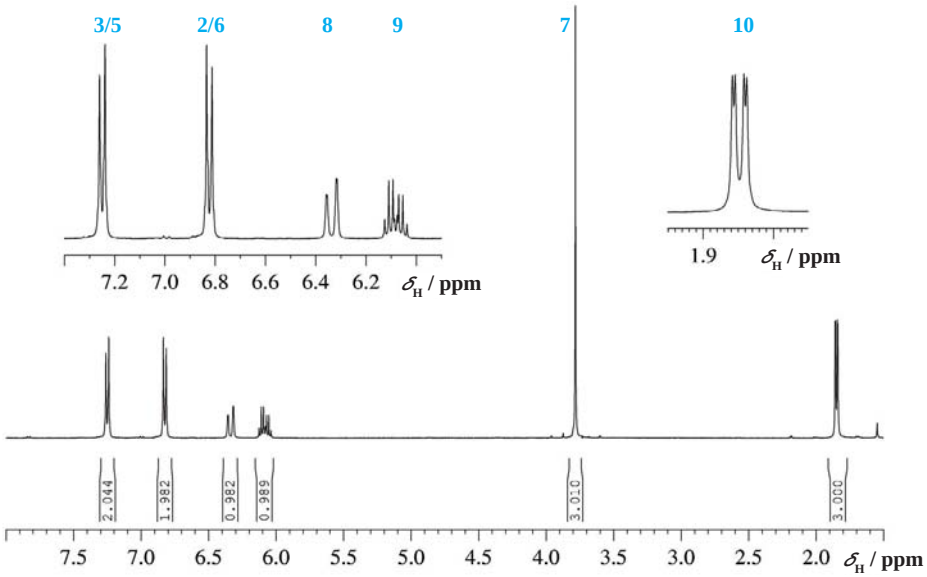


Fig. 2.1-7 ^{1}H NMR spectrum at 400 MHz in $CDCl_3$

In the ^{1}H NMR spectrum, we first find the typical AA'XX' pattern of a *para*-substituted benzene ring. The chemical shift of the two protons at 6.8 ppm indicates an oxygen substituent due the shielding effect of the mesomeric contribution of the free electron pair. The next pattern centred at 6.25 ppm is typical of a *trans*-double bond with a spin coupling constant of 15.7 Hz and an additional spin coupling to an attached methyl group. The methyl group signal itself nicely reveals a ^{3}J of 6.5 Hz and a ^{4}J (allylic spin coupling) of 1.6 Hz.

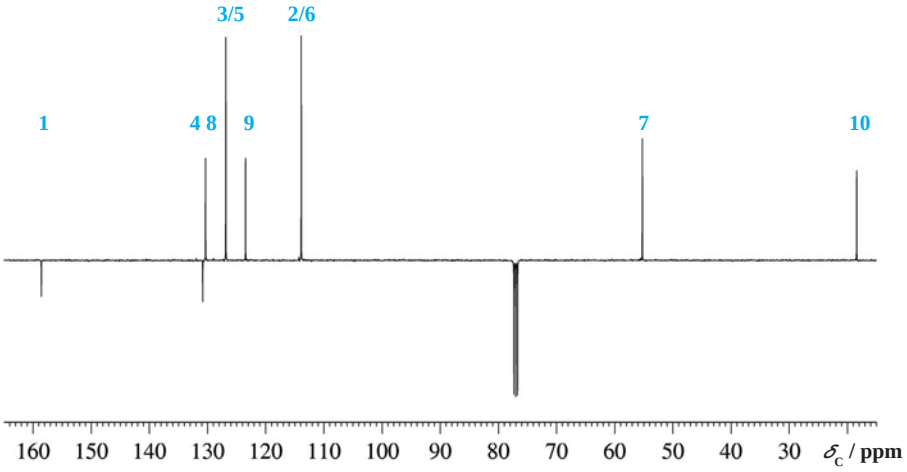


Fig. 2.1-8 APT ^{13}C NMR spectrum at 100 MHz in $CDCl_3$

The ^{13}C NMR spectrum reveals a typical pattern of a *para*-substituted aromatic compound and the assignment for the oxygen-substituted carbon atom C-1 at about 160 ppm is obvious. The only difficulty is the relative assignment of C-8 and C-9, but this easily follows by inspection of the HSQC spectrum, since in the proton spectrum the corresponding ^{1}H signals can be distuingished due to the coupling with the methyl group.

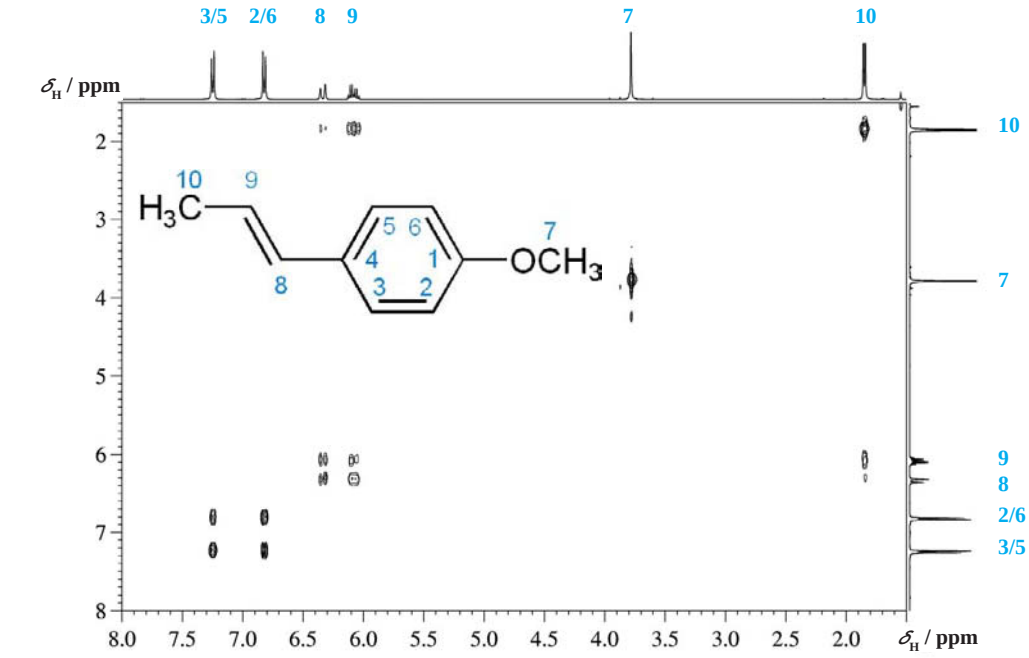


Fig. 2.1-9 COSY spectrum

The assignments discussed above are corroborated by both the COSY and NOESY spectra. Note that in the COSY spectrum a weak cross peak is visible due to the ^4J(H-10,H-8) spin coupling constant. For the NOESY spectrum it is interesting that the aromatic protons H-3/5 have NOE contacts to both olefinic protons H-8 and H-9, and this can be inspected with a molecular model. The protons H-2/6 show a cross peak to the methoxy group.

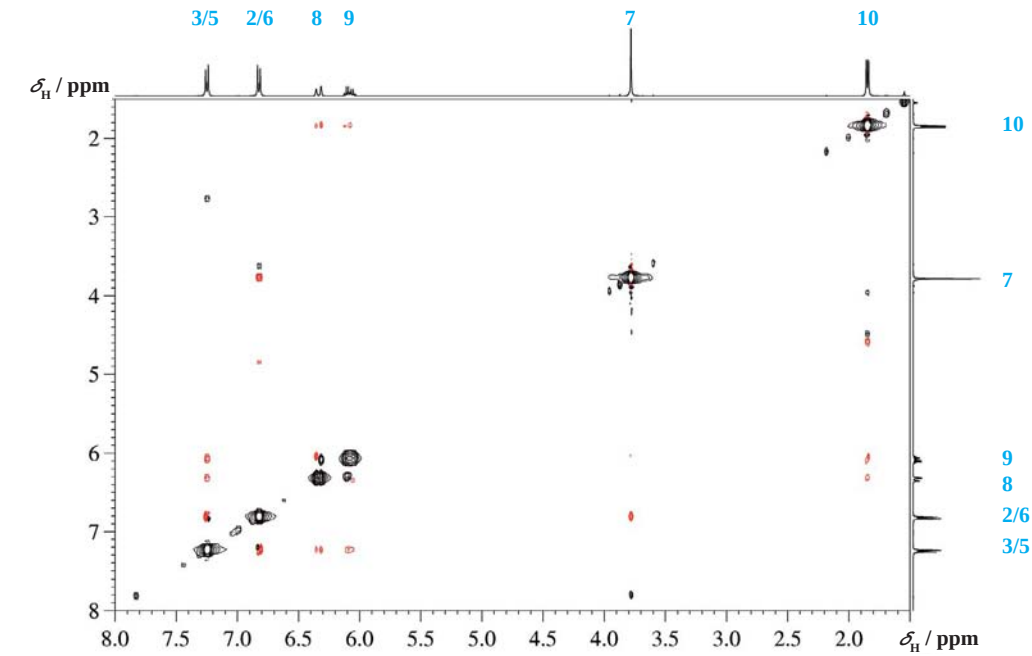


Fig. 2.1-10 NOESY spectrum

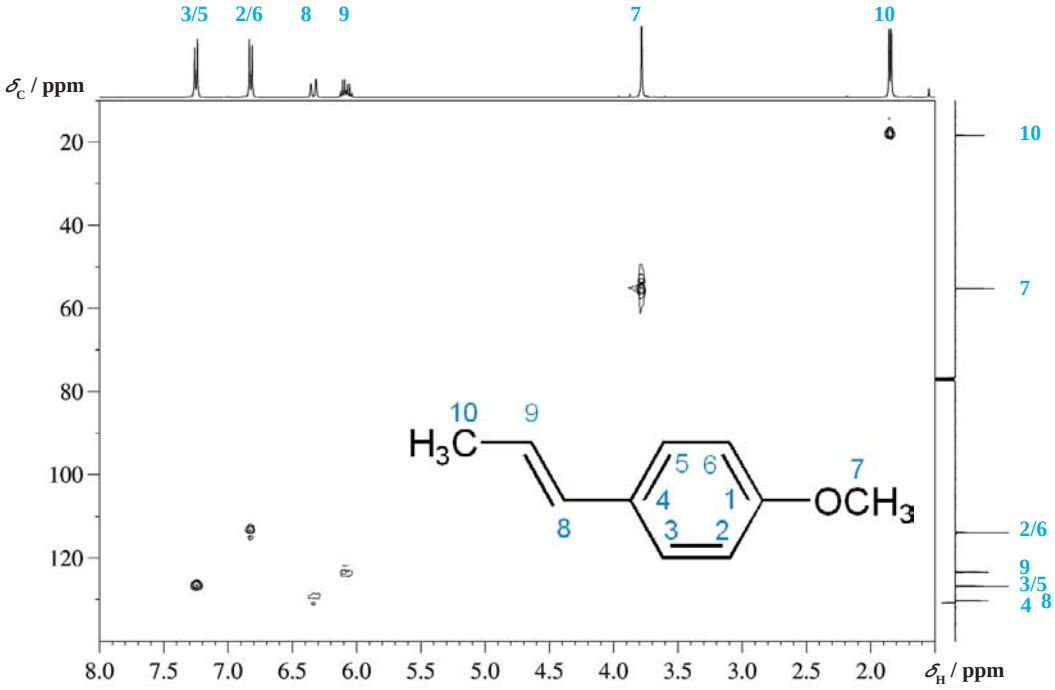


Fig. 2.1-11 HSQC spectrum

Inspection of the HSQC spectrum yields the relative sequence of the ^{13}C chemical shifts for C-3/5 and C-2/6. The signals of C-8 and C-9 also follow the sequence of the proton signals in this molecule. The expansion of the HMBC spectrum given is just a good learning example of the power of this method.

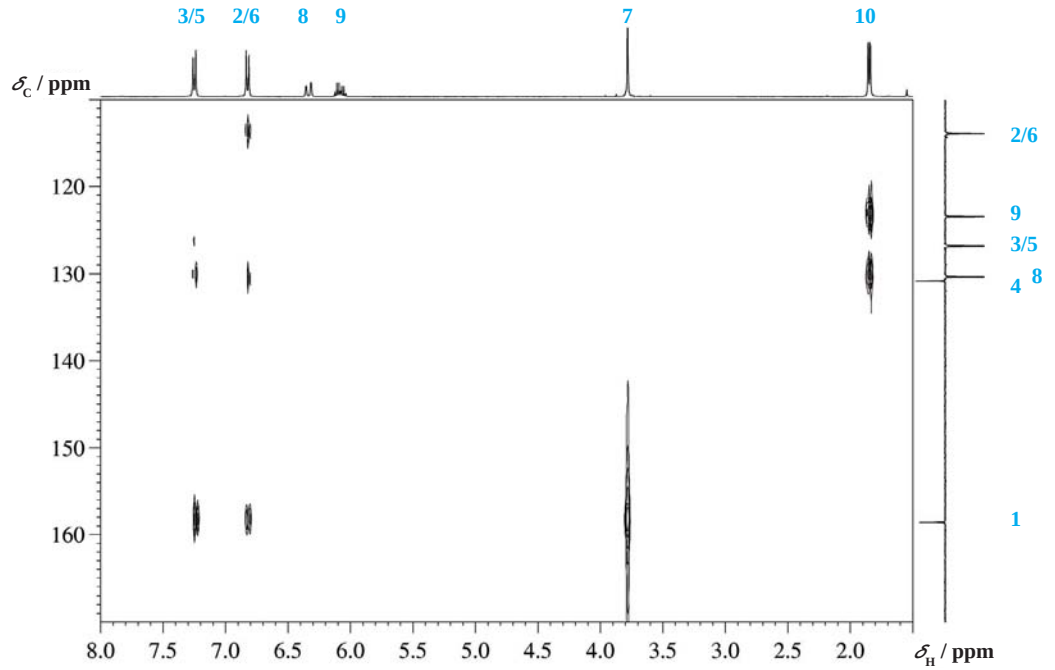


Fig. 2.1-12 HMBC spectrum

Anise-Almond Kisses

1 tube (7 ounces) almond paste
1/2 cup granulated sugar
1/2 teaspoon anise seed, crushed
1 extra-large egg white

Preheat the oven to 325 degrees. Using your fingertips, knead the sugar into the almond paste. Work in the crushed anise seed and then the egg white.

Place the mixture into a pastry bag fitted with a star tip. Pipe mounds to equal a heaping tablespoon spaced about an inch apart onto a baking sheet lined with a silicone-coated baking mat. Or simply use two spoons to drop mounds onto a greased baking sheet or parchment paper.

Bake until barely golden brown, about 15 minutes. Allow to cool on the baking sheet before removing.

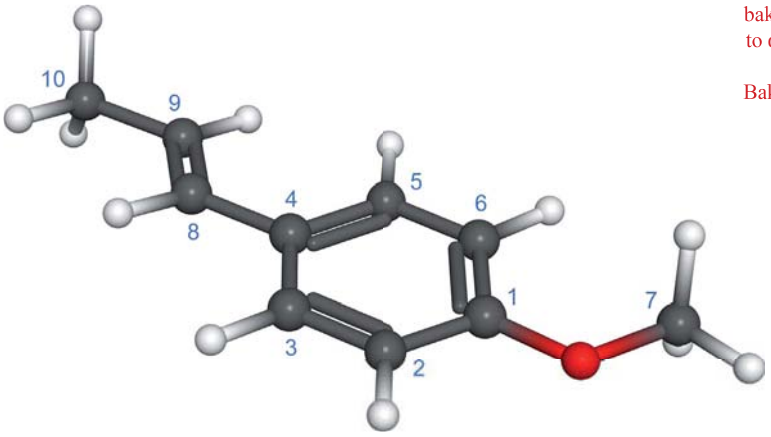


Fig. 2.1-13 Molecular model of anethole

^{13}C Signals δ / ppm	Type of Carbon	Assignment	Proton Signals δ / ppm, J / Hz
158.6	C_q	C-1	
130.8	C_q	C-4	
130.3	CH	C-8	6.34, ^3J(H-8,H-9) 15.7
126.9	CH	C-3/5	7.25
123.4	CH	C-9	6.08
113.9	CH	C-2/6	6.82
55.2	CH_3	C-7	3.78
18.4	CH_3	C-10	1.84, ^3J(H-10,H-9) 6.5, ^4J(H-10,H-8) 1.6

Table 2.1-1 NMR data for anethole

Les cuisines d'Hamilcar n'étant pas suffisantes, le Conseil leur avait envoyé des esclaves, de la vaisselle, des lits; et l'on voyait au milieu du jardin, comme sur un champ de bataille quand on brûle les morts, de grands feux clairs où rôtissaient des boeufs. Les pains saupoudrés d'anis alternaient avec les gros fromages plus lourds que des disques, et les cratères pleins de vin, et les canthares pleins d'eau auprès des corbeilles en filigrane d'or qui contenaient des fleurs. La joie de pouvoir enfin se gorger à l'aise dilatait tous les yeux çà et là, les chansons commençaient.

Gustave Flaubert (1821−1880),
Salammbô, Chap. 1

Fig. 2.1-14 *Lophantus anisatus*, another plant producing the aroma of anise

He was an exterminator in Chicago, a bartender in New York, a summons-server in Newark. In Paris he sat at cafe tables, watching the sullen French faces go by. In Athens he looked up from his ouzo at what he called the ugliest people in the world. In Istanbul he threaded his way through crowds of opium addicts and rug-sellers, looking for the facts. In English hotels he read Spengler and the Marquis de Sade. In Chicago he planned to hold up a Turkish bath, hesitated just for two minutes too long for a drink, and wound up with two dollars and had to make a run for it.

Jack Kerouac (1922−1969)
Addressing W. S Burroughs
in *On the Road*

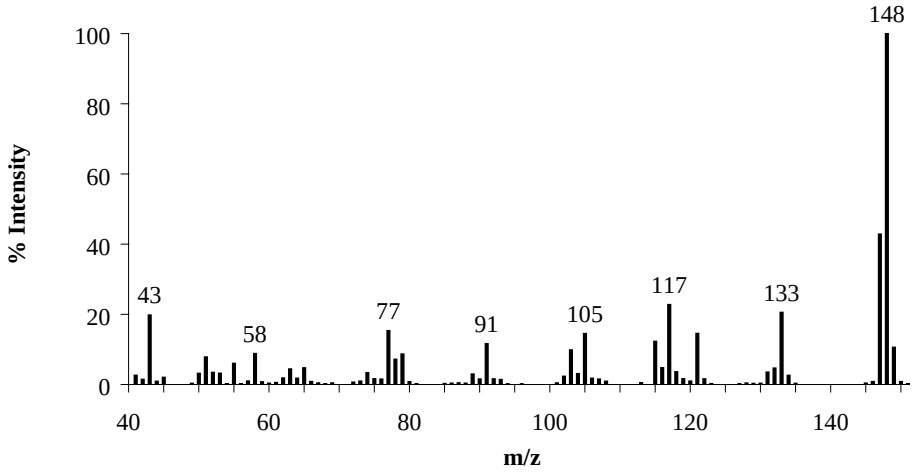
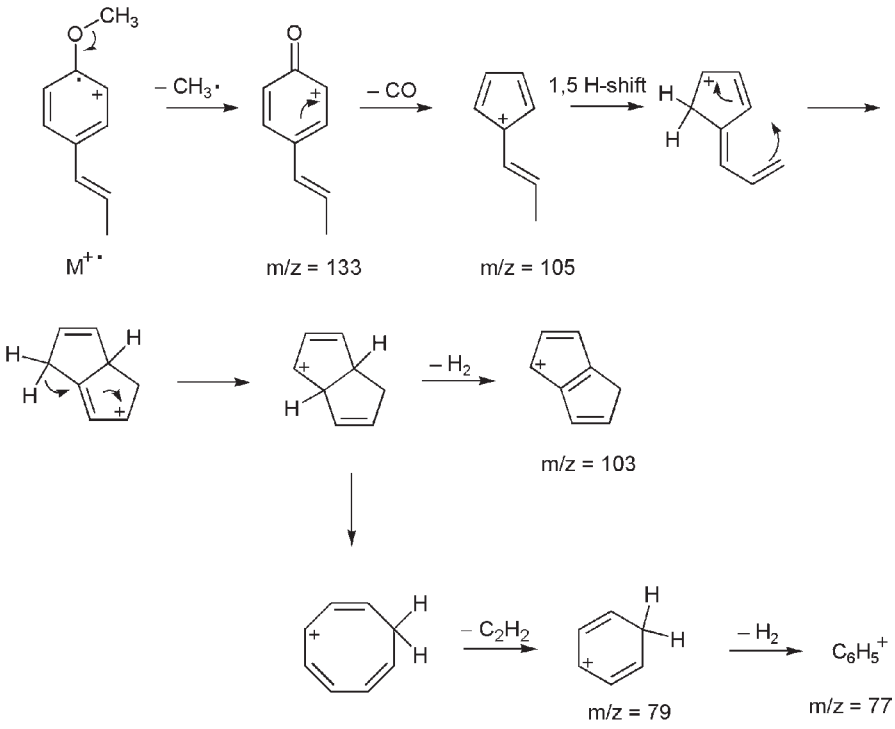


Fig. 2.1-15 Mass spectrum (EI)

The mass spectrum first reveals the loss of a methyl group. In addition, the peak at m/z = 117 could be due to a cleavage of the methoxy group. The signal at m/z =105 is caused by the typical loss of CO from aromatic methoxy compounds. This ion can rearrange and either split off H_2 to form the signal at m/z = 103, or split off ethyne to yield after additional loss of H_2 the ion at m/z = 77, which may be, at first sight, very surprising for a *para*-disubstituted aromatic compound.



Scheme 2.1-2 Fragmentation of anethole

5. Questions

A. Estragole, 1-methoxy-4-(2-propen-1-yl)benzene or simply *p*-allylanisole, is a double-bond isomer of anethole, occurring in tarragon, pine oil and oil of turpentine. Compare both structures and explain which is thermodynamically more stable.

B. Anisole, estragole and anethole show a common structural motif, the methoxy group, which is widespread among natural products. By which sequence of reactions does a methoxy substitution come about?

C. Compare the UV spectrum of anethole with that from eugenol given in this book. Why is ε of anethole significantly larger?

D. What is the pattern between 2200 and 1800 cm^{-1} in the IR spectrum called and what kind of transitions are these?

E. How can the doublet-like pattern of an aromatic AA'XX' proton spin system be distinguished from an AX spin system?

F. Is it possible to assign the configuration of the double bond from the NOESY spectrum alone?

G. Suggest a reason for the rather strong M–1 peak in the mass spectrum.

6. Own Observations

2.2 Eugenol

4-Allyl-2-methoxyphenol

From cloves
Syzygium aromaticum (Myrtaceae)

$C_{10}H_{12}O_2$, MW 164.20

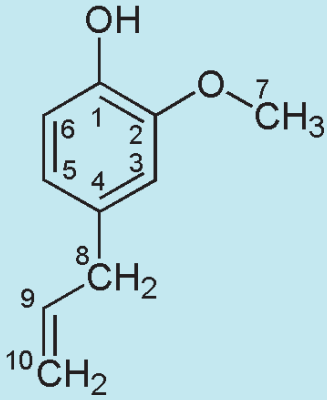
CAS RN 97-53-0, BRN 2044521

Pale yellow oil, bp 125 °C (1.9 kPa)

Eugenol is commercially available.

Synonymous names:
4-Allylguaiacol, Bioxeda

Level: medium



1. Background: How aromatic compounds got their name

Fig. 2.2-1 Originally a Swiss product for "true men", containing eugenol

Eugenol is a long-known natural product occurring in essential oils from clove, cinnamon, piment, oil of bay, laurel, basil and nutmeg. Eugenol is a clear, pale yellow oil with a pleasant scent and (in dilute aqueous solution) a spicy taste. It has a link to the history of organic chemistry. In 1825, Faraday isolated 3 g of a very strange liquid from town gas, in which the C:H ratio was as high as 1:1! (it was the first benzene). In 1865, Kekulé proposed a cyclic formula for it with alternating double bonds, which explained not all but quite a lot of the properties of such compounds with low H content. Soon, a name for the whole class was wanted. Because compounds such as eugenol, vanillin and anisole showed an aromatic flavour, the term aromatic compounds was used for a class of carbocycles with the feature of a cyclic conjugated system of π-electrons. Later, it was found that they all follow Hückel's ($4n$ + 2) rule.

Eugenol belongs to the group of phenylpropanes such as anethol, estragole and cinnamaldehyde, which are formed via the shikimate pathway and are frequently found in essential oils. Eugenol is used in perfumeries for spicy, clove-like and oriental-type fragrances. A molecular formula of $C_{10}H_{12}O_2$ leading to a boiling point of 251−254 °C ensures a distinct vapour pressure at ambient temperature necessary for a smell from the physical point of view. Eugenol is slightly soluble in water, a prerequisite for all other uses. It is used as a flavoring in the kitchen, e.g. for aromatized red wine and red cabbage. As with all phenols, eugenol is an antiseptic: think of thymol used in toothpaste and cresols used for disinfection. Similarly, eugenol is a constituent of mouthwash. Due to its local antiseptic and also analgesic properties, it is used in dentistry. Mixed with zinc oxide, it forms a cement used for fillings of the teeth. Finally, eugenol can be used as an insect attractant.

Fig. 2.2-2 Odol, a neologism formed from the Greek word for tooth and the Latin word for oil, was introduced in 1892 in Dresden, Germany

Together with other spice constituents, eugenol is under detailed investigation for its biological effects in the human body [1]. Erlenmeyer was the first to recognize the chemical nature of eugenol: he still called it *Eugensäure* (= eugenic acid) due to its acidic nature, which he used during purification [2]. As a chemical, eugenol was used to produce vanillin via isoeugenol (see Questions). It is classified as a hazardous material (classification Xn Harmful). Eugenol shows an allergenic potential and should therefore be handled with care. Avoid skin contact, as with all phenols. Never try it in pure form (it tastes extremely bitter). Overdosing by oral intake causes a range of serious symptoms, e.g. convulsions, dizziness and heartbeat acceleration.

2. Literature

[1] B. Laky, G. Hietsch, S. Knasmuller, "Gewürzinhaltsstoffe: kanzerogene und chemoprotektive Eigenschaften" [Constituents of spices: carcinogenic and chemopreventive properties] *Z. Ernährungsökol.* **2000**, *1*, 55−61.

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cancer therapy", *Cancer Lett.* **2004**, *215*, 129–140.

[3] E. Erlenmeyer, "Verhalten des Nelkenöls gegen Jodwasserstoff" [Behaviour of clove oil toward hydrogen iodide] *Z. Chem.* **1866**, *9*, 430–431.

[4] E. Stahl, W. Schild, "Isolierung von Eugenol aus Gewürznelken" [Isolation of eugenol from cloves]. In: "Isolierung und Charakterisierung von Naturstoffen", G. Fischer Verlag, Stuttgart, **1986**, pp. 90–92.

[5] F. von Massow, M. A. R. Smith, "Indirect carbon-13–hydrogen coupling in asymmetrically trisubstituted benzenes: a carbon-13 nuclear magnetic resonance study" *J. Chem. Soc., Perkin Trans. 2* **1976**, 977–980.

[6] V. Kovacik, J. Skamla, "Mass spectrometry of some model substances of lignin II" *Chem. Ber.* **1969**, *102*, 3623–3631.

Fig. 2.2-3 Cloves can contain up to 15% eugenol

3. Isolation

3.1 Principle

Eugenol is sparingly soluble in water, but dissolves well in organic solvents such as diethyl ether, petrol ether, chloroform and ethanol. Eugenol as a phenol is OH-acidic (pK_a 10.3) and can be deprotonated by NaOH solution. The ionic phenolate form is soluble in water; this property is used for separation from other organic constituents of the cloves.

The ground cloves are first treated in a Soxhlet apparatus with petrol ether to extract all ethereal oil components from the spice. From the extract obtained, all strongly acidic compounds such as carboxylic acids are removed by a first extraction with saturated $NaHCO_3$ solution, a very weak base which does not deprotonate eugenol. In a second extraction, eugenol is then selectively removed by NaOH extraction as phenolate from the organic into the aqueous phase. Acidification leads to eugenol as a cloudy precipitate in water, which is re-extracted into petrol ether. Final purification is possible by distillation in vacuo or even at ambient pressure, because eugenol has remarkable thermal stability.

"*Gariofiles nelchin* valde calidum est, et etiam quamdam humiditatem in se habet qua se suaviter extendit ut ipsa suavis humiditas mellis. Et si quis in capite, dolet, ita quod ei caput *dumet*, velut surdus sit, gariofiles sæpe comedat, et *dume* quæ in capite est minuit."

Hildegard Bingensis (1098–1178),
Physica – Lib. I. de Plantis,
Chap. XXVII

3.2 Method

Crushed cloves (50 g) are placed in the thimble of a Soxhlet apparatus and extracted with 650 mL of petrol ether (boiling range 50–80 °C) for 6 h. The cloudy extract is filtered and the filtrate is reduced on a rotary evaporator to obtain a clear yellow solution of 200 mL volume. Strongly acidic compounds are then removed by shaking with saturated $NaHCO_3$ solution (2 × 50 mL) in a separating funnel. The organic phase is extracted with 5% NaOH (4 × 50 mL) to transfer eugenol into the aqueous phase. To the combined NaOH extracts crushed ice (50 g) is added followed by concentrated HCl (30 mL), which is added slowly with stirring until pH 2 is reached. Test the acidic reaction with pH paper. Acidification is complete if addition of HCl does not cause further precipitation of eugenol as cloudy droplets. Eugenol is re-extracted with petrol ether (4 × 50 mL).

Fig. 2.2-4 A collection of a raw eugenol samples of a students' laboratory course was collected and distilled in vacuo. This 28 g sample was obtained starting from 400 g of cloves

3.3 Purification

The combined organic extracts are washed with saturated $NaHCO_3$ (50 mL) to remove traces of HCl and dried over $MgSO_4$. The solvent is removed in vacuo. A dark yellow oil of crude eugenol remains: 5.0 g, n_D 1.5385 (20 °C). Distillation in a micro distillation apparatus at ambient pressure using two heat guns yields 4.0 g of pale yellow eugenol: bp 251−254 °C, n_D 1.5410 (20 °C). Alternatively, distillation in vacuo is possible: bp 125−126 °C (1.9 kPa). On standing in a deep freezer, eugenol crystallizes (mp –9 °C).

4. Spectra and Comments

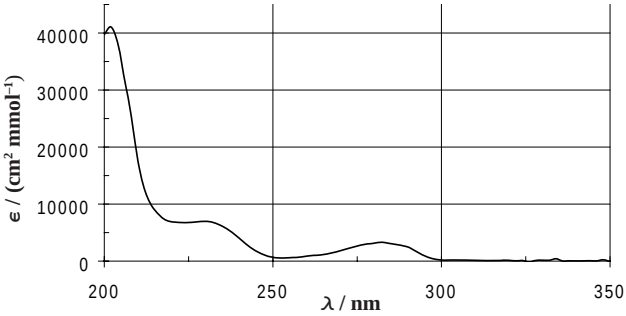


Fig. 2.2-5 UV spectrum in ethanol

As eugenol has a simple structure with only 10 carbon atoms, the spectroscopic structure elucidation is fairly straightforward. As can be seen from the photograph, the compound is colourless if freshly distilled in accordance with the UV spectrum.

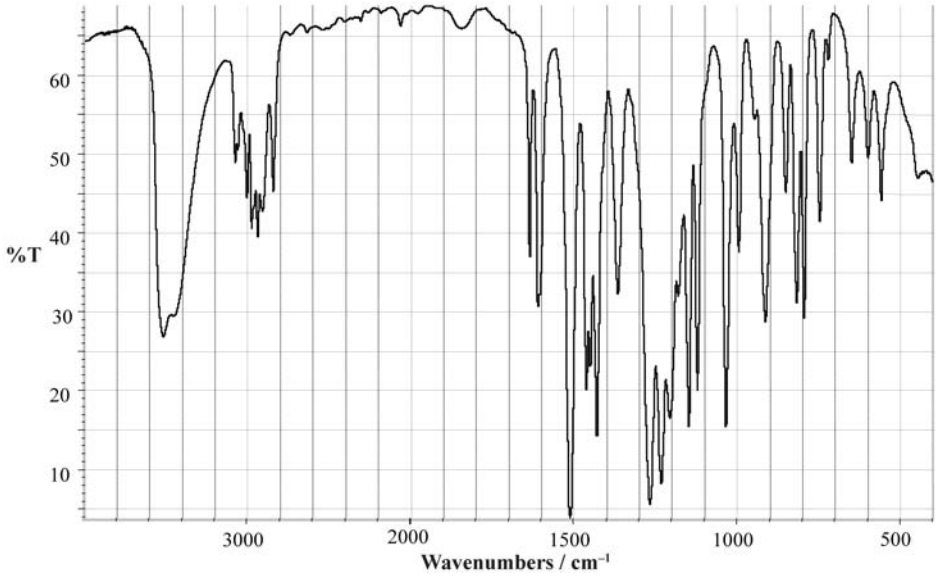


Fig. 2.2-6 IR spectrum as film

In the IR spectrum, the OH valence vibration at 3250 cm^{-1} dominates the spectrum followed by sp^2- and sp^3-type CH valence vibration bands. The splitting of the two bands in the C=C region at 1620 cm^{-1} is probably due to the presence of aromatic and olefinic double bonds.

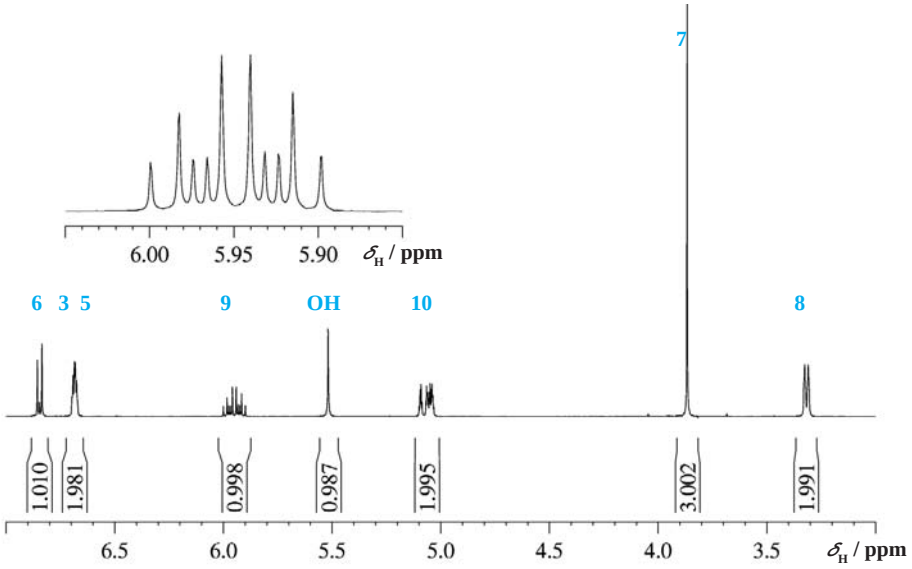


Fig. 2.2-7 ^{1}H NMR spectrum at 400 MHz in $CDCl_3$

In the ^{1}H NMR spectrum, the very typical pattern of a terminal vinyl group at 5.06 ppm is revealed displaying a large *trans*- and a smaller *cis*-olefinic vicinal spin coupling further complicated by allylic spin coupling to the methylene protons at C-8.

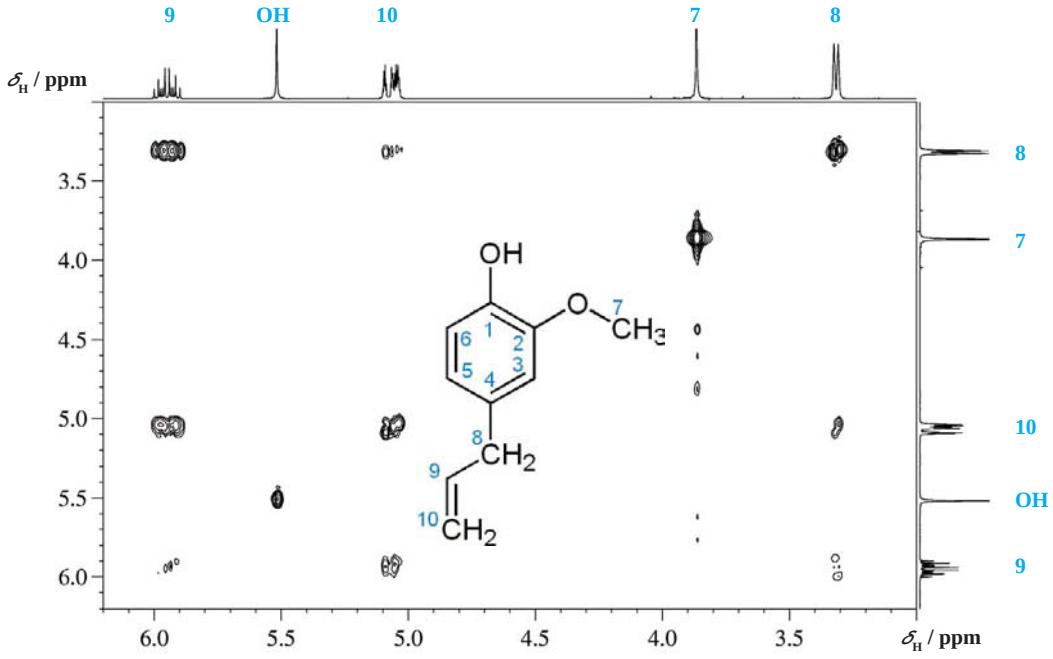


Fig. 2.2-10 COSY spectrum

The expansion shown from the COSY spectrum nicely displays the connectivities within the allylic moiety H-8, H-9 and H-10, demonstrating that in a COSY spectrum not only vicinal and geminal but also allylic spin coupling constants yield cross peaks.

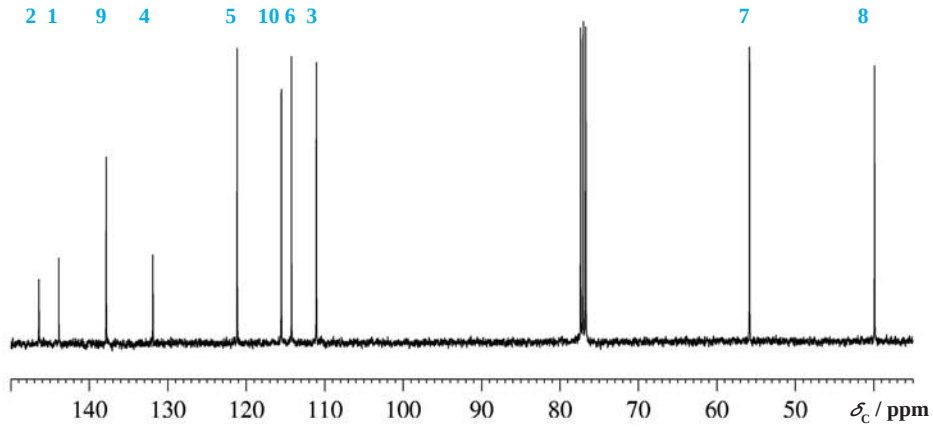


Fig. 2.2-9 ^{13}C NMR spectrum at 100 MHz in $CDCl_3$

The ^{13}C NMR spectrum displays 10 different signals showing that there is no symmetry in eugenol with its 10 carbon atoms. Whereas the assignment in the aliphatic part is straightforward, the assignment in the olefinic/aromatic part of the spectrum requires the study of the HSQC, HMBC and NOESY spectra given below.

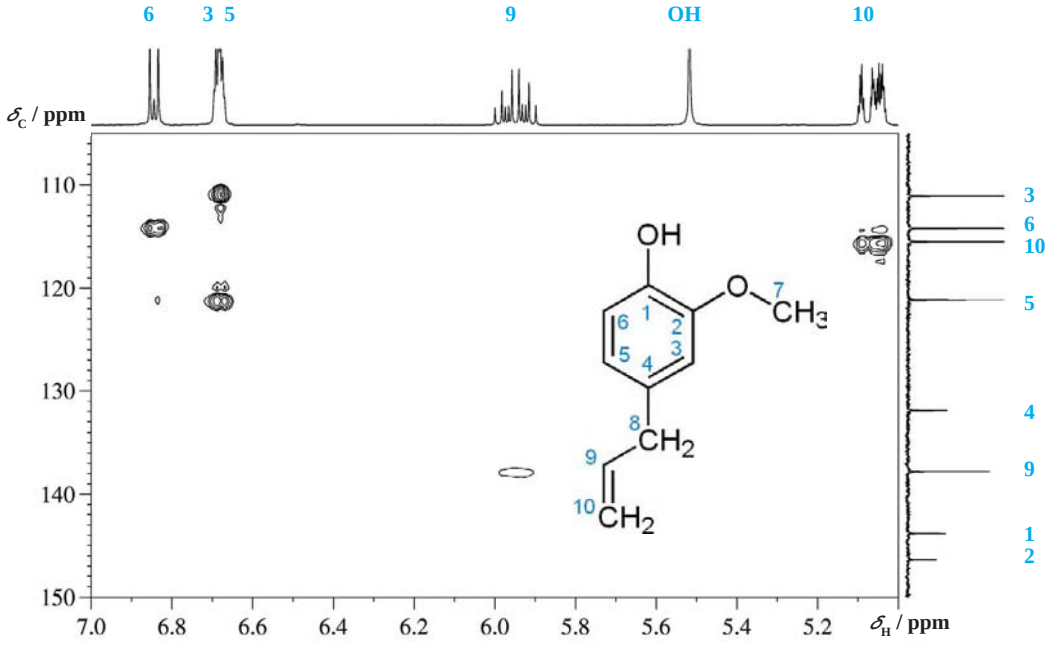


Fig. 2.2-10 Expansion of the HSQC spectrum in the olefinic and aromatic region

The HSQC spectrum reveals that two aromatic proton signals overlap each other, but with this information alone it is not yet possible to decide which is which carbon and proton signal.

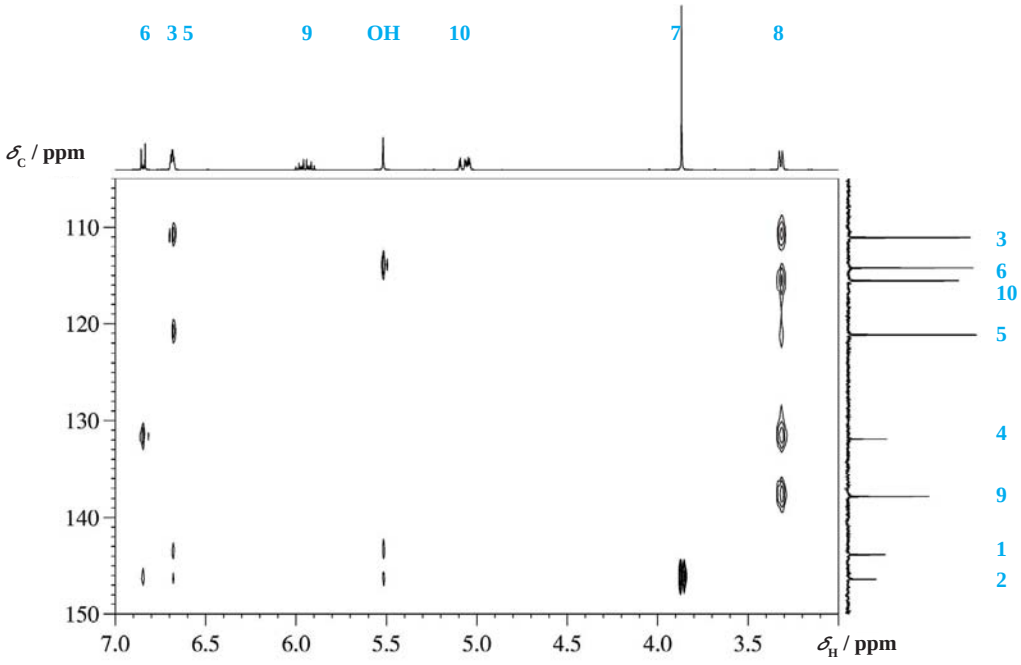


Fig. 2.2-11 Expansion of the HMBC spectrum

The HMBC spectrum was obtained with an effective $^1J_{CH}$ filter and was focused for a long-range CH coupling constant of 7 Hz. It is remarkable that the signal of the phenolic OH proton is rather sharp in $CDCl_3$ solution and even gives useful correlations in the HMBC spectrum. This is probably due to an intramolecular hydrogen bond to the oxygen atom of the methoxy group. The analysis of the HMBC spectrum starts best with the proton signal of the methoxy group, which shows a correlation to the carbon signal at 146.2 ppm and thus assigning this signal to C-2, which in turn fixes the signal at 143.9 ppm to C-1. The chemical shift of the remaining quaternary carbon atom C-4 is therefore at 131.9 ppm, in the HMBC spectrum seen as expected from the protons of methylene group H-8. Since the chemical shifts of C-9 and C-10 could be unequivocally identified in the HSQC-spectrum (137.8 and 115.5 ppm), only three more carbon atoms, C-3, C-5 and C-6, have to be assigned. However, at this stage, we are not even sure about the proton assignment in the aromatic region.

Fig. 2.2-12 The typical length of a clove is about 1.5 cm

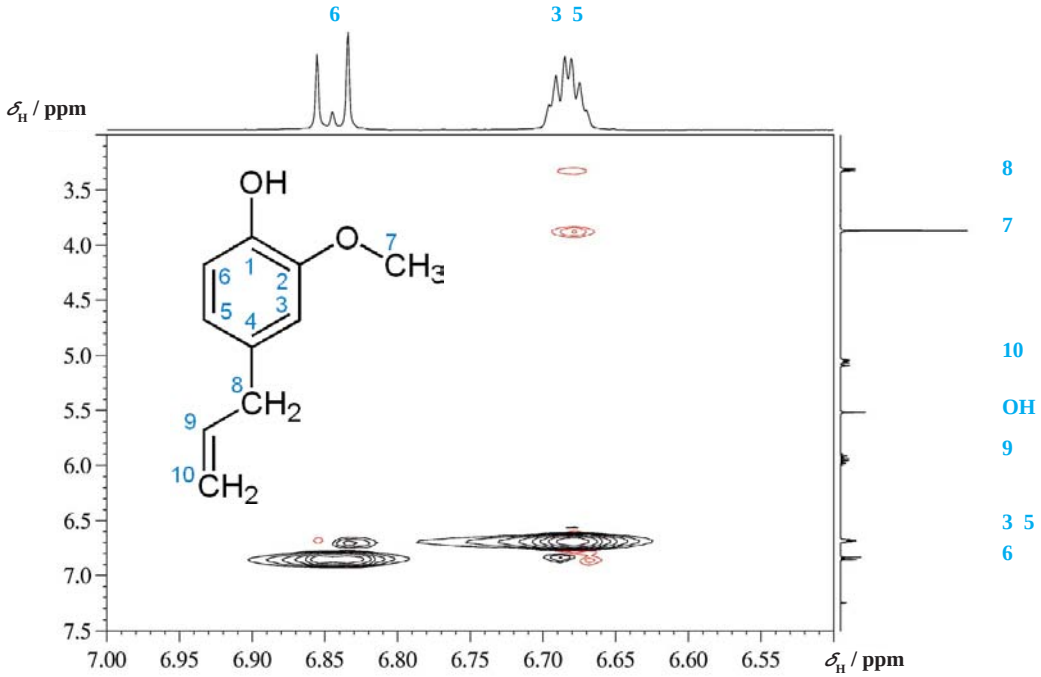


Fig. 2.2-13 Expansion of the NOESY spectrum in the aromatic region

The NOESY spectrum shown was obtained using a mixing time of 1 s. The NOESY cross signal connects the aromatic multiplet of 2H at 6.68 ppm with H-8 and H-7, thus H-6 must be the single proton at 6.85 ppm. This knowledge assigns the signal at 114.2 ppm in the HSQC spectrum to C-6.

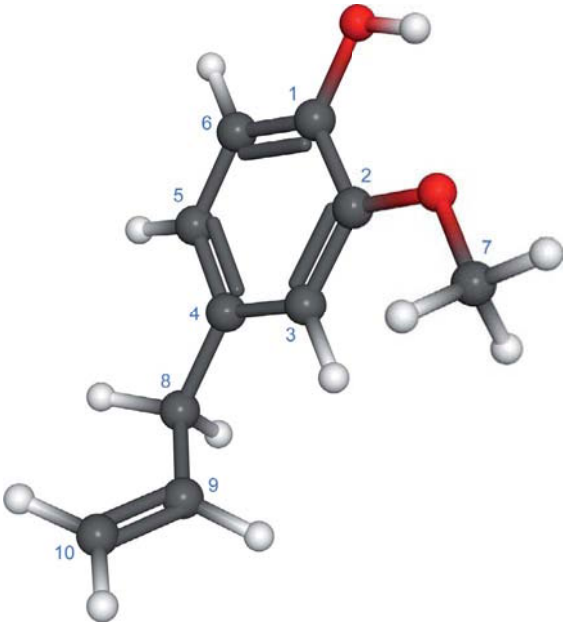


Fig. 2.2-14 Molecular model of eugenol

Fig. 2.2-15 Flowering cloves

^{13}C Signals δ / ppm	Type of Carbon	Assignment	Proton Signals δ / ppm, J / Hz
146.4	C_q	C-2	
143.9	C_q	C-1	
137.8	CH	C-9	5.95, J = 17.0, 9.7, 6.7
131.9	C_q	C-4	
121.2	CH	C-5	6.68
115.5	CH_2	C-10	5.06
114.2	CH	C-6	6.85, J = 8.4
111.1	CH	C-3	6.68
55.8	CH_3	C-7	3.87
39.9	CH_2	C-8	3.32, J = 6.7

Table 2.2-1 NMR data for eugenol

Fig. 2.2-16 Piment, another spice containing eugenol

"The armipotent Mars, of lances
the almighty,
Gave Hector a gift,
A gilt nutmeg.
A lemon.
Stuck with cloves.
No, cloven."

William Shakespeare
Love's Labors Lost, V, II, 647–649.

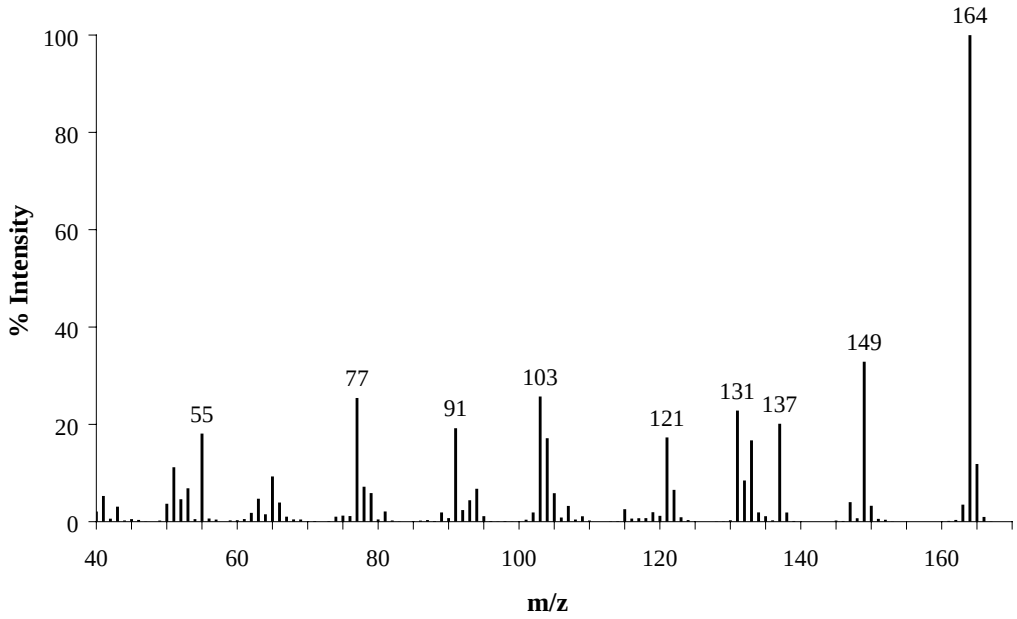


Fig. 2.2-17 Mass spectrum (EI)

The electron impact mass spectrum displays the molecular ion as the base peak, pointing to the relative stability of eugenol under these conditions. The signal at m/z =149 can be explained by a cleavage of the methyl group, whereas the loss of 27 mass units (signal at m/z = 137) points to the terminal vinyl group.

5. Questions

A. Eugenol undergoes a base-catalysed allyl−propenyl isomerization to form isoeugenol (2-methoxy-4-propenylphenol). What is the thermodynamic driving force for this rearrangement?

B. *ortho*-Eugenol (2-allyl-6-methoxyphenol), a constitutional isomer of eugenol, is accessible by a [3,3]-sigmatropic Claisen rearrangement. Which compound has to be heated at 230 °C to obtain *ortho*-eugenol?

C. Characterize the two bands in the UV spectrum and compare the ε values with those of anisole.

D. Which bands in the IR spectrum are most characteristic for an aromatic hydroxyl compound?

E. In the proton NMR spectrum, two of the three aromatic protons have nearly the same chemical shift. Interpret the given expansion of the NOESY spectrum to yield an unequivocal assignment.

F. Draw a "spin-key" for the multiplet at δ = 5.95 ppm; extract and assign the three spin coupling constants involved.

G. Since two of the aromatic protons have the same chemical shift, there is an ambiguity in the assignment of the protonated aromatic carbon atoms as shown in the HSQC spectrum. Show by careful analysis of the given expansion of the HMBC spectrum how a safe assignment of all carbon atoms can be reached.

H. How can one verify that the methoxy group is not on C-1?

I. Suggest a structure for the ion at m/z = 121 in the mass spectrum.

6. Own Observations

2.3 Chamazulene

7-Ethyl-1,4-dimethylazulene

From the essential oil of steam distilled German chamomile (camomile), i.e. from Chamomillae flores

Matricaria recutita L. (Asteraceae)
formerly *Chamomilla recutita*

$C_{14}H_{16}$, MW 184.28

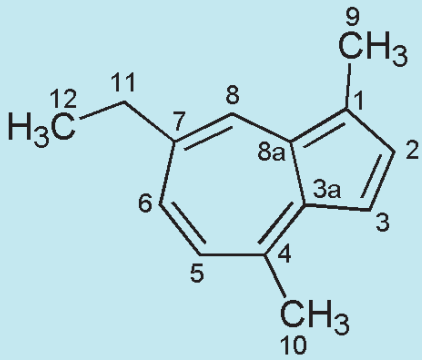
CAS RN 529-05-5, BRN 1306577

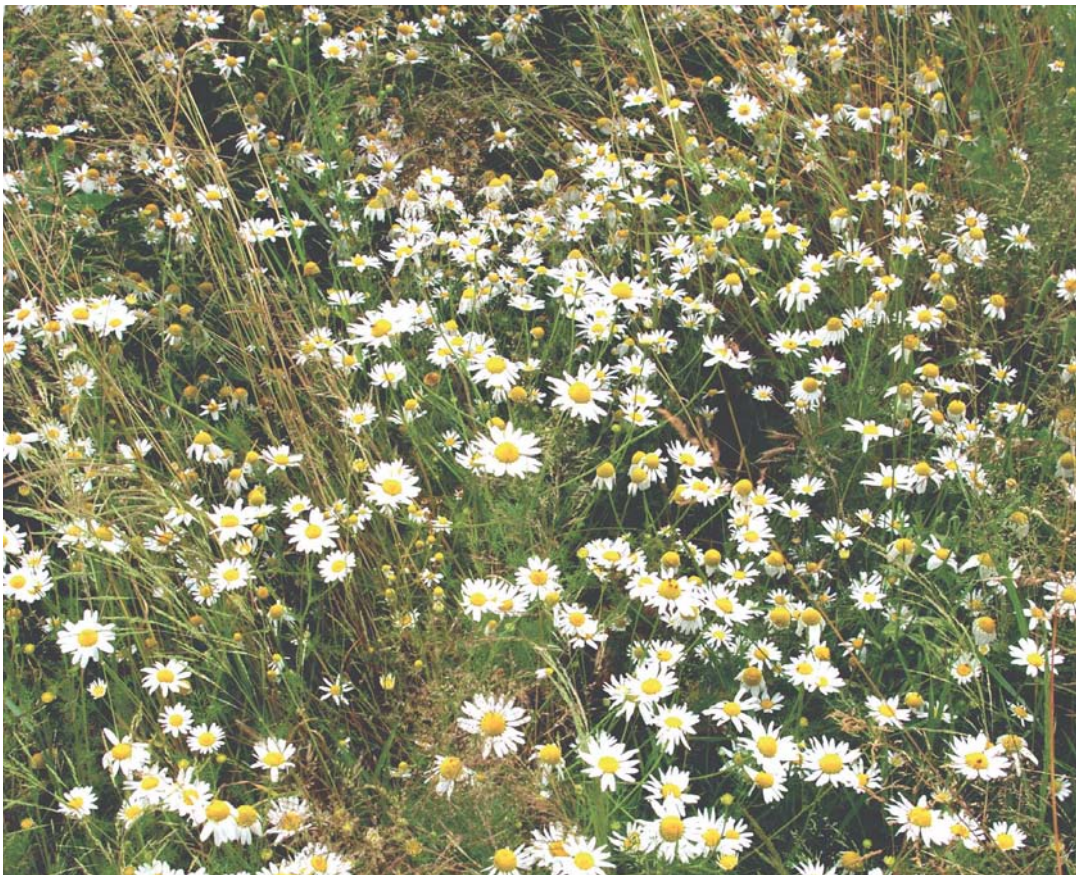
Dark blue viscous liquid, bp 158−160 °C (1.6 kPa)

Chamazulene is commercially available.

Synonymous names:
Camazulene, Chamazulen, Dimethulen, Dimethulene.

Level: medium



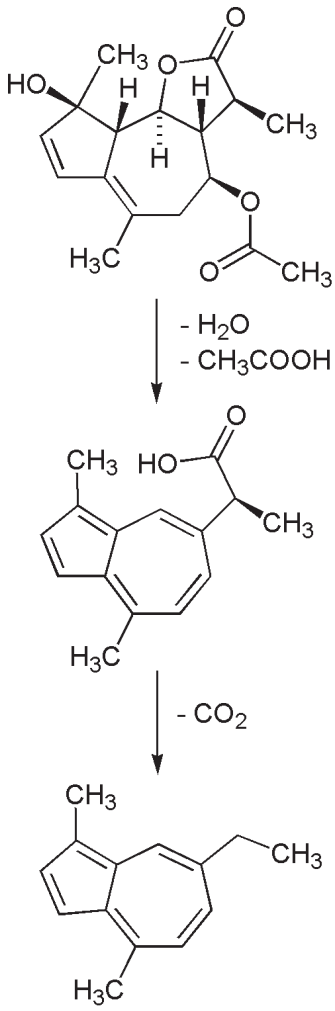


1. Background: In pursuit of a little something

In this chapter, a material is extracted that, for its many positive effects, is close to a panacea: Chamomillae flores, the flowers of *Matricaria recutita*, sort of a "plant doctor".

Our target, the hydrocarbon chamazulene, a dark blue liquid, is normally not listed among the natural constituents of these flowers, but instead is regarded as a transformation product of the natural precursor matricin (CAS RN 29041-35-8). Is this really correct? We have some doubt.

After what we have experienced during the isolation of chamazulene and matricin, we are convinced that also chamazulene itself is already present to a very small extent in an organic extract obtained with chloroform directly from dry Chamomillae flores. Of course, obtaining more chamazulene is possible only after a steam distillation of the flowers that transforms matricin completely into the steam volatile dark blue chamazulene (see the scheme).



Scheme 2.3-1 Degradation of matricin

Our first message regarding this isolation is born in own disasters. Make sure that the flowers of German chamomile that you buy do indeed contain matricin! It is a fact that within this species *Matricaria recutita*, a series of races has been bred or selected that differ enormously in the composition of their secondary plant metabolites. Some samples that we bought with innocent belief in different pharmacies did not contain any matricin at all. The second message is: realize that you are really in pursuit of a little something! A typical content of Chamomillae flores that contain matricin is below 0.1 g/kg. A paper by Flaskamp et al. in 1983 reported that 160 kg of chamomile flowers after steam distillation and several purification steps (extraction, chromatography, vacuum distillation) yielded only 11 g of chamazulene [1]. This is impressive and the value corresponds very well with our own yield of 4.9 mg from 60 g. Therefore, a suitable test for matricin is indispensable. This is easy and nice at the same time. It consists in the steam distillation of some Chamomillae flores. If the wonderful blue hydrocarbon appears in the distillate after a few minutes the presence of matricin is proved and the hunt for chamazulene or matricin is possible. A quick test is to extract 1 g of chamomile flowers with chloroform, to run a TLC of the extract and to heat it in the air stream of a heat gun. If matricin (colourless) is present, careful heating transforms it to a blue spot of chamazulene at a certain temperature. In this section we deal only with chamazulene. To isolate matricin is another and really sophisticated endeavour.

The correct chamomile denomination today is *Matricaria recutita*, a variation of the former *Chamomilla recutita*. The word chamomile is derived from the Greek χαμαίμηλον = earth-apple, which is based on χαμαί = on the ground and μήλον = apple. As a reason to give these names the apple-like odour of the flowers is mentioned. It seems to be a matter of taste if that is correct or requested. Matricaria is derived from the Latin *matrix* (uterine), which comes from a former use for curing women in puerperium. The Latin *recutita* stands for "trimmed". Azulene got its name from the Spanish word *azul* (blue) in 1863 by the French chemist Piesse [2]. Azulene, $C_{10}H_8$, i.e. bicyclo[5.3.0]decapentaene, is

a liquid and blue isomer of naphthalene. Hence chamazulene means "the blue from chamomile". Blue-coloured chamomile steam distillates have been known since the 15th century. Other widespread plants such as yarrow (*Achillea millefolium* L.) and wormwood (*Artemisia absinthium* L.) yield the same blue colour on steam distillation. Compared with the behaviour of the rest of the natural sources, this must have seemed miraculous for those who made it, if one keeps in mind the high reputation of the colour blue. The structure, however, remained unclear. There were no other blue hydrocarbons. In 1883, Hock assumed that the chamomile blue does not occur in the plant itself but instead a precursor exists [3]. The term "chamazulene" dates back to 1926 and the famous natural product chemist Ruzicka was the first to find the correct molecular formula for azulene. Koch was the first to prove the presence of a prochamazulene in chamomile [4]. The group of the Czech chemist Šorm discovered the structure of chamazulene in 1953 [5]. A few years later, the same group isolated the sensitive precursor matricin and assigned its constitution [6]. The configurational assignment as given above was done by means of NMR spectroscopy in 1982 [7]. Eventually, in 2001, the absolute configuration was firmly established by synthetic and spectroscopic studies of its degradation product chamazulene carboxylic acid (see the scheme) [8]. Once again, to find the structure of both compounds includes a story of more than 100 years' work.

Azulene is a nonbenzenoid aromatic compound. It is isomeric with naphthalene but has only half of its resonance energy. Azulene itself can be regarded as a fusion of an aromatic 6π-electron tropylium ion $[C_7H_7]^+$ and an aromatic 6π-electron cyclopentadienyl anion $[C_5H_5]^-$. Azulene is polar and has a dipole moment of 0.8 D. The nature of the blue colour can be understood in terms of a charge transfer between the two rings for which energy from the visible part of the spectrum is sufficient. The colour of derivatives is influenced by the substitution pattern. The lower stabilization of the nonbenzoid chamazulene means a higher reactivity, which is represented by instability towards the influence of oxygen, light and heat.

German chamomile is an annual herbaceous plant. It grows to a height of 15−60 cm and can be found naturally all over Europe and the temperate areas of Asia. It has been introduced in comparable climate zones of Northern America and Australia. The plant belongs to those that are well known to anybody from the strong aromatic smell of the yellow flowers. In case of confusion as to whether you have a corn chamomile plant (*Anthemis arvensis* L.) or not, test if the receptacle is hollow and lacks scales; if so, you have the true *M. recutita* chamomile species mentioned above. A harvest of up to 1000 kg/ha is possible. The yearly consumption of chamomile in Germany is about 3000 tons, with 500 tons used for drugs and the larger part for foodstuffs.

The many active ingredients of the flowers include the essential oil. It contains α-(–)-bisabolol, a monocyclic sesquiterpene alcohol, chamazulene, arising from the guaianolide matricin as described on steam distillation, farnesene, ene-yne-dispiro ethers and spathulenol,

Julchen ist hübsch kugelrund
Und schon ohne Wickelbund. −
Es ist Nacht. − Frau Doris ruht,
Während Knopp das Seine tut.

Aber Julchen in der Wiegen
Will partu nicht stille liegen.
Er bedenkt, daß die Kamille
Manchmal manche Schmerzen stille.

Wirkungslos ist dieser Tee.
Julchen macht: rabäh, rabäh!
Lieber Gott, wo mag's denn fehlen?
Oder sollte sonst was quälen?

O wie gern ist Knopp erbötig,
Nachzuhelfen, wo es nötig.
Aber weh, es will nicht glücken,
Und nun klopft er sanft den Rücken.

Oder will's vielleicht ins Bette,
Wo auf warmer Lagerstätte
Beide Eltern in der Näh?

Wilhelm Busch (1832−1908)
Tobias Knopp, Chap. 79

For a coffee mug of chamomile herbal tea, put two tablespoons (2–3 g) of chamomile flowers in a pot and infuse with boiling water. Cover and allow extraction for 10 min, and then strain through a sieve. (The recommended daily allowance is 9 g.)

Fig. 2.3-1 *Matricaria recutita* (chamomile)

Kamillentee wird bei Erkältungen, besonders wenn diese fieberartige Zustände begleiten, bei Grimmen, Krämpfen, starken Kongestionen usw. verwendet; die Kamillensäckchen sodann, die trefflichen Wärmer bei verschiedenen Zuständen, sind in jedem Haus so liebe Bekannte, dass es überflüssig erscheint, darüber ein weiteres zu sagen.

Sebastian Kneipp (1821–1987)
Pfarrer Kneipps Hausapotheke (1886)

a tricyclic sesquiterpene. Further ingredients are flavonoids such as apigenin and its glucoside responsible for the yellow colour, coumarins such as umbelliferone and phytosterols, e.g. varieties with up to 3% essential oil content have been bred. Chamomile has been used since ancient times. Applications are described from the Old Egyptian Empires. The Greek physician Galen, who had a major influence on later Western medical science, described it as a means against malaria. Also, Hippocrates recommended it, and later Pliny the Elder and Pedanius Dioscorides, who both practised in Rome.

The applications of chamomile are manifold; therefore, it is appreciated as a "plant doctor" that can even be used by laymen without danger when applied in moderation. Everybody knows the aromatic chamomile tea (see recipe in the margin).

The effects of the essential oil are antiphlogistic, spasmolytic, carminative, bactericidal and fungicidal. External uses (as a rinse in case of inflammation of the skin or mucose membranes) are usual, and also internal applications (against stomach ache, for gargling, against gingival inflammations). In former times, chamomile preparations were the first choice in the case of the barely understood "women's complaints". Chamomile is even rated as a mild sedative. Steam baths with chamomile extract are a means against sinusitis, and had to be experienced as a helpful inferno by one of the authors as a lad under his mother's strict supervision. Eventually, there are cosmetic uses as ingredients for skin creams and as a rinse for blonde hair. As with all bioactive compounds, overdoing it is not a good idea. Cases of allergic reactions to chamomile have been reported. Therefore, reliable chamomile products should be bought and the blue essential chamomile oil should never be used in pure form.

Of course, the main ingredients have been investigated for their individual bioactivity to find aims for suitable plant breeding. It has been found, e.g., that the antiphlogistic effect is caused by the group of lipophilic ingredients, in other words by the essential oil components α-(–)-bisabolol, ene-yne-dispiro ethers and chamazulene. Recently, it has been proved that the gastric fluid converts matricin to chamazulene carboxylic acid (see the scheme), which acts as an inhibitor of cyclooxygenase-2 and thus influences the eicosanoid biosynthesis from arachidonic acid [9]. From findings such as this and by locating synergetic effects of different compounds, the manifold effects of chamomile become better understandable.

2. Literature

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3. Isolation

3.1 Principle

Isolation of chamazulene (also a trial for the presence of matricin in the Camomile sample):

Chamomillae flores, the flowers of *Matricaria recutita* L., are subjected to steam distillation. After an initial period of a few minutes, the distillate not only contains colourless essential oils but also chamazulene, a blue constituent. Truly, this is a vision of delight for any chemist. The organic parts of the steam distillate are collected by extraction; their mass is only slightly less than 1% of the chamomile flowers. The blue hydrocarbon is separated by two column chromatographies. In the first run, using pure *n*-hexane as eluent, most of the essential oil constituents are removed in the forerun fractions from chamazulen and a very polar part which does not run at all but remains close to the start. In the second

"Das Tier heißt Maschurah (Die Berühmte). Um es vorher aufmerksam zu machen, mußt du diesen Namen zweimal nennen, worauf du dreimal hintereinander das Wort "Bubuna" (Kamille) sagst. Hast du das getan, so entwickelt es eine Eile, welche dir die stille Luft als Wind erscheinen läßt, und hört nicht eher auf, als bis du ihm das Wort "Yawahsch!" (Langsam, sachte) auch dreimal sagst. Da das Kamel das Pferd an Ausdauer überhaupt übertrifft, so hält meine Maschurah, die nun die deinige ist, auch unter dem Geheimnisse viel länger aus als ein Pferd, was dich aus großer Gefahr erretten kann und jede Verfolgung nutzlos machen wird. Hast du dir das alles gut gemerkt?"

"Ja; ich danke dir! Aber sag, warum hast du grad dieses Wort Bubuna gewählt?"

"Weil dieses Hedschihn eine große und ganz sonderbare Vorliebe für Kamillen hat. Ich habe darum, so oft ich es reite, stets einige von diesen Pflanzen in der Tasche. Ich reibe sie in der Hand, so daß sie nach ihnen riecht und liebkose dann das Maul und die Nase Maschurahs mit dieser Hand. Wenn du das tust, wirst du seine Freundschaft und Liebe schnell erwerben. Nur darfst du es keinem andern verraten, dem es dann durch Anwendung dieses Mittels gelänge, die Anhänglichkeit des Tieres auf sich zu lenken. Ich habe auch jetzt Kamillen mit und werde sie dir geben. Sie sind trocken geworden, duften aber noch stark genug."

Karl May (1842−1912)
Am Jenseits, El Aschdar

Fig. 2.3-2 Filtration

Fig. 2.3-3 Extract

Fig. 2.3-4 Column chromatography of chamazulene

run, the eluent is changed in a manner that makes chamazulene so fast in its elution that it can be separated from the more polar impurity that is its companion in the first run. A suitable solvent to achieve this final separation is chloroform–toluene (3:1, v/v).

Generally, it is worthwhile to do a lot of TLC work to get an idea of how solvents (pure single solvents should not be excluded in such tests!) influence the individual elution behaviour of compounds that are part of a mixture. The findings regarding the possible selectivity of the transport along the layer are often unpredictable – and just this is the chance for a successful separation. But never go to the column while the power of TLC has not been exhausted. Finally, in this case the amount of pure chamazulene obtained is about 1% of the essential oils, only. That means it is about 0.1 g/kg of the mass of the flowers used at the beginning. This ratio has to be kept in mind when starting with the matricin isolation.

3.2 Method

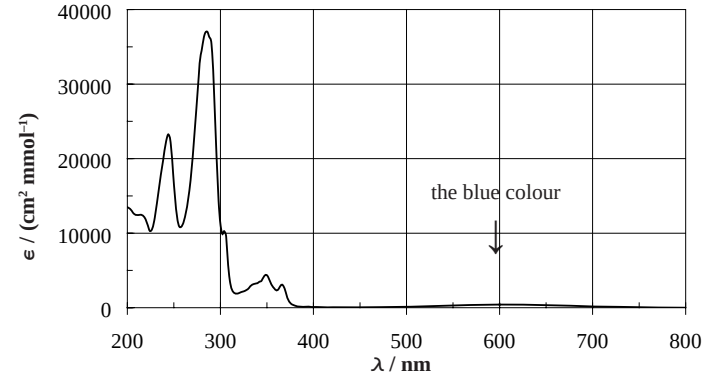
In a 2 L three-necked round-bottomed flask containing 1 L of distilled water, 60 g of Chamomillae flores, the flowers of *Matricaria recutita* L., are subjected to steam distillation. The blue chamazulene appears in the distillate after 10 min. The chamazulene formation ceases after 75 min. After this time, an amount of 2 L of steam distillate is collected in the receiver. It is cooled to RT and extracted with methyl *tert*-butyl ether (3 × 100 mL). The combined extracts are dried over $MgSO_4$, filtered and the solvent is removed in vacuo to leave 368 mg of an oily blue liquid. TLC in *n*-hexane shows the blue chamazulene spot at $R_f = 0.29$.

3.3 Purification

Conditions for the first column chromatography: column, 45 × 1.5 cm; stationary phase, silica gel 60 (0.040–0.063 mm); eluent: *n*-hexane. The liquid to separate is given at the top of the column within 1 mL of *n*-hexane solution. The *n*-hexane is passed through the column and first removes all the colourless compounds that are less polar than chamazulene. All blue fractions containing chamazulene are combined and the *n*-hexane is removed in vacuo to leave 8.7 mg of crude chamazulene. However, TLC shows that this material still contains a colourless impurity with $R_f = 0.05–0.1$ that quenches the activity of the $UV_{254\ nm}$ fluorescence indicator incorporated in the layer on the plates. Therefore, a further chromatography is necessary.

Conditions for the second column chromatography: column, 20 × 1.5 cm; stationary phase, silica gel 60 (0.040–0.063 mm); eluent, chloroform–toluene (3:1, v/v). The crude chamazulene sample is given at the top of the column within 1 mL of this eluent. The blue chamazulene fractions running close to the front of this chromatography are collected, combined and the solvent is removed carefully in vacuo. A TLC check shows that no impurity can be found, either by UV detection or by an oxidizing treatment with molybdophosphoric acid reagent. The mass of pure chamazulene is 4.9 mg in the form of a viscous, dark blue liquid. The boiling point given at the beginning of this section is from a database.

4. Spectra and Comments



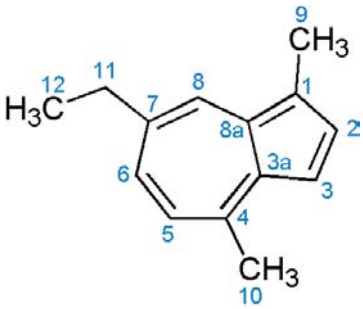


Fig. 2.3-5 UV spectrum in ethanol

The UV spectrum probably shows the most intense band of all molecules discussed in this book. Compared with the unsubstituted azulene, we find no fine structure due to vibrational losses caused by the alkyl groups. Compared with its aromatic sister compound naphthalene, there is a considerable red shift of the main absorption at 284 nm. The blue colour of chamazulene is caused by the broad absorption at 600 nm. Note that this peak has an ε value of 430, which can hardly be seen in this diagram because it is linear with respect to ε. Therefore, logarithmic UV diagrams are often displayed; for consistency with the other chapters, however, we refrain from a logarithmic scale.

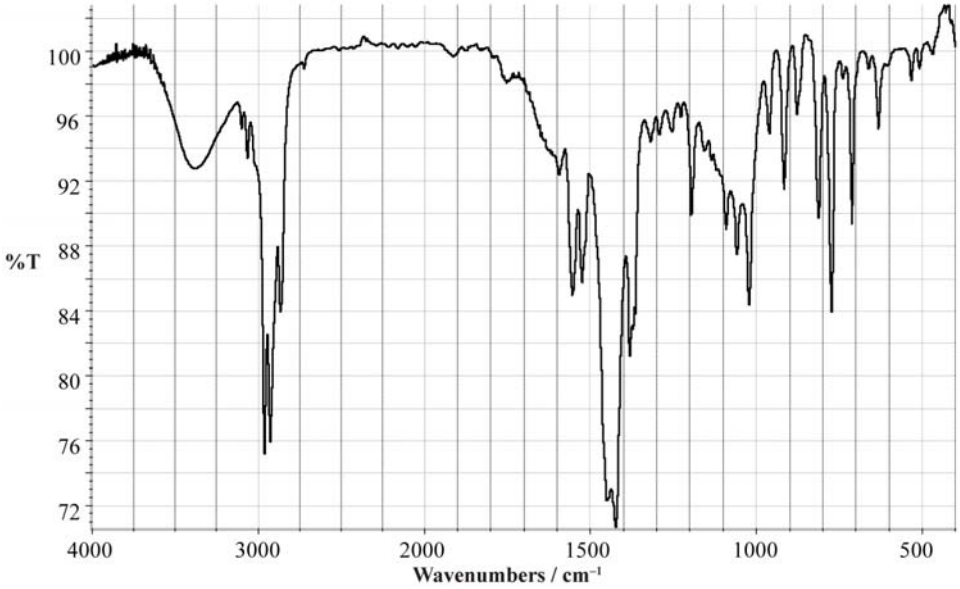


Fig. 2.3-6 IR spectrum as film

The broad signal at 3500 cm^{-1} stems from some residual humidity. Chamazulene gives the two very small sp^2 CH valence bands at about 3200 cm^{-1} and strong sp^3 CH vibrations below 3000 cm^{-1}. The aromatic overtone vibrations between 2000 and 1800 cm^{-1} can hardly be detected and also the C=C vibration at 1600 cm^{-1} is very weak. Both signal groups are considerably weaker than in a comparable alkyl-substituted naphthalene.

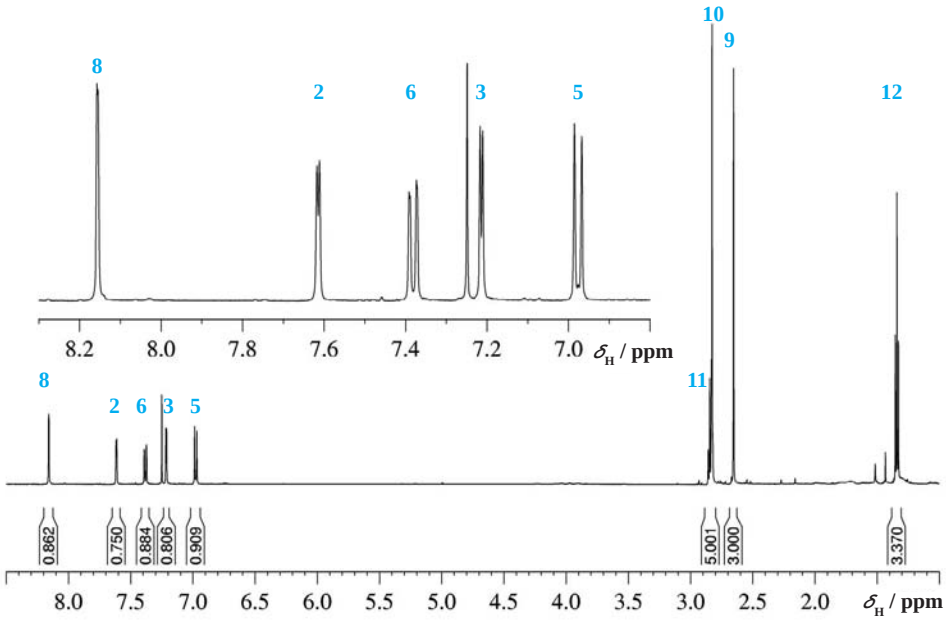


Fig. 2.3-7 ^{1}H NMR spectrum at 600 MHz in $CDCl_3$

Judging from ^{1}H NMR spectroscopy, chamazulene is a truly aromatic compound and not a cyclic polyolefin, since we find the resonances of the ring protons between 8 and 7 ppm and not below. Similarly, the methyl groups and the methylene group appear rather deshielded at 2.7 and 2.9 ppm, which must also be explained by a considerable "ring current". Apart from the signal of the solvent $CDCl_3$, we find in the aromatic region the required five signals, two pairs of doublets, one with a larger and one with a smaller spin coupling constant, and we have to decide which of these pairs belongs to the seven- and to the five-membered rings. The singlet at 8.15 ppm clearly belongs to the isolated proton H-8. In the aliphatic region, the ethyl group can be directly assigned with values of 1.3 and 2.8 ppm with the methylene group signal directly beneath the signal of one of the methyl groups. The relative assignment of the two methyl groups, however, has yet to be proved.

Dantur omnia mixta drachmae unius pondere contra serpentium omnium ictus. Pellunt mortuos partus, item menstrua in potu et urinam calculosque, inlita inflationes, iocinerum vitia, bilem subfusam, aegilopia, conmanducata ulcerum eruptiones manantes sanant. ex omnibus his generibus ad calculos efficacissima est quae florem purpureum habet, cuius et foliorum et fruticis amplitudo maiuscula est. Hanc proprie quidam eranthemim vocant.

Plinius Maior (23−79)
Naturalis Historia Liber, XXII, 54

Fig. 2.3-8 Steam distillation apparatus. From the left: electric steam generator, safety flask (heated), flask with chamomile flowers and hot water (heated), condenser, receiver

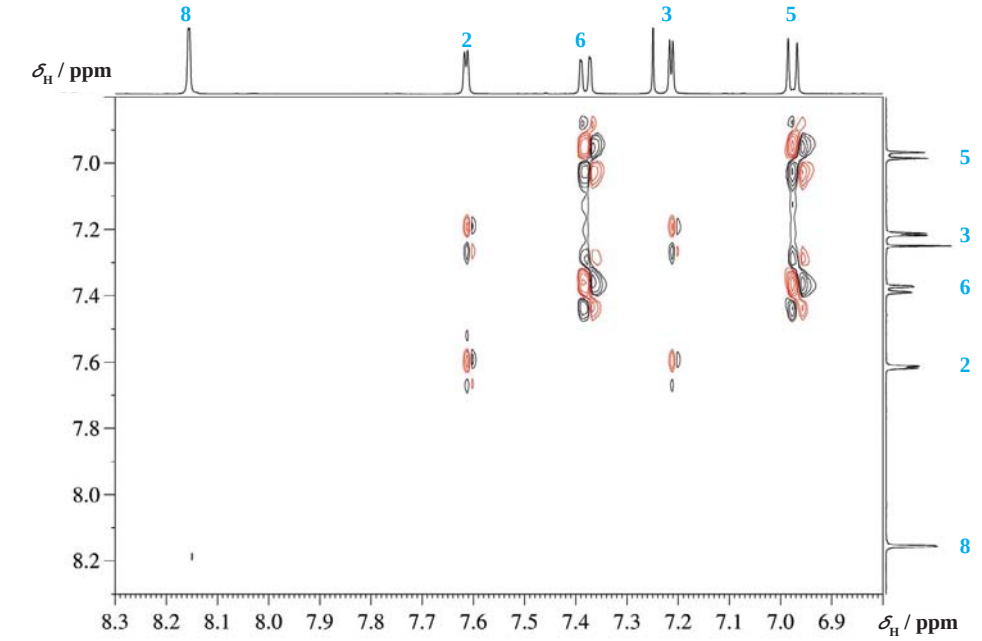


Fig. 2.3-9 COSY spectrum in the aromatic region

The double quantum filtered COSY spectrum gives no further help for the assignment. In the expansion of the aromatic region it shows only the connectivities between the aforementioned pairs of doublets. The expansion in the aliphatic region is shown here for another reason. The COSY pattern of the ethyl group is a very nice AX spin pattern, although the ethyl group forms an A_3X_2 spin system. This is a perfect educational example of how a double quantum filtered COSY spectrum reduces the multiplicity of the cross and diagonal peaks.

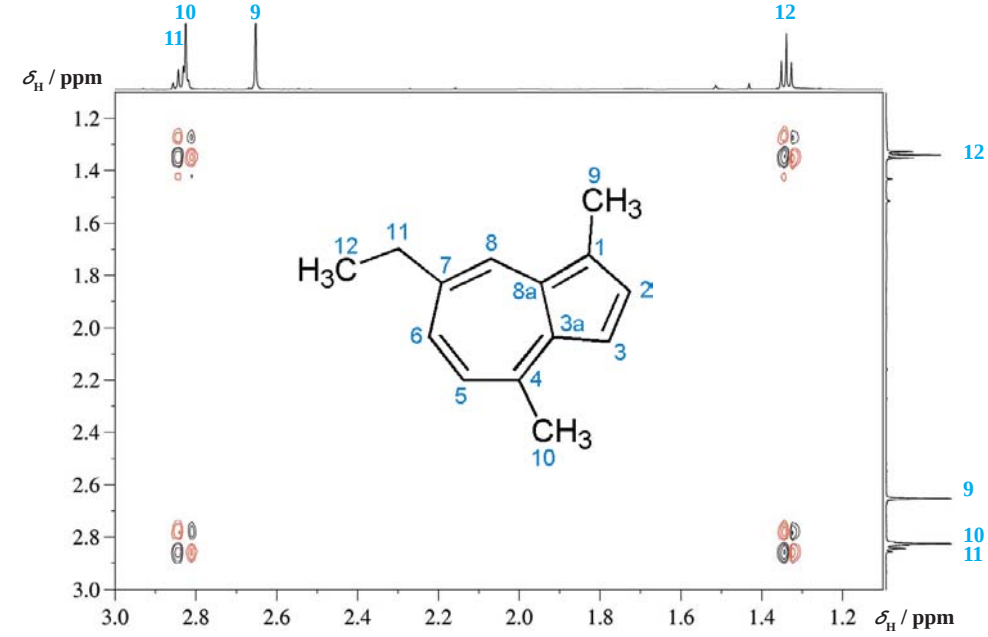


Fig. 2.3-10 COSY spectrum in the aliphatic region

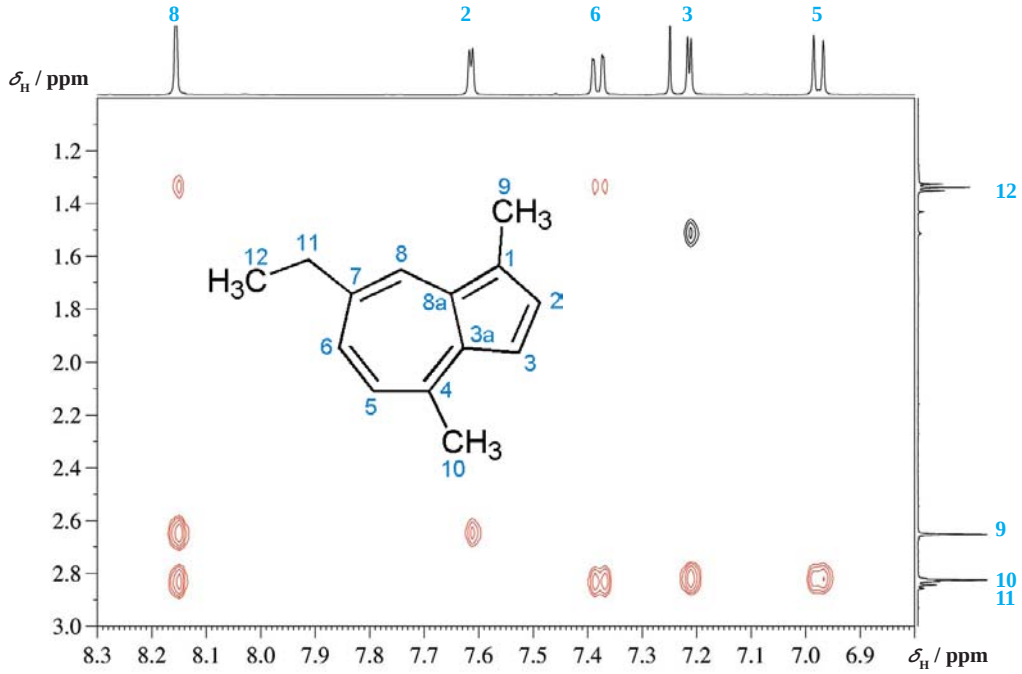


Fig. 2.3-11 NOESY spectrum

All open proton assignment questions for this compound are solved by the inspection of a NOESY expansion, which connects the aromatic with the aliphatic region. The methyl group H-12 displays an NOE cross peak to H-8 and to the doublet with the larger coupling constant at 7.4 ppm, which therefore must be H-6. Therefore, the pair of doublets with the larger spin coupling belong to the seven-membered ring. Both H-8 and H-6 also show a cross peak to the methylene group H-11. H-8 and the doublet with the smaller spin coupling at 7.6 ppm show a cross peak to the methyl group at 2.65 ppm. Therefore, this methyl group is assigned to H-9 and the proton at 7.6 ppm to H-2. Finally, the methyl group at 2.9 ppm, H-10, is seen from H-3 at 7.2 ppm and H-5 at 6.95 ppm.

Falstaff: Peace, good pint-pot; peace, good tickle-brain. Harry, I do not only marvel where thou spendest thy time, but also how thou art accompanied: for though the camomile, the more it is trodden on the faster it grows, yet youth, the more it is wasted the sooner it wears.

William Shakespeare (1564–1616)
King Henry IV, Part I, II, 4

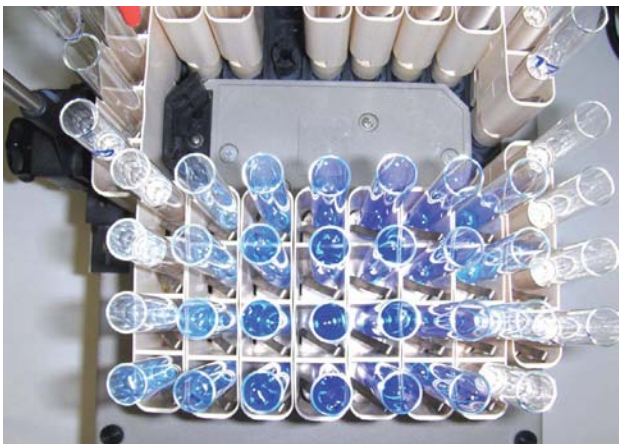


Fig. 2.3-12 Chromatographic fractions containing chamazulene

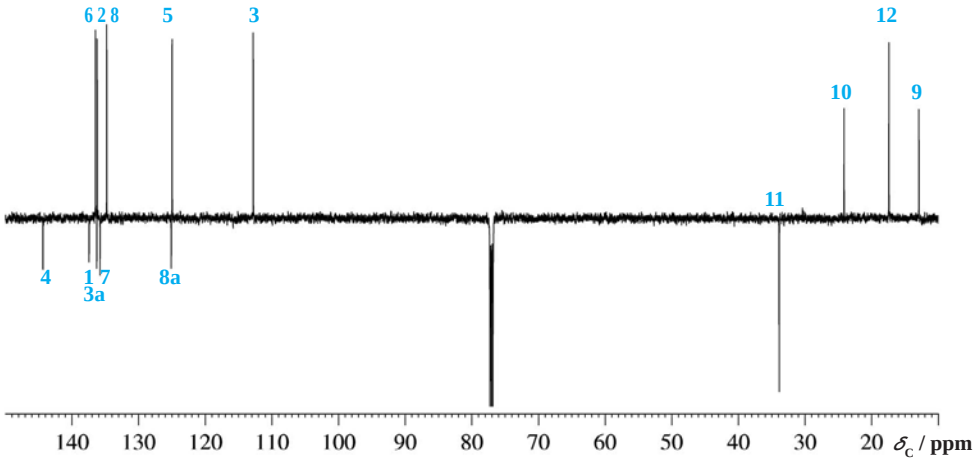


Fig. 2.3-13 APT ^{13}C NMR spectrum at 150 MHz in $CDCl_3$

The edited ^{13}C NMR spectrum displays in the aromatic region five CH signals and five signals of quaternary carbons; from the carbon spectrum alone, however, a safe assignment is not possible. In the aliphatic region we find three methyl group signals and only the most shielded signal can be assigned with certainty to C-12 at 12.9 ppm. Of course, the methylene group can be directly identified.

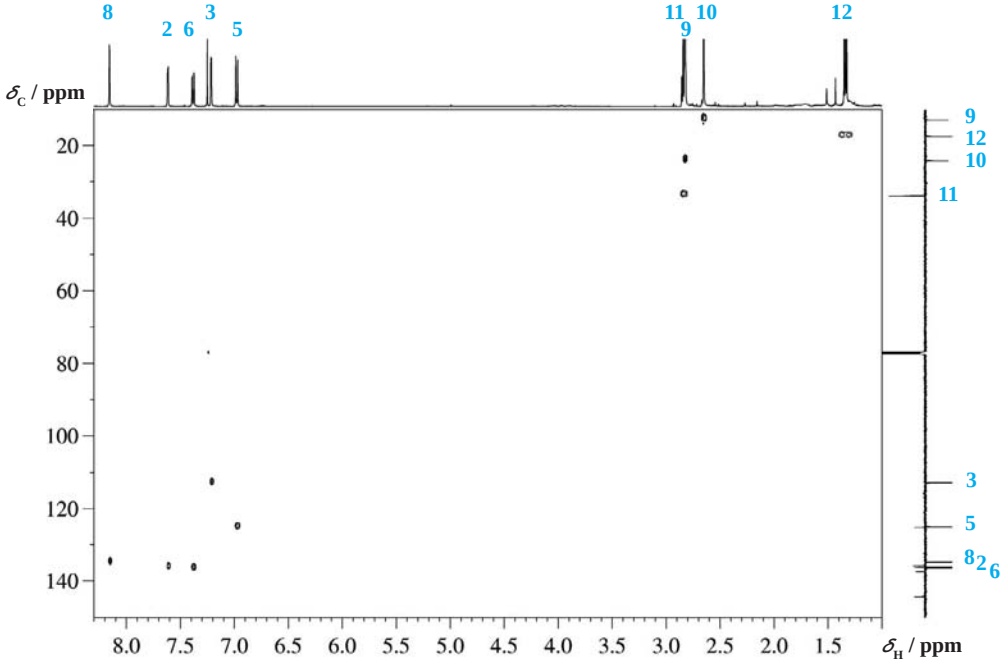


Fig. 2.3-14 HSQC spectrum

Since we have the proton spectrum fully assigned, we can use the HSQC spectrum to assign the signals of the protonated carbon atoms with ease. The resolution of the 2D spectrum is just enough to distinguish between the slightly separated signals of C-6 and C-2. It is interesting that the signal of the methyl group C-9 attached to the five-membered ring is 7 ppm more shielded than the signal of the methyl group C-10 at the seven-membered ring.

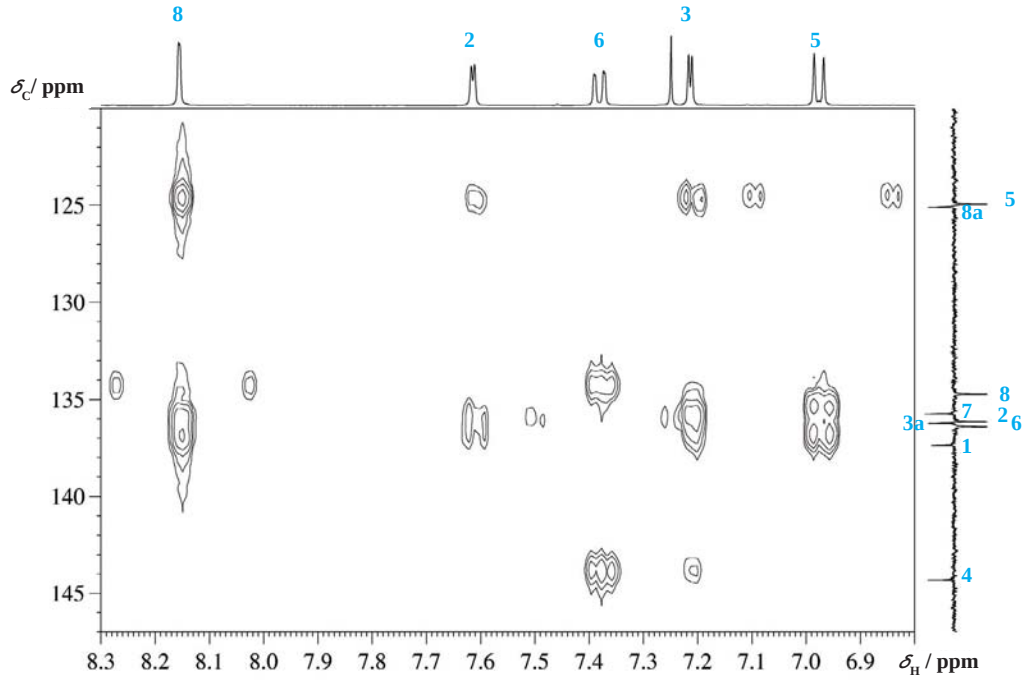


Fig. 2.3-15 HMBC spectrum in the aromatic region

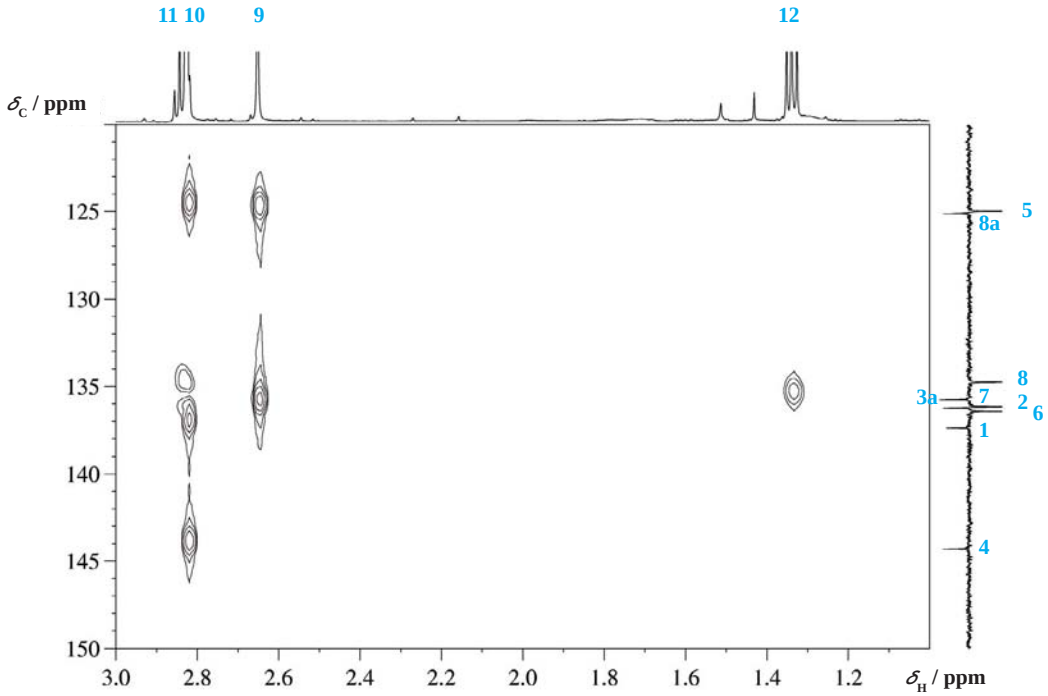


Fig. 2.3-16 HMBC spectrum in the aliphatic region

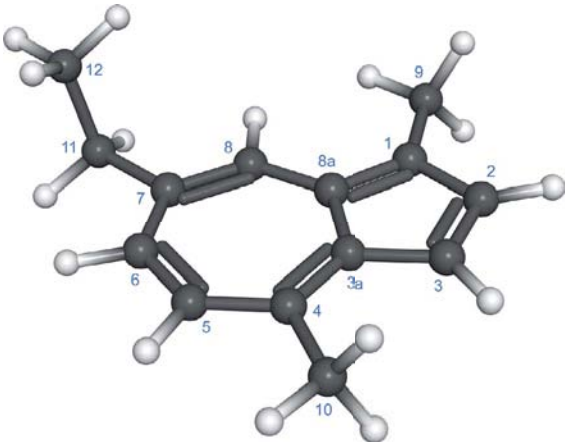


Fig. 2.3-17 Molecular model of chamazulene

Fig. 2.3-18 Stamp with chamomile flowers

The HMBC spectrum is finally only used to assign the signals of the quaternary carbon atoms. We rely here on the predominance of $^3J_{CH}$ in aromatic systems. The most deshielded signal at 144.3 ppm is assigned to C-4 due to the cross peaks from H-6 and H-3. In contrast, the most shielded signal at 125.1 ppm is assigned to C-8a due to three cross peaks from H-8, H-2 and H-3. The remaining quaternary signals are very close together and a correct assignment is difficult. We assign the signal at 136.2 ppm to C-3a due to the cross peak with H-8, the signal at 137.4 ppm to C-1 based on the cross peak from H-8 and H-3 and the remaining signal at 135.7 ppm to C-7 based on the cross peak from H-5. These assignments are corroborated from the analysis of the second HMBC expansion showing the cross peaks from the alkyl side chains. Thus the proton signals of the methyl group H-12 and that of the methylene group H-11 show a cross peak to C-7. Similarly, the protons of methyl group H-10 are connected to C-4, C-3a and C-5. Finally, the protons of methyl group H-9 should show a cross peak to C-8a, C-1 and C-2.

With the assignment given, one is tempted to check whether the charge distribution often discussed for azulenes is revealed by the carbon chemical shifts. This is, of course, dangerous due to the different substitutions and bond angles; however, if one takes only the two chemical shifts of the CH carbon atoms in the five-membered ring one can calculate an average chemical shift of 124.5 ppm, whereas the average of C-8, C-6 and C-5 in the seven-membered ring gives 132 ppm, hence as suggested from the polar mesomeric structure, the seven-membered ring is more deshielded.

Fig. 2.3-19 Flowering chamomiles

Die blaue Blume

Ich suche die blaue Blume,
Ich suche und finde sie nie,
Mir träumt, daß in der Blume
Mein gutes Glück mir blüh.

Ich wandre mit meiner Harfe
Durch Länder, Städt und Au'n,
Ob nirgends in der Runde
Die blaue Blume zu schaun.

Ich wandre schon seit lange,
Hab lang gehofft, vertraut,
Doch ach, noch nirgends hab ich
Die blaue Blum geschaut.

Joseph von Eichendorff (1788−1857)

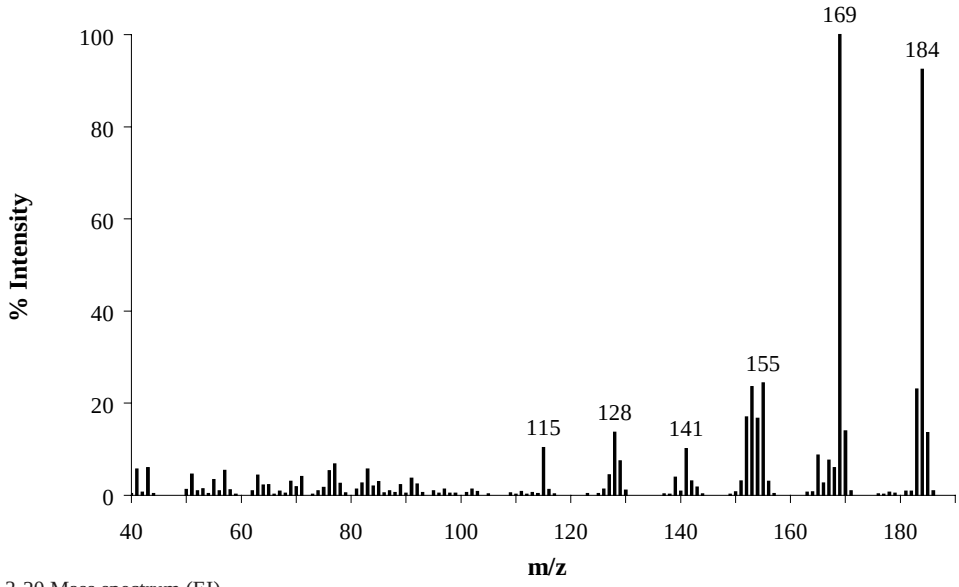
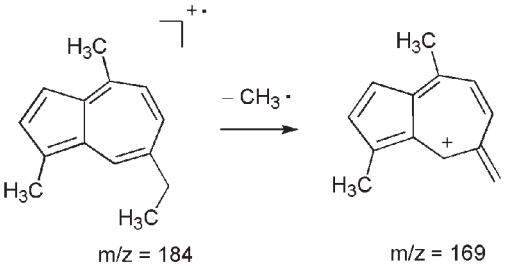


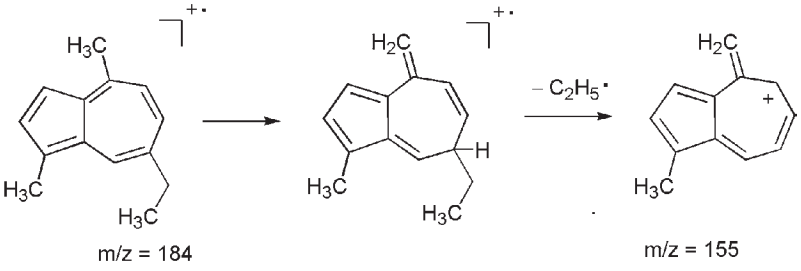
Fig. 2.3-20 Mass spectrum (EI)

The base peak of the mass spectrum is caused by the loss of a methyl radical and this can easily drawn as given below:



Scheme 2.3-2 Fragmentation of chamazulene

A 1,5-hydrogen shift explains the subsequent cleavage of an ethyl radical as shown below:



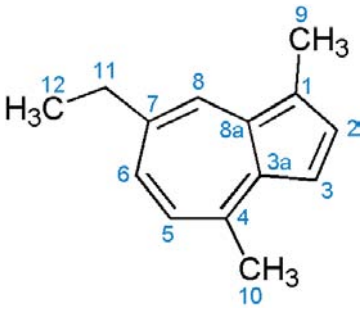
Scheme 2.3-3 Further fragmentation of chamazulene

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz
144.3	C_q	C-4	
137.4	C_q	C-1	
136.4	CH	C-6	7.39, $J_{6,5}$ = 10.3
136.2	C_q	C-3a	
136.2	CH	C-2	7.62, $J_{2,3}$ = 3.6
135.7	C_q	C-7	
134.7	CH	C-8	8.16
125.1	C_q	C-8a	
125.0	CH	C-5	6.98, $J_{5,6}$ = 10.3
112.8	CH	C-3	7.22, $J_{3,2}$ = 3.6
33.8	CH_2	C-11	2.85, $J_{11,12}$ = 7.6
24.1	CH_3	C-10	2.83
17.4	CH_3	C-12	1.35, $J_{12,11}$ = 7.6
12.9	CH_3	C-9	2.66

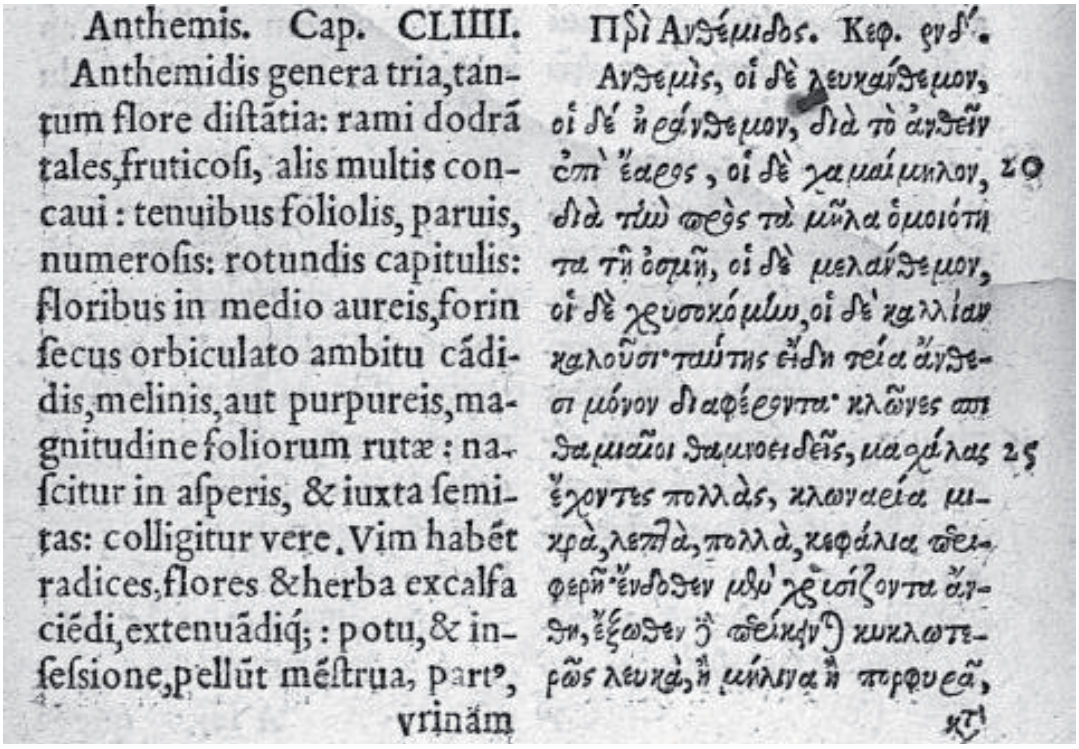
Table 2.3-1 NMR data for chamzulene

Multa inde praeclara remedia parantur, atq; inter alia ex floribus imprimis aruensis oleum caerulei coloris optime in colicis doloribus.

Joachim Camerarius (1534–1598)
Hortus Medicus et Philosophicus



Scheme 2.3-4


Anthemis. Cap. CLIIII.
Anthemidis genera tria, tantum flore diſtãtia: rami dodrãtales, fruticoſi, alis multis concaui: tenuibus foliolis, paruis, numeroſis: rotundis capitulis: floribus in medio aureis, forin ſecus orbiculato ambitu cãdidis, melinis, aut purpureis, magnitudine foliorum rutæ: naſcitur in aſperis, & iuxta ſemitas: colligitur vere. Vim habẽt radices, flores & herba excalfaciẽdi, extenuãdiq;: potu, & inſeſsione, pellũt mẽſtrua, partũ, vrinam

Πρὶ Ἀνθέμιδος. Κεφ. ρνδ'.
Ἀνθεμὶς, οἱ δὲ λευκάνθεμον, οἱ δέ ἠεράνθεμον, διὰ τὸ ἀνθεῖν ἐπὶ ἔαρος, οἱ δὲ χαμαίμηλον, διὰ τὴν πρὸς τὰ μῆλα ὁμοιότητα τὰ τῇ ὀσμῇ, οἱ δὲ μελάνθεμον, οἱ δὲ χρυσοκόμην, οἱ δὲ καλλίαν καλοῦσι· ταύτης εἴδη τρία ἄνθεσι μόνον διαφέροντα· κλῶνες αὐτῆς μαιάδες, μαιοειδεῖς, μαλακὰς ἔχοντες πολλὰς, κλωναρίοις μικρὰ, λεπτὰ, πολλὰ, κεφάλια φέρει· ἔνδοθεν μὲν χρυσίζοντα ἄνθη, ἔξωθεν δὲ κύκλῳ κυκλοτερῶς λευκὰ, ἢ μήλινα ἢ πορφυρᾶ, κτλ

Fig. 2.3-21 Pedanius Dioscoridie (40–90)
Materia Medica Dioscoridis Libri Octo Graece et Latine, 1549, Bibliotheca Albertina, University of Leipzig

5. Questions

A. Matricin, molecular formula $C_{17}H_{22}O_5$, is an acetylated sesquiterpene lactone. Indicate the three isoprene units present. Do the same with chamazulene, $C_{14}H_{16}$. Which carbon of the sesquiterpene skeleton is lost?

B. Guaiazulene (CAS RN 489-84-9) and vetivazulene (CAS RN 529-08-8) are two isomeric solid blue bicyclic sesquiterpenes, $C_{15}H_{18}$, that are structural relatives of chamazulene. Draw their structures, give their names and search for their origin and possible uses.

C. The synthesis of the azulene was difficult for more than 50 years because early attempts required a dehydrogenation procedure under harsh conditions that diminished the yield. How the problem was resolved can be studied on pages 301−302 of *Angew. Chem.* **1955**, *67*. The letters published there in the context of K. Ziegler, K. Hafner, "A rational synthesis of azulene" (Eine rationelle Synthese des Azulens), *ibid*., are part of a science thriller that nowadays seems both charming and old-fashioned. Search for the principle breakthrough in their synthesis of azulene. Give it in a scheme and discuss briefly.

D. Explain why an absorption in the red region (600 nm) gives a deep blue colour of the compound. Draw a structure which explains the "charge transfer band" in the UV spectrum.

E. Can one assign the two small IR absorptions above 3000 cm^{-1} to the five- and seven-membered rings?

F. The two methyl groups attached to the five- and seven-membered rings have considerable chemical shift differences. Can they be related to resonances structures in chamazulene?

G. Give an explanation for the considerable M−1 peak in the mass spectrum

6. Own Observations

2.4 Tetrahydrocannabinol

(6a*R*,10a*R*)-6a,7,8,10a-Tetrahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol

From marijuana
Cannabis sativa L. var. *sativa*
(Cannabaceae)

$C_{21}H_{30}O_2$, MW 314.46

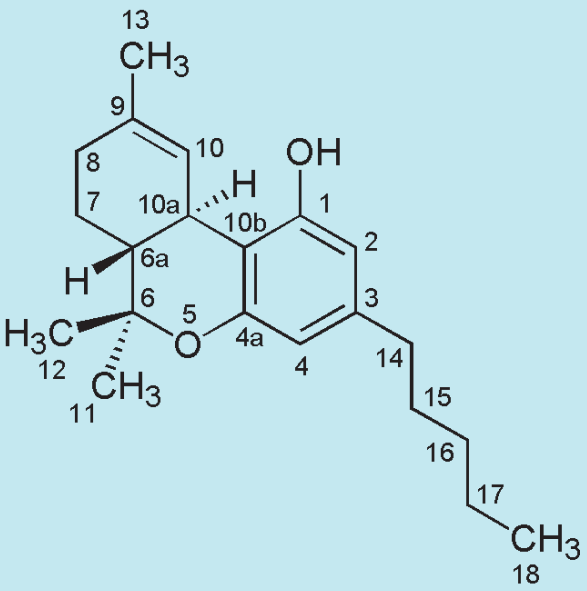
CAS RN 1972-08-3, BRN 1623667

$[\alpha]_D^{22}$ –162.5 ° (*c* 0.0065 g/mL, $CHCl_3$)

Pale yellow oil, bp 175–177 °C (7 Pa)

Tetrahydrocannabinol is commercially available.

Synonymous names:
Cannabinol, Dronabinol, Marinol, Δ^9-Tetrahydrocannabinol, *trans*-(−)-Δ^9-Tetrahydrocannabinol, *trans*-Δ^9-Tetrahydrocannabinol, Δ^9-THC, (−)-Δ^1-Tetrahydrocannabinol. (The numbers ^{9} and ^{1} are derived from different nomenclatures and are not contradictory.)



Level: easy

Caution!

Explicitly, we want to stress that the only intention of this section is to describe the isolation of an interesting natural product with high bioactivity that gave the impetus for the development of useful medications. Concerning the problem of provisioning with the required starting material, we want insistently to emphasize that the reader should carefully take notice of the particular laws of his or her home country that are valid for the purchase, possession and commerce of the special natural sources of THC.

1. Background: Birth of a medicine from a drug

ταύτης ὧν οἱ Σκύθαι τῆς καννάβιος τὸ σπέρμα ἐπεὰν λάβωσι, ὑποδύνουσι ὑπὸ τοὺς πίλους καὶ ἔπειτα ἐπιβάλλουσι τὸ σπέρμα ἐπὶ τοὺς διαφανέας λίθους τῷ πυρί· τὸ δὲ θυμιᾶται ἐπιβάλλομενον καὶ ἀτμίδα παρέχεται τοσαύτην ὥστε Ἑλληνικὴ οὐδεμία ἄν μιν πυρίη ἀποκρατήσειε. οἱ δὲ Σκύθαι ἀγάμενοι τη πυρίη ὠρύονται· τοῦτό σφι ἀντὶ λουτροῦ ἐστι· οὐ γὰρ δὲ λούονται ὕδατι τὸ παράπαν τὸ σῶμα.

Ἡερόδοτος [Herodotus]
(480–425 BC)
The Histories, Book IV

As in some other cases, the first problem is not to write a whole book on the topic *Cannabis sativa* L., one of the species within the genus hemp (*Cannabis*). Whereas *Cannabis* has a Greek root (κάνναβις) that is borrowed from the Scythians, *sativa* is Latin and stands for "cultivated". Hemp plants belong to the earliest domesticated plants known. Their usability is extremely manifold. An excellent overview on all aspects of hemp growing and use is given in a multilingual book [1]. Another book describes the chemistry of the cannabinoids in detail [2].

The oldest known use is as a source of resistant fibres. Together with flax, hemp allowed the first woven textiles to be made. This dates back about 5 millennia in the area of ancient China. Later, the first jeans tailored by Levi Strauss in the middle of the 19th century for gold-diggers were made of resistant sailcloth, woven from hemp fibres. It is of interest that banknote paper was made from hemp fibres in the 19th century.

Fig. 2.4-1 Female plant of cannabis

Fig. 2.4-2 Male plant

Hemp seeds, botanically nuts, are made up one-third of fatty oil rich in two essential fatty acids, linoleic and linolenic acid, and contain about 20% proteins. No wonder that the ancient Chinese considered hemp as a cereal for its nutritional value. Still today, cold pressed and refined hemp oil is used in body care products and paints and can also be used in the kitchen. Neither hemp seeds nor hemp oil contain amounts of Δ^9-THC that may cause a psychoactive drug effect or intoxication.

In ancient times, the plant was distributed from Central Asia around the world via India and Mesopotamia. The oldest European findings are about 5000 years old (in what is now Germany). The ancient Greeks used hemp fibre textiles, as mentioned by Herodot (450 BC). The strings of mediaeval longbows were made from hemp fibres that were able to withstand the enormous tension during the firing of an arrow. However, the use was wider. Hemp was also a plant to make medicine from, to obtain oil from and to achieve psychoactive effects. Pliny the Elder and Pedanius Dioscorides from the Roman Empire described hemp as a painkiller. In the 9th century, Charlemagne listed the hemp plant in his famous regulation *Capitulare de villis vel curtis imperii* that set rules on how to manage ideally the manors of his kingdom. Canvas and ropes for sailing ships were made from hemp fibres. Finally, a first European paper mill using hemp was built in Nuremberg in the 13th century. The Gutenberg Bible, the most famous *incunabulum*, was printed in 1452 on hemp paper in Mainz. However, it should not be forgotten that this was only a re-invention of an art that had been kept as a national secret by the Chinese for about 1500 years by then. Clearly, not many plants are so closely affiliated with human civilization as hemp.

Botanically, cannabis plants are annual flowering herbs and dioecious plants, i.e. male and female plants exist.

Hemp plants can be grown from the temperate to the tropical climate zones around the world and grow up to 4 m in height. The plants have ornamental, characteristic serrate leaves, being very far from any

other plants in their appearance (compare photographs). The flowers are wind-pollinated. Compared with weeds, young hemp plants grow extraordinarily fast and leave other plants literally in the shade. Therefore, neither manual weeding nor agrochemical weed control is necessary. Hemp also does not emaciate the soil.

Today, two branches of cannabis production exist. Industrial hemp growing includes cultivars bred for industrial non-drug use and is directed at making fibres or hemp seed oil. Such strains are low in phytocannabinoids, have minute THC levels (less than 0.3%) and may be grown by farmers who have a licence for hemp cultivation.

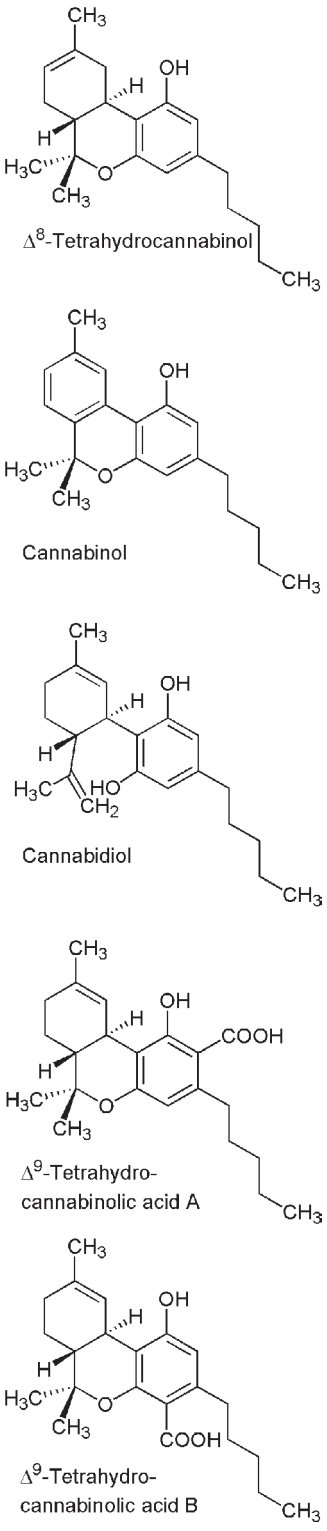
The growing of psychoactive cannabis, on the other hand, is directed at obtaining products rich in cannabinoids, especially in the product of this section, Δ^9-THC (content about 20% in types that are cultivated for medical use). The non-medical use of such types is what may cause legal problems, addiction and, in the worst case, trigger a permanent psychosis, if an individual has a corresponding unlucky disposition. In this grey area, two different products are known: marijuana, consisting of the dried mature flowers and subtending leaves of pistillate hemp plants, is the first one. Whereas this can be seen as the herbal form, the other product, hashish, is resinous and made from the THC-rich glandular hairs (so-called trichomes) together with flower and leaf particles. Such "hash" is preferably made from female plants.

It is stored in compressed blocks, but hashish oil is also known ("honey oil", not really an oil, more exactly a resin extract) that is made by solvent extraction and is very rich in psychoactive ingredients. Whereas hashish has an Arabic root, meaning "grass", the word marijuana comes from Mexican slang and arose as a code word for hemp during the American hemp prohibition campaign in the 1930s. The THC content in marijuana samples may differ widely from 1% to about 20%. Hashish is illegal but in great demand in Western Europe, and Morocco is a main supplier. Note also the overview of some of the other 70 naturally occurring cannabinoids in the margin.

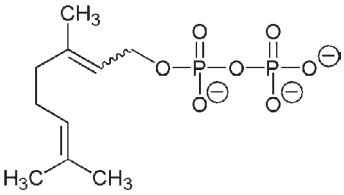
The structure of Δ^9-THC as a terpenoid benzopyran derivative was determined in 1964 [3]. For a liquid compound such as this, spectroscopic techniques such as NMR and mass spectrometry, just developing at that time, were especially helpful for structural assignments.

The plant biosynthesis starts from *n*-capronic acid, from which olivetolic acid is formed. It is first condensed with geranyl pyrophosphate or neryl pyrophosphate under enzymatic catalysis and then oxidatively cyclized by an oxidocyclase to form Δ^9-THC [4].

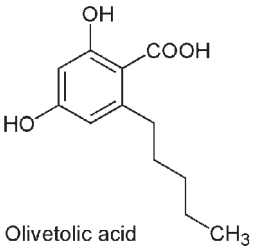
With the aim of writing a book about natural products, we do not intend to subject our readers to a campaign in respect of the deleterious psychoactive effects that arise from cannabis smoking. On the other hand, this does not mean promoting the consumption of cannabis products in an illegal manner here. Of course, it is at least interesting to consider the history of cannabis as a drug, but this is not the place to tell about the "killer weed" campaign, the cannabis prohibition and the



Scheme 2.4-1 Cannabinoid structures

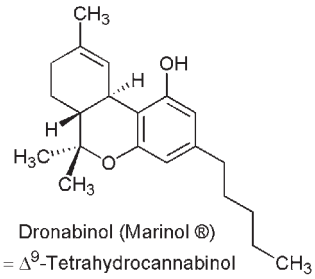


Geranyl pyrophosphate: (*E*)-config.
Neryl pyrophosphate: (*Z*)-config.

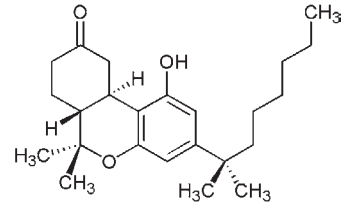


Olivetolic acid

Scheme 24-2



Dronabinol (Marinol ®)
= Δ^9-Tetrahydrocannabinol



Nabilone

Scheme 2.4-3

"Marihuana Tax Act" in the 1930s in the USA; this can be found elsewhere [1]. Nowadays, many of the previously excited dust has settled. What remained is a natural product that by some superior physiological effects gave the impetus for the development of medications, which are very helpful for seriously ill patients. The effects accessible are mentioned below.

In contrast to opiates, cannabinoids received rather late approval as medications, from the 1980s on. Their main application is in support of the treatment of cancer and AIDS. The medical use of cannabinoids is based upon two compounds that can both be made synthetically. The first is Δ^9-THC itself, which has the INN (International Nonproprietary Name) dronabinol and is sold under the registered trademark Marinol® of Solvay Pharmaceuticals. Dronabinol acts as an antiemetic and as an appetite stimulant, helpful for both chemotherapy and AIDS patients. Dronabinol also reduces the intraocular pressure.

Synthetic approaches to Δ^9-THC have been developed. In principle, as for any chiral compound, two methods are possible, i.e. to start from a chiral pool member or to develop an enantioselective synthesis. Both strategies have been pursued successfully. Suitable chiral pool compounds to start with are (4*R*)-(+)-limonene (see section 5.1) or (+)-2-carene that serve as precursors of (+)-*p*-mentha-2,8-dien-1-ol. The deciding step is its addition to olivetol (5-*n*-pentyl-resorcinol) followed by a Lewis acid-catalysed cyclization [5]. Evans et al. developed an enantioselective synthesis based upon using an auxiliary technique involving a chiral oxazolidinone. It has the principal advantage of making Δ^9-THC as readily accessible as was described for its enantiomer [6].

The second compound is nabilone, which is a synthetic cannabinoid mimicking the properties of THC. Eli Lilly has developed nabilone as an antiemetic and as an adjunct analgesic against neuropathic pain. The compound is on the market as the racemate. Nabilone is helpful in the treatment of nausea and vomiting caused as unwanted side effects during cancer chemotherapy. Also, it acts as an appetizing agent and is helpful against anorexia and weight loss in AIDS patients. Furthermore, it is useful in the management of chronic pain. In general, this medical use of THC and analogues has led to a more differentiated discussion about hemp constituents than ever before.

2. Literature

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3. Isolation

3.1 Principle

The marijuana ingredient Δ^9-THC is highly lipophilic, as can be seen from its formula. Therefore, it can be extracted by chloroform−methanol. After removal of this solvent, it is substituted by 90% methanol, *n*-hexane is added and both liquids are shaken in a separating funnel. This step leads to a selective transfer of Δ^9-THC and the corresponding cannabinolic acids into *n*-hexane. The acids form most of the crude extract obtained. A suitable means to separate the cannabinolic acids from the distinctly less acidic phenolic Δ^9-THC is extraction of the former from an ethereal solution of the crude extract with aqueous $NaHCO_3$ solution, a very weak base, but basic enough to deprotonate the carboxylic acids and to remove them in anionic form. Workup of the ethereal phase shows that its mass has been reduced to about 1/7. The final purification was done by preparative thin-layer chromatography, leading to pure Δ^9-THC that forms only about 10% of the crude marijuana extract. The amount of Δ^9-THC obtained allowed for the measurement of all spectra and the optical rotatory power. Values for bp and n_D given are taken from databases.

Fig. 2.4-3 Hemp seeds

Cannabis in silvis primum nata est, nigrior foliis et asperior. semen eius extinguere genituram virorum dicitur. sucus ex eo vermiculos aurium et quodcumque animal intraverit eicit, sed cum dolore capitis, tantaque vis ei est, ut aquae infusus coagulare eam dicatur; et ideo iumentorum alvo succurrit potus in aqua. radix articulos contractos emollit in aqua cocta, item podagras et similes impetus. ambustis cruda inlinitur, sed saepius mutatur, priusquam arescat.

Plinius Major (23−79)
Naturalis Historia Liber,
Book XX, 97.

Fig. 2.4-4 Germinating seeds of cannabis

3.2 Method

Marijuana (2.53 g) is pulverized in a mortar. The powder obtained is stirred with a mixture of chloroform–methanol (1:1, v/v) (200 mL) in a beaker and filtered by suction. The solvent is removed completely from the filtrate in vacuo. The dark brown oil that remains (1.0 g) is dissolved in methanol–water (9:1, v/v) (10 mL) and extracted with *n*-hexane (4 × 10 mL). The combined hexane phases are reduced to dryness in vacuo. The orange oil that remains (700 mg) is dissolved in diethyl ether (50 mL) and extracted with saturated $NaHCO_3$ solution (7 × 30 mL). The aqueous phases are discarded. The ether is removed from the organic phase in vacuo. Oil remains (100 mg).

3.3 Purification

The oil is further purified by preparative TLC on self-made plates [layer prepared with Merck silica gel for preparative TLC at a thickness of 1 mm, eluent: *n*-hexane–diethyl ether (1:1, v/v); note: the use of commercial plates would require a larger number of plates due to their lesser thickness]. The upper zone is removed with a razor blade and the Δ^9-THC is dissolved with diethyl ether (4 × 5 mL). The ether is removed in vacuo and the material remaining is subjected to the same procedure again. Eventually, Δ^9-THC is obtained as clear, pale yellow oil from which all of the spectra shown here can be taken.

Optical rotatory power: $[\alpha]_D^{22}$ –162.5° (*c* 0.0065 g/mL, $CHCl_3$).

De Hanff

Hanff calidum existit, et cum aer nec multum calidus nec multum frigidus crescit, et ita etiam natura ipsius est, et semen eius sanitatem habet et sanabile est sanis hominibus ad comedendam, et in stomacho eorum leve est et utile ita quod slim de stomacho eius aliquantulum aufert, et faciliter digeri potest, atque inolos humores minuit, et humores bonos fortes facit.

Hildegard Bingensis (1098–1178),
Physica – Lib. I. de Plantis, Chap. XI

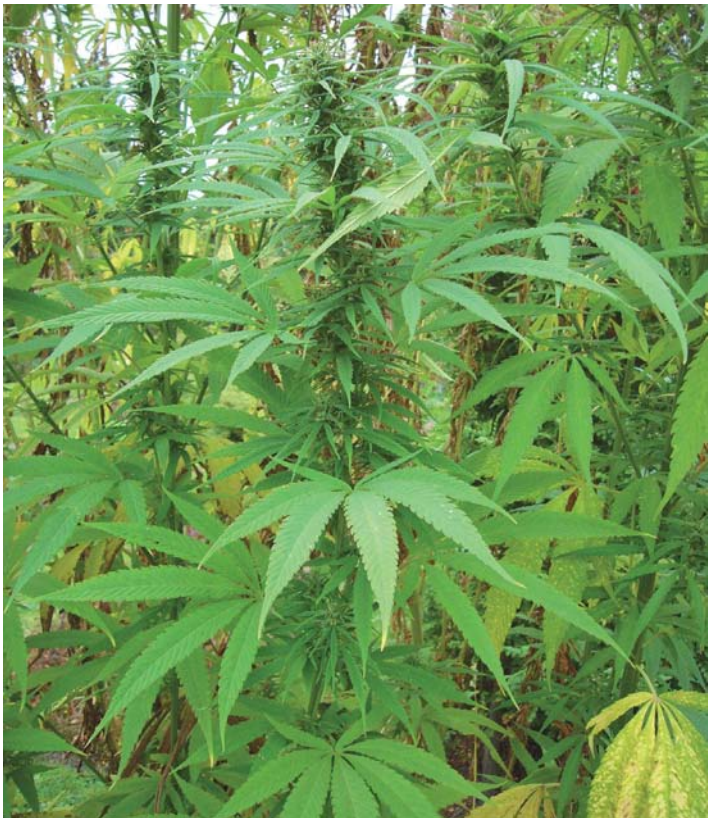


Fig. 2.4-5 A hemp forest

4. Spectra and Comments

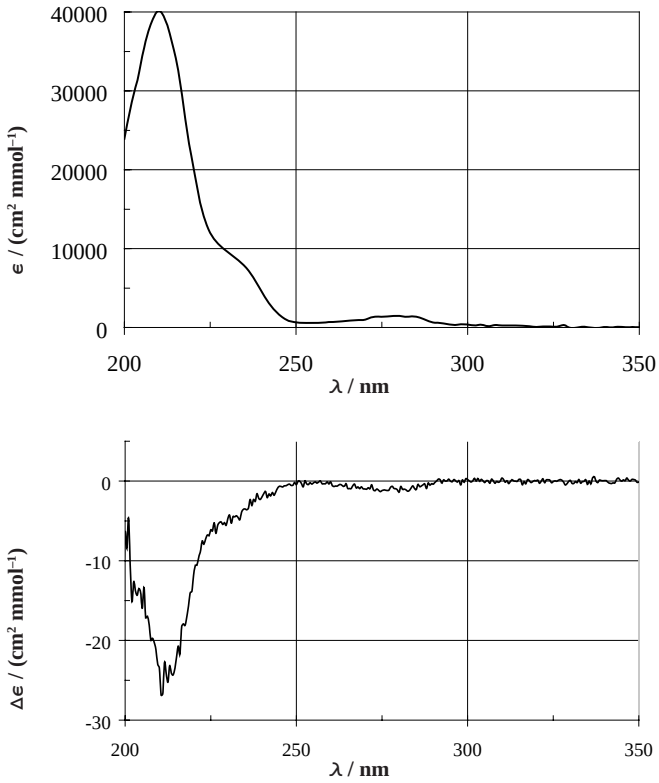
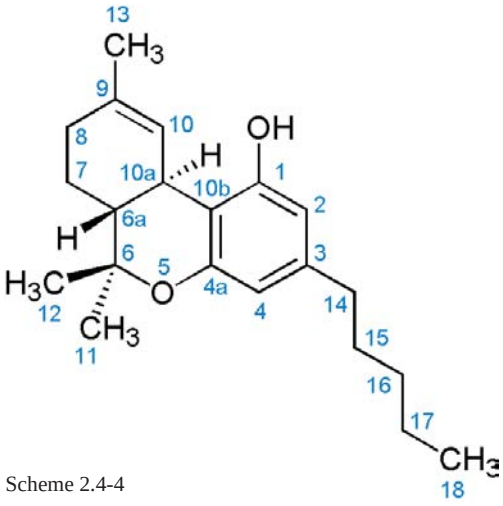


Fig. 2.4-6 UV and CD spectra in ethanol

The UV spectrum shows a fairly normal pattern for an aromatic compound with two oxygen atoms attached to the π-system. The strong maximum at 210 nm with ε = 40 000 cm^2 $mmol^{-1}$ has a significant shoulder at 230 nm, followed by a weak absorption at 280 nm. Only the main band at 210 nm shows a strong and negative Cotton effect in the CD spectrum.



Scheme 2.4-4

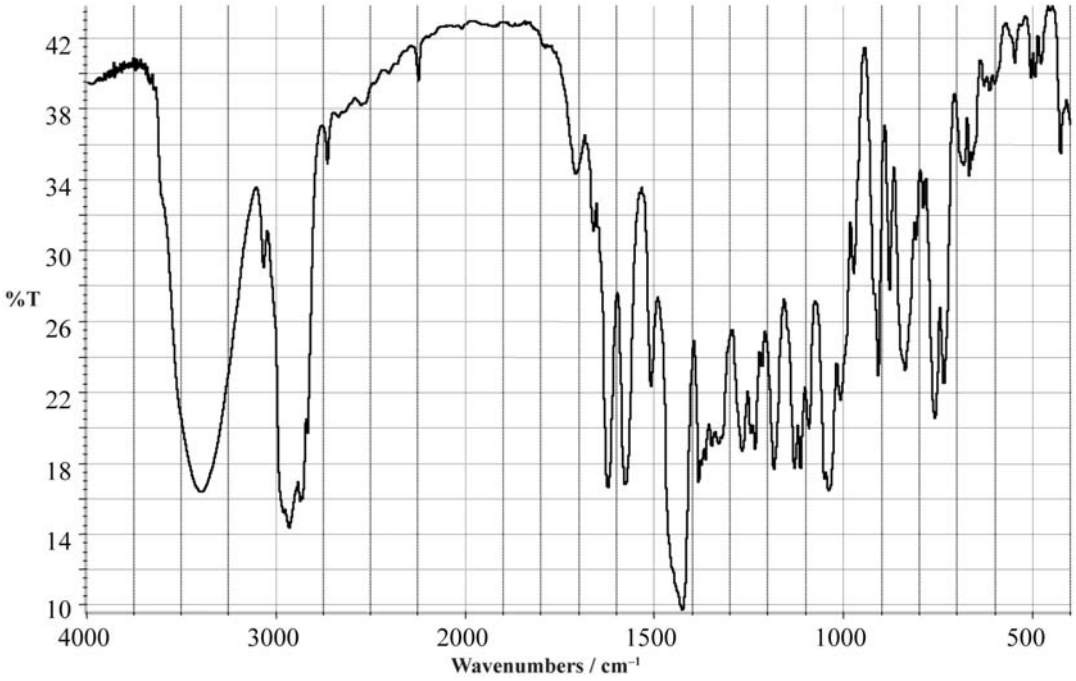


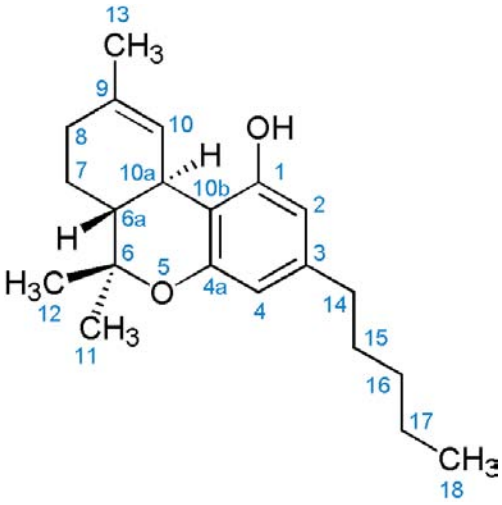
Fig. 2.4-8 IR spectrum in KBr

Dominating in the IR spectrum are the OH and CH valence bands of the phenolic OH group and the many aliphatic sp^3 hybridized CH_x groups. In the double bond region we observe two bands at 1620 and 1580 cm^{-1}, probably from the aromatic and olefinic C=C moieties.

Fig. 2.4-9 Hemp in garden pots

La plupart des novices, au premier degré d'initiation, se plaignent de la lenteur des effets; ils les attendent avec une impatience puérile, et la drogue n'agissant pas assez uite à leur gré, ils se livrent à des fanfaronnades d'incrédulité qui sont fort réjouissantes pour les vieux initiés qui savent comment le haschisch se gouverne. Les premières atteintes, comme les symptômes d'un orage longtemps indécis, apparaissent et se multiplient au sein même de cette incrédulité. C'est d'abord une certaine hilarité, saugrenue, irrésistible, qui s'empare de vous. Ces accès de gaieté non motivée, dont vous êtes presque honteux, se reproduisent fréquemment, et coupent des intervalles de stupeur pendant lesquels vous cherchez en vain à vous recueillir. Les mots les plus simples, les idées les plus triviales prennent une physionomie bizarre et nouvelle; vous vous étonnez même de les avoir jusqu'à présent trouvés si simples. Des ressemblances et des rapprochements incongrus, impossibles à prévoir, des jeux de mots interminables, des ébauches de comique, jaillissent continuellement de votre cerveau. Le démon vous a envahi; il est inutile de regimber contre cette hilarité, douloureuse comme un chatouillement. De temps en temps vous riez de vous-même, de votre niaiserie et de votre folie, et vos camarades, si vous en avez, rient également de votre état et du leur; mais, comme ils sont sans malice, vous êtes sans rancune.

Charles Baudelaire (1821−1867)
Les Paradis Artificiels



Scheme 2.4-5

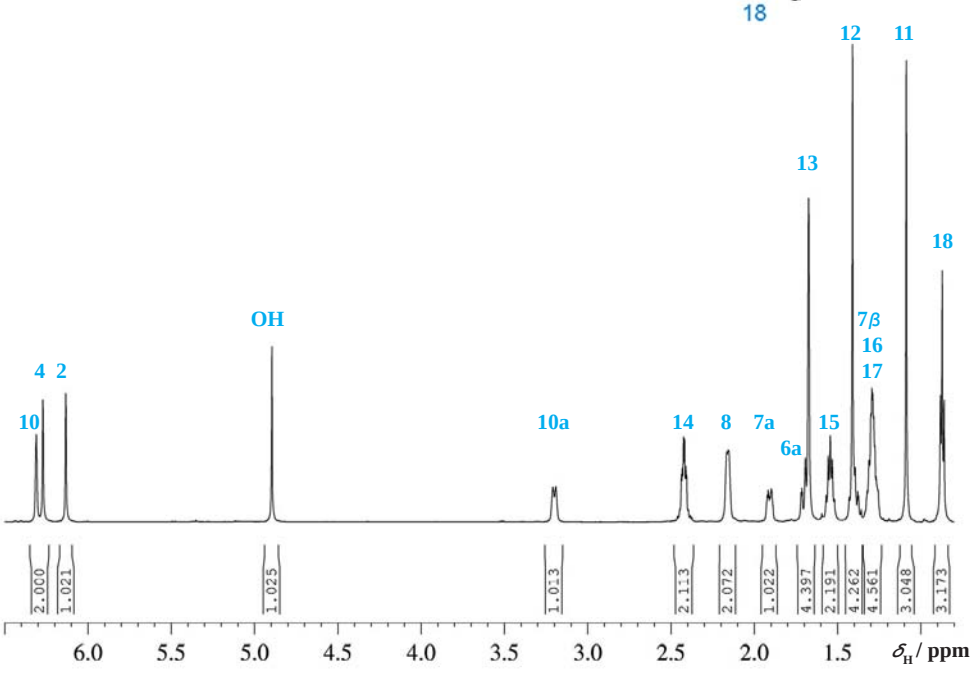


Fig. 2.4-10 ^{1}H NMR spectrum at 600 MHz in $CDCl_3$

The proton NMR spectrum of Δ^9-THC is rather crowded in the aliphatic but very empty in the aromatic/olefinic region. Here we find three singlets between 6.5 and 6 ppm, which must belong to H-2, H-4 and H-10; however, an individual assignment is not possible at this stage. As will be seen later in the HSQC spectrum, the singlet at about 5 ppm is not connected to a carbon atom and therefore we assign this to the phenolic hydroxyl group, which is surprisingly shielded. Looking at the structure of Δ^9-THC, the most deshielded aliphatic proton should be H-10a due to its benzylic and at the same time allylic position and therefore we assign the signal at 3.2 ppm to H-10a. The compound contains four methyl groups, two of which can be directly assigned. Clearly, the triplet at 0.87 ppm belongs to H-18, whereas the singlet at 1.67 ppm is assigned to H-13 due to its position at an sp^2 centre. The remaining two singlets at 1.41 and 1.09 ppm belong to H-12 and H-11; however, again, an individual assignment is not yet possible.

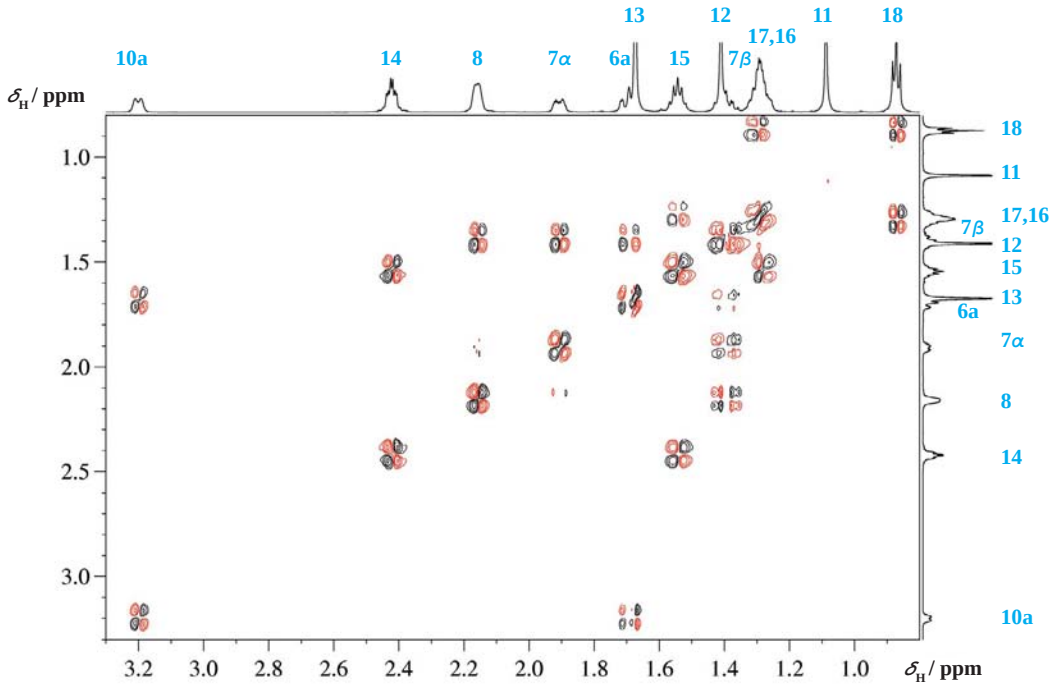
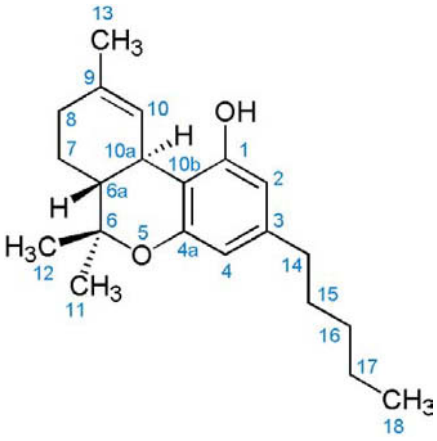


Fig. 2.4-11 COSY spectrum

Although the COSY spectrum does not give any information about the three singlets in the aromatic/olefinic region, it gives much insight into the aliphatic protons. Starting from the methyl group H-18 at 0.87 ppm, we find a cross peak to a broad signal of four protons at 1.29 ppm, which therefore are assigned to H-17 and H-16. From this signal we find the next cross peak leading to a signal of two protons at 1.54 ppm, accordingly assigned to H-15, which in turn leads to the signal at 2.42 ppm assigned to H-14 with its typical chemical shift of a methylene group attached to a benzene ring. Since we have already assigned the signal at 3.2 ppm to H-10a, we can identify the signal at 1.69 ppm at the base of the signal of the methyl group H-13 as H-6a. A cross peak from H-6a leads us to one of the signals of H-7 at 1.39 ppm and to the foot of another methyl group. The two protons H-7 are strongly diastereotopic and the partner of H-7 can again be found via the COSY cross peak leading to the signal at 1.91 ppm. In addition, the former signal of H-7 at 1.39 ppm leads us also to a signal of two protons at 2.17 ppm, which therefore belong to H-8.



Scheme 2.4-6

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz
154.7	C_q	C-4a	
154.2	C_q	C-1	
142.8	C_q	C-3	
134.3	C_q	C-9	
123.8	CH	C-10	6.31
110.1	CH	C-4	6.27
109.1	C_q	C-10b	
107.6	CH	C-2	6.13
77.3	C_q	C-6	
45.8	CH	C-6a	1.69
35.5	CH_2	C-14	2.42
33.6	CH	C-10a	3.20, $J_{10a,6a}$ = 10.1
31.5	CH_2	C-16	1.29
31.2	CH_2	C-8	2.17
30.7	CH_2	C-15	1.54
27.6	CH_3	C-12	1.41
25.0	CH_2	C-7	H-7α 1.91, H-7β 1.39, $J_{7\alpha,7\beta}$ = –12
23.4	CH_3	C-13	1.67
22.5	CH_2	C-17	1.29
19.3	CH_3	C-11	1.09
14.0	CH_3		0.87, $J_{18,17}$ = 7.0
			OH: 4.89

Table 2.4-1 NMR data for tetrahydrocannabinol

"Old Shatterhand hat wohl gesprochen. Wir wollen die Pfeife des Friedens mit ihm rauchen."

Hierauf setzten sie sich mit uns an das Wasser. Er zog eine Pfeife hervor, deren lieblich-niederträchtige Penetranz meine Nase schon von weitem empörte, und stopfte sie mit einer Mischung, welche aus zerstoßenen roten Rüben, Hanfblättern, geschnittenen Eicheln und Sauerampfer zu bestehen schien, versetzte sie in Brand, stand auf, tat einen Zug, blies den Rauch gen Himmel und gegen die Erde und sagte:

"Da oben wohnt der gute Geist, und hier auf der Erde wachsen die Pflanzen und die Tiere, welche er für die Krieger der Kiowas bestimmt hat."

Hierauf tat er vier weitere Züge und fuhr fort, nachdem er den Rauch nach Norden, Süden, Osten und Westen geblasen hatte:

"Nach diesen Gegenden hin wohnen die roten und weißen Männer, welche diese Tiere und Pflanzen unrechtmäßiger Weise für sich behalten. Wir werden sie aber aufsuchen und uns nehmen, was uns gehört. Ich habe gesprochen. Howgh!"

Karl May (1842−1912)
Winnetou I, Chapter 3

Fig. 2.4-12 Hemp plant in a botanical garden

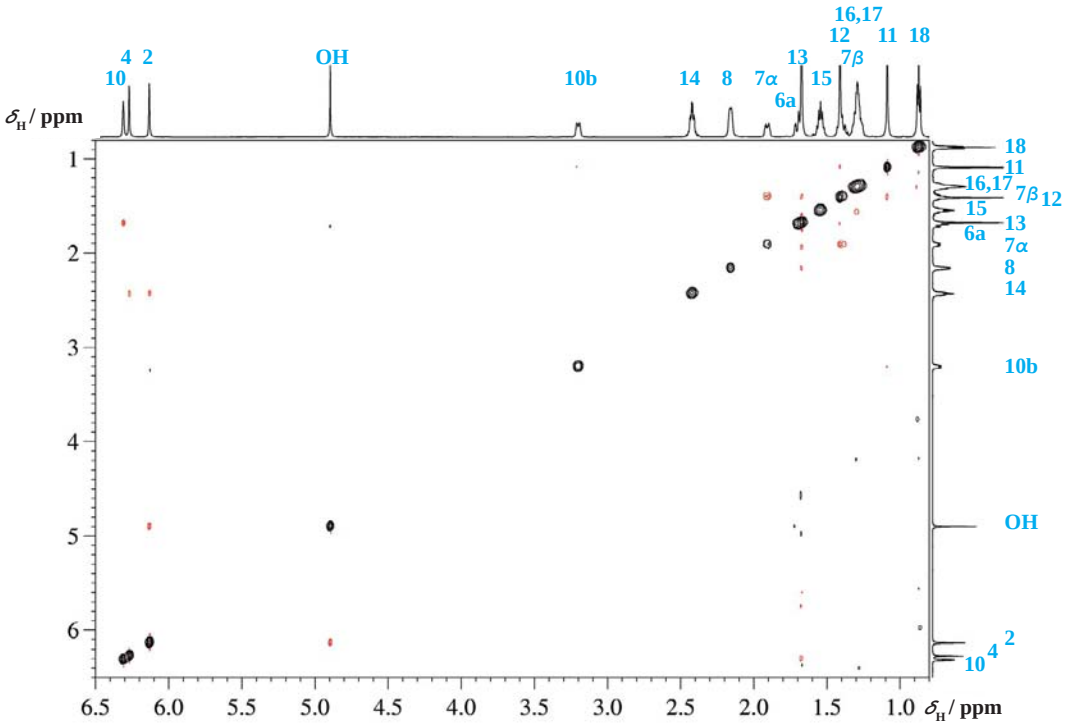


Fig. 2.4-13 NOESY spectrum

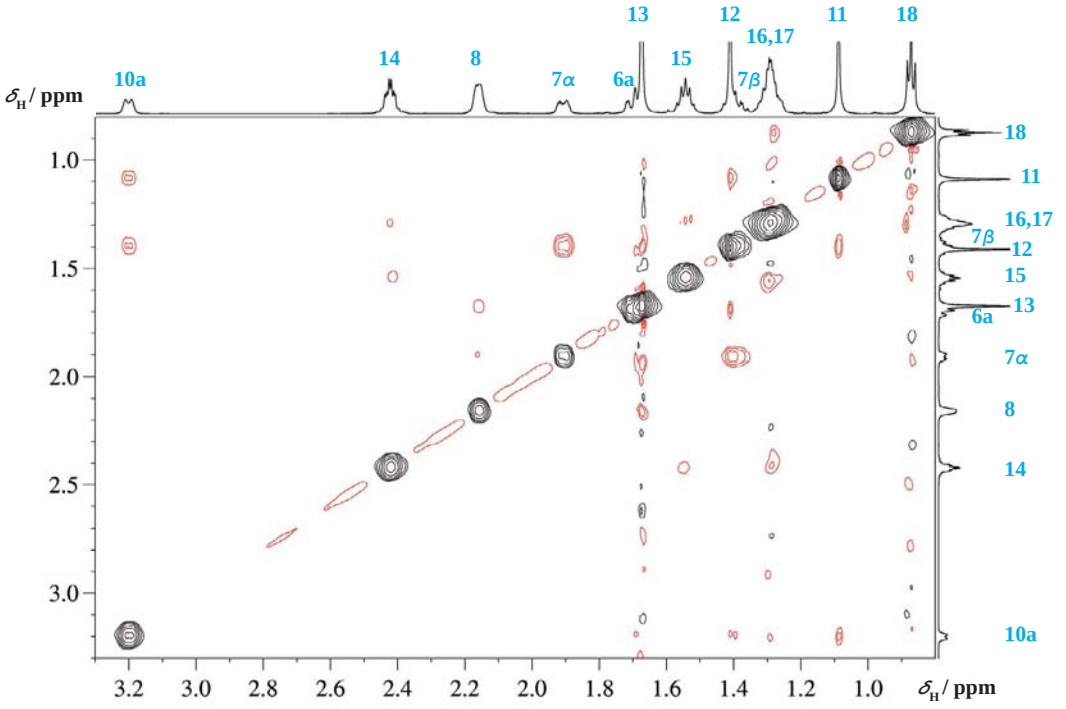


Fig. 2.4-14 Expansion of the NOESY spectrum

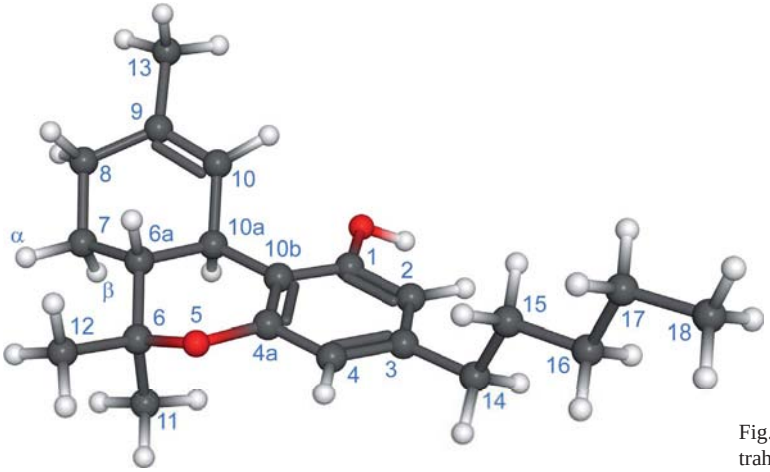


Fig. 2.4-16 Molecular model of tetrahydrocannabinol

Looking at the proton spectrum, we realize that of the six methylene groups of Δ^9-THC only one, C-7, displays proton signals with a considerable chemical shift difference. In principle, all methylene protons are diastereotopic due to the two stereogenic centres of the molecule; however, the aliphatic side chain is too far away and the H-8 protons also fall together in one multiplet. Therefore, we use the NOESY spectrum to assign the two protons H-7α an H-7β and also the methyl groups H-12 and H-11. First of all, however, the NOESY spectrum is helpful in obtaining an individual assignment of the three singlets between 6.5 and 6 ppm. In the NOESY overview spectrum we find a cross peak between the OH signal at 4.89 ppm and the signal at 6.13 ppm, which therefore must be H-2. Both H-2 and H-4 show a NOESY cross peak to H-14, and this assigns the signal at 6.27 ppm to H-4. Finally, we find for the signal at 6.31 ppm a cross peak to the methyl group signal H-13 and this confirms the assignment for H-10.

Close inspection of the NOESY expansion reveals that the signal of H-6a displays a NOESY cross peak to the methyl group signal at 1.41 ppm but not to that at 1.09 ppm. Therefore, the former is assigned to H-12. Both methyl group signals of H-12 and H-11 are interconnected by a NOESY cross peak, but close inspection of the peak shape shows that H-11 also displays a cross peak to the H-7 signal at 1.39 ppm, which is therefore assigned to H-7β; H-6a has a faint cross peak to H-7α at 1.91 ppm.

После третьего блюда Лысевич говорил, обращаясь к Анне Акимовне:
– Женщина fin de siecle, – я разумею молодую и, конечно, богатую, – должна быть независима, умна, изящна, интеллигентна, смела и немножко развратна. Развратна в меру, немножко, потому что, согласитесь, сытость есть уже утомление. Вы, милая моя, должны не прозябать, не жить, как все, а смаковать жизнь, а легкий разврат есть соус жизни. Заройтесь в цветы с одуряющим ароматом, задыхайтесь в мускусе, ешьте гашиш, а главное, любите, любите и любите... На первых порах я на вашем месте завел бы себе семерых мужчин, по числу дней в неделе, и одного назвал бы Понедельником, другого – Вторником, третьего – Средой и т. д., чтобы каждый знал свой день.
Этот разговор волновал Анну Акимовну. Она ничего не ела и только выпила рюмку вина.

Anton Chekhov (1860–1904)
Бабье царство

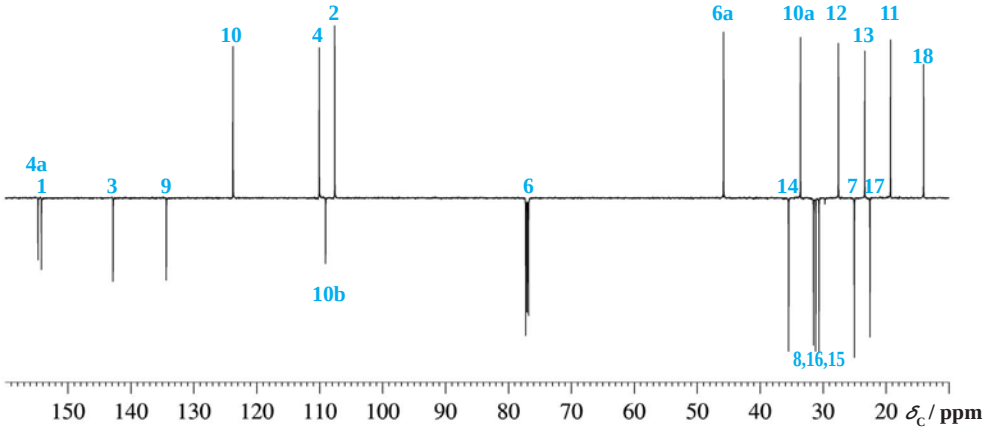


Fig. 2.4-19 APT ^{13}C NMR spectrum at 100 MHz in $CDCl_3$

The APT ^{13}C NMR spectrum nicely displays the required 21 signals, five quaternary carbon atoms and three CH groups in the olefinic/aromatic region, six signals for the four methyl groups and the two CH groups in the aliphatic region as well as six methylene groups. Close inspection of the solvent signal reveals that there is an additional signal underneath, which can be directly assigned to C-6, due to its vicinity to oxygen and being quaternary. The two quaternary signals at about 154 ppm clearly belong to C-1 and C-4a, without the possibility of distinguishing between them at present. The same is true for the following pair C-3 and C-9 at 142.8 and 134.3 ppm, whereas the quaternary signal at 110.1 ppm can be safely assigned to C-10b due to its β-position to both oxygen substituents. This signal is embraced by two CH group signals which are also in a β-position to an oxygen substituent and therefore belong to C-4 and C-2, which leaves the assignment for C-10 for the signal at 123.8 ppm. It is best not to speculate here about the assignment of the aliphatic carbon signals but to use both HSQC and HMBC spectra to obtain a safe assignment.

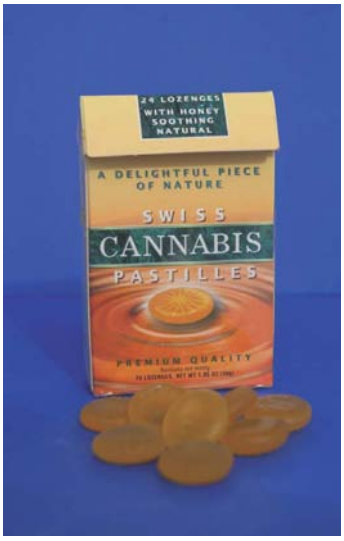
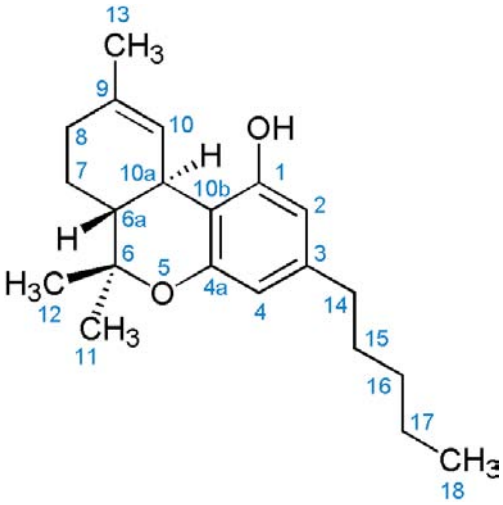


Fig. 2.4-18 Fake pastilles

Fig. 2.4-19 Flyer found in a disco



Scheme 2.4-7

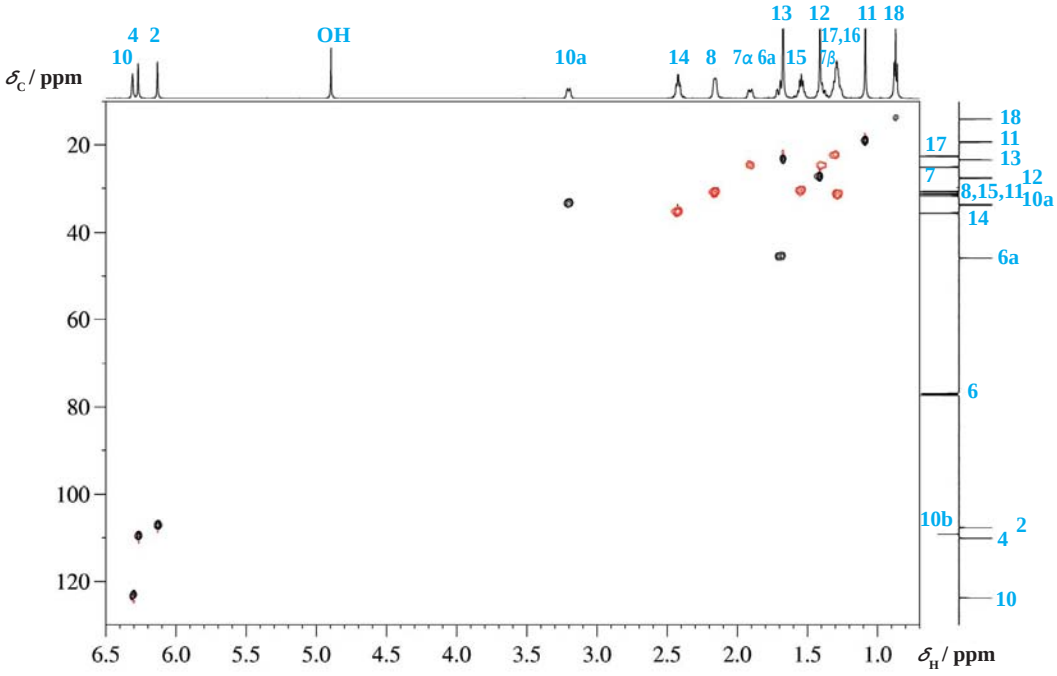


Fig. 2.4-20 HSQC spectrum

The CH_x edited HSQC spectrum shows again that only one methylene group has significantly different proton chemical shifts. Since the proton shifts are all known, the assignment of the carbon signals is straightforward. The only problem is the relative assignment of C-16 and C-17, since the proton chemical shifts fall together, whereas the carbon chemical shifts are clearly different. Since C-17 has two α- and one β-substituent, whereas C-16 has two α- and two β-substituents, C-16 has to be more deshielded and is assigned to the signal at 31.5 ppm.

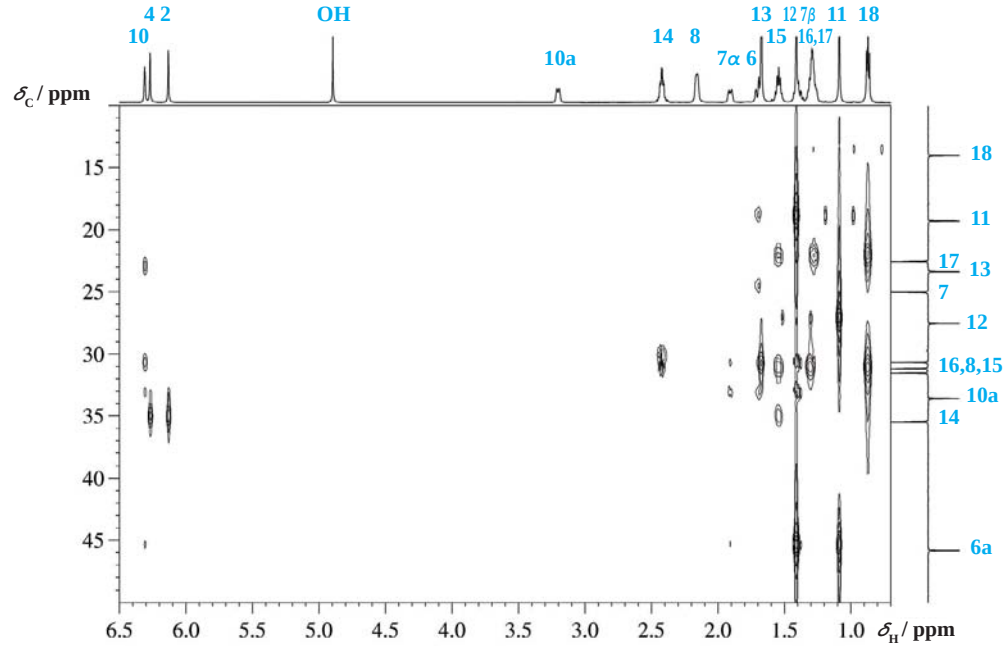


Fig. 2.4-21 HMBC spectrum in the aliphatic carbon region

As for the other compounds in this book, the HMBC spectrum provides the final check on whether all given assignments are consistent with the structure and especially the quaternary carbon atoms are correctly assigned. The aromatic expansion given demonstrates that C-6 is seen from both methyl group signals H-12 and H-11 and from H-6a. The relative assignment of C-2 and C-4 is based on the cross peak from the OH group, whereas the relative assignment of C-1 and C-4a is based on the cross peaks from H-4 and H-2. The aliphatic expansion of the HMBC spectrum will be addressed in the Questions section.

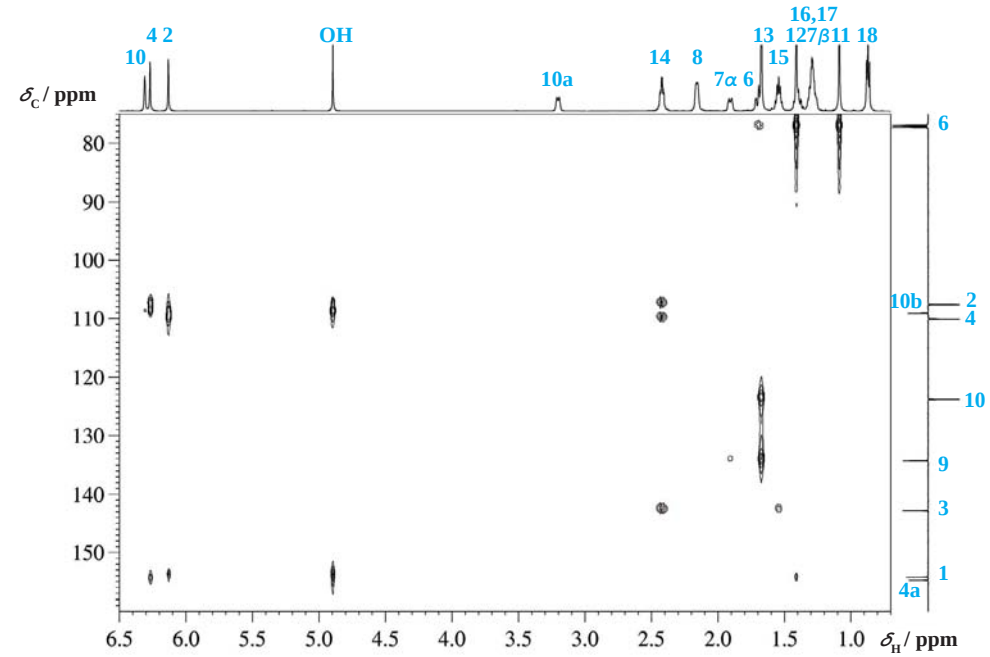
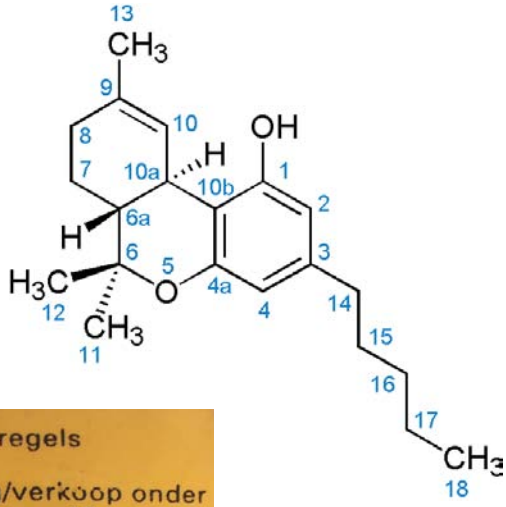


Fig. 2.4-22 HMBC spectrum in the aromatic carbon region

Fig. 2.4-23 Marihuana



Scheme 2.4-8

Fig. 2.4-24 Leaflet obtained in a Dutch coffee schop

You know I've smoked a lot of grass
O' Lord, I've popped a lot of pills
But I never touched nothin'
That my spirit could kill
You know, I've seen a lot of people
walkin' 'round
With tombstones in their eyes
But the pusher don't care
Ah, if you live or if you die
God damn, The Pusher
God damn, I say The Pusher
I said God damn, God damn The
Pusher man

You know the dealer, the dealer is a
man
With the love grass in his hand
Oh but the pusher is a monster
Good God, he's not a natural man
The dealer for a nickel
Lord, will sell you lots of sweet
dreams
Ah, but the pusher ruin your body
Lord, he'll leave your, he'll leave your
mind to scream
God damn, The Pusher
God damn, God damn the Pusher
I said God damn, God, God damn The
Pusher man

Well, now if I were the president of
this land
You know, I'd declare total war on
The Pusher man
I'd cut him if he stands, and I'd shoot
him if he'd run
Yes I'd kill him with my Bible and my
razor and my gun
God damn The Pusher
Gad damn The Pusher
I said God damn, God damn The
Pusher man

Rockband Steppenwolf
Best known from the movie
"Easy Rider" (1969)

Fig. 2.4-25 Hashish

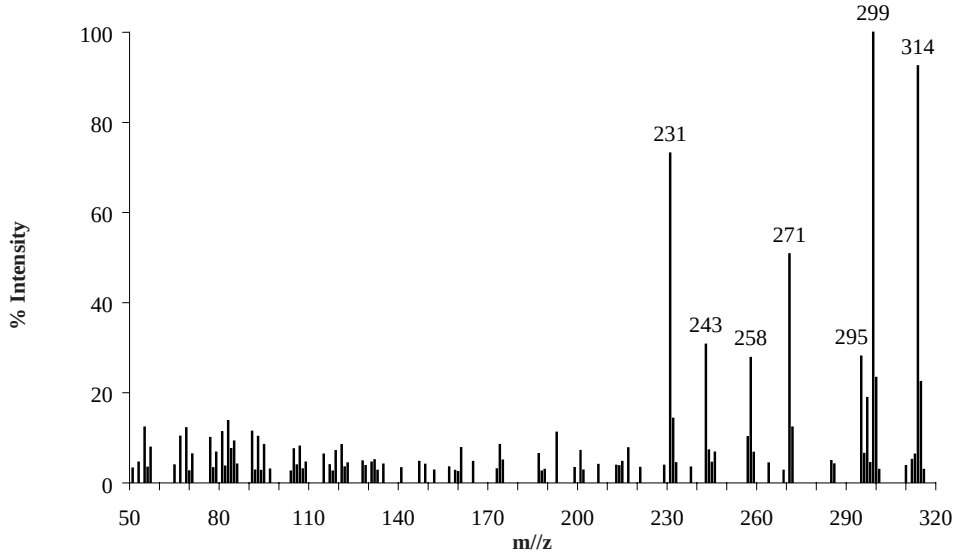
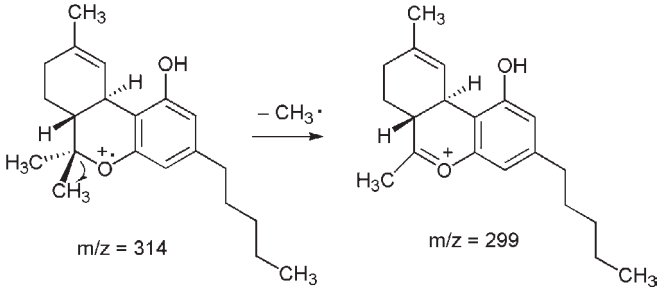


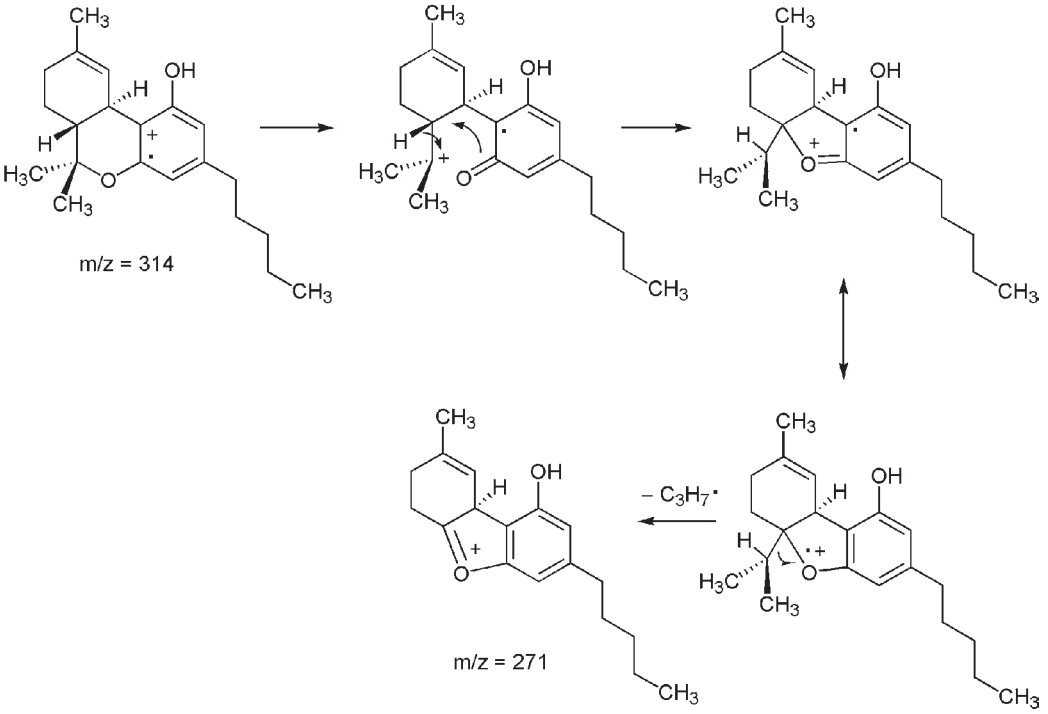
Fig. 2.4-28 Mass spectrum (EI)

The EI mass spectrum shows a surprisingly strong molecular ion peak at m/z = 314. This ion loses a methyl radical to form the ion at m/z = 299, which can easily be explained by an α-cleavage after ionization at the ether oxygen.



Scheme 2.4-9 Fragmentation of tetrahydrocannabinol

The ion at m/z = 271 corresponds to a loss of 43 mass units. Ionization in the aromatic ring and benzylic-type cleavage forms a radical ion which undergoes a hydrogen transfer and subsequent elimination of an isopropyl radical, as indicated in the following scheme:



Scheme 2.4-10 Further fragmentation of tetrahydrocannabinol

5. Questions

A. Which of the principles applicable during the isolation of alkaloids cannot be used for THC isolation?

B. Which property is used to separate all cannabinoids from the other marijuana constituents? Which property is used to remove the tetrahydrocannabinolic acids from Δ^9-THC?

C. What happens with the two tetrahydrocannabinolic acids on heating?

D. The discovery of two endogenous cannabinoid receptors, CB1 and CB2, in the nervous system and in the periphery in the 1990s led to two questions: Is there an endogenous cannabinoid to interact with this receptor? If not, what compound is it then? This discussion took place against the background that there is real endogenous morphine in the human body that may interact with a kind of opiate receptors. Search for the chemical nature of the endogenous key for the cannabinoid receptors.

E. Use of marijuana or hashish can be analytically verified for the rather long time of about one month because constituents of both or their metabolites are stored long-term in the body. Make a suggestion for the structural reason for this fact.

F. Is there a relationship between the sign of the Cotton effect in the CD spectrum and the sign of the polarimetry value?

G. In Δ^9-THC the olefinic signal of H-10 is more deshielded than the aromatic signals of H-2 and H-4. Is there no aromatic ring current?

H. If the signal of C-6 completely coincided with the solvent signal, how could one make it visible?

I. In the NOESY expansion, the signal of H-10a displays two cross peaks to H-11 and H-7β. Is this consistent with the stereochemistry?

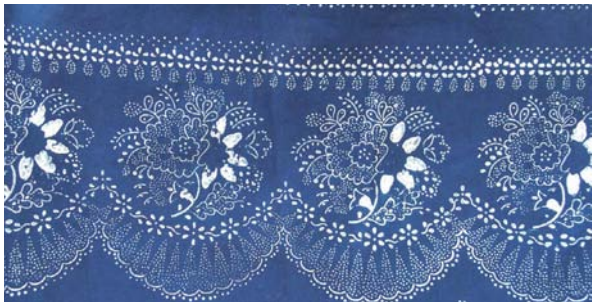
J. Discuss the four HMBC signals for H-10, H-4 and H-2 in the second HMBC expansion.

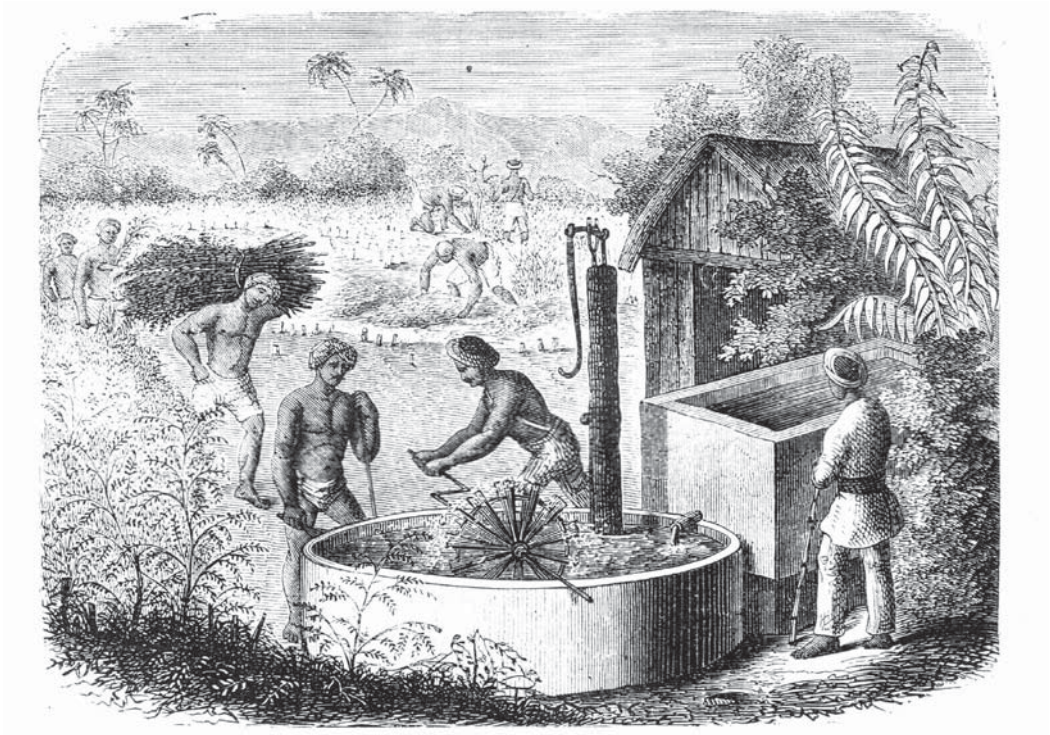
K. Discuss the HMBC correlation peaks of the four methyl groups.

L. Suggest a mechanism leading to the ion with m/z = 231.

6. Own Observations

Chapter 3 Dyestuffs and Coloured Compounds





Indigo production in Bengal

In the field of dyestuffs, four scientists made very important contributions which won them the Nobel Prize:

1915

Richard Martin Willstätter
(Germany, 1872 − 1942)

Germany, Munich University

“for his researches on plant pigments, especially chlorophyll”

1930

Hans Fischer
(Germany, 1881 − 1945)

Germany, Technical University, Munich

“for his researches into the constitution of haemin and chlorophyll and especially for his synthesis of haemin”

1937

Paul Karrer
(Switzerland, 1889 − 1971)

Switzerland, Zürich University

“for his investigations on carotenoids, flavins and vitamins A and B2”

1938

Richard Kuhn
(Austria, 1900 − 1967)

Germany, Heidelberg University and Kaiser-Wilhelm-Institute, Heidelberg

“for his work on carotenoids and vitamins”

3.1 Lawsone

2-Hydroxy-1,4-naphthalenedione

From the dried leaves of the henna plant
Lawsonia inermis L. (Lythraceae)

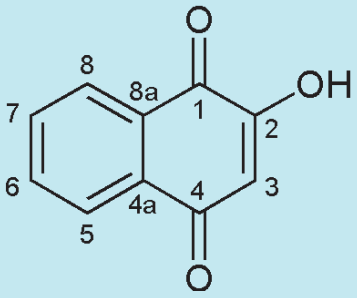
$C_{10}H_6O_3$, MW 174.15

CAS RN 83-72-7, BRN 1565260

Orange crystals, mp 193−195 °C

Lawsone is commercially available.

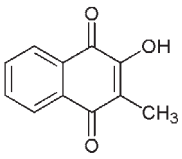
Synonymous names:
2-Hydroxy-1,4-naphthoquinone, CI 75480,
CI Natural Orange 6, Flower of Paradise, Henna (dye),
Hennotannic acid, Mehendi, Mendi, Pakarli



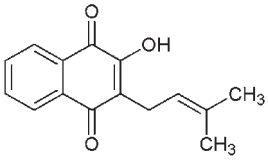
Level: medium

Caution!

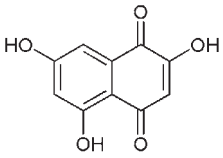
Lawsone reacts on contact with the skin to form a permanent stain that lasts until the skin is shed. Therefore, it is strongly recommended to work tidily with both aqueous and organic solutions containing lawsone to avoid a tenacious discoloration of the skin lasting several weeks. Wearing resilient protective gloves is advisable. An unwanted fixing of lawsone in the laboratory is very different from henna body art.



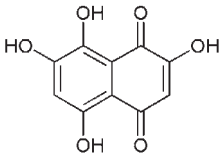
Phthiocol



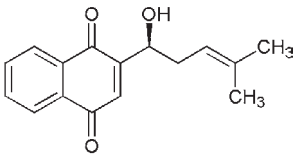
Lapachol



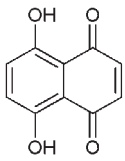
Flaviolin



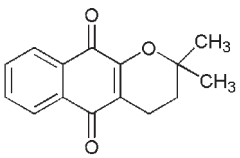
Mompain



Alkannin



Naphthazarine



Lapachone

Scheme 3.1-1 Natural naphthoquinones

1. Background: An exceptionally good dye for proteins

If you look at the structure of this molecule, you will find it small and simple at first glance. Indeed, the peculiarities of this compound are hidden. It is no wonder that it was made earlier in the laboratory than found in a natural source. Already the first to describe it in 1870 noticed its remarkable acidity and even named it “Naphthalinsäure” (naphthalinic acid) although they knew it belonged to the quinones [1]. Another expression of this property is the trivial name hennotannic acid. Interesting from the chemist’s point of view are the redox properties of naphthoquinones such as lawsone.

The driving force to deal with such naphthoquinone dyes was the upcoming dyestuffs industry. At the end of the 19th century it became the mother of industrial chemistry. About 50 years later, an Italian chemist discovered that the colouring matter in henna leaves had the same structure [2]. He gave the compound the trivial name lawsone due to its origin, the henna plant *Lawsonia inermis* L.. That it got the name *Lawsonia* is in turn based upon the fact that the henna plant was named by the great Swedish botanist Carl von Linné (1707−1778), who established the modern binomial nomenclature and is therefore known as the father of modern taxonomy. All plant names which have the abbreviation L. at the end evoke the name Linné. The reason why Linné gave the name *Lawsonia* to a genus within the family Lythraceae is that he wanted to thank the Scottish doctor of medicine Isaac Lawson, one of his investors who supported the publication of *Systema Naturae*, a book on the hierarchical classification of Nature in three large divisions, namely the plant, the animal and the mineral kingdoms, in 1735. The Latin term *inermis* means defenceless, i.e. a plant without spines. This is true for the young plant; mature plants have spines and were regarded as a separate species in Linné’s times.

There are several other relative natural 1,4-naphthoquinones, as shown in the margin. Another well-known compound is the subject of our question C. Lawsone occurs in the henna plant leaves in the form of glycosidic precursors that have to be cleaved prior to isolation. It was such henna constituents that were the basis to claim a combination of 1,2,4-trihydroxynaphthalene glycosides and glycosidases as a suitable means for hair dyeing [3].

The henna plant is a tall flowering shrub or tree about 5 m in height, native to tropical and subtropical regions of Africa, Asia and northern Australia (tropical savannah, the tropical arid zone, oases in the Sahara). Henna shrubs need light and warmth and are regarded as rather pest resistant. Henna flowers exhale a distinctive scent, appreciated in the Orient for millennia, a reason why henna also makes an ornamental bush in oriental house gardens.

The practical use of henna leaf powder as a dye for colouring hair and nails and for decoration of parts of the body temporarily is known as Mehndi (also known as Mehandi or Mehendi) traditionally in India, Pakistan, Iran and North Africa. The tradition is very old. Egyptian mummies decorated with henna paintings have been found. Naturally

made henna colorations are considered as harmless if they only contain lawsone and no chemical additives (see below). Some native tribes in North Africa apply henna paste on the skin without any decoration as a protection against the sun. This is possible, because lawsone strongly absorbs UV radiation and so do its covalent reaction products with the protein keratin in the skin.

Henna penetrates the dead cells of the horny outermost layer of the skin. To obtain a paste with colouring properties requires that dried and ground henna leaves are treated in a special manner including a warm and aqueous medium (see section 3.1 below) that ensures deglycosylation and aglycone (= lawsone) formation. The paste has a peculiar smell that is not to everyone's liking. How long the henna paste affects the skin is decided by the depth of the reddish brown coloration and how long it will be visible. Usually 6–8 h are required to achieve a satisfactory result. An oriental wedding is a typical event where the bride is decorated with lacy Mehndi body art. The deeper ritual reason for tinting the skin of the hands and feet with henna is the desire to avert the evil eye from a person, an ancient idea in Arabia. Keeping in mind the chemical structure, it is easy to understand why not only the skin but also silk and wool can be well dyed with henna. Similarly, the use of henna leaf extract as a general protein stain for both polyacrylamide gels and protein blots is comprehensible [4].

Mehndi decorations became known and fashionable in Western countries since the American singer–songwriter Madonna released the single "Frozen" in 1999 together with a video clip showing her with Arabian henna tattoos. However, tattooing with henna should be done with the utmost caution. Beware especially of a kind of so-called "black henna". This is a mixture of henna with synthetic *para*-phenylenediamine (PPD) intended to create a black stain. Many cases have been reported in which this PPD additive has caused an immediate severe allergic contact dermatitis reaction or induced a later allergy. However, there is another kind of harmless "black henna" containing only henna and indigo. Therefore, accurate information is essential.

Since ancient times, henna leaves have been used in traditional medicine as an astringent, antiseptic and antipyretic. Henna was used in ancient times also to treat serious diseases (leprosy, smallpox, chickenpox, tumours) by Arabian doctors. More resently, henna has been investigated and some physiological effects have been confirmed, e.g. bactericidal and fungicidal action (by its tanning effect). Henna itself is not an allergen, nor could rumours be proved that it might be a carcinogen. The wound-healing process is supported by a henna leaf extract – a fact that was already known to African healers, and was recently confirmed [5]. Recently, lawsone was found to be suitable as a reagent for the detection of latent fingermarks on paper, which is still an extremely important requirement in criminology as contact evidence. Lawsone, in this context, could serve as a substitute for ninhydrin, used hitherto [6].

Fig. 3.1-1 Young henna plant

She said, it hath reached me, O auspicious King, that the young merchant continued, when I entered and took a seat, the lady at once came in crowned with a diadem of pearls and jewels; her face dotted with artificial moles in indigo, her eyebrows pencilled with Kohl and her hands and feet reddened with Henna. When she saw me she smiled in my face and took me to her embrace and clasped me to her breast; then she put her mouth to my mouth and sucked my tongue (and I did likewise) and said, "Can it be true, O my little darkling, thou art come to me?" adding, "Welcome and good cheer to thee! By Allah, from the day I saw thee sleep hath not been sweet to me nor hath food been pleasant." Quoth I, "Such hath also been my case: and I am thy slave, thy negro slave."

The Book of the Thousand Nights and a Night, 26th Night
Translated from the Arabian by
Richard F. Burton

2. Literature

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[7] M. Shahid Khan, Z. H. Khan, "Ab initio and semiempirical study of structure and electronic spectra of hydroxy substituted naphthoquinones", *Spectrochimica Acta Part A* **2005**, *61*, 777−790.

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[11] K. P. Zeller, "Mass spectra of quinones", in S. Patai (Ed.), "The Chemistry of Quinonoid Compounds", Part 1, **1974**, pp. 231−255; K. P. Zeller, R. Müller, *ibid*, Vol 2, Part 1, **1988**, pp. 87−110.

Fig. 3.1-2 Branch of the henna plant

I walked around and tried to see if anybody walked behind me in any particular way. Then I sought out a restaurant that didn't smell of frying grease and found one with a purple neon sign and a cocktail bar behind a reed curtain. A male cutie with henna'd hair drooped at a bungalow grand piano and tickled the keys lasciviously and sang Stairway to the Stars in a voice with half the steps missing. I gobbled a dry martini and hurried back through the reed curtain to the dining room.

Raymond Chandler (1888−1959)
Farewell, My Lovely, 34

3. Isolation

3.1 Principle

Lawsone does not occur as such in henna plant leaves, but in the form of glycosidic precursors that have to be cleaved prior to isolation of the aglycone. It is a kind of miracle that the dried and powdered leaves do contain an intact glycosidase even after years, able to split the glycosidic bond, when brought into contact with hot water. Therefore, the henna leaf powder suspension is stirred for serveral hours in water at 70 °C.*

Because lawsone is not readily soluble in water but acidic (pK_a 4), at the end $NaHCO_3$ is added to make the aqueous phase weakly basic (pH 7.5) and to bring any lawsone into solution before the suspension is filtered. The filtrate is then acidified and lawsone is extracted into diethyl ether. From the solid crude product remaining after removal of the ether, lawsone is separated in a highly pure form by means of column chromatography. This material can be further purified by recrystallization from glacial acetic acid or by vacuum sublimation. Depending on the shape and size of the crystals formed, the colour of lawsone differs from an intense orange–yellow to dark red–brown. On the TLC plate in the eluent mentioned in all cases an intense dark orange spot can be observed.

[*] We want to thank Prof. Dr. L. Wessjohann, Leibniz Institute of Plant Biochemistry, Halle, Germany, for a kind discussion of this matter. A paper on these glycosides is in preparation by his group.

3.2 Method

Powder of dried henna leaves (40 g) is placed in a large beaker and distilled water (2 L) is added together with a magnetic stirring rod. The suspension is stirred on a magnetic stirrer with heating while the temperature is kept at 70 °C. After 45 min, the colour of the green suspension turns to brown. The increasing content of lawsone in the aqueous phase can be seen by TLC on standard silica gel plates with the eluent methanol–ethyl acetate (1:2, v/v) + 0.5% acetic acid. The R_f of the dark orange lawsone spot is 0.6. After 4 h, solid $NaHCO_3$ (8.4 g) is added. The suspension is filtered by gravity overnight over three large glass funnels with filter paper (diameter 30 cm). This kind of filtration is slow but works reliably. Attempts to force the pace by suction filtration are not advisable because then colloidal particles will rapidly plug the pores of the filter. The filtrates are combined and acidified to pH 3 by addition of 0.12 M HCl. The brown extract undergoes a clarification in this step and turns slightly cloudy. The swollen plant material is discarded. The filtrate is extracted with diethyl ether (4 × 200 mL). In the final extraction, the ether turns to a very pale yellow, indicating the end of extraction. The aqueous phase does not change its brown colour during extraction but turns clear and can be discarded after the extraction. The combined ethereal phases are washed with water (3 × 50 mL) and dried over $MgSO_4$. The ether is removed completely in vacuo to leave a reddish brown solid (660 mg) as crude product.

3.3 Purification

The crude lawsone is purified by column chromatography. Conditions (not optimized): column 40 × 3 cm; stationary phase, silica gel 60 (45 g, 0.040–0.063 mm); eluent, ethanol–ethyl acetate (1:2, v/v) (by using ethanol instead of methanol, any dissolution of tiny silica gel particles is suppressed). The crude product is dissolved in 10 mL of the eluent, placed on the top of the column and the elution is started. It is easily observable by the different coloured zones formed. Fractions of 10 mL are taken; in the region of the lawsone zone the fraction size is reduced to 3 mL. The composition of all fractions is checked by TLC.

Pour ses yeux, s'ils ne justifiaient pas entièrement ce qu'en disait la crédulité populaire, ils étaient au moins d'une étrangeté admirable; des sourcils bruns dont les extrémités seffilaient gracieusement comme les pointes de l'arc d'Eros, et que rejoignait une ligne de henné, à la mode asiatique, de longues franges de cils aux ombres soyeuses, contrastaient vivement avec les deux étoiles de saphir roulant sur un ciel d'argent bruni qui leur servaient de prunelles. Ces prunelles, dont la pupille était plus noire que l'atrament, avaient dans l'iris de singulières variations de nuances; du saphir elles passaient à la turquoise, de la turquoise à 'laiguemarine, de l'aigue-marine à l'ambre jaune, et quelquefois, comme un lac limpide dont le fond serait seméde pierreries, laissaient entrevoir, à des profondeurs incalculables, des sables d'or et de diamant, sur lesquels des fibrilles vertes frétillaient et se tordaient en serpents d'émeraudes.

T. Gautier (1811–1872)
Le Roi Candaule

The first lawsone fractions contain the tailing of a compound with lower polarity, unfortunately, which means a decrease in lawsone yield. Therefore, the next fractions 28–34 are combined and the solvent is completely removed in vacuo, including the use of a rotary vane pump. These fractions contain small amounts of a less polar impurity and are recrystallized from glacial acetic acid to yield 33 mg of brown crystals (mp 193–195 °C) after filtration, washing with *n*-hexane and drying in vacuo. Under the microscope, a sublimation process can be seen at 170 °C; the final melting is dark red. The next fractions 35–38 did not show any spots other than lawsone in TLC and yielded 23 mg of a red-brown powder (mp 191–193 °C). Fractions 39–48 are worked up and the output is subjected to vacuum sublimation (heating bath temperature increased from 140 to 160 °C within 1 h; pressure from 0.4 to 0.04 mbar). A dark yellow sublimate is formed (5 mg, mp 191–193 °C), pure according to TLC. All three products are checked by NMR for their purity. The recrystallized sample is used to measure the spectra given here.

Note: the chromatography described here can surely be optimized in respect of the lawsone yield: by separating a smaller amount of crude product, or by using a longer column or by using another eluent. The crucial problem of increasing the yield is to elute completely the impurity less polar than lawsone before lawsone itself is eluted.

Fig. 3.1-3 Neutral and alkaline solution of lawsone

Fig. 3.1-4 Henna powder used for isolation of lawsone

4. Spectra and Comments

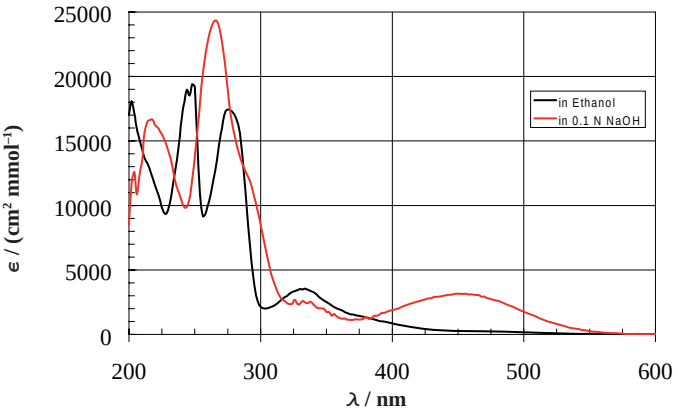


Fig. 3.1-5 UV spectrum in ethanol

The UV spectrum of lawsone in ethanol is of course similar to that of 1.4-naphthoquinone, but the main band of 1.4-naphthoquinone at 245 nm is split in lawsone into two π–π* transitions at 248 and 276 nm. Remarkable is the long tail of the band at 334 nm reaching far into the visible region, which is responsible for the yellowish colour of lawsone. It was suggested [6] that the HOMO has a large contribution from the hydroxyl group. If one removes the acidic proton and measures the UV spectrum in 0.1 N NaOH, the compound gives an orange colour and in the spectrum an additional band at about 450 nm appears (bathochromic shift).

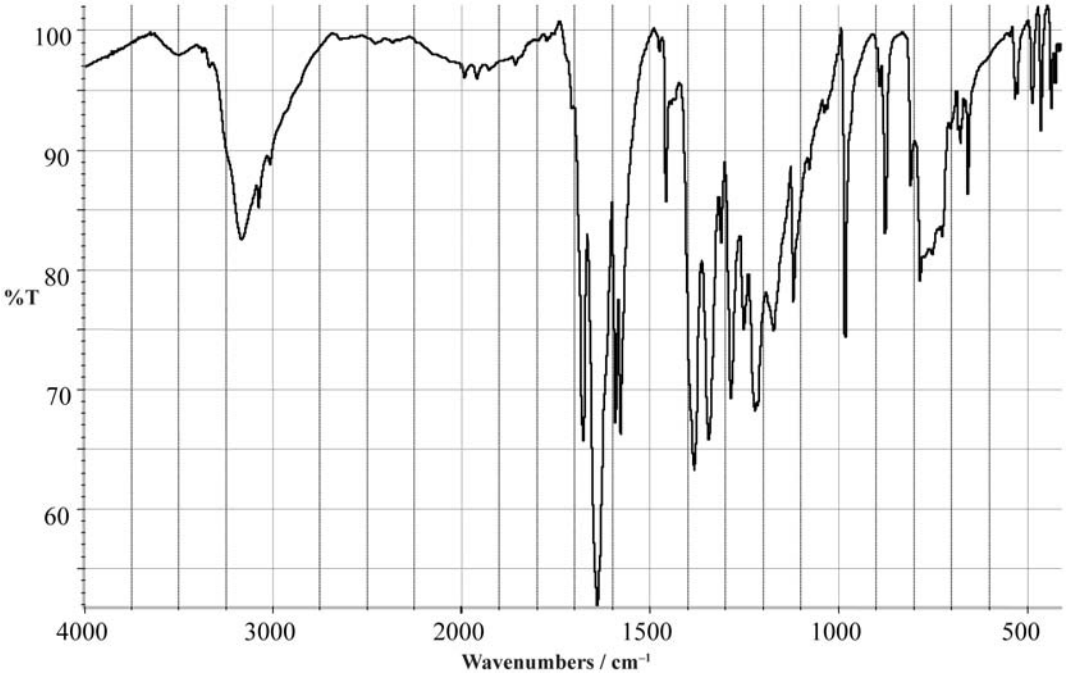
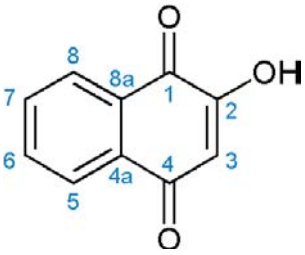


Fig. 3.1-6 IR spectrum in KBr

There is considerable discussion in the literature [7] as to whether in lawsone a significant hydrogen bond exists. The IR spectrum reveals a broad band for the OH valence vibration which overlays the CH vibrations. The carbonyl stretching frequency is split with values of 1680 and 1640 cm^{-1} and this could be due to some contribution of an internal hydrogen bond. The C=C vibrational bands of the naphthalene ring appear at their usual location between 1580 and 1600 cm^{-1}.



Scheme 3.1-2

Fig. 3.1-7 Henna leaves in spring

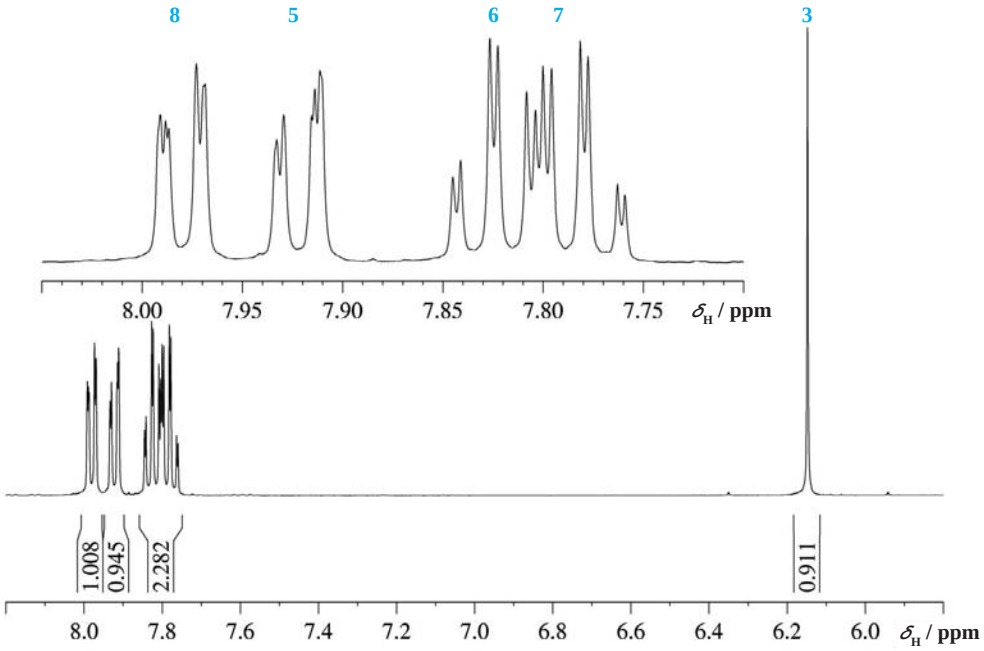


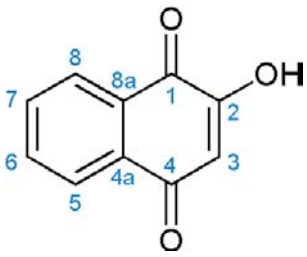
Fig. 3.1-8 ^{1}H NMR spectrum at 400 MHz in DMSO-d_6

In the proton spectrum we find a very typical aromatic ABCD spin system of the naphthalene ring system which consists of two doublets and two triplets where each multiplet is further split by a small spin coupling. Whereas it is clear that the doublet-type signals belong to H-5 and H-8 and the triplet-type signals to H-6 and H-7, their individual assignment cannot be deduced from the ^{1}H NMR spectrum. The signal of H-3 appears as a sharp singlet at 6.15 ppm. The OH group signal (not shown) is at 13 ppm in DMSO-d_6 and this indicates its rather acidic nature.

I have compared you, my love, to a steed in Pharaoh's chariots.
Your cheeks are beautiful with earrings, your neck with
strings of jewels.
We will make you earrings of gold, with studs of silver.
Beloved
While the king sat at his table, my perfume spread its fragrance.
My beloved is to me a sachet of myrrh, that lies between my breasts.
My beloved is to me a cluster of henna blossoms from the vineyards
of En Gedi.

A locked up garden is my sister, my bride; a locked up spring,
a sealed fountain.
Your shoots are an orchard of pomegranates, with precious fruits:
henna with spikenard plants,
spikenard and saffron, calamus and cinnamon, with every kind
of incense tree; myrrh and aloes, with all the best spices,
a fountain of gardens, a well of living waters, flowing streams
from Lebanon.

Old Testament, Song of King Solomon, 1; 9−14 and 4, 12−15



Scheme 3.1-3

Amid the eddy of these dream-fragments, amid the smell of henna and the twanging of the guitar, amid the waves of air charged with fragrant spray, I would catch like a flash of lightning the momentary glimpse of a fair damsel. She it was who had saffron-coloured paijamas, white ruddy soft feet in gold-embroidered slippers with curved toes, a close-fitting bodice wrought with gold, a red cap, from which a golden frill fell on her snowy brow and cheeks.
She had maddened me. In pursuit of her I wandered from room to room, from path to path among the bewildering maze of alleys in the enchanted dreamland of the nether world of sleep.

Rabindranath Tagore (1861−1941)
The Hungry Stones and Other Stories

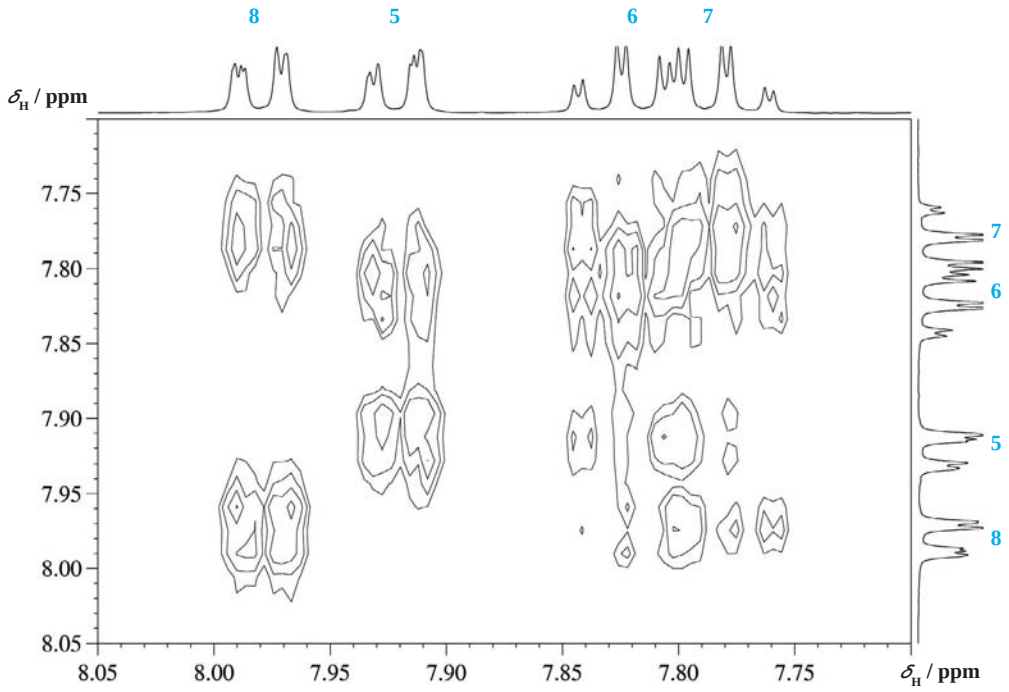


Fig. 3.1-9 COSY spectrum

The COSY spectrum only reveals the connectivity between the doublet and triplet signals, and one cannot decide which signal belongs to either H-5 or H-8, or to H-6 or H-7.

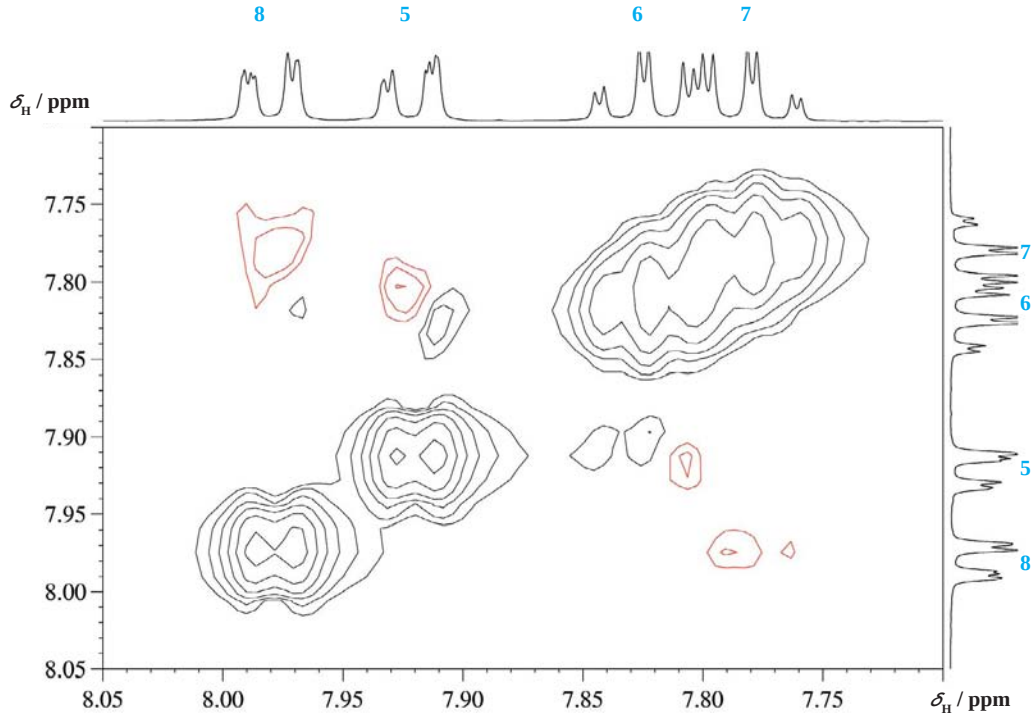


Fig. 3.1-10 NOESY spectrum

Similarly to the COSY spectrum the NOESY spectrum is in this case of no special value. The distance from H-3 to H-5 is too large to help in the assignment. Otherwise, the NOESY spectrum corroborates the information already obtained from the COSY spectrum.

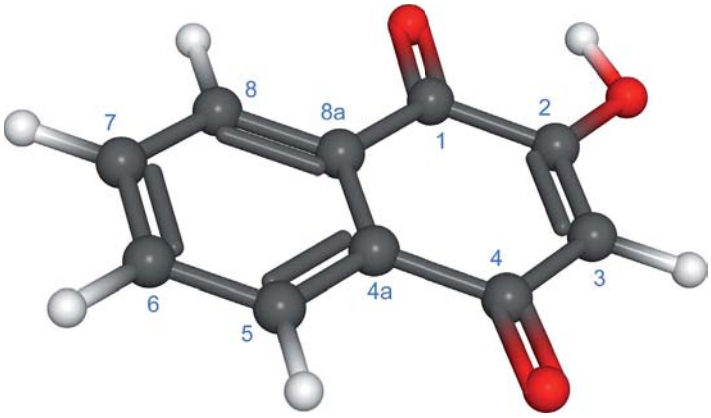


Fig. 3.1-11 Molecular model of lawsone

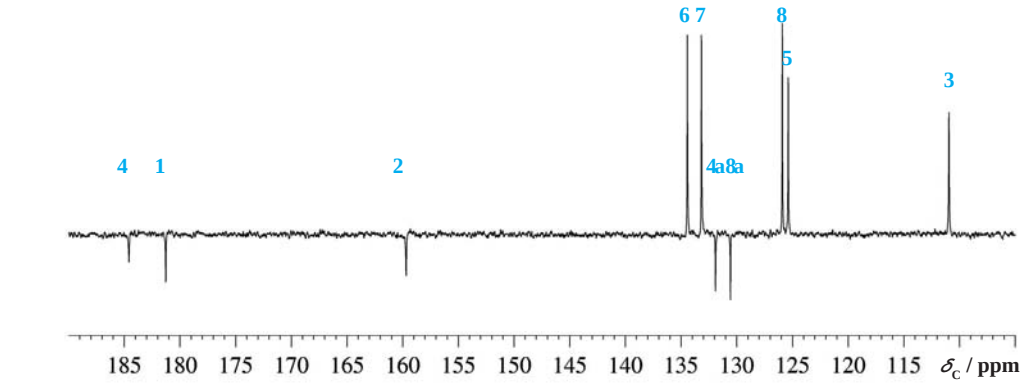


Fig. 3.1-12 APT ^{13}C NMR spectrum in DMSO-d_6

The ^{13}C NMR spectrum shows three quaternary carbon atoms at 184.6, 181.3 and 159.7 ppm. The two signals at 184.6 and 181.3 ppm clearly belong to the carbonyl C-1 and C-4 atoms. One might speculate about their individual assignment and common chemical shift theory would assign the more deshielded signal to C-4, whereas the signal of C-2 clearly appears at 159.7 ppm. The two remaining quaternary carbon atoms C-4a and C-8a resonate very closely together at 131.9 and 130.6 ppm and we do not attempt here to assign them individually. Similarly, the four CH moieties of the naphthalene ring are difficult to assign individually, whereas it is fairly safe to assign the signal at 110 ppm to C-3 due to its β-position with respect to the hydroxyl group.

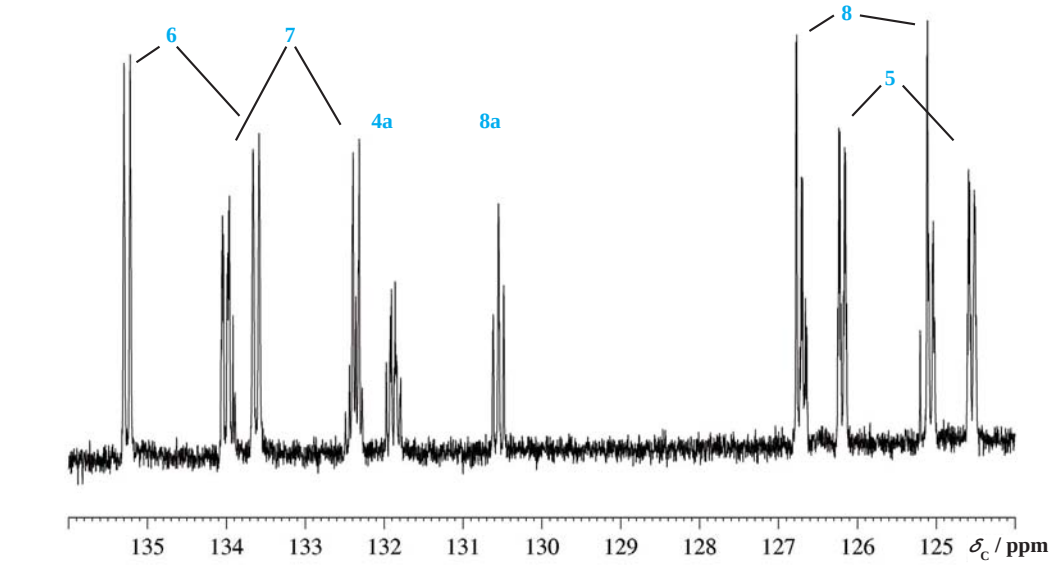


Fig. 3.1-13 Gated decoupled ^{13}C NMR spectrum

The gated decoupled ^{13}C NMR-spectrum, currently a rather forgotten technique, shows a beautifully resolved aromatic spin system. The assignments could be taken from this directly with the appropriate knowledge on CH coupled spin systems. We use it here only for the distinction between C-4a and C-8a. Their individual assignment can be obtained by comparing the two multiplets at 131.9 and 130.6 ppm (see Question J).

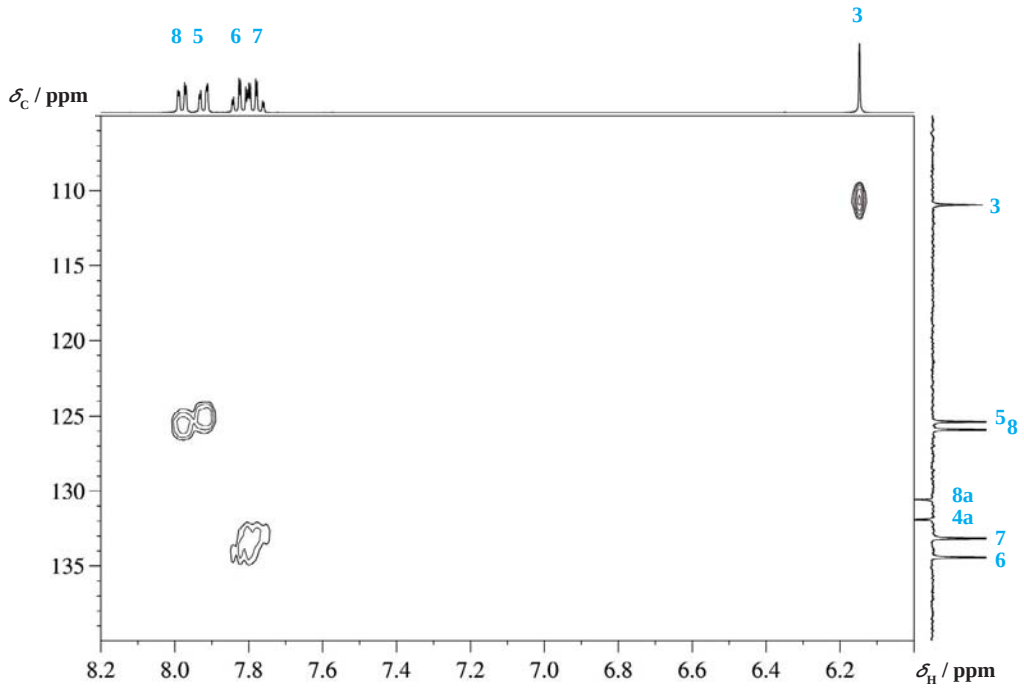


Fig. 3.1-14 HSQC spectrum

The HSQC spectrum first confirms our assignment for C-3 due to its correlation signal with H-3. We also see that the two proton doublets belong to the carbon signals at about 125 ppm and the two proton triplets to those at about 134 ppm, but we are still not able to perform a safe individual assignment.

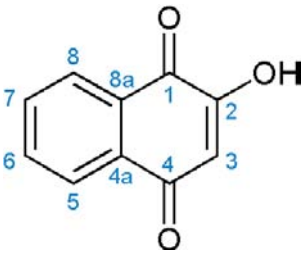
Taanach revint près d'elle; et quand elle eut disposé deux candélabres dont les lumières brûlaient dans les boules de cristal pleines d'eau, elle teignit de lausonia l'intérieur de ses mains, passa du vermillon sur ses joues, de l'antimoine au bord de ses paupières, et allongea ses sourcils avec un mélange de gomme, de musc, d'ébène et de pattes de mouches écrasées.

Salammbô, assise dans une chaise à montants d'ivoire, s'abandonnait aux soins de l'esclave. Mais ces attouchements, l'odeur des aromates et les jeûnes qu'elle avait subis, l'énervaient. Elle devint si pâle que Taanach s'arrêta.

"Continue!" dit Salammbô, et, se roidissant contre elle-même, elle se ranima tout à coup.

Gustave Flaubert (1821−1880)
Salammbô

Fig. 3.1-15 A henna bush



Scheme-3.1-4

Her eyelashes, though dark as night, were tinged
(It is the country's custom), but in vain;
For those large black eyes were so blackly fringed,
The glossy rebels mock'd the jetty stain,
And in their native beauty stood avenged:
Her nails were touch'd with henna; but again
The power of art was turn'd to nothing, for
They could not look more rosy than before.

The henna should be deeply dyed to make
The skin relieved appear more fairly fair;
She had no need of this, day ne'er will break
On mountain tops more heavenly white than her.

Lord Byron (1788−1824)
Don Juan

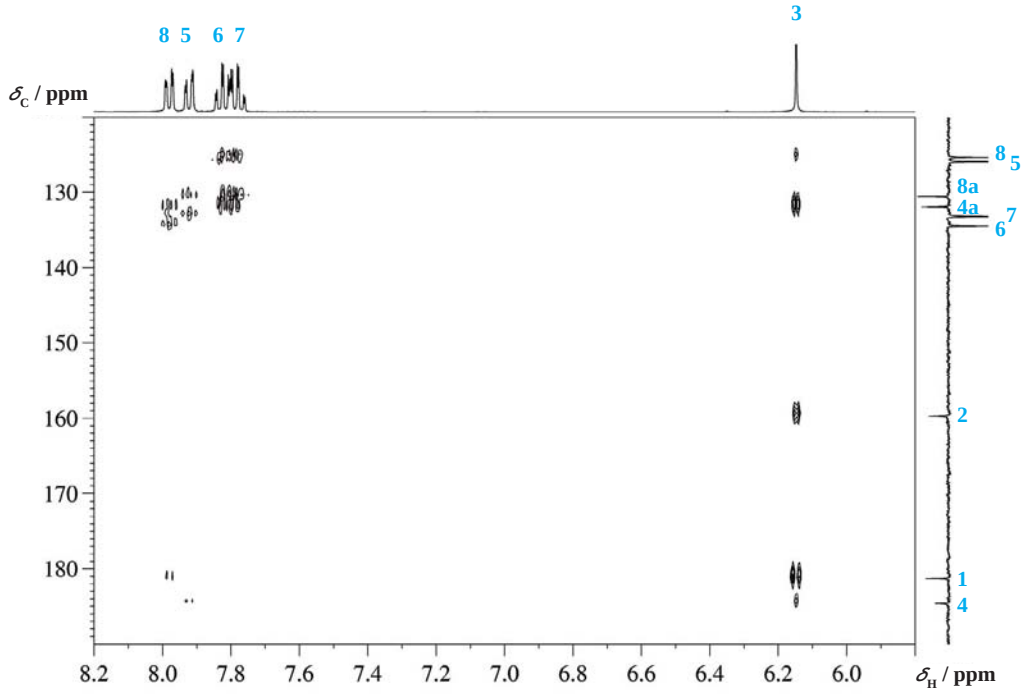
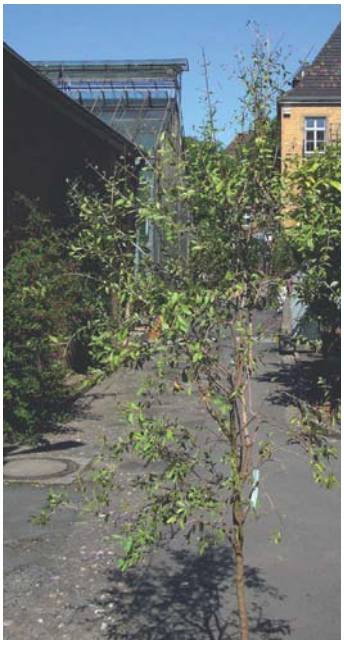


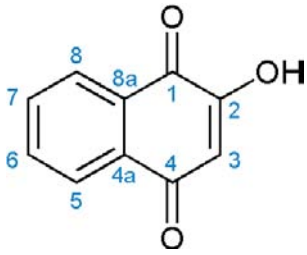
Fig. 3.1-16 HMBC spectrum

The HMBC spectrum finally resolves all unanswered questions. Inspection of the correlation signals for H-3 reveals a stronger and a weaker signal to the two carbonyl C-atoms. Clearly, the stronger coupling is via three bonds to C-1 and the weaker via two bonds to C-4, which firmly assigns the two carbonyl signals in accordance with our earlier suggestion. Since we know now that the more deshielded carbonyl C-atom at 184.6 ppm belongs to C-4, we can assign the proton doublet signal at 7.9 ppm to H-5 and the more deshielded at 8.0 ppm to H-8 on the basis of their correlation signals with the carbonyl C-atoms. The relative assignment for H-6 and H-7, and that for C-6 and C-7, follows then by consideration of the COSY and HSQC results.

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz	$^{1}J_{C,H}$ / Hz
184.6	C_q	C-4		
181.3	C_q	C-1		
159.7	C_q	C-2		
134.4	CH	C-6	7.82, $J_{6,5}$ = 7.47, $J_{6,7}$ = 7.47, $J_{6,8}$ = 1.41	164.8
133.2	CH	C-7	7.78, $J_{7,8}$ = 7.41, $J_{7,6}$ = 7.47, $J_{7,5}$ = 1.41	166.0
131.9	C_q	C-4a		
130.6	C_q	C-8a		
125.9	CH	C-8	7.98, $J_{8,7}$ = 7.39, $J_{8,6}$ = 1.53	160.2
125.4	CH	C-5	7.92, $J_{5,6}$ = 7.45, $J_{5,7}$ = 1.43	165.4
110.9	CH	C-3	6.15	163.3

Table 3.1-1 NMR data for lawsone





Scheme 3.1-5

Fig. 3.1-17 Henna on a street

He held up the flap that gave access to the booth, and Susie went in.
Margaret and Arthur Burdon, somewhat against their will, were obliged to follow. The native closed the opening behind them. They found themselves in a dirty little tent, ill-lit by two smoking lamps; a dozen stools were placed in a circle on the bare ground. In one corner sat a fellah woman, motionless, in ample robes of dingy black. Her face was hidden by a long veil, which was held in place by a queer ornament of brass in the middle of the forehead, between the eyes. These alone were visible, large and sombre, and the lashes were darkened with kohl: her fingers were brightly stained with henna. She moved slightly as the visitors entered, and the man gave her his drum. She began to rub it with her hands, curiously, and made a droning sound, which was odd and mysterious. There was a peculiar odour in the place, so that Dr Porhoet was for a moment transported to the evil-smelling streets of Cairo. It was an acrid mixture of incense, of attar of roses, with every imaginable putrescence. It choked the two women, and Susie asked for a cigarette. The native grinned when he heard the English tongue. He showed a row of sparkling and beautiful teeth.

W. Somerset Maugham (1874–1965)
The Magician

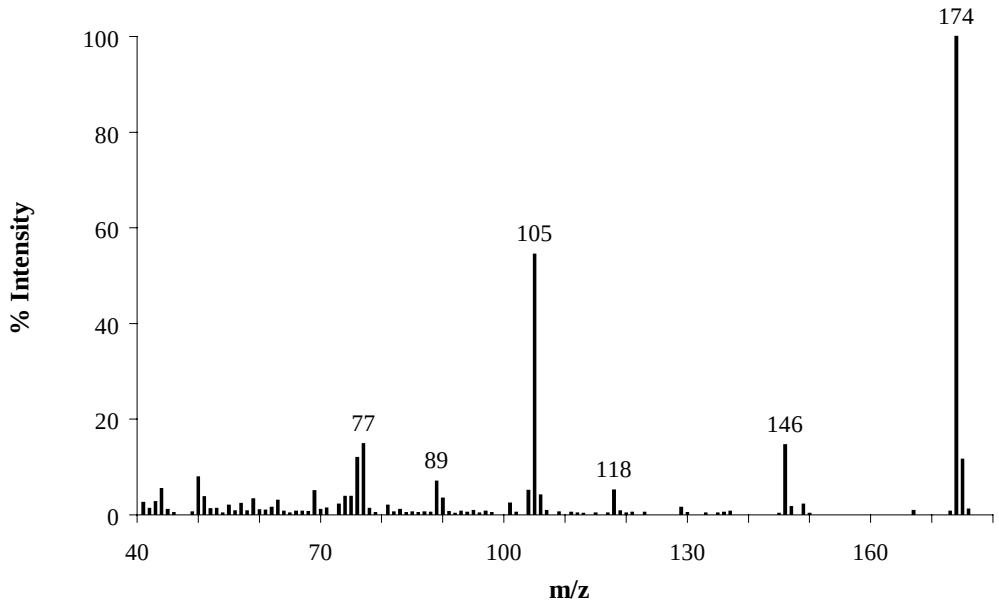
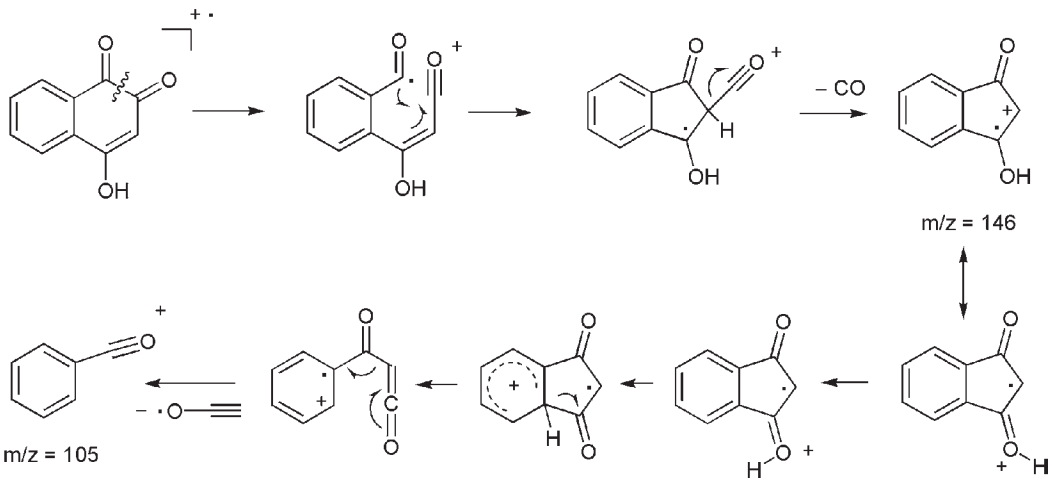


Fig. 3.1-18 Mass spectrum (EI)

The mass spectrum gives the molecular ion as the base peak, pointing to the stability of this naphthoquinone. There has been a discussion in the literature on the loss of CO leading to the ion with m/z = 146. Contrary to the first assumption that one of the carbonyl groups is eliminated, it was shown by ^{13}C labelling that mainly C-2 is lost, as indicated in the scheme below. The ion with m/z = 146 can form the benzoyl ion with m/z = 105, which finally loses CO to give the phenyl ion with m/z = 77.



Scheme 3.1-6 Fragmentation of lawsone

5. Questions

A. What is the structural reason for the low pK_a value of 4 of lawsone? Compare its acidity with that of acetic acid.

B. Rate the redox properties of a compound such as lawsone. Which vitamins contain a related structural pattern? What is their biological function?

C. The green husks of the common walnut tree (*Juglans regia* L.) contain a regioisomer of lawsone known for its remarkable allelopathic properties, i.e. the ability to suppress or even inhibit the growth of other plants in the vicinity of the walnut tree. Whereas this is no problem for humans, it should be kept in mind that collecting freshly dropped walnuts still enclosed by their husk will likely end in discoloured fingers by the same effect of staining as described for lawsone, as experienced by one of the authors as a child. Give the name and structure of this compound.

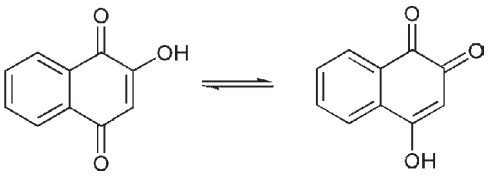
D. On searching for lawsone you may encounter the acronym HNQ. What does it mean?

E. Explain chemically why lawsone from henna is especially suitable for dyeing wool and silk fibres, by which it is fixed well.

F. Draw a formula which explains the new band in the UV spectrum under alkaline conditions.

G. There are several small bands in the IR spectrum between 1800 and 2000 cm^{-1}. What are these called in the laboratory jargon and what do they indicate?

H. There has been a suggestion that lawsone is in equilibrium with a tautomeric form as shown. Discuss the likelihood of this form on the basis of the UV spectrum.



Scheme 3.1-7

I. Give a reason why C-4 is more deshielded than C-1.

J. We have assigned C-4a and C-8a on the basis of their multiplets in the gated decoupled ^{13}C NMR spectrum. Explain in detail.

K. Suggest a structure for the ions with m/z = 118 and 89.

6. Own Observations

3.2 Curcumin

(1*E*,4*Z*,6*E*)-5-Hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one

From turmeric
Curcuma longa L. (Zingiberaceae)

$C_{21}H_{20}O_6$: MW 368.39

CAS RN 458-37-7, BRN 1894001, 2016701, 2306965

Orange crystals, mp 182−184°C

Curcumin is commercially available.

Synonymous names:
1,7-Bis(4-hydroxy-3-methoxyphenyl)-(1*E*,6*E*)-1,6-heptadiene-3,5-dione, CI Natural Yellow 3, CI 75300, Diferuloylmethane, E 100, Indian Saffron, Turmeric, Yellow Root

Level: medium

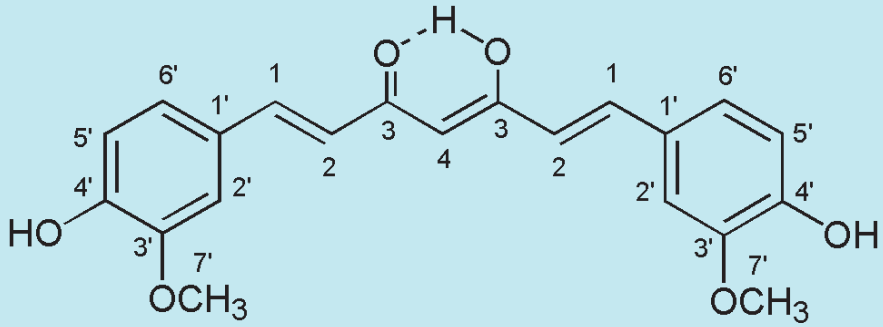


Fig. 3.2-1 *Curcuma longa* plants in a greenhouse

"Ah," said the Uncle, "but in India we learn how to freeze our blood and boil it at the same time."
In those hot longitudes, perhaps, the blood is always near boiling-point, which accounts for Indian tempers, though not for the curry and pepper they eat. But I must not wander; there is no curry at all in this story. About temper I will not say.

Edith Nesbit (1858−1924)
The Woodbegoods

1. Background:
An edible indicator only – for hardboiled ones

Again, this is a chapter that deals with something one can eat. For 4000 years, turmeric has been known as an Indian spice. Turmeric is a tropical perennial plant from the ginger family (Zingiberaceae). It has a short stem with large longish leaves. It bears egg-shaped or elongated rhizomes, often branched and of a brownish−yellow colour. Still today, India produces and consumes most of it, 80% of the world's crop. It is a basic constituent of curry powders and blended with cumin, coriander and fenugreek. Besides contributing to the taste by its essential oils, turmeric powder gives the mixture its characteristic orange colour due to curcumin, our compound of interest. It is said that any Indian housewife and cook has their own recipe for an individual curry powder, using other ingredients such as fennel seed, mustard seed, ginger, clove, cardamom, cinnamon, pepper, nutmeg, garlic, etc. Of course, each composition is a secret. In India, turmeric is also considered as auspicious and therefore it is part of religious rituals.

Turmeric has conquered the ethnic borders of Southeast Asia as a culinary delight, imparting both a pleasant colour and smell to dishes. Turmeric is also used as "Indian saffron" to give other foods such as mustard a yellow colour because it is much cheaper than real saffron. The EU classification for curcumin as a food dyestuff is E 100. Its properties as a fabric dye are insufficent because it is not lightfast enough and unstable under alkaline conditions. However, the food dyestuff properties have been important enough to ensure turmeric a listing in the Colour Index as Natural Yellow 3, or CI 75300. Curcumin is contained in the turmeric rhizome together with some close structural relatives at a level of 3−4 %. The amount of essential oils is up to 6% (terpenoids, steam distillable − if desired).

The most important trading centre for turmeric is in the city of Sangli in the Indian state of Maharashtra. It can be used in the kitchen in dried and powdered form (India) or fresh and ground (Thailand). A further advantage of the main ingredient curcumin is that the addition of 1−2% of turmeric to stored cereal is an efficient means to protect it from pest attacks.

Curcumin was of interest early as a natural product [1,2]. Interestingly, already in the early second paper [2], still without a structural description of curcumin, two analytical aspects are mentioned. The first is that "Curcumapapier" (curcuma paper) is mentioned, which was used as an early indicator paper, the turning point being at pH 8−9 with a transition from yellow (acidic) to brown (basic). The second is that the possibility of verifying borates by formation of a coloured complex (called rosocyanine) using curcumin was known and is described. It took some decades until, after a long scientific debate, the correct constitution was suggested [3]. Its elucidation was strongly impelled by the knowledge gathered and required by colour and dyestuff chemistry, which was one of the main industrial driving forces for chemistry at that time. Eventually, the structure was proved by synthesis [4].

Biosynthetically, one aromatic ring of curcumin originates from the shikimate pathway, and the other from the acetate/malonate pathway of flavonoid biosynthesis.

The possibilities of using turmeric in medicine have been known in India and China for millennia and have been used practically in Ayurvedic and similar systems of traditional medicine. The diseases cured with turmeric include anorexia, biliary disorders, cough, hepatic disorders, diabetic wounds, sinusitis and rheumatism. Only in the last decade have scientists outside India also recognized the potential of turmeric. This realization initiated a real explosion in research on turmeric ingredients in combination with the desire to find other applications.

Curcumin has been found to show, e.g., antioxidant properties, anti-inflammatory effects, hypotensive, hypocholesteraemic and anti-cancer properties (manifold, through induction of apoptosis), haemostatic properties and anti-amyloid properties following oral or topical administration. It has been shown to reduce the severity of cystic fibrosis, Alzheimer's and Parkinson's disease and even stroke. Its mechanisms of action include inhibition of cell signalling pathways and effects on enzymes such as cyclooxygenase and glutathione-S-transferase. Oral administration is well tolerated – not unexpected if one thinks of its use as spice through the ages. The bioavailability is relatively low – this can be understood in terms of its hydrophobicity. After absorption curcumin is rapidly metabolized, with a glucuronide as one of the main metabolites. Contraindications are known: people suffering from gallstones, bile duct obstructions, stomach ulcers or stomach hyperacidity are advised to abstain from turmeric. More than 3500 papers have dealt with curcumin, among them over 200 reviews. A massive increase in interest in this natural product from a readily available source (not an unimportant aspect!) can be observed from around 2000 on. Representatively, a few reviews [5-8] can be cited.

In summary, turmeric and its main component curcumin have a widespread profile of biological activity; however, as with many natural products, it is the dose that decides the effect, none of them can be regarded as the philosopher's stone.

The place was full of English soldiery as they passed. English bugles woke them in the morning; at nightfall they went to bed to the note of the British fife and drum: all the country and Europe was in arms, and the greatest event of history pending: and honest Peggy O'Dowd, whom it concerned as well as another, went on prattling about Ballinafad, and the horses in the stables at Glenmalony, and the clar't drunk there; and Jos Sedley interposed about curry and rice at Dumdum; and Amelia thought about her husband, and how best she should show her love for him; as if these were the great topics of the world.

William Makepeace Thackeray
(1811−1863)
Vanity Fair

2. Literature

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[2] "Curcumin", *Ann. Chem. Pharm*. **1842**, *44*, 297−298.

[3] J. Milobedzka, S. v. Kostanecki, V. Lampe, "Zur Kenntnis des Curcumins" [On the knowledge of curcumin] *Ber. Dtsch. Chem. Ges.* **1910**, *43*, 2163−2170.

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[5] F. Campbell, G. P. Collett, "Chemopreventive properties of curcumin" *Future Oncology*, **2005**, *1*, 405−414.

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[10] A. Arrieta, L. Beyer, E. Kleinpeter, J. Lehmann, M. Dargatz, "Synthesis and proton NMR spectroscopic investigations of new curcumin analogs" *Chemiker-Zeitung* **1992**, *334*, 696−700.

3. Isolation

3.1 Principle

Curcumin as a diarylheptanoid shows a similar orange−yellow colour to the carotinoid capsanthin. In both cases the colour arises from an extensively conjugated π-system, which includes two aromatic rings in the case of curcumin. Therefore, in this case light absorption does not lead to isomerization of double bonds as with capsanthin. Hence light protection during isolation is not necessary. Although curcumin contains three hydroxyl groups, in solution it is lipophilic enough to be extracted with methylene chloride by Soxhlet extraction from turmeric powder. The residue obtained is subjected to two column chromatographies to yield pure crystalline curcumin.

"Siehste", sagte sie, schüttete etwas Curry in die heiße Pfanne, schnitt dann mit dem Messer eine Kalbswurst in Scheiben hinein, sagte "Weißwurst, grausam, und dann noch süßer Senf. Das veddelt einen doch." Sie schüttelte sich demonstrativ: Brrr, klackste Ketchup in die Pfanne; rührte; gab noch etwas schwarzen Pfeffer darüber und schob dann die Wurstscheiben auf den gefältelten Pappteller: "Das is reell. Hat was mitm Wind zu tun. Glaub mir Scharfer Wind braucht scharfe Sachen."
Ihr Schnellimbiß stand wirklich an einer windigen Ecke. Die Plastikbahne war dort, wo sie am Stand festgezurrt war, eingerissen, und hin und wieder, bei stärkeren Böen, kippte eine der großen Plastik-Eistüten um. Das waren Reklametische, auf deren abgeplattetem Eis man die Frikadellen und, wie gesagt, diese ganz einmalige Currywurst essen konnte.
"Ich mach die Bude dicht, endgültig."

Uwe Timm (1940−)
Die Erfindung der Currywurst

3.2 Method

Turmeric powder (50 g) is placed in the thimble of a Soxhlet extractor and extracted for 4 h with methylene chloride (500 mL). The content remaining in the thimble has a mass of 45 g after drying. The extract obtained is filtered. The clear orange filtrate is reduced to a volume of 20 mL in vacuo. This concentrate is allowed to stand in a refrigerator at 4 °C overnight. Crude curcumin crystallizes and is filtered off through a sintered glass filter funnel to yield 398 mg of orange crystals with mp 175−178 °C, which TLC shows not to be pure yet.

TLC conditions are: stationary phase, silica gel 60_{F254} plates (Merck); eluent, dichloromethane−methanol (99:1, v/v); R_f of curcumin = 0.45, R_f of main side components = 0.20 and 0.08.

3.3 Purification

The crude curcumin thus obtained is subjected to two column chromatographies.

Conditions: first run: column, 60 × 1.5 cm; stationary phase, silica gel 60 (45 g, 0.040–0.063 mm); eluent, dichloromethane–methanol (99.3: 0.7, v/v), 750 mL.

Crude curcumin (398 mg) is added to 5 mL of eluent; fractions of 1 mL are collected.

Fractions of curcumin obtained are analysed by TLC on silica gel 60_{F254} plates in the TLC eluent. All fractions containing mainly curcumin are collected, the solution is reduced to dryness in vacuo and the remaining residue recrystallized from ethanol (10 mL) to yield orange crystals (125 mg, mp 178–182 °C). However, TLC shows them still to contain a small amount of the first side component, obviously by a tailing effect of curcumin.

Therefore, a second run is necessary with all curcumin obtained from the first run. A column of 40 × 4 cm is used with the elution system as above. Fractions of 1 mL are collected and all containing curcumin (according to TLC) were combined and worked up as above to yield pure curcumin as orange crystals (75 mg, mp 182–184 °C, in accordance with literature values).

4. Spectra and Comments

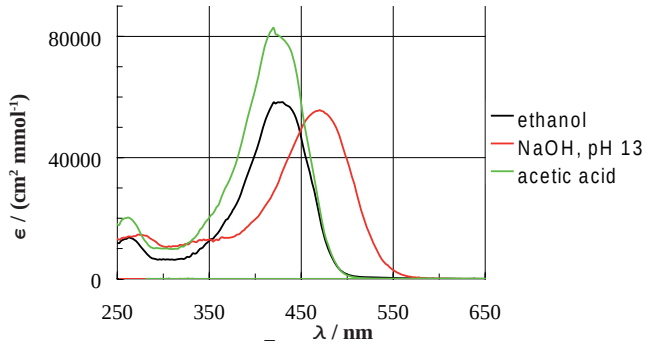


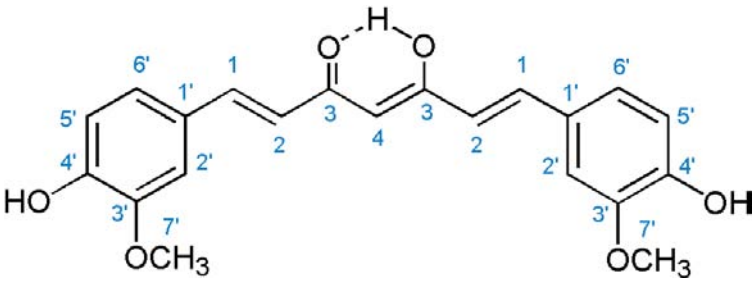
Fig. 3.2-3 UV spectrum in acetic acid, ethanol and NaOH

The UV spectrum is strongly pH dependent. On increasing the pH, the absorption of the band at 430 nm experiences a bathochromic shift. The colour of the solution changes from orange to red, but these solutions at high pH are unstable and must be measured directly.

Have you ever observed the remarkable adherence to set forms of speech which characterizes the talkers of arrant nonsense! Precisely the same sheepish following of one given example distinguishes the ordering of genteel dinners. When we gave a dinner at home, we had gravy soup, turbot and lobster-sauce, haunch of mutton, boiled fowls and tongue, lukewarm oyster-patties and sticky curry for side-dishes; wild duck, cabinet-pudding, jelly, cream and tartlets. All excellent things, except when you have to eat them continually. We lived upon them entirely in the season. Every one of our hospitable friends gave us a return dinner, which was a perfect copy of ours–just as ours was a perfect copy of theirs, last year.

Wilkie Collins (1824–1889)
A Rogue's Live

Fig. 3.2-2 Turmeric powder used for isolation of curcumin



Scheme 3.2-1

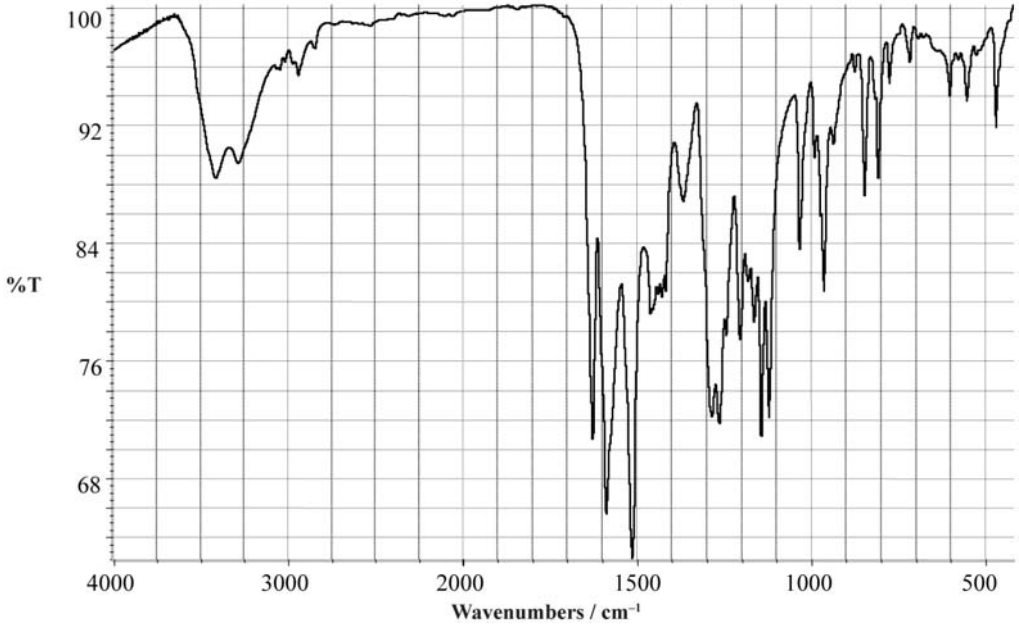


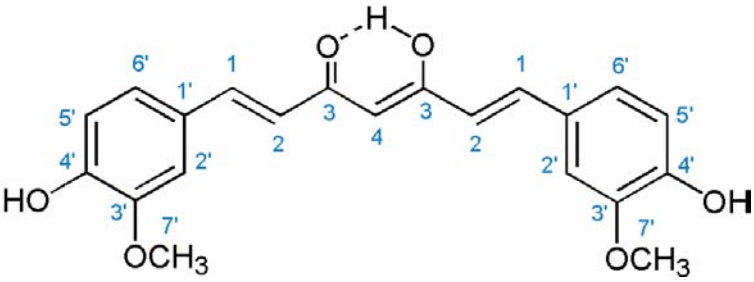
Fig. 3.2-4 IR spectrum in KBr

The IR spectrum displays the typical OH stretching band for phenolic compounds. In the double bond region one would expect a C=O and the aromatic C=C vibration. Two narrow bands are seen at 1630 cm^{-1} and at 1600 cm^{-1}. Comparison with acetylacetone shows two similar bands at this frequency.

Nature has implanted an exquisite sympathy on this subject, which extends through all her works. It is an invariable attribute of the female heart, to melt at the cry of early helplessness, and to take an instinctive interest in the distresses of the parent and the young. On the present occasion, the ladies of the family were full of pity and commiseration; and I shall never forget the look that lady Lillycraft gave the general, on his observing that the young birds would make an excellent curry, or an especial good rook-pie.

Washington Irving (1783−1859)
Bracebridge Hall or the Humorists: Family Misfortunes

Fig. 3.2-5 The colour of spices is a central feature of an oriental bazaar



Scheme 3.2-2

Die Krähe

Die Krähe lacht. Die Krähe weiß,
Was hinter Vogelscheuchen steckt,
Und daß sie nicht wie Huhn mit Reis
Und Curry schmeckt.

Joachim Ringelnatz (1883−1934)

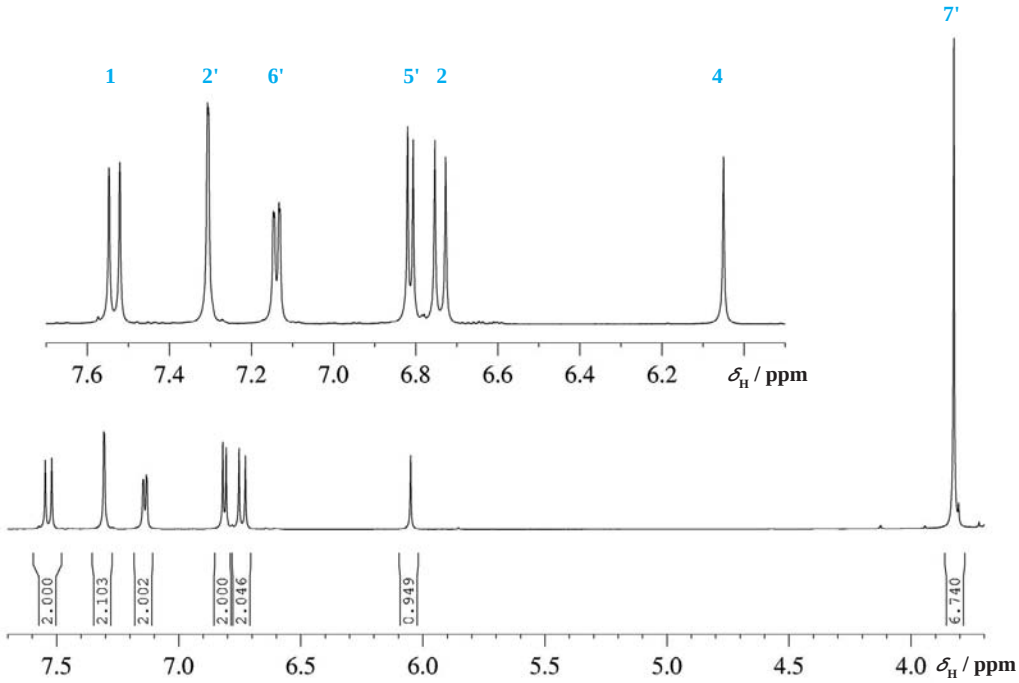
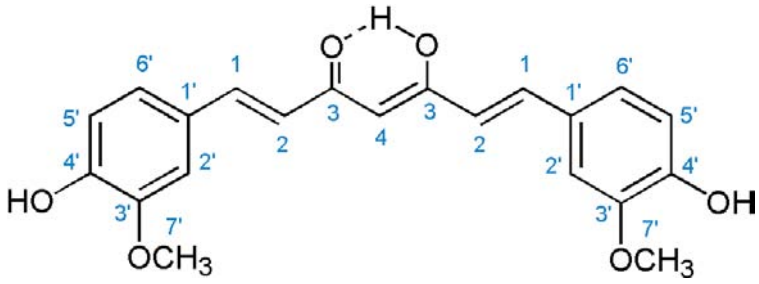


Fig. 3.2-6 ^{1}H NMR spectrum at 600 MHz in DMSO-d_6

The proton NMR spectrum shows in the aromatic region an AX spin system at δ_H = 7.53 and 6.74 ppm with a spin coupling of 15.8 Hz, which can easily be assigned to the protons H-1 and H-2 of the *trans* olefinic bond. This AX spin system embraces the aromatic protons 2', 5' and 6' of the 1,3,4-trisubstituted aromatic ring systems. As also seen in the spectra of eugenol (section 2.2), these protons display a very typical pattern and their individual assignment is obvious due to the small spin coupling between H-2' and H-6'. The singlet at 6.05 ppm integrating for only one proton is assigned to H-4 and confirms the symmetric structure as drawn in the enolic form. The assignment for the methoxy group signal at 3.82 ppm is obvious. The enolic OH proton is in exchange with the phenolic OH protons and residual water of the solvent (not shown).

Scheme 3.2-3



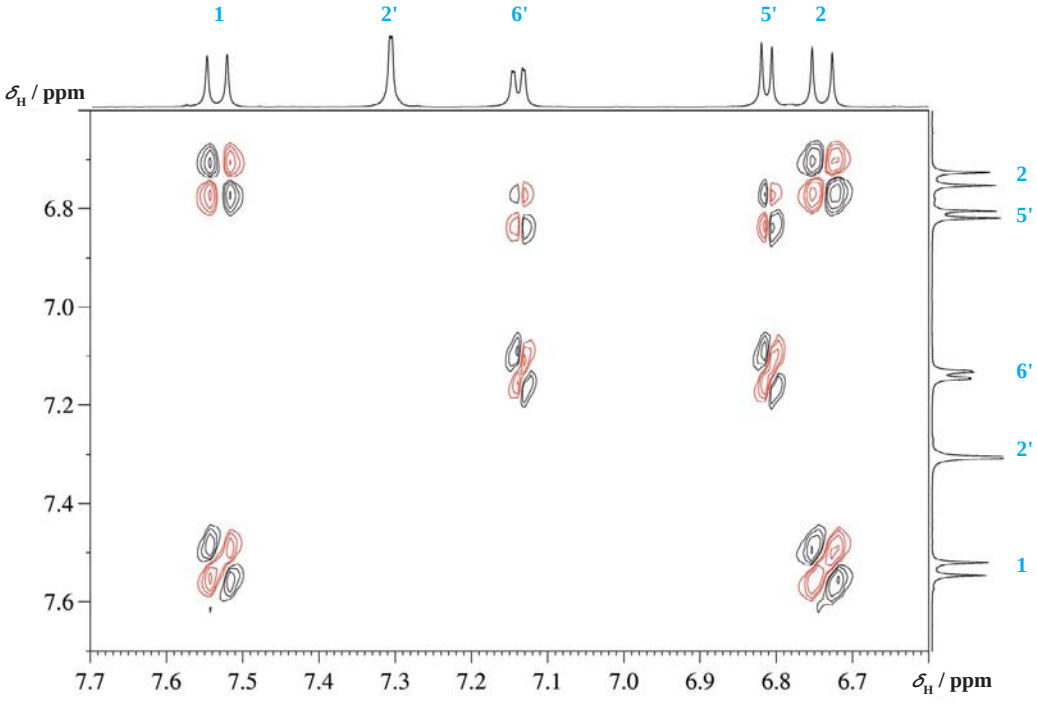


Fig. 3.2-7 Expansion of the DQF-COSY spectrum

The spin couplings described are nicely revealed also in the COSY spectrum. To display the cross peaks for the small spin coupling between H-2' and H-6' would cause an overflow for the other signals; however, these cross peaks can be easily observed on a computer screen or enhanced by using long-range COSY.

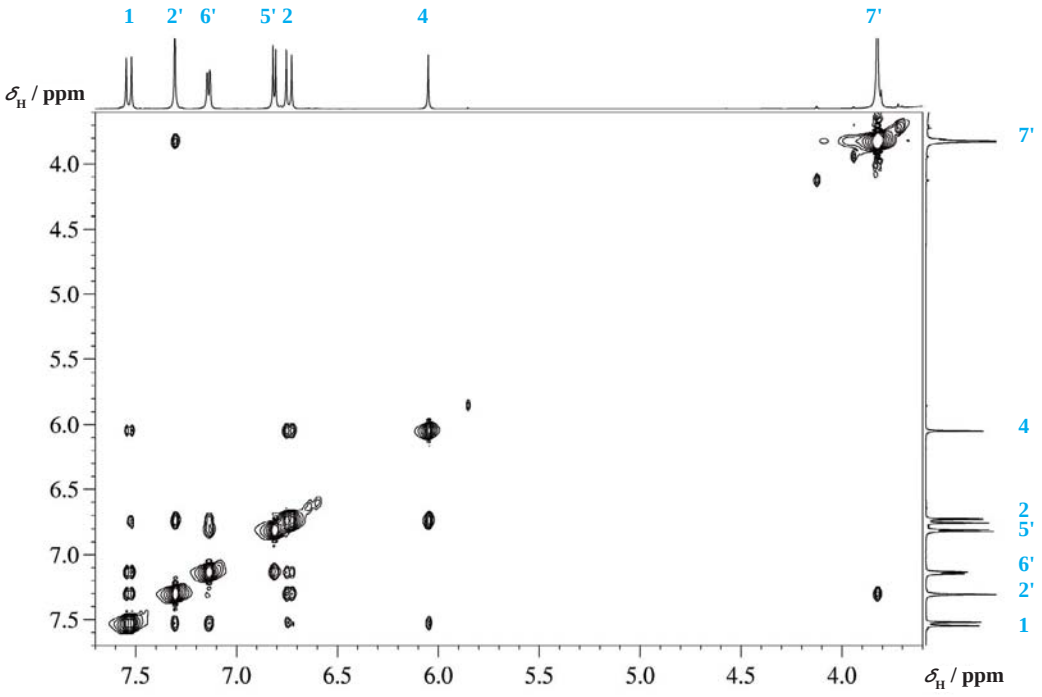


Fig. 3.2-8 NOESY spectrum

In the NOESY spectrum all signals have the same sign as the diagonal, therefore they are printed here with the same colour. For a molecule of this size this is unusual, even in DMSO solution. An explanation for this is an ongoing keto−enol equilibrium, where normal NMR detects only the predominant enol form, but NOESY is able to detect the exchange by the sign of the cross peaks.

The NOESY spectrum confirms that the methoxy group is located in the vicinity of H-2' and also shows a strong NOE between H-4 and H-2. The significant cross peaks between the *trans* olefinic protons H-1 and H-2 and the cross peaks between H-4 and H-1 are probably induced by the keto−enol equilibrium.

Fig. 3.2-9 Turmeric rhizome cut in halves

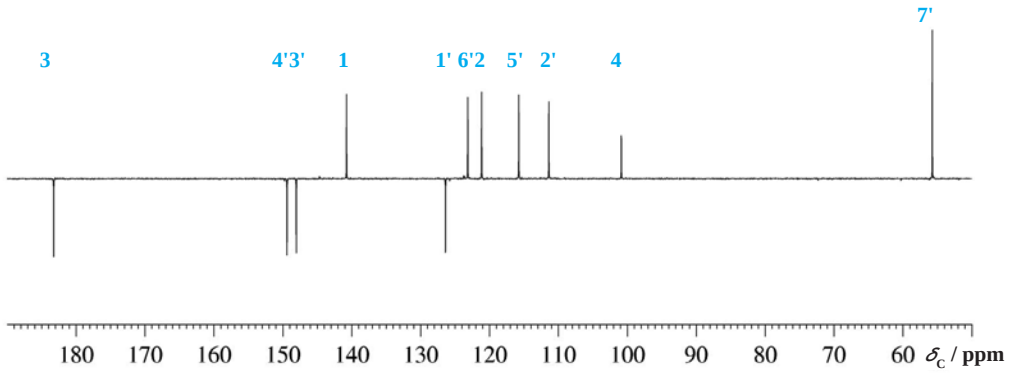


Fig. 3.2-10 APT ^{13}C NMR spectrum at 150 MHz in DMSO-d_6

In the carbon NMR spectrum the two obviously assignable signals are from the carbonyl atom C-3 at 183 ppm and the CH_3O group C-7' at 55.7 ppm. Note that the chemical shift value of C-3 is also indicative for the enolic form.The other CH signals will be easily assigned via the HSQC spectrum, since the proton assignment is already complete. The remaining three quaternary carbons will be assigned with the help of the HMBC spectrum. Again, the total number of signals indicates a symmetric structure.

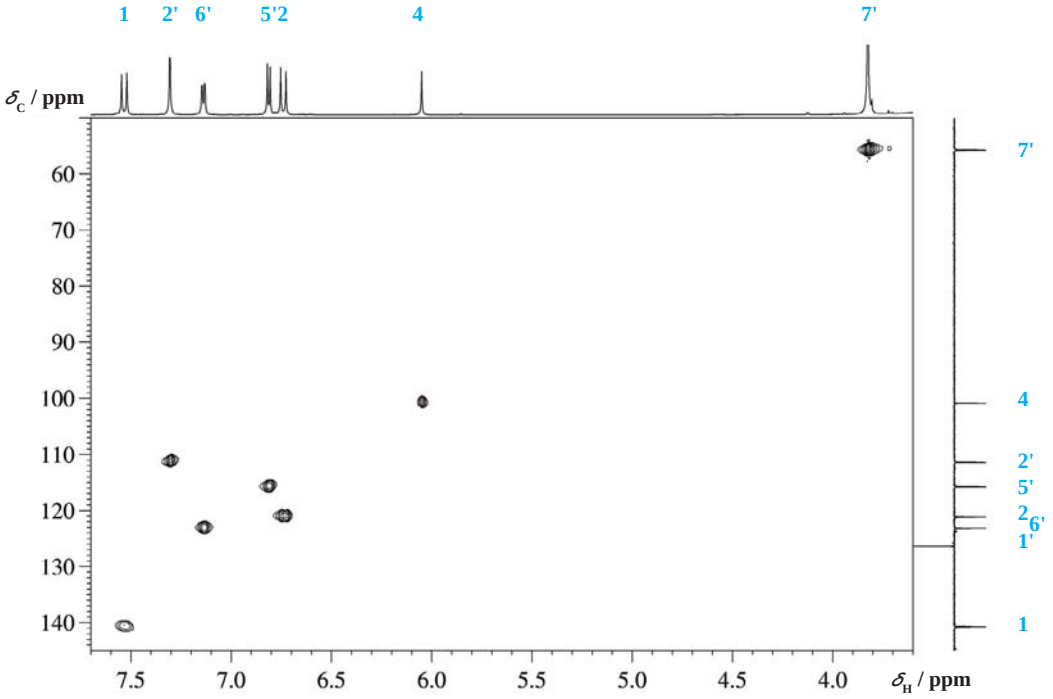


Fig. 3.2-11 Expansion of the HSQC spectrum

As the HSQC spectrum reveals, the most shielded olefinic carbon signal stems from C-4. This is most likely also caused by the keto−enol equilibrium, by which this carbon exchanges with a methylene group signal. The signals of carbon atoms C-1 and C-2 have the usual order found in α/β unsaturated ketones.

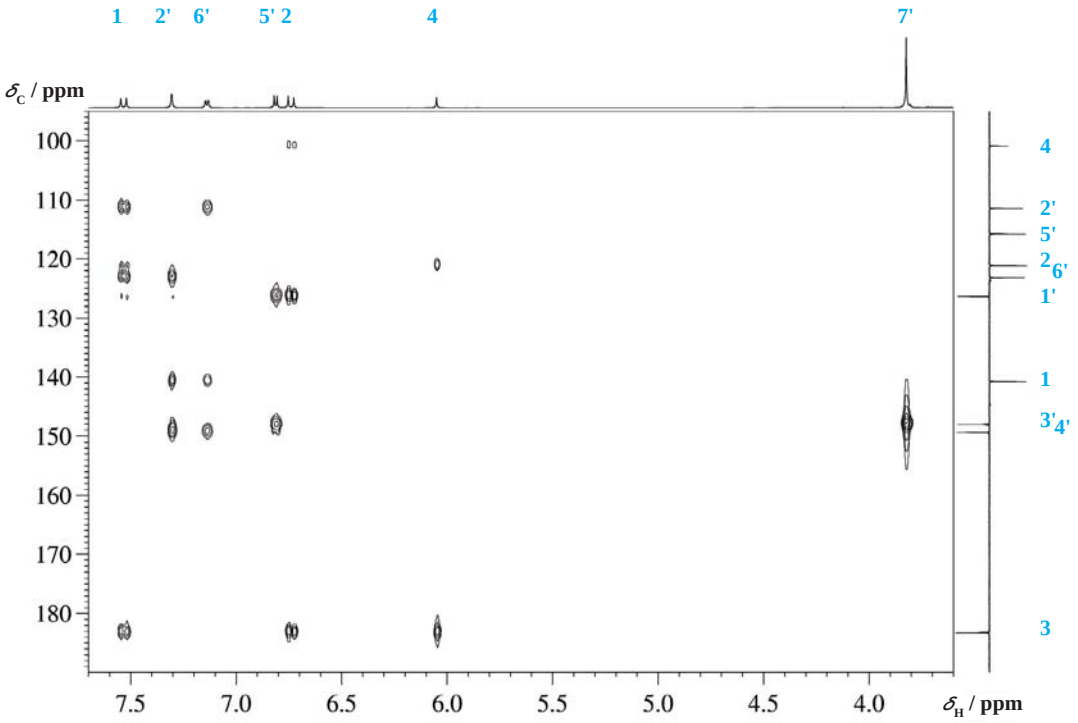


Fig. 3.2-12 Expansion of the HMBC spectrum

The HMBC spectrum clarifies the assignment of the quaternary carbon signals. C-3' at 148 ppm is seen from the OCH_3 protons and from H-5', whereas proton 2' and 6' have a ^3J (C,H) spin coupling to C-4' at 149.4 ppm. C-1' at 126.4 ppm displays the two expected connectivities to H-5' and H-2. Although no longer needed in this case, C-3 is seen by protons H-1, H-2 and H-4.

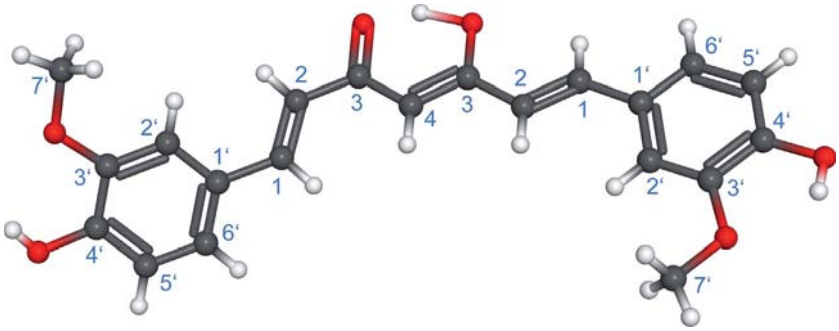


Fig. 3.2-13 Molecular model of curcumin

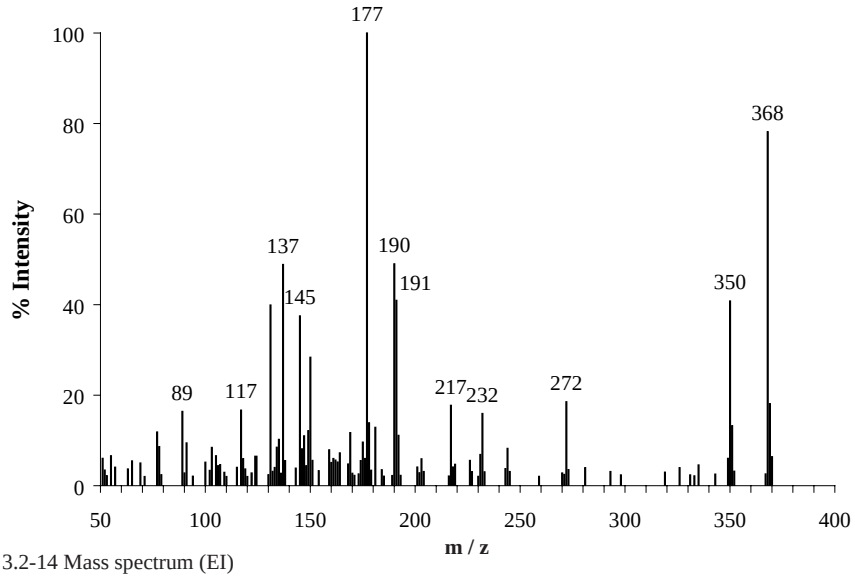
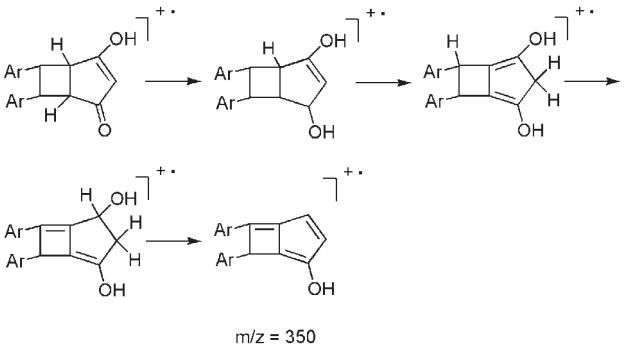


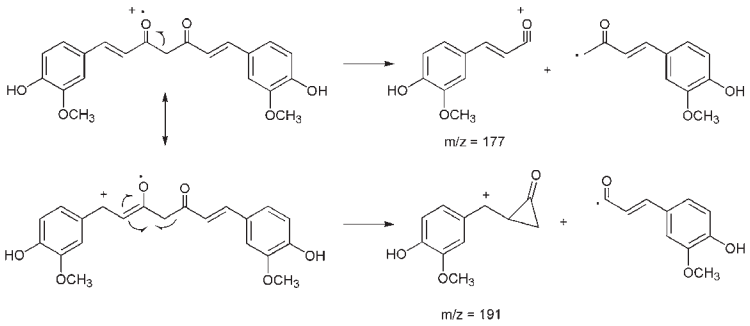
Fig. 3.2-14 Mass spectrum (EI)

The mass spectrum displays the molecular ion at m/z = 368 and subsequently the loss of water. A possible mechanism for this cleavage was suggested by Prof. K. P. Zeller (University of Tübingen) and involves first the formation of a cyclobutane ring:



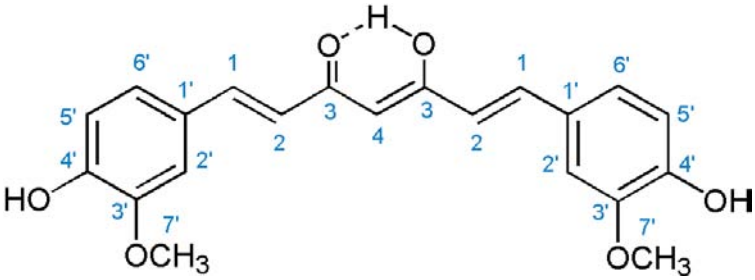
Scheme 3.2-4 Fragmentation of curcumin

If one assumes ionization at one of the carbonyl groups, the signal at m/z = 177 is caused by α-cleavage by breaking the C-3–C-4 bond and this forms the base peak. The peak at m/z = 191 then resembles the other part of the molecule and can be explained as shown below:



Scheme 3.2-5 Further fragmentation of curcumin

Fig. 3.2-15 Currywurst − a regional fast food speciality, consisting of a sliced frying sausage spiced with curry. The song in the margin describes the ritual of eating a currywurst at a snack stand after work.



Scheme 3.2-6

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz
183.2	C_q	C-3	
149.4	C_q	C-4'	
148.0	C_q	C-3'	
140.7	CH	C-1	7.54, J = 15.8
126.4	C_q	C-1'	
123.1	CH	C-6'	7.14, J = 8.2, 1.7
121.1	CH	C-2	6.74, J = 15.8
115.7	CH	C-5'	6.81, J = 8.2
111.4	CH	C-2'	7.31, J = 1.7
100.8	CH	C-4	6.05
55.7	CH_3	C-7	3.82

Table 3.2-1 NMR data for curcumin

CURRYWURST

gehse inne stadt
wat macht dich da satt
'ne currywurst

kommse vonne schicht
wat schönret gibt et nich
als wie currywurst

mit pommes dabei
ach, dann gebense gleich zwei
mal currywurst

bisse richtig down
brauchse wat zu kaun
'ne currywurst

Willi, komm geh mit
ich krieg appetit
auf currywurst

ich brauch wat in bauch
für mein schwager hier auch
noch ne currywurst

Willi, is dat schön
wie wir zwei hier stehn
mit currywurst

Willi, wat is mit dir
trinkse noch n' bier
zur currywurst

ker scharf is die wurst
mensch dat gibt'n durst,
die currywurst

bisse dann richtig blau
wird dir ganz schön flau
von currywurst

rutscht dat ding dir aus
gehse dann nach haus
voll currywurst

aufm hemd auffer jacke
ker wat ist dat ne kacke
alles voll currywurst

komm Willi
bitte, bitte, komm geh mit nach hause
hörma ich kriegse wenn ich so nach
hause komm
Willi, Willi, bitte, du bisn kerl nach
mein geschmack
Willi, Willi komm geh mit, bitte Willi

Herbert Grönemeyer (1956−),
see and listen: http://www.youtube.
com/watch?v=Ha88NCLFMwQ

5. Questions

A. Based on tradition, all databases show the formula of the keto form of curcumin, which is not a realistic expression of the structure of the molecule at all. Give two arguments from the theory of chemical bonding why the enol form as shown at the beginning of this chapter is distinctly stabilized compared with the diketo form.

B. Which kind of isomerism can be observed at a pair of keto–enol tautomers? Which form is the more polar one? Design a non-spectroscopic experiment for a pair of keto and enol forms of a β-diketone by which this can be shown experimentally.

C. What is the structural reason for the colour of the enol form? Would you expect the diketo form to have an absorption in the visible part of the spectrum, too?

D. Explain the pH dependence of the UV spectrum.

E. H-1 displays in the NOESY spectrum strong cross peaks to both H-6' and H-2', and the same is true for the interaction of H-2 with H-2' and H-6'. Explain.

F. Discuss all the other cross peaks in the HMBC spectrum.

G. The signal at m/z = 137 has not been commented upon. Explain.

H. There is an explanation for the ion at m/z = 272 in the literature involving a metathesis reaction. Try to find this pathway.

6. Own Observations

3.3 Brazileine

3,6a,10-Trihydroxy-6a,7-dihydrobenz[*b*]indeno[1,2-*d*]pyran-9(6*H*)-one

From brazilwood (Pernambuco wood)

Caesalpina echinata Lam. (Caesalpinia)

$C_{16}H_{12}O_5$, MW 284.26

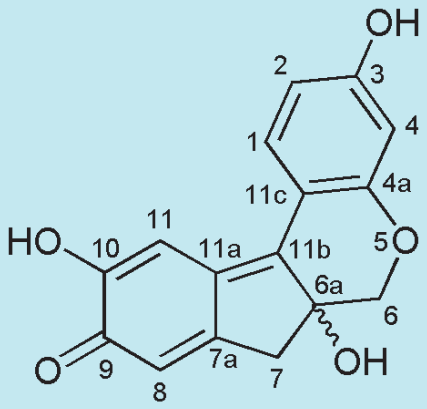
CAS RN 600-76-0, BRN 8071909

Grey−black microcrystals, not melting until 360 °C (no mp reported), $[\alpha]_D^{24}$ –227.3° (*c* 0.0011 g/mL, acetone)

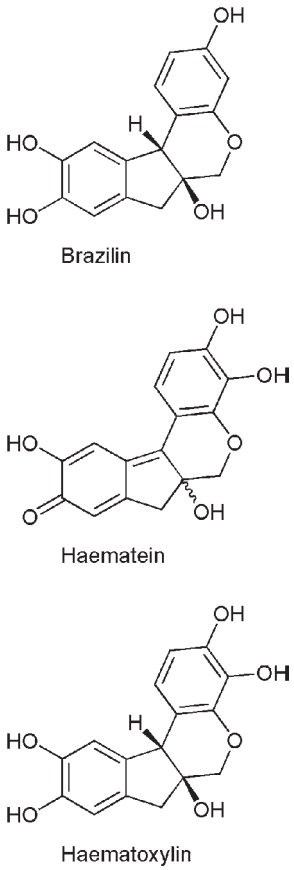
Brazilein is not commercially available.

Synonymous names:
Brasilein, CI 75280, CI Natural Red 24

Level: medium



Caution!
You are handling colouring solutions in this method. It is recommended to work carefully and avoid unnecessary contamination of skin and clothing.



Scheme 3.3-1 Neoflavanoids

1. Background:
Learn two words of Portuguese at least: *Pau Brasil*

Brazilein is a neoflavanoid dye and one among four closely related compounds. Its natural precursor is the reduced form called brazilin. A close relative is haematein (CI 75290, CI Natural Black 1), its 4-hydroxy derivative, which in turn develops on oxidation from haematoxylin as its reduced form (see formulae in the margin). We will discuss all four here.

T. Swain, Editor of *Phytochemistry*, coined the term neoflavanoids in 1964 in delimitation of flavanoid dyes. All four compounds have found practical application, first in staining; their long known physiological effects are nowadays under detailed investigation to develop novel pharmaceutical applications. Their discovery in the wood of certain trees is linked with the colonial history of Central and South America and of Southeast Asia. The intensive exploitation of the natural sources by ruthless felling the trees in huge numbers led to the situation that some of the species have now to be regarded as endangered. Especially brazilwood is nearly extinct in its original range.

Brazilin and brazilein occur in so-called redwoods. This term includes two species of the family Fabaceae, the East Indian redwood or sappanwood tree (*Caesalpinia sappan*), occurring in Southeast Asia and the Malay Archipelago, and the South American redwood (*Caesalpinia echinata* Lam.), native to Brazil. Redwood dyes were used by the Maya, Inca and Aztec cultures long before the European discovery of the Americas. Already in ancient times, recipes were developed to extract brazilin from redwood sawdust by soaking with lye. Addition of alum caused precipitation of a red powder, useful as a kind of lake pigment that was used, for example, in the painting of handwritten books in the Middle Ages. Of course, silk and wool have also been dyed. However, redwood stains show only a limited fastness to light.

Haematoxylin and haematein occur in the logwood tree (*Haematoxylum campechianum* L.), native to the Gulf of Campeche region in Yucatan, Mexico, but growing throughout Central America. The State of Belize originated from camps of British woodcutters of the 17th century. In 1860, the French logwood import was about 52 000 tons. One example of use was in the dyeing of uniform cloths.

The botanical names have very different origins. The name *Caesalpinia* was given in honour of Andrea Cesalpino (1519−1603), an Italian physician and one of the most important botanists before Carl von Linné. The word *echinata* stems from the Greek ἐχῖνος, meaning urchin, due to the spiny bark of the tree. Obviously, brazilin and brazilein have a link to the country Brazil, and it might seem that the name of the wood is derived from the country – but the opposite is true, the country was named after a tree! The story is like as follows: the first Portuguese explorers landing in 1500 on the coast of present-day Brazil found redwood trees (*C. echinata*). They knew of that kind of coloured wood from the botanically closest relative, the sapanwood tree, the powder of which was traded as a valuable source of a red dye by caravans via the

Silk Road from Asia through the Middle East to Europe. Of course, this led to an enormous price. During the Renaissance, the dyestuff was only used for luxury textiles such as velvet. Immediately, they recognized the immense value of their discovery. Felling and shipping of this profitable wood was a monopoly granted by the Portuguese king. The wood got the name *Pau Brasil* in Portuguese as a combination of *pau* (Portuguese for wood) and brasil derived from *brasa* (Portuguese for ember or glowing, reflecting the deep red colour of the wood). Eventually, the whole country of *Pau Brasil* was called *Terra do Brasil* (Brazil). The tree is Brazil's national tree and today under conservation.

Haematoxylum has the meaning of bloodwood, composed from the Greek words "αἷμα" for blood and "ξύλον" for wood. The reason for this is that haematein is a coloured compound useful for blue–violet dyeing, especially in combination with mordants. If you want to buy a bag of wood shavings, the best approach is to use not the trivial name but the botanical name. For example, "Pernambuco wood" is a term correct for *C. echinata*, but also used mistakenly for other redwoods. It may be interesting to know that the same wood as used here is used for making bows for string instruments such as violins. It is appreciated for its high shine.

A look at the structures substantiantes why pure haematoxylin and brazilin are only pale yellow in colour whereas haematein and brazilein have an intense reddish brown or black colour: haematoxylin and brazilin contain two separate chromophores, which in the neutral state hardly absorb visible light. Compared with this, in their oxidized counterparts a quinonoid chromophore is conjugated with an aromatic one, and both are substituted with auxochromic hydroxy groups that effect a bathochromic shift of the λ_{max} value. The French chemist M. E. Chevreul was the first to isolate haematoxylin from logwood at the beginning of the 19th century [1]. It is understandable that he was also eligible as a director of the Gobelin tapestry works where he studied colour contrasts in artworks as a scientist.

Both haematein and brazilein are weak organic acids (pK_a ca. 6).They can be used as acid–base indicators because their anionic form is of a deeper colour than the neutral form. For protonated haematein λ_{max} = 445 nm (yellow) and for the deprotonated form λ_{max} = 560 nm (purple). Compare our spectra for brazilein and note the bathochromic effect of deprotonation.

All four compounds have the structural feature of units capable of complexation with polyvalent metal ions, such as one or two catechol units (brazilein and haematein) and enol units. Therefore, they are excellently suitable as mordant dyes. In this technique a fibre is first stained with a mordant and then dyed with a suitable dye (see Question B). The colour finally achieved depends on the kind of metal ion used as mordant. The mechanisms of the centuries-old mordant staining with haematein and brazilein have been thoroughly investigated. It has been found that the lack of an OH group in brazilein reduces the fastness of the stain [2].

Quomodo poteris de bresilio operari. – Accipe patellam æream, et brasilium intus rade, quantum tibi visum fuerit. Postea imple eam urina, pulveriza desuper alumen, et sic una nocte dimittes. In crastino super carbones mitte, unam aut duas undias bullire facies, et retrahe ab igne patellam, et pone parumper de viva calce cum brisillio et alumen, et insimul move, et ita dimittas; dum spissum fuerit, et aqua desuper natauerit, projice foras, et reliquum ad solem permitte siccum fieri, et serva quantum volueris. De hoc colore in ligno et in muro operari poteris, mirabelius tamen in pergamenis.

Heraclius (8th–10th Century)
De Coloribus et Artibus Romanorum

Fig. 3.3-1 Young redwood tree

Whereas dyeing with redwood and logwood lost its importance with the decline of natural resources and the rapid development of synthetic dyes at the end of the 19th century, some special applications have kept their value. One example is from histology. Without staining it would in many cases be nearly impossible to observe any differences in cell morphology. Therefore, staining is necessary to enhance the contrast in the tissue being examined. A combination of haematoxylin and eosin is used as a standard stain. Whereas eosin causes a deep pink cytoplasm, haematoxylin colours nuclei blue, selectively.

Fig. 3.3-2 Brazilwood chips after extraction

The first report on brazilin from brazilwood dates back to 1876 [3]. A first proof of the redox connection between brazilin and brazilein and the quinonoid nature of brazilein was reported in 1903 [4]. The correct constitution was known with the question of the stereochemistry still unanswered [5]. The absolute configuration of the chiral centres 6a (*S*) and 11b (*R*) in enantiopure natural brazilin was determined together with that of a series of other sappanwood constituents in 1987 by forming diastereomeric derivatives, measuring their CD spectra and interpreting them according to Horeaus' rule [6].

The question of the absolute configuration of the oxidized forms, haematein and brazilein, remains. Although some sources give a description of the configuration at 6a, it is our understanding that it is not really experimentally proven what is correct. One may argue that brazilein arising from oxidation of brazilin will likely adopt a 6a*S* configuration because the oxidation does not seem to influence this position. Strictly, that may seem likely, but it is not valid evidence. Obviously, therefore, also the SciFinder database only gives the constitutional formulae for brazilein and haematein. Nevertheless, brazilein is chiral, as shown by its optical rotatory power. However, due to the structural peculiarities of this compound, there is no way of running an NMR experiment free of derivatization that could decide this question. It is, of course, conceivable what kind of derivative has to be made prior to an NMR measurement to find a valid answer (see Question C), however, such is not the subject of this book.

In the traditional medicine of the peoples living in the areas of redwood and logwood growth, several medical applications were known. After the long period of using the constituents described for dyeing, now medical research is intensively investigating them in respect of their physiological potential with the aim of developing novel medications from these natural leads. Only one of many recent examples can be cited here [7].

2. Literature

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[3] C. Liebermann, O. Burg, “Ueber das Brasilin” [On brazilin] *Ber. Dtsch. Chem. Ges*. **1876**, *9*, 1883−1888.

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[8] H. O. Yang, Y. H. Choi, “A mass production method of brazilein from *Caesalpinia sappan* L.” PCT Int. Appl. (2007), Application: WO 2006-KR5082 20061129.

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[10] H. G. M. Edwards, L. F. C. de Oliveira, M. Nesbitt “Fourier-transform Raman characterization of brazilwood trees and substitutes” *Analyst* **2003**, *128*, 82−87.

[11] D. S. Kim, N.-I. Baek, S. R. Oh, K. Y. Jung, I. S. Lee, H.-K. Lee “NMR-assignment of brazilein” *Phytochemistry* **1997**, *46*, 177−178.

3. Isolation

3.1 Principle

Wood is a very complex material. Therefore, it is not a good idea to “attack” it with excessive power, e.g. with a boiling solvent. Too much unwanted compounds would be forced into solution by doing so. It is a better idea to let a solvent soak up the compound of interest over a longer period of time. Of course, this is not at all selective regarding the brazilein that it is intended to isolate, but it is not as non-selective as the first heavy treatment would be. For brazilein, methanol is a suitable solvent, when used in larger amounts. Brazilein is a hybrid of polar and nonpolar structural elements, with the former dominating the behaviour. It is astonishing how much of the wood can be dissolved in methanol: 41.9 g from the 300 g of brazilwood. Because it is a tricky task to obtain brazilein rapidly from the first extract, we recommend using the second crop of brazilein and purifying it by recrystallization as described.

Em seguida, foram-se os mareantes para as naus, deixando em terra dois degredados e no dia immediato, 2 de maio, a frota fez-se de véla para o Cabo da Boa Esperança, tendo regressado ao reino uma das caravelas, capitaneada por Gaspar de Lemos, para levar ao rei a noticia do reconhecimento officialmente feito da terra do Brazil e da sua posse para a corôa portugueza.
A essa terra, que era conhecida pelo nome de Terra dos Papagaios e que Cabral denominou Vera Cruz, poz d. Manoel, em 1502, o nome de Santa Cruz, que foi posteriormente substituido pelo de Brazil, devido ao grande commercio do pau brazil que ella produzia.
Dando conta, em carta, ao rei da Hespanha do reconhecimento do Brazil feito por Cabral, disse d. Manoel: “o capitão deixou alli dois degredados á mercê de Deus.” Um dos pilotos da frota explicou depois que esses degredados puzeram-se a chorar e que logo os naturaes os animaram, mostrando ter piedade delles.

Manuel Ferreira Garcia Redondo
(1854−1916)
O Descobrimento do Brazil

August 6th. − In the afternoon we stood out to sea, with the intention of making a direct course to the Cape de Verd Islands. Unfavourable winds, however, delayed us, and on the 12th we ran into Pernambuco, − a large city on the coast of Brazil, in latitude 8 degs. south. We anchored outside the reef; but in a short time a pilot came on board and took us into the inner harbour, where we lay close to the town.

Pernambuco is built on some narrow and low sand-banks, which are separated from each other by shoal channels of salt water. The three parts of the town are connected together by two long bridges built on wooden piles. The town is in all parts disgusting, the streets being narrow, ill-paved, and filthy; the houses, tall and gloomy. The season of heavy rains had hardly come to an end, and hence the surrounding country, which is scarcely raised above the level of the sea, was flooded with water; and I failed in all my attempts to take walks.

The flat swampy land on which Pernambuco stands is surrounded, at the distance of a few miles, by a semicircle of low hills, or rather by the edge of a country elevated perhaps two hundred feet above the sea. The old city of Olinda stands on one extremity of this range. One day I took a canoe, and proceeded up one of the channels to visit it; I found the old town from its situation both sweeter and cleaner than that of Pernambuco. I must here commemorate what happened for the first time during our nearly five years' wandering, namely, having met with a want of politeness. I was refused in a sullen manner at two different houses, and obtained with difficulty from a third, permission to pass through their gardens to an uncultivated hill, for the purpose of viewing the country. I feel glad that this happened in the land of the Brazilians, for I bear them no good will − a land also of slavery, and therefore of moral debasement.

Charles Darwin (1809−1882)
The Voyage of the Beagle, Chapter 21

Note carfully the following circumstance: compounds with a very high melting point often tend to crystallize in form of tiny micro-crystals, appearing like a strange dust that does not seem to represent the desired compound. Here, from a clear orange solution, such a grey−black precipitate slowly forms and it is not what you should discard but keep.

Yet another compound in this book shows a similar behaviour: indigo on recrystallization from acetic acid.

3.2 Method

This procedure is inspired by a method for the extraction of brazilein from related *Caesalpina sappan* [8].

Brazilwood chips (*Caesalpina echinata* Lam.) (300 g) are placed in a 2 L beaker with methanol (1700 mL) and allowed to stand for extraction for 2 d. The mixture is occasionally agitated with a glass rod. The dark red mixture is then filtered through a Buchner funnel. The dark red−orange filtrate is reduced to dryness in vacuo. A dark red, tenacious solid remains (27.6 g). The procedure is repeated twice. The coloration of the filtrate becomes weaker each time. The yield of crude extracts is 10.4 g (second run) and 3.9 g (third run). TLC shows no considerable change in the composition of the extracted compounds.

3.3 Purification

The material obtained in the first extraction is redissolved in methanol (500 mL). The intense red solution is concentrated on a rotary evaporator (bath temperature 35 °C) with a rotation speed of 100 rpm within 1.5 h to a volume of 150 mL and then allowed to stand in a crystallization bowl for 3 d. Tiny grey−black microcrystals of brazilein separate and are filtered off by suction, an operation that needs time due to the very viscous liquid phase. The crystals are washed with a few mL of ice-cold methanol and dried in vacuo. Their mass is 490 mg.

The solid from the second extraction is treated in a different manner. It is dissolved in methanol (100 mL) and allowed to stand to crystallize in a bowl for 2 d. During this time, a kind of black dust seems to precipitate. However, these tiny crystals consist of brazilein and are filtered, washed and dried as described above. Another crop of brazilein is obtained (340 mg). Its purity is the same as that of the first portion. A 200 mg amount of material from the second crop is recrystallized from methanol (10 mL) to yield 75 mg of brazilein. It does not melt until 360 °C; $[\alpha]_D^{24}$ –227.3° (*c* 0.0011 g/mL, acetone). Analyses were performed using this brazilein (see note below).

The solid from the third extraction is dissolved in methanol (110 mL) and allowed to stand to crystallize as described above; 34 mg brazilein of lower purity are thus obtained. On cleaning the glassware, note the considerable solvatochromy of brazilein. A few drops of a methanolic solution of brazilein turn deep red on dilution with water.

For a purity check by TLC, ethyl acetate is a suitable solvent with standard silica gel foils for TLC. As a reference, a spot of the first

extract solution is used. Three main spots are observable. At R_f 0.55 a colourless compound is present (visible under 254 nm UV irradiation). Its colour turns to orange within 30 min (assumption: brazilin). At R_f 0.38 a dark orange spot of brazilein appears that is visible all the time. At R_f 0.26 a colourless spot (visible only under 254 nm UV irradiation) exists, which does not become coloured on standing. In the preparation of the samples for analyses, the following hint is important. Brazilein includes firmly a small amount of methanol on crystallization, as can be seen from the NMR spectrum (suitable solvent: DMSO-d_6). This is an inclusion only and not a covalent reaction product. The methanol can be removed nearly completely from the brazilein crystals in a heated flask (90 °C) using a rotary vane pump (10^{-2} mbar) for several hours. In this book, there is only one compound with a similar lasting problem (removal of acetic acid from hesperidine). Another method is to redissolve several milligrams of crystalline brazileine in a few mL of methanol and to remove the solvent immediately with a rotary evaporator followed by a rotary vane pump.

4. Spectra and Comments

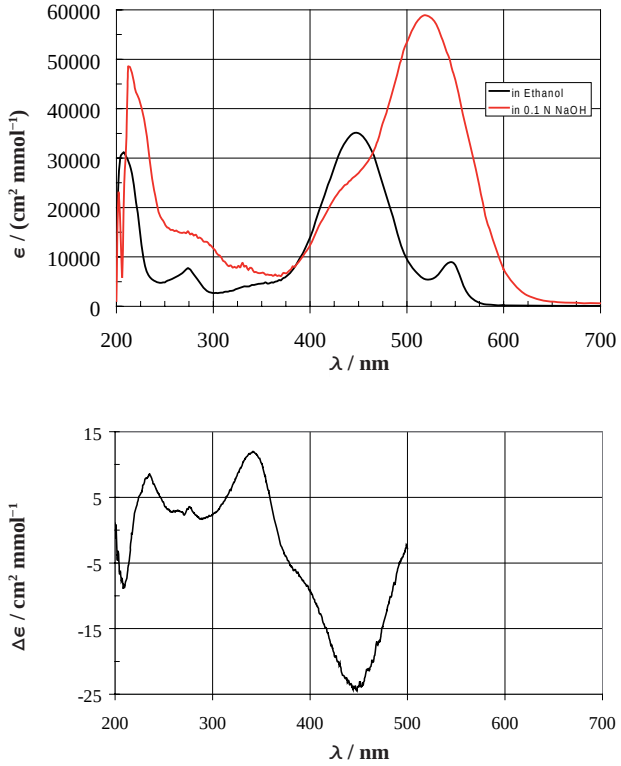
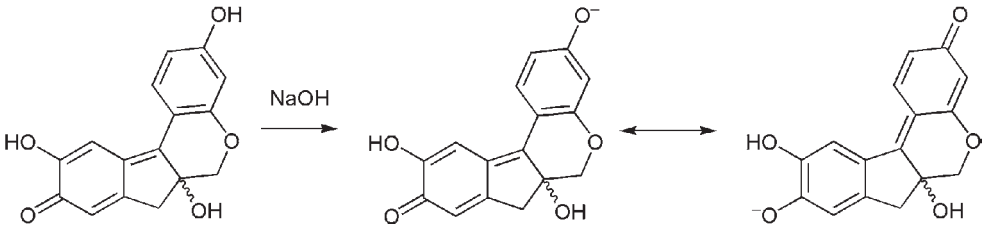


Fig. 3.3-3 UV and CD spectra

Fig. 3.3-4 Pau Brazil

As is to be expected for a weak organic acid with a phenolic OH group, the UV spectrum is pH dependent. As shown, we observe in ethanol a spectrum with a maximum at about 450 nm and this undergoes a significant red shift to a λ_{max} at 525 nm in 0.1 N NaOH. At the same time, ε increases from 35 000 to 60 000 cm^{-1} $mmol^{-1}$. On deprotonation at the OH group of C-3 we can form an extended mesomeric π-system which covers the whole molecule, as indicated in the formula. The behaviour is similar to that already described for lawsone, although in lawsone the same effect is much less expressed. The Cotton effect of the main band in the CD spectrum is very strong and negative.



Scheme 3.3-2

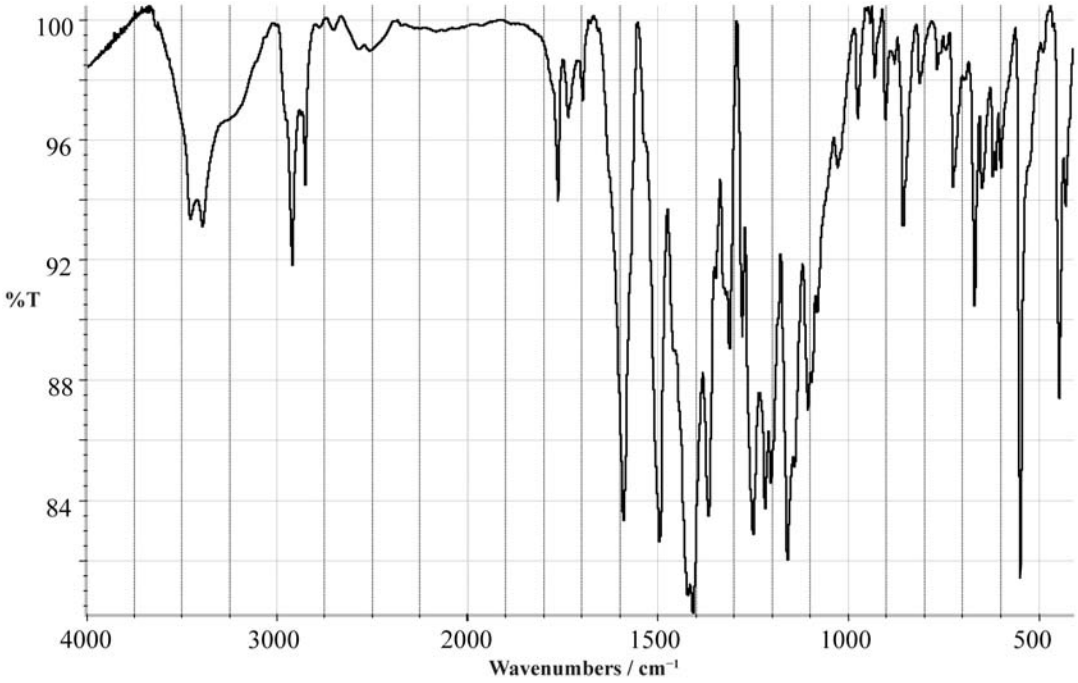


Fig. 3.3-5 IR spectrum in KBr

The three different OH groups of brazilein give rise to a split OH valence band. Surprisingly, there are no CH valence bands of sp^2 hybridized carbon atoms to be seen, but only those of the two methylene groups below 3000 cm^{-1}. The carbonyl absorption is split and at a remarkably low frequency at about 1650 cm^{-1} and is very weak. This may be due to the situation as an α/β unsaturated carbonyl group with an additional chelation by the *ortho*-hydroxyl group. The C=C double bond vibration at 1600 cm^{-1} bond is rather intense.

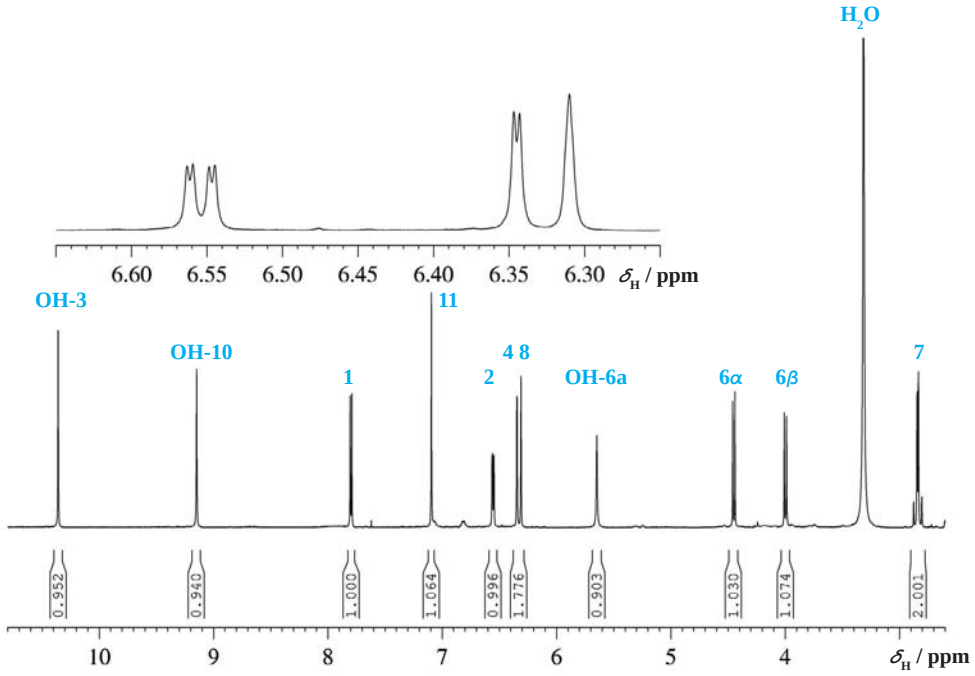
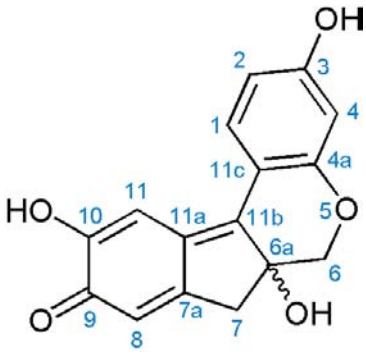


Fig. 3.3-6 ^{1}H NMR spectrum at 600 MHz in DMSO-d_6

In the proton NMR spectrum, recorded in DMSO, we find first four rather broad signals which are from the three exchanging OH groups and the residual water in DMSO. Two of the OH group signals are above 9 ppm and therefore these OH groups are rather acidic. The signals belong to the OH groups on C-10 and C-3. The OH group at the quaternary aliphatic carbon C-6a resonates at 5.65 ppm, whereas the residual water signal appears at 3.3 ppm. Between 6 and 8 ppm we observe the typical spin system of a 1,2,4-trisubstituted aromatic compound with a tiny splitting of 2.3 Hz for H-4 at 6.35 ppm, a doublet for H-1 at 7.79 ppm and a dd signal for H-2 at 6.55 ppm. Next we find two singlets at 6.31 and 7.09 ppm which belong to H-8 and H-11 without the possibility of an individual assignment yet. Finally, we see an AX spin system centred at 4.25 ppm, which stems from the two diastereotopic methylene protons 6α and 6β, and another AB spin system centred at 2 ppm from the protons 7α and 7β. Again, their individual assignment is not yet possible.



Scheme 3.3-3

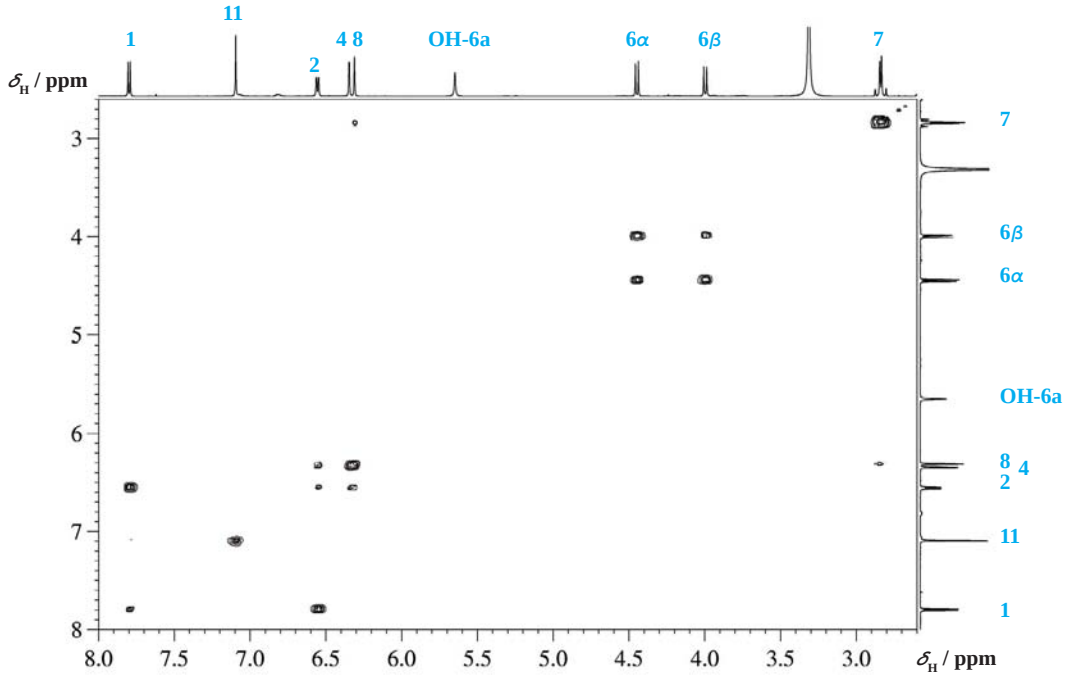


Fig. 3.3-7 Long-range COSY spectrum

Since there are only three coupled spin systems in brazilein which have no further connection to each other, the normal COSY spectrum is rather trivial and gives no other assignment information. However, the long-range version with an extra delay of 50 ms after the evolution time shown here reveals very nicely the situation in the 1,2,4-trisubstituted aromatic ring, considering the differences in the spin coupling constants. Furthermore, an interesting long-range connectivity can be seen between H-8 and H-7. If one increases the long-range delay to 200 ms, one can even find correlation signals between H-11 and H-1.

Three weeks later, with a bigger record of mountain lions and bear than Hastings' to his credit, Billy emerged from Curry County and drove across the line into California. At once Saxon found herself among the redwoods. But they were redwoods unbelievable. Billy stopped the wagon, got out, and paced around one.
"Forty-five feet," he announced. "That's fifteen in diameter. And they're all like it only bigger. No; there's a runt. It's only about nine feet through. An' they're hundreds of feet tall."
"When I die, Billy, you must bury me in a redwood grove," Saxon adjured.
"I ain't goin' to let you die before I do," he assured her. "An' then we'll leave it in our wills for us both to be buried that way."

Jack London (1876–1916)
Valley of the Moon, Book 3,
Chapter 16

Fig. 3.3-8 Redwood powder

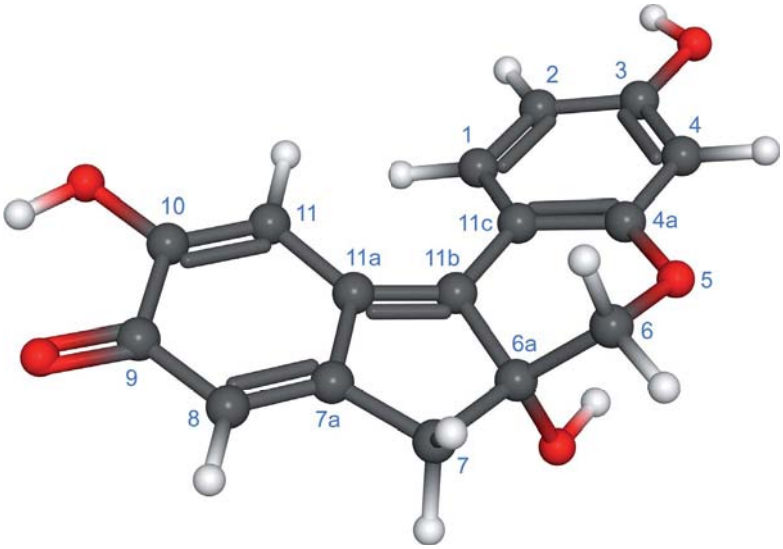


Fig. 3.3-9 Molecular model of brazileine

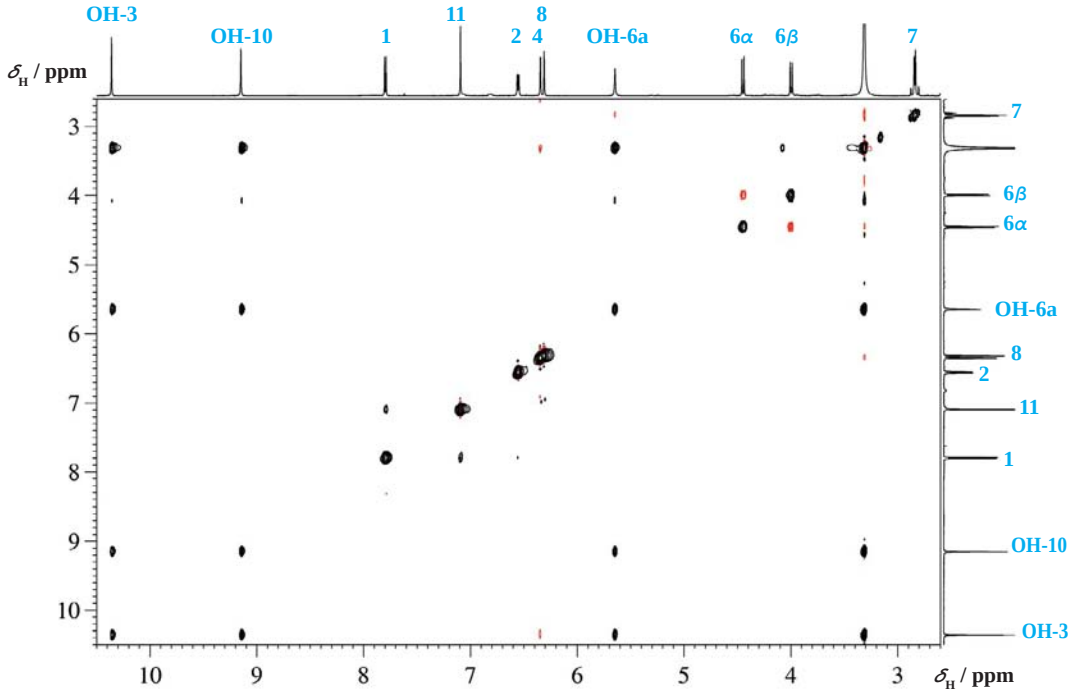


Fig. 3.3-10 NOESY spectrum

Like the COSY spectrum, the NOESY spectrum is of minor value in this case. Predominant are the strong exchange peaks which connect all OH group signals including the signal of the residual water. Note that due to the rather high viscosity of the DMSO, some of the all cross peaks (not only the exchange peaks) have the same sign as the diagonal.

Fig. 3.3-11 Lacquer painting

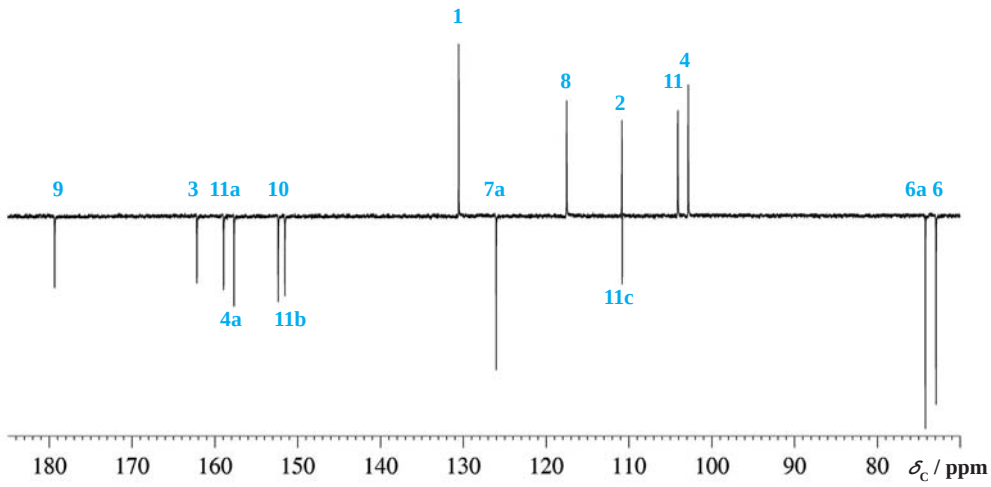


Fig. 3.3-12 APT ^{13}C NMR spectrum

Brazilein contains nine quaternary carbon atoms, eight of which are in the olefinic/aromatic and carbonyl region. Five aromatic/olefinic CH group signals should be present and two signals of the methylene groups C-6 and C-7. The signal of C-6 comes very close that of C-6a, since both are connected to an oxygen atom. The signal of C-7 can be found directly underneath the DMSO signals. We can safely assign the most deshielded signal at 179.3 ppm to C-9 and the signal at 162.1 ppm to C-3. The assignment of the other signals has to await the discussion of the HSQC and HMBC spectra.

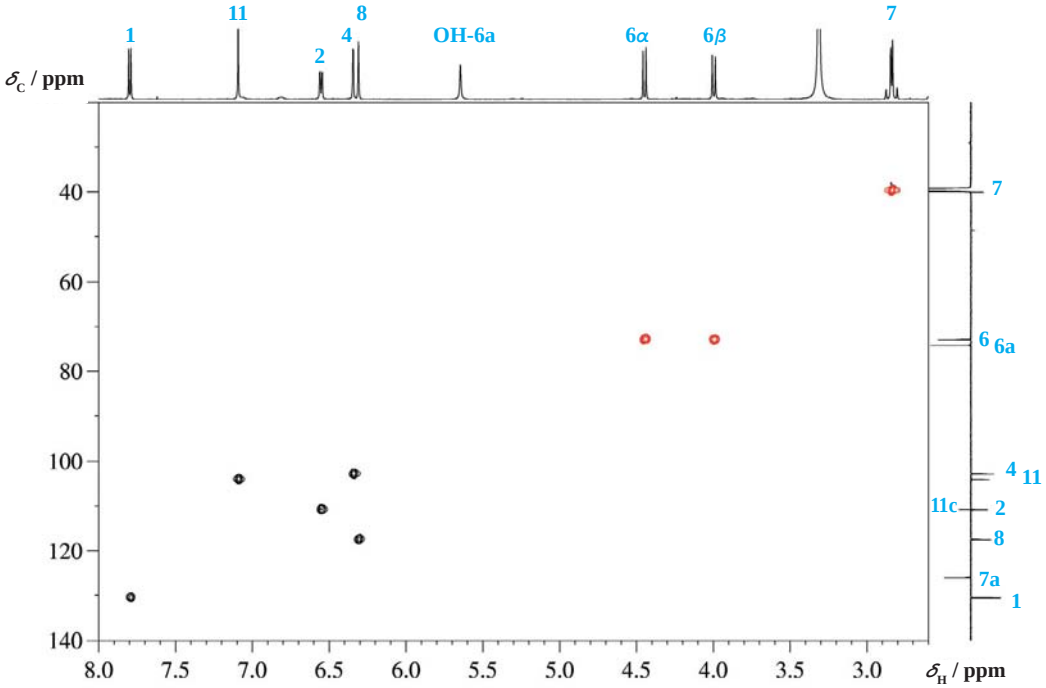
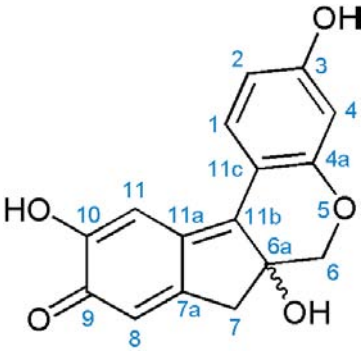


Fig. 3.3-13 HSQC spectrum

The CH edited HSQC spectrum immediatedly reveals the two methylene groups and demonstrates that the signal of C-7 lies underneath the DMSO signals. Since the proton spectra of H-1, H-2 and H-4 have been assigned due to their typical spin coupling patterns, their corresponding carbon signals are found at 130.5, 110.8 and 104.0 ppm. The HSQC spectrum is not able to give an individual assignment for the signals of C-8 and C-11.



Scheme 3.3-4

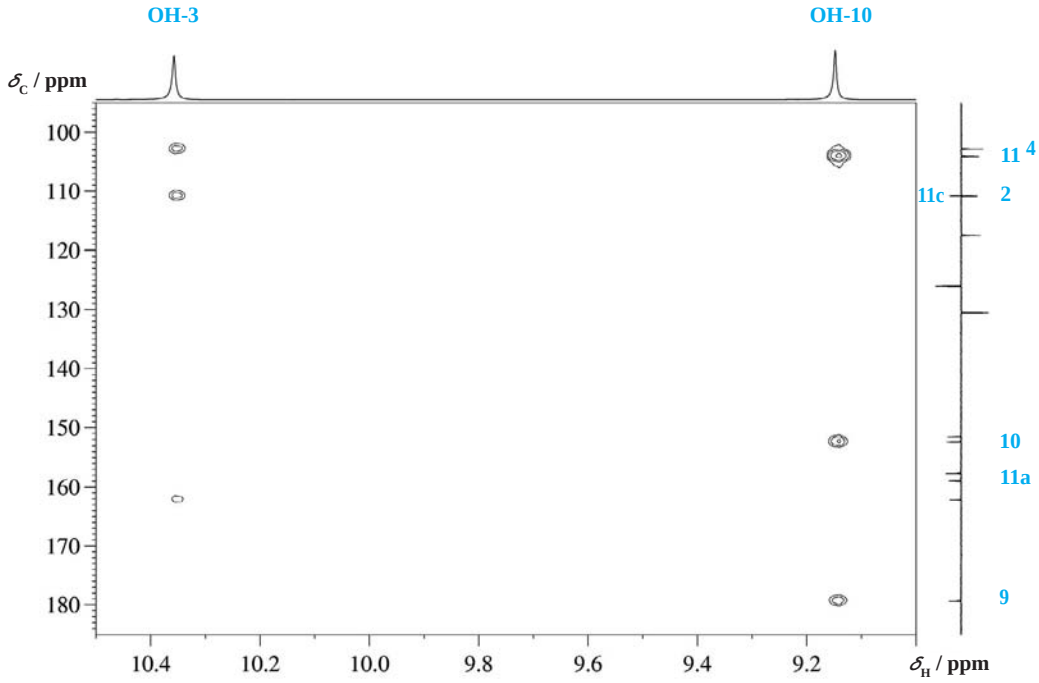


Fig. 3.3-14 HMBC spectrum for OH-3 and OH-10

The HMBC spectrum reveals for this compound a rather strong power of structural insight. We start with the information obtained from the three OH groups. The most deshielded OH group signal at 10.36 ppm belongs, as expected, to the phenolic OH group at C-3, since we find a correlation peak to C-3 at 162.1 ppm; furthermore, we find the two correlation peaks to C-2 at 110.8 and C-4 at 104.0 ppm. The second OH group signal at 9.15 ppm has a correlation signal to the carbonyl C-9 at 179.3 ppm and is therefore safely assigned to the OH group at C-10, and this carbon atom C-10 is also assigned according to the cross peak at 152.3 ppm. In addition, C-11 is found at 102.8 ppm. The OH group at C-6a is identified by its cross peak with C-6a itself and helps to find the signal of C-11b at 151.5 ppm. The analysis of the HMBC cross peak of the spin system of the protons H-1, H-2 and H-4 is a scholarly example of such a trisubstituted aromatic system and we leave this for the reader in the Questions section. Similarly, analysis of the cross peaks for the two singlets at 7.09 and 6.31 ppm reveals their individual assignment to H-11 and H-8.

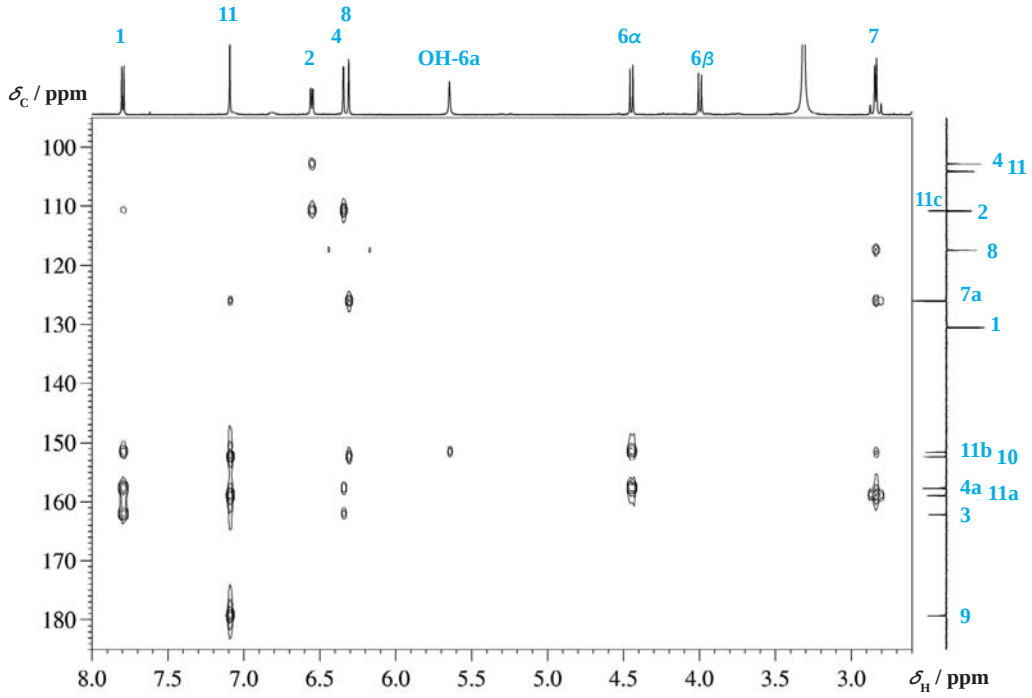


Fig. 3.3-15 HMBC spectrum for the olefinic and aromatic carbon atoms

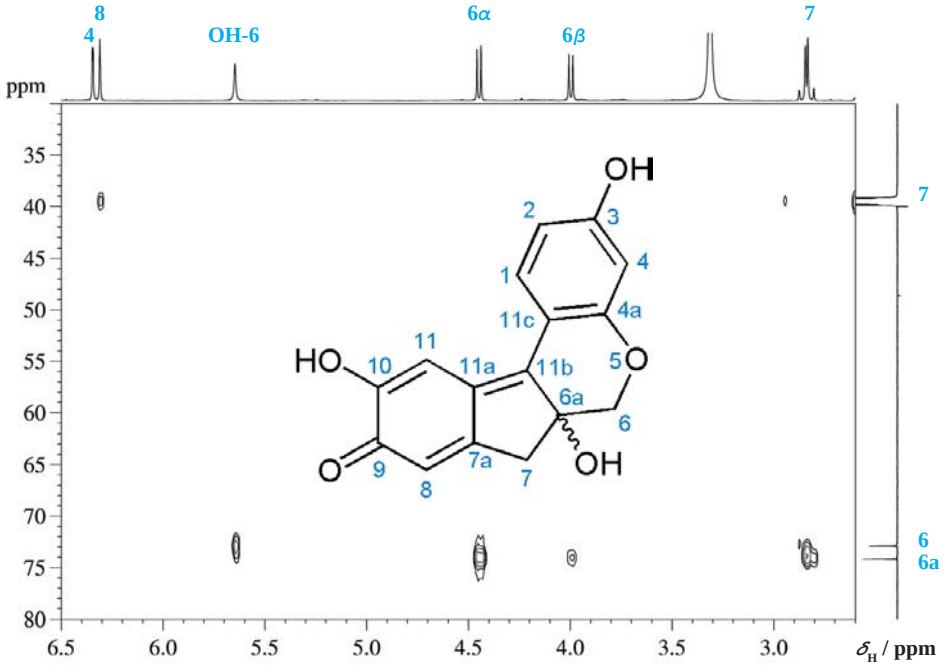


Fig. 3.3-16 HMBC spectrum for the aliphatic carbon atoms

Fig. 3.3-17 Stem of Pau Brazil

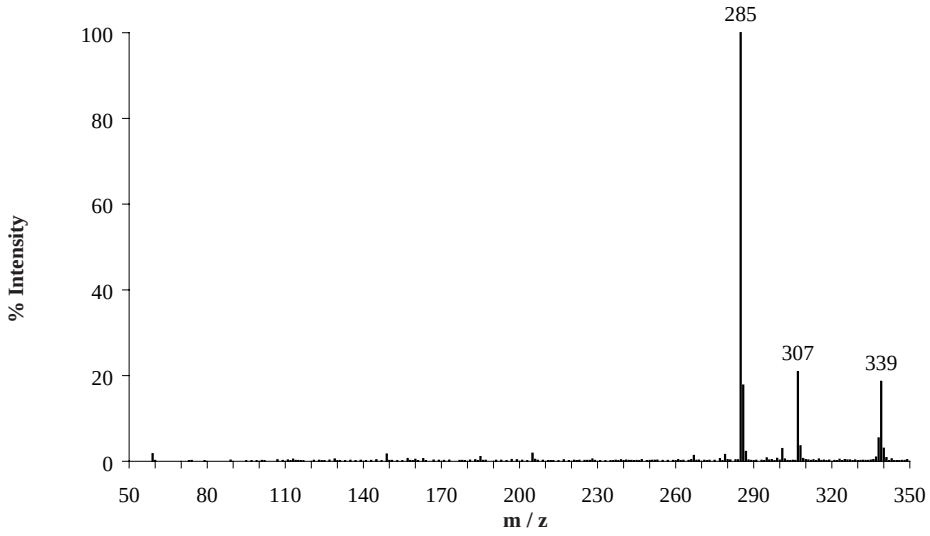


Fig. 3.3-18 Mass spectrum (ESI)

Brazilein is a good example on which to perform mass spectrometry with electrospray ionization, in both the positive and negative ion modes. The spectrum shown was recorded in the positive ion mode and displays the ion $[M+H]^+$ with m/z = 285. In addition, an adduct with sodium can be seen with an m/z value of 307. In the negative ion mode (not shown), only the ion $[M-H]^-$ at m/z = 283 can be detected.

We also recorded a tandem mass spectrum by isolating this ion and fragmenting it with the use of helium, as shown below.

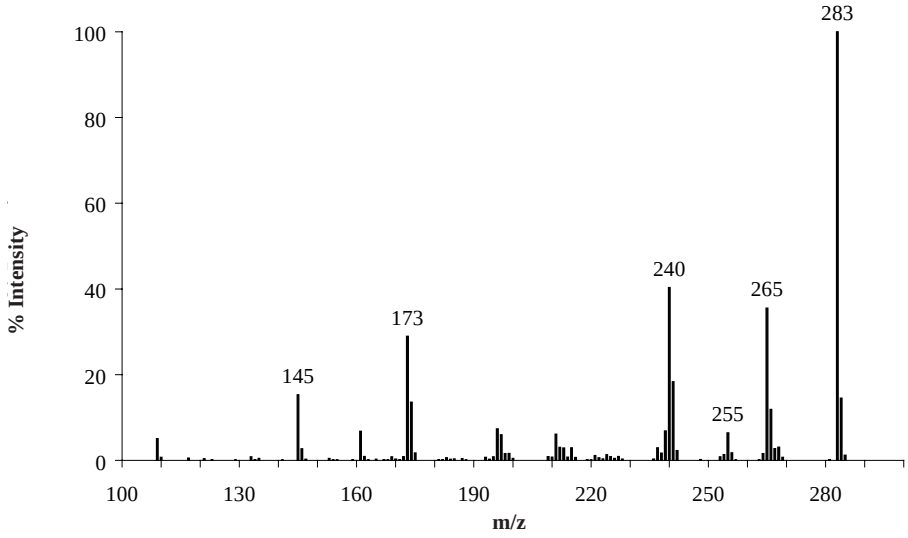
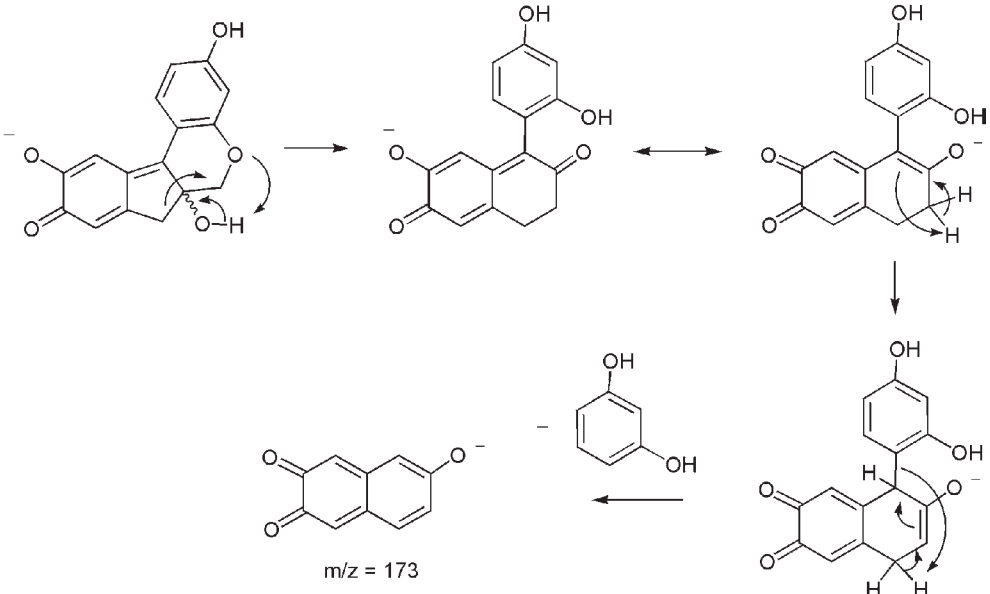


Fig. 3.3-19 Tandem mass spectrum (ESI)

The quasi-molecular ion loses water and CO to form the ions with m/z = 265 and 255. The peak at m/z = 173 may be rationalized at the loss of $C_6H_6O_2$ as given follows:



Scheme 3.3-5 Further fragmentation of brazileine

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz]
179.3	C_q	C-9	
162.1	C_q	C-3	
158.9	C_q	C-11a	
157.7	C_q	C-4a	
152.3	C_q	C-10	
151.5	C_q	C-11b	
130.5	CH	C-1	7.79, $J_{1,2}$ = 8.8
126.0	C_q	C-7a	
117.5	CH	C-8	6.31
110.8	CH	C-2	6.55, $J_{2,1}$ = 8.8, $J_{2,4}$ = 2.3
110.8	C_q	C-11c	
104.0	CH	C-11	7.09
102.8	CH	C-4	6.35, $J_{4,2}$ = 2.3
74.2	CH_q	C-6a	
72.9	CH_2	C-6	4.45, 4.0, J = −11.6
39.2	CH_2	C-7	2.84, J = −17.9
			OH at C-3: 10.36
			OH at C-10: 9.15
			OH at C-6: 5.65

Table 3.3-1 NMR data for brazileine

It was at Para also that we engaged Gomez and Manuel, two half-breeds from up the river, just come down with a cargo of redwood. They were swarthy fellows, bearded and fierce, as active and wiry as panthers. Both of them had spent their lives in those upper waters of the Amazon which we were about to explore, and it was this recommendation which had caused Lord John to engage them. One of them, Gomez, had the further advantage that he could speak excellent English. These men were willing to act as our personal servants, to cook, to row, or to make themselves useful in any way at a payment of fifteen dollars a month. Besides these, we had engaged three Mojo Indians from Bolivia, who are the most skilful at fishing and boat work of all the river tribes. The chief of these we called Mojo, after his tribe, and the others are known as Jose and Fernando. Three white men, then, two half-breeds, one negro, and three Indians made up the personnel of the little expedition which lay waiting for its instructions at Manaos before starting upon its singular quest.

Arthur Conan Doyle (1859−1930)
The Lost World, Chapter 7

5. Questions

A. What is the structural difference between a flavanoid dye and a neoflavanoid dye? Give your answer with two constitutional formulae for flavan and neoflavan as the basic compounds of the two classes. Give the systematic names for both instead their trivial names.

Flavanoid dyes include flavones, flavonols, flavanones, isoflavones, chalcones and aurones, and include most of the yellow natural dyes suitable for dyeing textiles. Give the structural patterns for these six skeletons.

Search for the structures of the following three compounds and assign them to the corresponding classes of above: luteolin, quercetin and hesperetin (the aglucone of hesperidine, see section 4.4).

B. What is the chemical principle of mordant dyeing? Describe the reaction principle. What is suitable as a mordant?

C. Why is brazilein as such inaccessible for an NMR decision on the absolute configuration? Suggest a derivatization that could allow for a successful NMR experiment.

D. Brazilein provides an example of both an AX and an AB spin system within one molecule. Inform yourself about the rules on how to extract the relevant chemical shift and spin coupling information from these two types of spin systems and perform this on the two expansions given below (600 MHz).

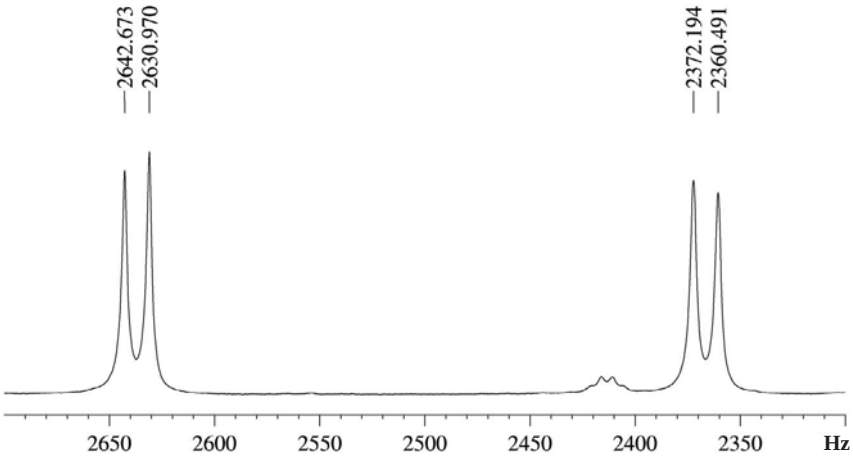


Fig. 3.3-20 ^{1}H expansion of the signals at 4.2 ppm

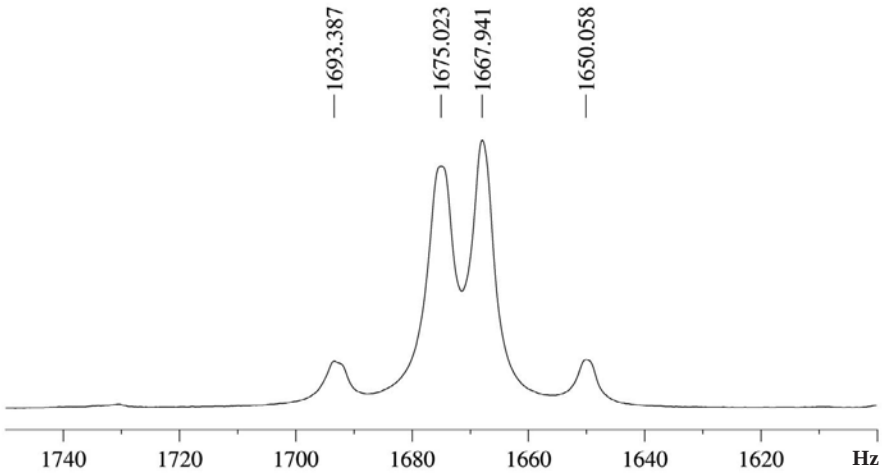


Fig. 3.3-21 ^{1}H expansion of the signals at 2.8 ppm

E. List all the connectivities seen in the HMBC spectrum for the protons H-1, H-2 and H-4.

F. Extract the same information for the protons H-8 and H-11.

6. Own Observations

3.4 Indigo

2-(1,3-Dihydro-3-oxo-2*H*-indol-2-ylidene)-1,2-dihydro-3*H*-indol-3-one

From woad
Isatis tinctoria L. (Brassicaceae)

$C_{16}H_{10}N_2O_2$, MW 262.26

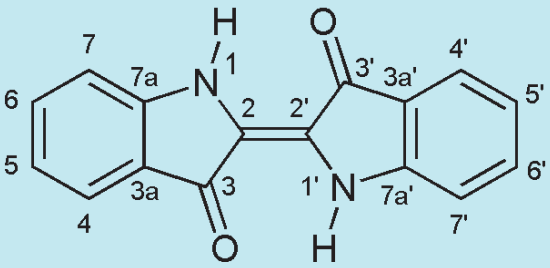
CAS RN 482-89-3, BRN 88275

Blue microcrystalline powder,
mp 390 °C (decomposition)

Indigo is commercially available.

Synonymous names:
Indigo Pure BASF, CI 73000; CI Natural Blue 1; CI Pigment Blue 66; CI Vat Blue 1; Indigo Blue; Indigotin, Indigotine, Natural Blue 1; Pigment Indigo; Vat Blue 1; [Δ2,2'-Biindoline]-3,3'-dione; [Δ2,2'(3*H*,3'*H*)-Biindole]-3,3'-dione

Level: medium



1. Background: A royal blue

Omnes vero se Britanni vitro inficiunt, quod caeruleum efficit colorem, atque hoc horridiores sunt in pugna aspectu; capilloque sunt promisso atque omni parte corporis rasa praeter caput et labrum superius.

C. I. Caesar (100−44 BC)
De Bello Gallico V,14

During the Gallic Wars (58–50 BC): *Uaaah, uaaah!* Hundreds of muscular warriors with blue painted faces were running out of the forest on to the battlefield. Roman troops, although being sure to be equipped with superior arms, must have been shocked just by this ugly sight when they tried to conquer Britannia. Blue faces, but how? With Roman words they used *vitrum*, with our words it was indigo from a plant named woad, which has been used for this formidable war paint.

The colour blue always was a special, sometimes royal one, e.g. at the times of the Pharaohs. Blue-coloured clothes were not at all as easy to make by a dying process as yellow-, brown- or orange-coloured ones. Therefore, such wardrobe was expensive, and sometimes its use was restricted to members of the upper class. Blue could be part of a sacred, holy symbol due to the blue-coloured sky, which can be found in old frescos (a typical one is shown in the margin).

Fig. 3.4-1 Fresco in a Roman chapel in the Pyrenees showing the Lord in the so-called Mandorla filled with blue colour as a holy sign.

᾿Ανθρακίνου βαφή

ὡς τάλαντον εἰς πίθον ἐμβαλεῖν ἐν ἡλίῳ κείμενον, χωροῦντα μὴ ἔλασσον μετρητῶν ιε καὶ ἐπισάξαι καλῶς. εἶτ᾽ ἐπιχέοντα οὖρον, ἕως ἂν ὑπερέχῃ τὸ ὑγρόν,ἡλιάζειν, τῇ δὲ ἐχομένη ἀναποιῆσαι συνπατοῦντα ἐν ἡλίῳ, ἕως ἀνα--δευθῇ καλῶς. τοῦτο δὲ χρὴ ποιεῖν ἐφ᾽ ἡμέρας γ.

Papyrus Graecus Holmiensis (ca. 300−400 AC) from a grave in Theben

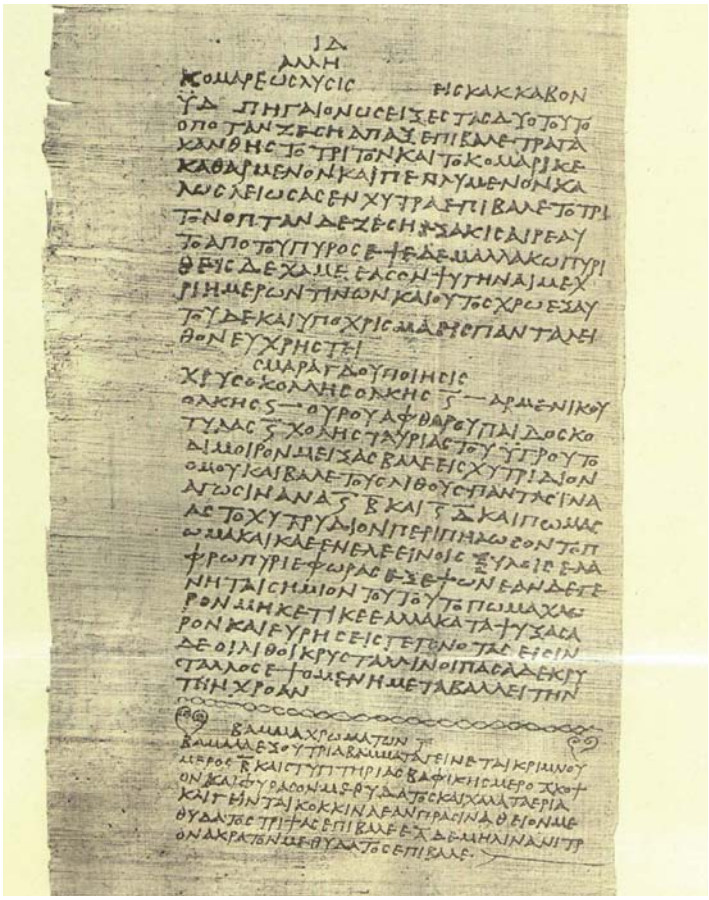


Fig. 3.4-2 Sheet from Papyrus Graecus Holmiensis

Turbans of Touareg nomads in the Sahara Desert are formed from a sheet dyed blue with indigo. Although blue-coloured blossoms are not

rare in Nature (think of cornflowers, bellflowers, sage and gentian), such colours are unsuitable for dyeing procedures. Coincidentally, the matter is not different in the world of blue inorganic pigments: blue is also a rare colour there. There are not many blue pigments in Nature and none is better than ultramarine, which can be made from lapis lazuli found in Afghanistan.

This situation prompted a lot of consequences. Those who knew the tricky art of dyeing a fibre or textile blue were specialists and artisans rather than craftsman. They could fix the price. Correspondingly, the single steps and conditions of the dyeing method were kept secret within the dyeing guild. Dyeing was a trade which was appreciated by the public and despised at the same time: the former for the nice appearance that one could get by wearing a fashionable coloured item of clothing, the latter by the dreadful stench often produced by the materials in the dyeing drums, like stale urine. Therefore, clothes dyed in blue had their price! It illustrates the wealth that could be accumulated that the small German town of Erfurt, a centre of blue dyeing with indigo from woad, was able to maintain a university since 1392 mainly from the income from this trade. It earned a lot of money by dyeing blue the uniforms of several European armies. Within this town still today you can find addresses such as *Färberwaidweg* (Woad Way).

Indigo dyeing with woad in Europe is an old business going back to the 13th century when cultivation of woad was common throughout Europe, with centres in France (Toulouse) and Germany (Thuringia, Hessen). However, the use of the dye is much older and woven textiles have been found dating back to the pre-Roman iron age (around 600 BC). The Greeks and Romans also knew of indigo. For dyeing of textiles endemic woad was used, whereas precious indigo imported from India was used for paintings owing to its character as an insoluble blue pigment. The word *indigo* is derived from the Indian river *Indus* where the first culture dyeing with indigo settled.

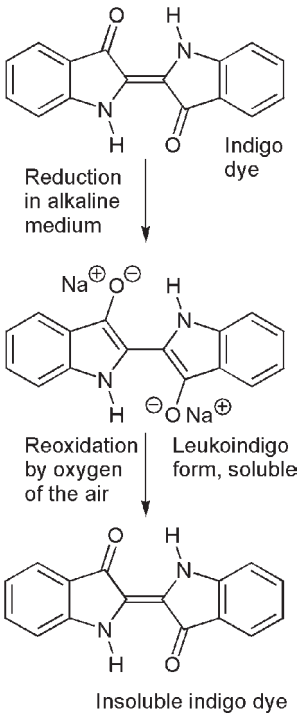
European dyers cultivated the woad plant (see photographs), which was used as a source of the final dye indigo. Woad is a biennial plant. In the first year the plant grows up to 30 cm in height, forming a large rosette of leaves only, without flowers. In the second year it forms a stem with yellow flowers similar to rape, which forms small black pods containing tiny lengthy seeds similar to grass seeds. In its first year the plant is a suitable precursor ready for processing into a material containing indigo particles for dyeing.

In the prime of woad cultivation around 1550 about 3700 hectares of woad were grown in 300 villages around Erfurt in Thuringia. Harvesting a hectare of woad in a day required 40 work forces. Hence, seasonal workers had to be hired. How was woad transformed into a dye in the past? Woad leaves were harvested in the early autumn. The leaves were washed in a creek and spread out on meadows for drying and flagging. The flagged leaves were then crushed to a mash by means of a woad mill comparable to a grain mill. A millstone was of 1.65 m diameter and turned in upright position over a circular layer of woad leaves. From the

Fig. 3.4-3 Woad leaves in the first year, cut off and washed

Fig. 3.4-4 A woad field in Thuringia

Fig. 3.4-5 Woad seeds (a) with hulls (left) (b) threshed (right). The size of a seed is about that of a grass seed



Scheme 3.4-1 Vat dyeing

Fig. 3.4-6 A blue printed shopping bag made from cotton

woad pulp obtained countrywomen formed woad balls of the size of a fist. These balls were dried, , superposable and were sold by farmers as roll woad at the market in the town to woad traders. A farmer was not allowed to process woad leaves beyond that semifinish stage. Woad traders stockpiled the roll woad in woad warehouses. During the winter months, the woad dyeing powder was manufactured by woad servants. The woad balls were smashed with big hammers and banked to large piles that were wetted with water. This caused a fermentation process based on microorganisms adhering at the leaves that was lasting several weeks and accompanied by the release of much heat and vapour. Correct treatment of the piles during fermentation was essential for the quality of the final vat dying powder obtained. Controlling the temperature by maintaining the suitable humidity was crucial. Therefore, woad piles in fermentation had to be torn apart, turned, wetted and heaped up again. During this process the colourless glycosidic indigo precursors (see below) were cleaved and transformed into crude indigo particles dispersed among the woad leave pieces. The fermented woad was dove-coloured, sieved, packed and sold in barrels. To enrich indigo as a reasonably pure dyestuff did not belong to the business because the content of glycosidic indigo precursors in woad is not as high as in the true indigo plant Indigofera tinctoria L. of India (see below).

The tricky dyeing manipulations were kept as professional secret. Woad powder was treated with bran, madder root, urine and potash at a temperature no higher than 50 °C to reduce and dissolve the solid and insoluble indigo and form a solution of leukoindigo, a reduced and yellow soluble derivative of indigo. Certainly at the end of this step, most of the plant material will have been removed as useless to obtain a manageable solution for dyeing. Any goods for this so-called vat dyeing were then immersed in the vat to allow soaking of the fabric with the leukoindigo form. Then, the cloths were taken out and hung in the air. This process caused air oxidation to form the blue indigo dye. It was called Blaumachen in German (= making the blue). Because this took some time during which nothing had to be done Blaumachen became a German expression for taking off.

The dyeing method itself is still known and conventional today at a special arts and craft level, called *Blaudruck* (= blue printing) in Germany. However, it should be noted that obtaining an intense blue coloration requires up to 20 fold repeats of this vat dipping and air oxidation treatment of the fabric. Another secret of the dyeing process was how it was possible to cover certain parts of the cloth with a special mass that prevented dyeing and allowed patterns to be generate.

It is informative to compare how the true indigo plant *Indigofera tinctoria* L. from India was treated to obtain indigo. In this plant, the content of glycosidic indigo precursors is about 30 times higher than in woad; 1 ton of leaves gave rise to 20 kg of dyestuff in the form of an enriched solid indigo powder, after removal of most of the plant material. The fermentative treatment was similar to that in woad processing. By doing this in an aqueous slurry, it was possible for the indigo particles formed to settle at the bottom because the density of indigo is above 1 g/cm^3.

Thus, the dyestuff indigo could be obtained as a concentrated matter. Indigo from such Far East sources was shipped to Europe early after the discovery in India. Dyers' guilds in France and Germany tried to outlaw this protect to their own business. However, it was a hopeless venture if one compares the superior dye content of Indian indigo with that of woad balls coming from the British colonies, where the *Indigofera* plant was cultivated, Bengal indigo contained up to 55% indigo, Java indigo even up to 80%. Looking at the high dye content and the comfort in handling, it is easy to understand that the imported British indigo was eventually mainly used for dyeing in Europe. This led to a distinct decline of the European woad cultivation in the 18th century.

Fig. 3.4-7 A woad plant after the second year with ripe seeds

In summary, European indigo dyeing was influenced by two historical shockwaves. The first was the introduction of the superior Indian indigo concentrate imported from the British colonies until around 1900. The second was the invention of a synthesis for indigo and its industrial implementation in 1897. This event led to the decline of the mass cultivation of the true indigo plant in East India until the beginning of World War I.

Today, woad cultivation has seen a certain renaissance just in the heart of one of the old woad-producing centres around Erfurt in the German state of Thuringia. However, this ecological woad relaunch is not directed at the production of indigo but at the creation of a new kind of wood preservation. Woad leaves are harvested in the same manner as in former times to obtain indigo and squeezed to give a juice which has the ability to protect wood from the attack of destroying fungi. One of the authors, in a search for woad leaves to isolate indigo, learned at a farm that this is already a multi-cubic metre business. Furthermore, it is intended to press an oil from woad seeds which can be used for cosmetic purposes.

Interestingly, due to the Jeans fashion, it is the natural dyestuff indigo, which − as a synthesized dye − is the most used of all dyes for woven fabrics. The amount synthesized per year is about 30 000 tons. To dye a pair of blue jeans requires only 10 g of indigo. Probably you will know that indigo was first used as a dye for resistant professional Denim wear in the middle of the 19th century. The first consumers were gold-diggers wearing tough trousers of the Levi Strauss Company reinforced with studs. However, during the industrial revolution, many very brilliant and colour-fast dyes were invented which were superior to indigo in this respect. Therefore, the destiny of indigo was in the balance in the middle of the 20th century after 50 years of synthetic indigo. It is odd but true that major indigo producers such as BASF were at the point of considering whether the synthesis of a dye such as indigo might be regarded as obsolete because indigo colourings are not very colour-fast, i.e. the colour fades as is well known. A rescue for indigo arose from an unexpected trend. It reached everyday clothing after World War II and was disseminated by heroes of a new generation such as James Dean wearing blue jeans. From then on, the former holy colour blue became a colour for really everyone.

What's the use of wearing braces?
Vests and pants and boots with laces?
Spats and hats you buy in places
Down the Brompton Road?
What's the use of shirts of cotton?
Studs that always get forgotten?
These affairs are simply rotten,
Better far is woad.
Woad's the stuff to show men.
Woad to scare your foemen.
Boil it to a brilliant hue
And rub it on your back and your abdomen.
Ancient Briton ne'er did hit on
Anything as good as woad to fit on
Neck or knees or where you sit on.
Tailors you be blowed!!
Romans came across the channel
All dressed up in tin and flannel
Half a pint of woad per man'll
Dress us more than these.
Saxons you can waste your stitches
Building beds for bugs in britches
We have woad to clothe us which is
Not a nest for fleas
Romans keep your armours.
Saxons your pyjamas.
Hairy coats were made for goats,
Gorillas, yaks, retriever dogs and llamas.
Tramp up Snowdon with your woad on,
Never mind if you get rained or blowed on
Never want a button sewed on.
Go it Ancient Bs!!

The Woad Ode, anonymous, 1921

The fading, hitherto seen as a drawback, had appeal and was considered an advantage. How does this slow fading effect come about? Indigo is not an unstable chemical compound, apart from of its reducibility to a leuko form, a stress which does not occur in the everyday life, however. Indigo is also not a thermally unstable compound: compare the high melting point of 390 °C, even above the boiling point of mercury. Indigo is also stable against light. The fading effect has a simple physicochemical reason. In contrast to many other types of dyestuffs indigo has only a very low fixation to cotton. None of the possibilities that the cellulose fibre of the cotton offers to achieve colour fastness is used during the vat dyeing process. Tiny indigo particles when developed by air oxidation are only sandwiched between the fibres. In addition to this mechanical inclusion, there is chemically no other support than the weak van der Waals bonding that slightly connects the dye and fibre. Therefore, blue jeans show a low fastness to rubbing. This means that indigo particles can leave the fabric rather easily under mechanical stress, e.g. in a washing machine, which improves their reputation with each use. This is one of the few examples where a consumer item with an obviously imperfect property is still highly appreciated, more than colour-fast blue jeans dyed with an indanthrene blue dyestuff would ever be.

Finally, after all these stories, let us look at studies on the indigo structure and its precursors, on their transformation into the dyestuff and on syntheses for indigo in the laboratory and on an industrial scale.

The first to deal with indigo was the German pharmacist Unverdorben, who was an expert in the dry distillation of organic material. In 1826 he obtained a liquid by the dry distillation of Indian indigo in the presence of caustic lime, it was the first aniline! Despite this, the constitution of indigo was far from being understood. Certainly, indigo belongs to the group of compounds which pushed both the efforts to establish a system for theoretically understanding organic structures and to produce them on an industrial scale, if necessary. Other chemists found anthranilic acid and isatin (1*H*-indole-2,3-dione) as degradation products. At this state of the art, the German chemist von Baeyer began his studies on the constitution of indigo in 1865. This is not the place to tell this story, which was subsequently reported by himself [1], but it took him 18 years to find the correct symmetric constitution. However, still the question of whether indigo is in a *cis*- or *trans*-arrangement was not decided, and interestingly not essential in the search for a technical synthesis. The academic question of the stereochemistry was answerd only in 1928 by X-ray crystallographic means, showing indigo to have a *trans*-configuration. Tyrian purple proved to be 6,6'-dibromoindigotine [2].

The search for the indigo precursors in different plants was similar difficult and subject to errors when judged from a present-day position. Plants such as woad or the true indigo produce colourless glycosidic indigo precursors as secondary metabolites which are stored in the cell. The assumption that their benefit for the plant may be protection from pest attacks seems reasonable when looking at the wood protection effect of woad sap mentioned above. All known glycosides are derivatives of the aglucone indoxyl (1,2-dihydro-3*H*-indol-3-one; in

its enol form: 1*H*-indol-3-ol). Biosynthetically, indoxyl is made by hydroxylation of free indole [3], which in turn is formed in the cell by enzymatic degradation of the amino acid tryptophan. Interestingly, this finding is also a rather recent one. The glycosidic indigo precursors have really elusive structures and could not be proved until 2004 by a group from Thuringia [4]. Indican (1*H*-indol-3-yl-β-D-glucoside) is the indigo precursor in the true indigo plant. On the other hand, the woad plant was shown to contain two different glycosidic precursors, isatan A, and isatan B. However, the widely distributed assumption that the latter is indoxyl-5-ketogluconate had to be revised [4]. The major indigo precursor is Isatan A, i.e. 1*H*-indol-3-yl 6'-*O*-(carboxyacetyl)-β-D-ribohex-3-ulopyranoside, with isatan B as its precursor, 1*H*-indol-3-yl-β-D-ribohex-3'-ulopyranoside (see formulae in the margin) leading to isatan B by malonylation. Both compounds are keto sugars and were shown to exist in the aqueous solution of the cell as hydrates. As can be seen, isatan B, one of the rare ribohex-3-ulopyranose derivatives among natural products, is a direct derivative of indican, which obviously is formed by an oxidoreductase specific for oxidation of the 3-OH group of indican.

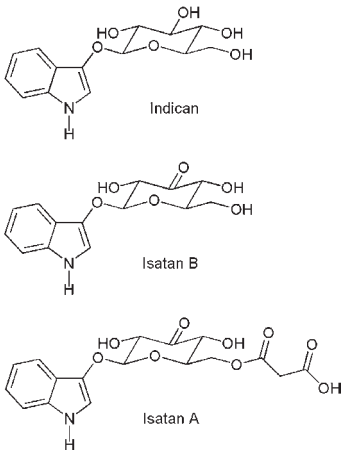
An indigo synthesis according to von Baeyer starting with 2-nitrobenzaldehyde, acetone and base such as sodium hydroxide is extremely elegant and fast. One can use it to obtain a nice massive indigo precipitation in the laboratory within 1 min. However, it cannot be transferred to the technical scale due to the high price of 2-nitrobenzaldehyde. The technical indigo synthesis requires a cheap and efficient access to indoxyl as a suitable precursor that rapidly dimerizes oxidatively under the influence of air oxygen to form indigo.

The technical synthesis of indigo is an extreme example of perserverance combined with venturing a lot of money. As described in detail [5], it took 17 years and 18 million Goldmark invested by BASF and Hoechst in the imperial Germany at the end of the 19th century (this sum is equivalent to several billion US dollars today) to eventually bring about a reliable and efficient procedure to make indigo on a technical scale (see also [6]).

The so-called second Heumann indigo synthesis (Heumann was a Swiss chemist from Zürich) of that time consisted in heating *N*-(2-carboxyphenyl)glycine in an alkali metal hydroxide melt which leads to cyclization to indoxyl that undergoes oxidative cyclization to indigo. Despite the breakthrough the synthesis was − under the pressure of competition between BASF and Hoechst − still the subject of attempts to improve it, which meant simplifying it and putting it on the basis of cheap bulk chemicals. Hence, in 1925, an alternative process was launched starting with the synthesis of phenylglycine nitrile from aniline, formaldehyde and hydrocyanic acid. Hydrolysis of this nitrile leads to phenylglycine (the indigo precursor of the so called first Heumann indigo synthesis). Its sodium salt was cyclized by heating it in a eutectic melt of NaOH and KOH at 220 °C, the new trick being an addition of sodium amide to enhance the basicity of the mixture (an invention of Pfleger). Thus, the disodium salt of the enol form of indoxyl was

A blue disaster

One of the authors remembers very well to have got − more or less as a joke by his supervisor − as a student the task of synthesizing the indigo precursor indoxyl following the pathway of the technical indigo synthesis, including the eutectic NaOH/KOH melting in a large Sintolan® cup heated with three Bunsen burners. However, once indoxyl is formed it was impossible to isolate it in a reasonably pure form because the driving force for the oxidative dimerization is so strong. All flasks and beakers were covered with indigo and so was the surface of all solutions.



Scheme 3.4-2 Indigo presursors

obtained. Hydrolysis and oxidation led to indigo with an overall yield of 84%. This still is the current procedure for indigo synthesis.

Finally, this section should not be closed without mentioning that indigo is also a compound which has attracted attention due to its physiological effects. The impetus to use it medically came mainly from traditional Chinese medicine [7]. Hence it is studied today together with its constitutional isomer indirubin [3-(1,3-dihydro-3-oxo-2*H*-indol-2-ylidene-1,3-dihydro-2*H*-indol-2-one], as a possible additive compound in cancer therapy or as a means helpful against a form of leukaemia i.e. acute promyelotic leukaemia.

2. Literature

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Do it yourself! Prepare some indigo in a minute:

Place 1 g of 2-nitrobenzaldehyde in a 100 mL Erlenmeyer flask and dissolve it in 20 mL of acetone. Add 40 mL of distilled water and 5 mL of 2 M NaOH solution. Stopper the flask and secure with a clamp. Shake well for half a minute. Observe!
The solution turns yellow after ca. 30 s, then green, then deep blue−violet due to the indigo which is precipated. After 5 min, open the flask, isolate the indigo by suction filtration and wash it with water and ethanol. The dry mass will be around 600 mg. This material can be used for vat dyeing. In case of interest consult [5] for the complex mechanism of this indigo formation.

Fig. 3.4-8 Indigo poster by BASF

He had often remarked that sea water was blue, and he had frequently caused pails to be lowered, and the water brought on deck, to see if he could come at any of this blueing matter – for indigo was both scarce and dear in his part of the world, but he never could make out anything by the experiment; from which he concluded that, on the whull, there was pretty much no such thing as color, at all.

James Fenimore Cooper (1789–1851)
The Monikins, XXVI

3. Isolation

Preliminary remarks: woad usually cannot be bought from an official source. Hence a private supplier must be found. The authors are grateful to Mr. Friebel, a farmer at Landgut Kornhochheim near Erfurt, who explained the background of contemporary woad cultivation, undergoing a certain renaissance at present. The woad used for this experiment was supplied by him and was harvested in mid-September from plants sown in the same year (see photographs).

Furthermore, we are grateful to Mr. Feige, a retired master house painter from the village Neudietendorf, for his stories around woad as a renewable resource and the use of woad juice preparations for painting and protection purposes, and also for cosmetic applications.

As mentioned above, dyeing with woad was not based on isolated indigo. Therefore, no procedures for isolating indigo from woad on a macroscopic scale have been reported. The method described does not rely on the classical rotting process mentioned by the woad traders. To try this, far more woad leaves would have been necessary. Furthermore, the method described here is not optimized! In principle, although woad is a plant which is not as rich in indigo precursors as *Indigofera tinctoria* L. is, the possible yield should be distinctly higher than ours. We would greatly appreciate any comments on improving the procedure given below.

3.1 Principle

This isolation procedure tries to make use of the enzymatic degradation of glycosidic indigo precursors to indoxyl, which forms indigo. Formation of the dye is accompanied by a clear darkening of the leaf pulp from green to a very dark, nearly black, colour. This behaviour is also a simple test possible for farmers with leaves from the field to establish whether the plants are ready for harvesting. Insoluble indigo is then removed from the plant material by conversion into water-soluble leukoindigo with the help of sodium dithionite and a base.

Care has to be taken with the temperature of this leukoindigo solution which should not exceed 50 °C to prevent decomposition of the leuko dye. This solution, suitable for vat dyeing, is then brought in contact

Figs. 3.4-9, 10, 11
To initate the ancient process of a woad mill, here woad leaves have been cut up and crushed with a kitchen blender – with limited success ...

with air to achieve − at least to a certain degree – reoxidation to indigo pigment, which is removed by filtration and recrystallized from acetic acid.

Various procedures have been tested to achieve crushing of the relatively tough woad leaves to as as fine a degree as possible. E.g., woad leaves have been cut with a kitchen knife and with a kitchen blender to imitate the action of the former woad mill and to obtain a woad pulp. Then the pulp obtained was allowed to stand wet in a tray for several days, which soon led to a strong stench. Another access mode consisted in freezing fresh woad leaves in a deep freezer at –18 °C. After unfreezing them, the mass was similarly allowed to stand in a tray for several days and then worked up in the same manner. The intention was to use the circumstance that slowly frozen and thawed plant cells undergo complete destruction due to the volume expansion of the ice formed from cell water. In other cases, we have found that such material ensures good conditions for enzymatic cleavage of glycosides.

From the results, it is impossible to decide whether the first or second pathway yields a better result: rather, they both give a poor yield regarding the mass of indigo, but at least its purity after recrystallization from glacial acetic acid is very good. Guess how much indigo there is that you can see as blue foam on the surface of the liquid in the beaker shown in the margin. It is only 3 mg, when dry!

3.2 Method

Woad leaves harvested from the field in mid-September are sorted out to be free of weed, washed so as to contain no soil particles and frozen in 1 kg portions in plastic bags in a deep freezer at –18 °C. One such portion is put in a plastic tray and allowed to defrost overnight. On standing for another day without further manipulations, an obvious darkening of the leaves to a very dark green with a black shade can be observed. This is sign of glycoside decomposition and oxidation. All the plant material is then subjected to the formation of leukoindigo by reduction in an alkaline medium. For this is transferred in equal portions into two 2 L beakers with 1500 mL of a solution of sodium hydroxide (15 g) and sodium dithionite (3.5 g) in water in each beaker. The beakers are warmed in a water bath to 50 °C while stirring with a glass rod. A yellow solution forms which becomes green in a few minutes. When intensely stirred some foam arises. Soon, the first indigo particles are formed by air oxidation and float to the surface, where they are carried by the foam (see photograph in the margin).

Fig. 3.4-12 Tiny indigo particles totalling not more than 3 mg are contained in the fluffy blue foam

The solution is allowed to act on the woad mass for 30 min. The temperature must not be higher than 50°C to avoid decomposition of the leuko dye form. Removal of the plant particles is not simple. We found the following procedure to be acceptable. First, remove the indigo foam by skimming with large spoon and place it in a smaller beaker containing distilled water. Let the indigo settle to the bottom and decant any other material together with part of the water. The remaining mixture from the vat process is filtered by suction through a large

Buchner funnel, but without a filter paper (which would soon become clogged with colloidal plant particles). In contrast, without filter paper, a turbid filtrate is obtained, which at least is free from all rough plant particles and suitable for further processing. The filtrate is distributed into two 2 L beakers and a weak stream of compressed air is introduced for 30 min. Another portion of indigo is formed and removed by skimming from the foam from the surface. All other solutions (green) are discarded.

The indigo obtained is then stirred with 250 mL of distilled water and filtered by suction through a small glass filter funnel of very fine porosity; alternatively, a very hard filter paper may be used. The tiny filter cake obtained is washed with 250 mL of distilled water. After drying, 3 mg of a deep blue–violet mass are obtained.

3.3 Purification

Indigo is not soluble in most solvents commonly used for recrystallization. However, boiling acetic acid does sparingly dissolve indigo. To dissolve 3 mg, ca. 50 mL of glacial acetic acid are necessary. Dissolution occurs on heating under reflux for a few minutes. To avoid loss of material, two hints should be noted: if only a glass filter funnel is used, some boiling acetic acid should be poured through it to dissolve any indigo adhering between the glass spheres; if the filter paper method is used, both the cake and the filter should be subjected to boiling in glacial acetic acid. Formation of tiny microcrystalline indigo crystals does not occur rapidly, but on standing overnight. The dark blue–violet indigo thus obtained has to be carefully dried using a high-vacuum pump to remove traces of acetic acid and obtain clean NMR spectra. The final yield is 2 mg.

3.4 Alternative method to isolate indigo from Indian indigo powder

This material can be bought in shops specializing in natural colourings and similar natural raw materials. It has been made in India since ancient times from the true Indigo plant *Indigofera tinctoria* L. (Fabaceae). It is the material that led to the breakdown of European woad cultivation. The commercial natural indigo is a middle blue powder, not as dark blue–violet as synthetic indigo. Nevertheless, based on our reading of the history of this Indian indigo (see above), we were at first convinced that we would have at least 50% indigo at hand and that it would be easy to isolate indigo from this source in a large amount compared with woad. However, the opposite was true, as reported below:

A 1.0 g amount of indigo natural colouring powder from the true indigo plant *Indigofera tinctoria* is refluxed in glacial acetic acid (750 mL) for 1 h to ensure dissolution of as much indigo as possible. The blue suspension is filtered hot and the undissolved pale brown material is weighed when it has been dried in air and finally in vacuo. Its mass is 973 mg. On standing overnight, from the blue filtrate only 22 mg of dark blue crude indigo crystallizes in microcrystalline form. This difference explains the weak coloration of the raw material purchased and is an expression of its poor quality. Hopefully, the reader on repeating this

Le train était parti à l'heure réglementaire. Il emportait un certain nombre de voyageurs, quelqeues officiers, des fonctionnaires civils et des négociants en opium et en indigo, que leur commerce appelait dans la partie orientale de la péninsule.

Jules Verne (1828–1905)
Le Tour du Monde en Quatre-Vingts jours, XI

Fig. 3.4-13 Traditional Persian carpets belong to the best in the world. They are exclusively tied from wool or silk thread dyed with natural dyestuffs, some of them are shown in this picture from the Carpet Museum of Iran in Teheran.

method can use a dyestuff powder that has a higher indigo content than described here. Recrystallization from glacial acetic acid (25 mL) yields only 1 mg of blue–violet microcrystals of indigo. These crystals have to be carefully dried using a rotary vane pump to remove traces of acetic acid. Their solubility is poor in any NMR solvent. They are pure according to ^{1}H NMR spectroscopy.

4. Spectra and Comments

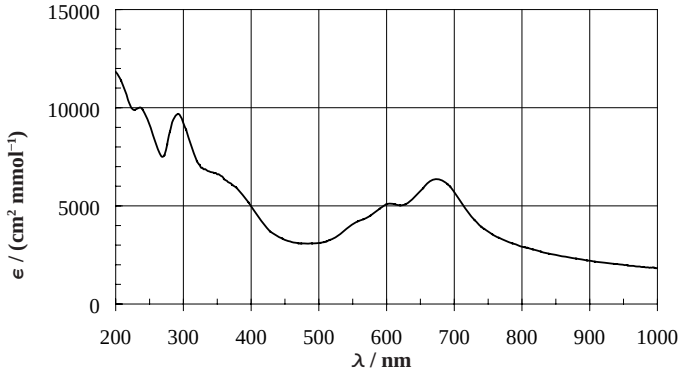
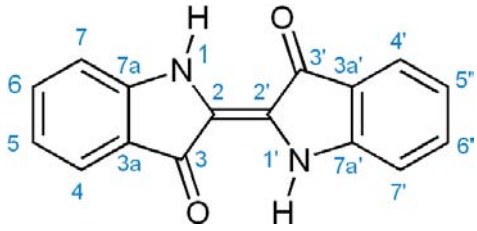


Fig. 3.4-14 UV spectrum in acetonitrile

The spectrum displays two distinct maxima, one in the UV region at 290 nm and the other in the visible region at 670 nm. The former is typical of an aromatic ketone and or an aromatic amine and indigo contains both substructures. The blue region of the spectrum around 400 nm does not show a significant absorption, and therefore with the main absorption of 670 nm in the red region the dyestuff appears deep blue to the eye. Acetonitrile was chosen as the solvent since the solubility in ethanol proved to be too low.

La salle commençait à se remplir, on tirait les lorgnettes de leurs étuis, et les abonnés, s'apercevant de loin, se faisaient des salutations. Ils venaient se délasser dans les beaux-arts des inquiétudes de la vente; mais, n'oubliant point les affaires, ils causaient encore cotons, trois-six ou indigo. On voyait là des têtes de vieux, inexpressives et pacifiques, et qui, blanchâtres de chevelure et de teint, ressemblaient à des médailles d'argent ternies par une vapeur de plomb.

Gustave Flaubert (1821–1880)
Madame Bovary, XXIV



Scheme 3.4-3

Fig. 3.4-15 An adult woad plant after growing for 6 months; the dyestuff precursors are contained in the long leaves

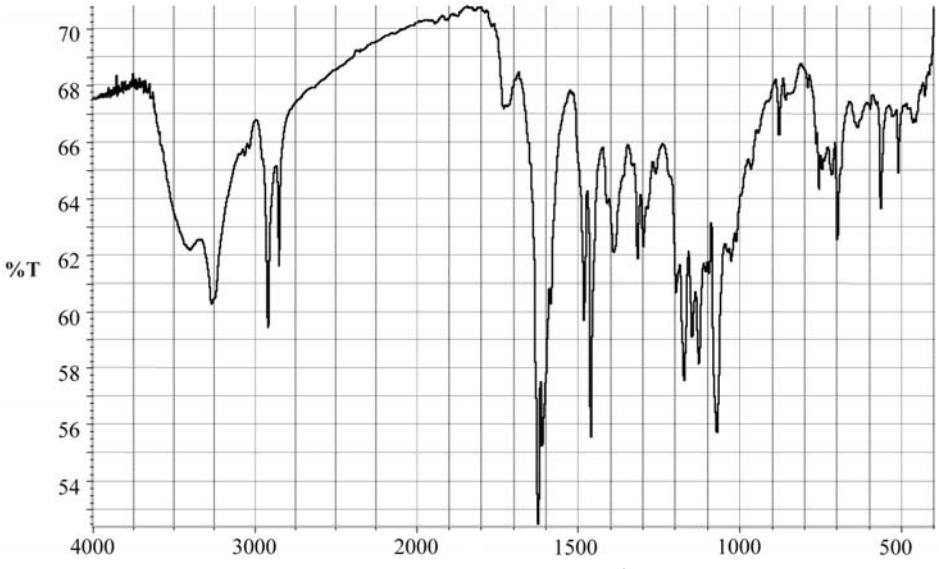


Fig. 3.4-16 IR spectrum in KBr

The IR spectrum displays a rather sharp NH valence vibration at 3200 cm^{-1}. There are two very small CH valence vibrations visible at 3100 cm^{-1}, whereas the bands below 3000 cm^{-1} probably stem from aliphatic impurities. There are mesomeric formulae possible for indigo which interpret the molecule as a vinylogous amide and the strongly split amide band at 1620 cm^{-1} probably points to this assumption.

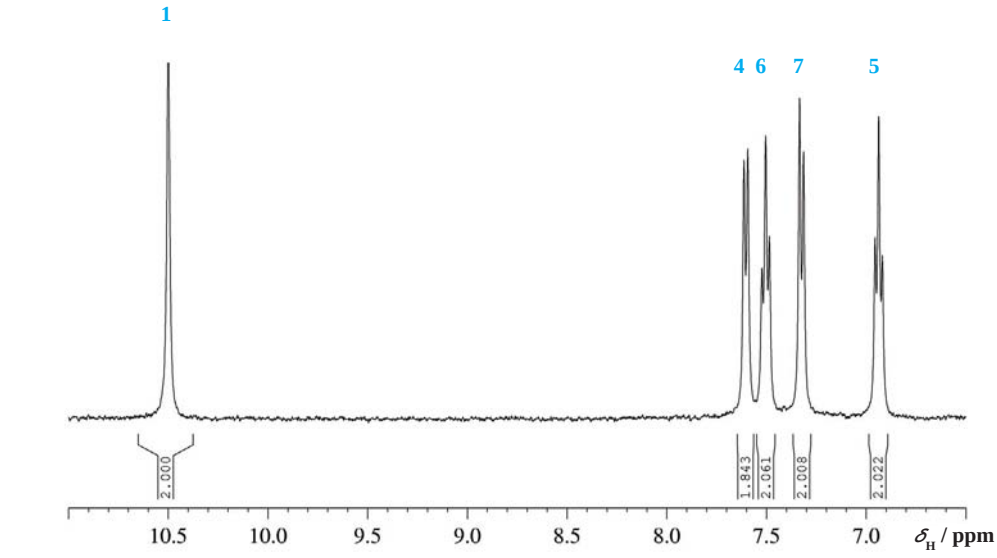
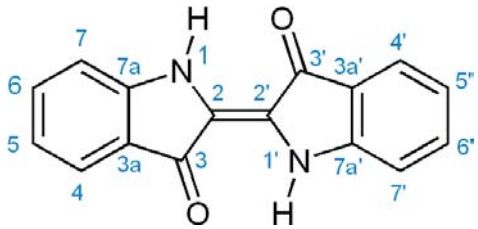


Fig. 3.4-17 ^{1}H NMR spectrum at 400 MHz in DMSO-d_6

Indigo is only sparingly soluble in DMSO and therefore the NMR spectra are not of high quality. In the proton spectrum the NH proton at 10.5 ppm can immediately be assigned. The four aromatic protons form the typical ABCD spectrum with two doublet-like and two triplet-like signals. The former belong to the protons H-4/4' and H-7/7' and the latter to the protons H-5/5' and H-6/6'. Their individual assignment, however, needs information from the NOESY spectrum.



Scheme 3.4-4

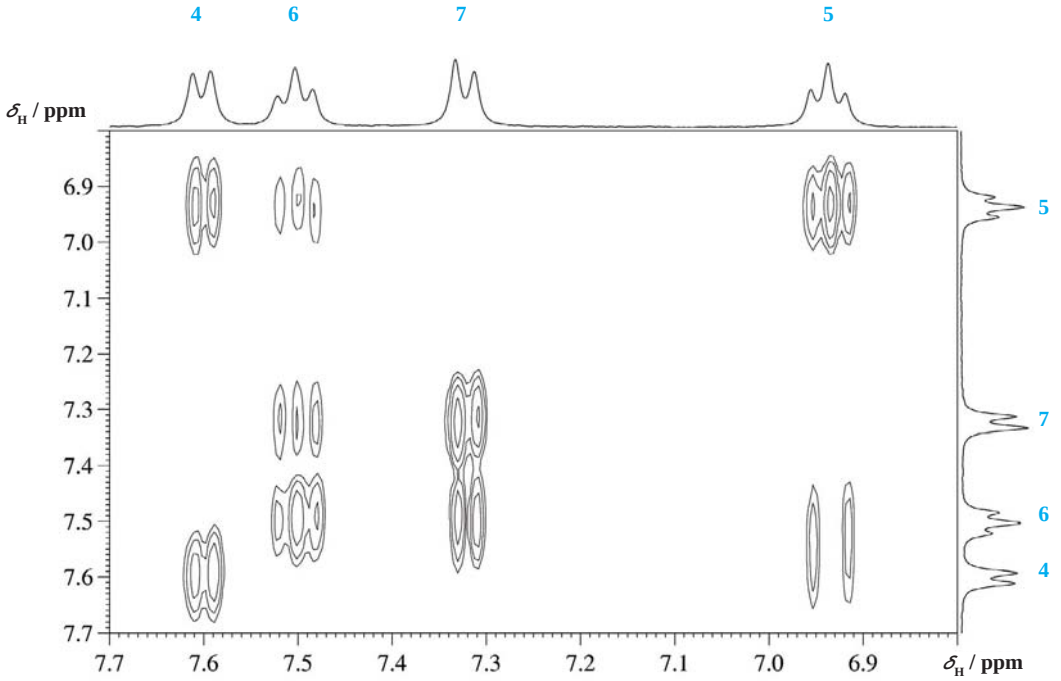


Fig. 3.4-18 COSY spectrum

The COSY spectrum gives a scholarly example of how this technique clarifies the connectivity within such an aromatic ABCD spectrum. Thus, the most deshielded doublet at 7.6 ppm is coupled to the most shielded triplet at 6.93 ppm and this in turn is coupled to the most deshielded triplet at 7.5 ppm, which finally is connected to the doublet at 7.32 ppm.

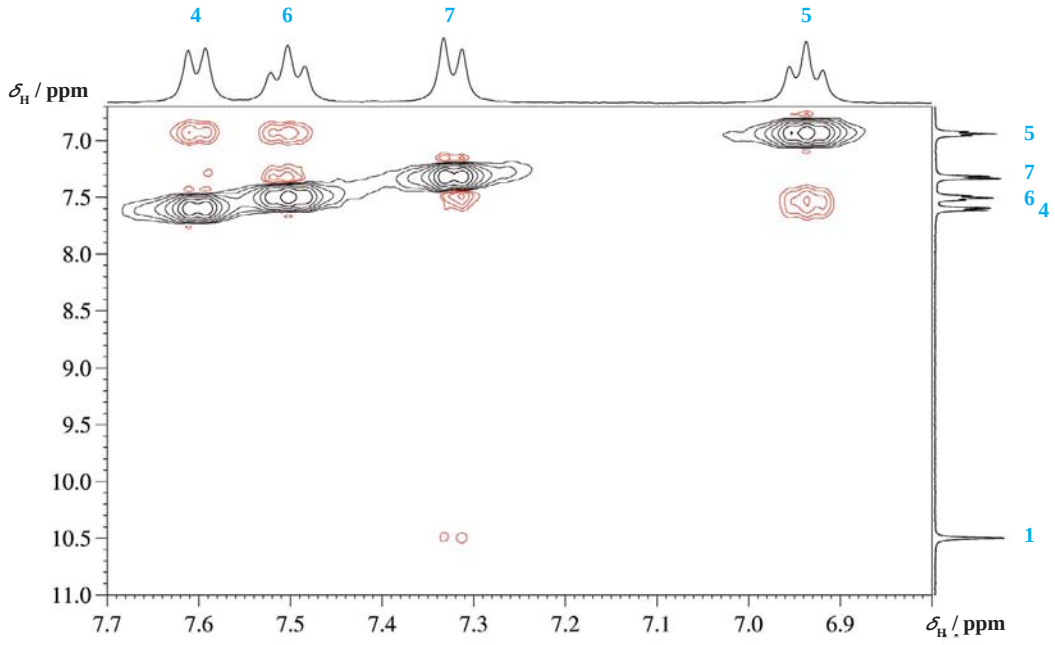


Fig. 3.4-19 NOESY spectrum

The NOESY spectrum reveals that the more shielded doublet at 7.32 ppm is in vicinity of the NH group. This is in accordance with the common knowledge of the electron-withdrawing effect of the keto group and the electron-releasing effect of an NH group when coupled to an aromatic ring system. Since we can now assign all proton signals, one may interpret why the triplet of H-5/5' is most shielded and the triplet of H-6/6' is more deshielded.

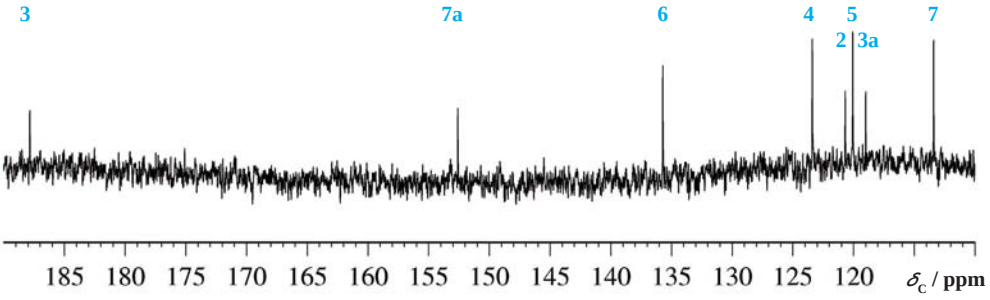


Fig. 3.4-20 ^{13}C NMR spectrum at 175 MHz in DMSO-d_6

Because of the limited solubility of indigo the recording of an old-fashioned ^{13}C NMR spectrum needed a very sensitive instrument. Due to the intrinsic symmetry of the molecule, we expect eight signals. Of these, only two can be assigned directly from their chemical shift values, namely the carbonyl atom C-3/3' at 187.8 ppm and the nitrogen-bearing carbon C-7a/7a' at 152.6 ppm. The assignment of the others has to await the help of the HSQC and HMBC spectra.

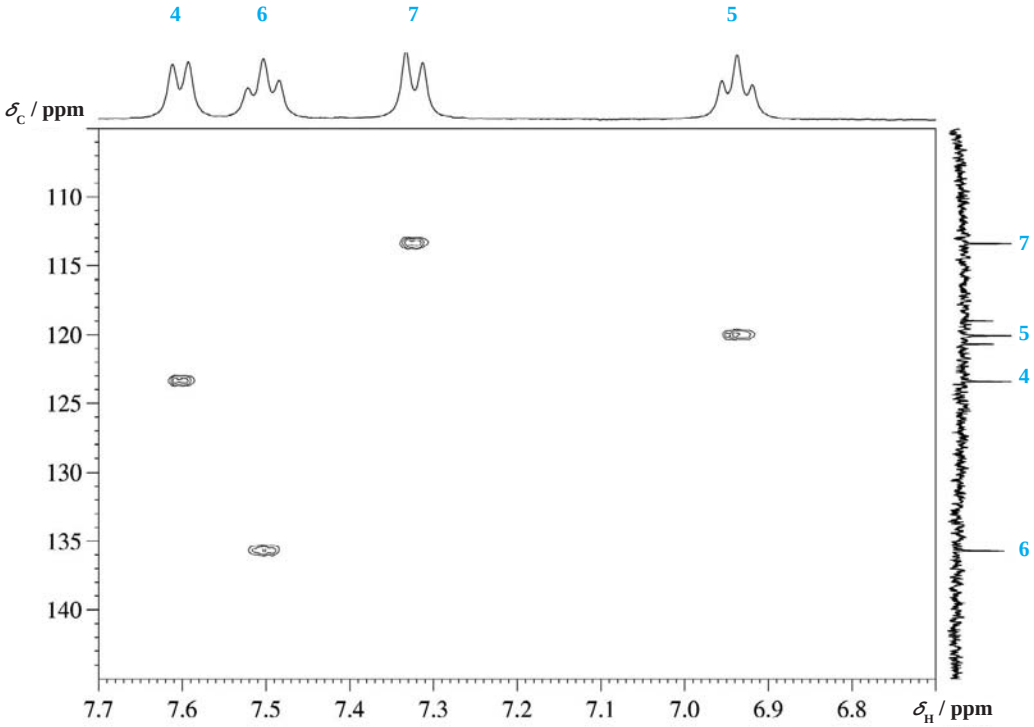


Fig. 3.4-21 HSQC spectrum

The HSQC spectrum immediately fixes the assignment of the protonated carbon atoms, because we have already safely assigned the proton spectrum. It is interesting, that the relative sequence of the carbon chemical shifts does not follow that of the protons.

Fig. 3.4-22 Molecular model of indigo

Sie hörte hinter sich ein spritzendes Geräusch, als hätte jemand aus dem Wasser sich ans Ufer geschwungen. Ein Schauer lief ihr über den Rücken, sie wußte sich plötzlich nicht mehr allein und drehte sich jäh um. Ein großer Knabe stand da, zwischen ihr und dem Wasser, gedrungen stark. Sie hätte glauben können, den Färber vor sich zu sehen: die breitbeinige Gestalt, die gebuckelte Stirne, das krause schwarze Haar; er trug ein Gewand von wunderbar blauer Farbe, nicht so, als hätte man ein weißes Gewebe in die Küpe gelegt, darin sich die Stärke des Indigo und des Waid vermischten, sondern so, als wäre die Bläue des Meeresgrundes selbst hervorgerissen und um seinen Leib gelegt worden.

Hugo von Hofmannsthal (1874−1929)
Die Frau ohne Schatten

Fig. 3.4-23 Close-up of woad seeds at the end of the plant's two-year growth period

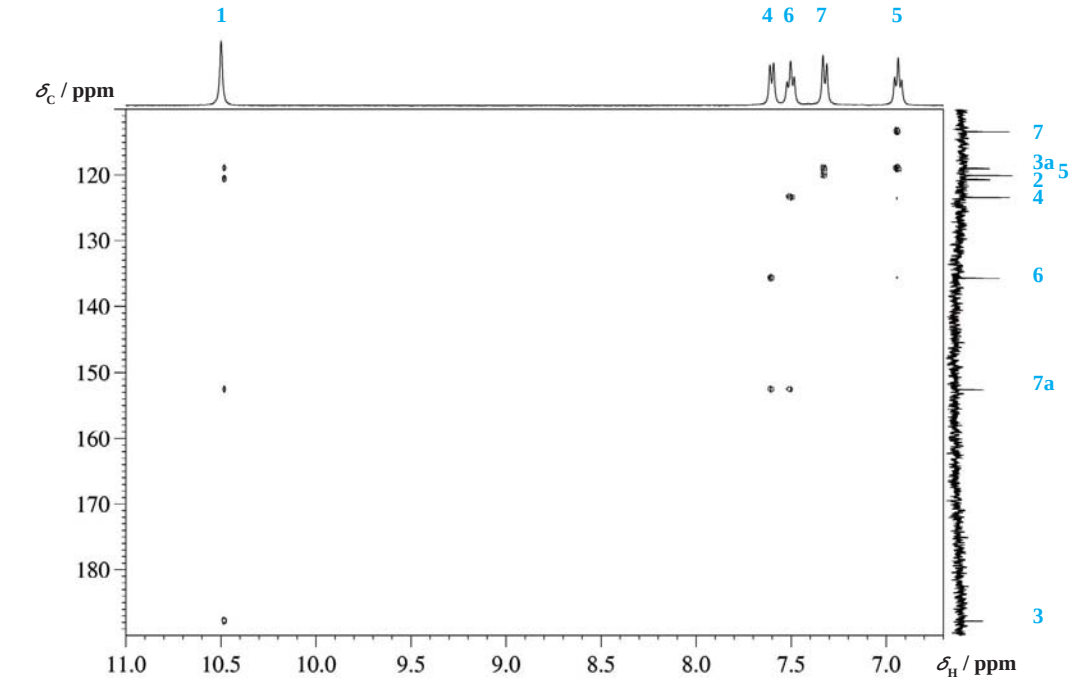


Fig. 3.4-24 HMBC spectrum

Again, the HMBC spectrum is a scholarly example of the power of this technique. We have at this stage only two open questions, namely the assignment of the two quaternary signals of C-3a/3a' and C-2/2'. The NH proton sees all four quaternary signals, of which we have already assigned two, hence the two signals at 119.0 and 120.7 ppm must stem from C-2/2' and C-3a/3a'. The final decision is made from the signal of H-5, which can only be connected to H-3a at 119.0 ppm.

It was of special interest to record the ^{15}N NMR chemical shift data, since they should provide confirmation of whether indigo can be regarded as a vinylogous amide. However, due to the very limited solubility, this attempt was not successful.

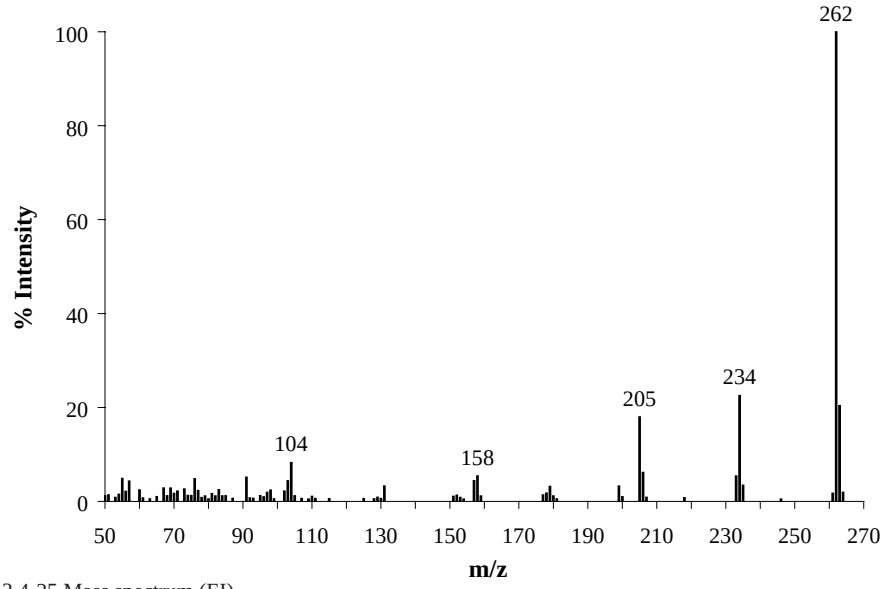
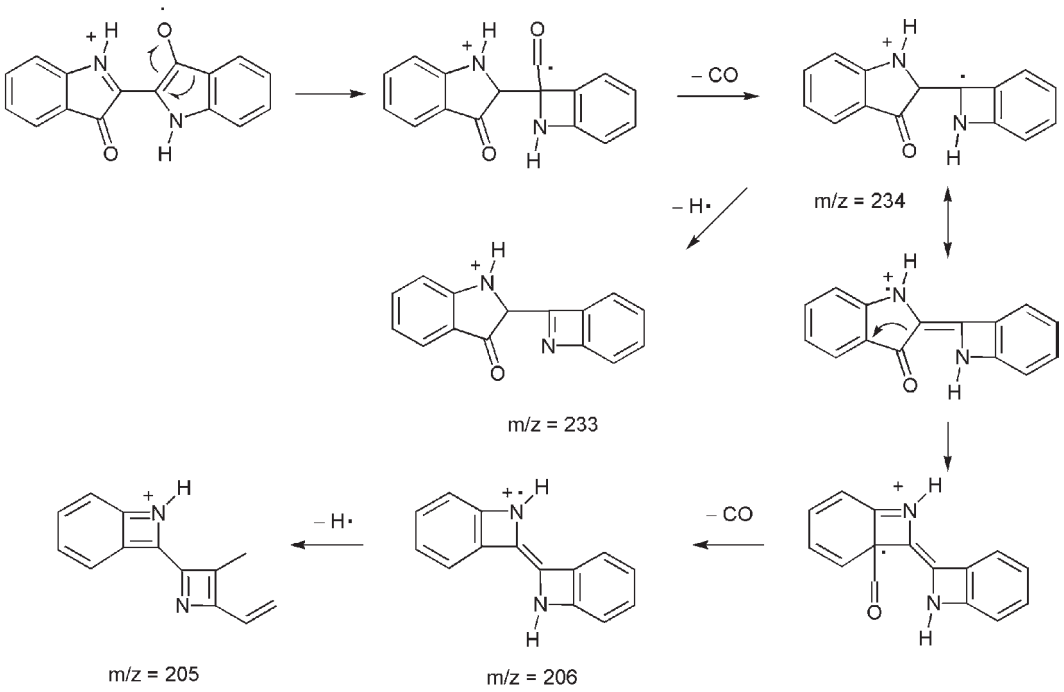


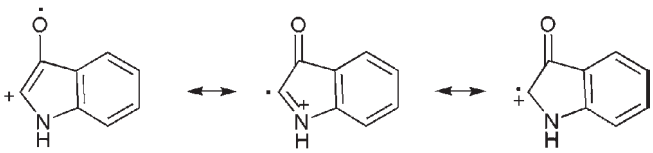
Fig. 3.4-25 Mass spectrum (EI)

The EI mass spectrum shows the molecular ion as the base peak, indicating the stability of indigo even after ionization. We have seen this behaviour for other heterocyclic compounds described in this book, such as caffeine and theobromine. The first fragment ion at m/z = 234 is obviously due to a loss of CO from the molecular ion. This process might be repeated and with an additional loss of hydrogen the fragment ion at m/z = 205 is formed:



Scheme 3.4-5 Fragmentation of indigo

As noted, remeasured and confirmed by the analysis of the ^{13}C isotope peaks by Prof. K. P. Zeller, University of Tübingen, the small peak at m/z = 131 should be interpreted both as M^{2+} and as $M/2^{+\cdot}$. The structure of the latter ion can be drawn as follows:



m/z 131

Scheme 3.4-6 Mass fragment 131

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz
187.8	C_q	C-3/3'	
152.6	C_q	C-7a/7a'	
135.7	CH	C-6/6'	7.50
123.4	CH	C-4/4'	7.60
120.7	C_q	C-2/2'	
120.1	CH	C-5/5'	6.93
119.0	C_q	C-3a/3a'	
113.4	CH	C-7/7'	7.32
			NH: 10.5

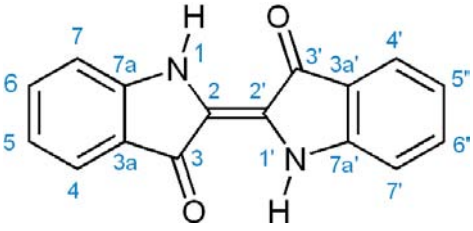
Table 3.4-1 NMR data for indigo

The Blue Jackal

A jackal by the name of Chandarava was living in the forest. Driven by hunger, he decided to risk going to the outskirts of a small village near the forest. When the dogs spotted him, they surrounded him, barked and started to bite him with their sharp teeth.
He ran away as fast as he could, he dodged them and jumped, but the dogs kept coming after him. Running for his life, he entered the house of a vat dyer and plunged into a big tub full of indigo solution. When he crawled back out of the tub, his coat had turned blue, and the dogs didn't recognize him as jackal any more, so they dispersed.

Chandárava also went back into the forest. But the blue color never faded.

Indian Fairy Tale



Scheme 3.4-7

5. Questions

A. Draw the formula of Tyrian purple using the name given in the Background section. Search for the time at which in history this compound was isolated and from what natural source. For what purposes has it been used? Have a look at the structure and make a comment on why this dyestuff will not be available from a plant rooted in the soil.

B. Search for the term Akashin A, to find the citation and structure of a bioactive *N*-glycosidic derivative of 5,5'-dichloroindigo isolated in 2002 from a terrestrial microbial source.

C. Draw the structure of indirubine according to the name given in the Background section. Indirubine is a compound of value for further studies due to its physiological activity. It was first synthesized by von Baeyer from an ethanolic solution of indoxyl and isatin in the presence of sodium carbonate in 1881.

D. Which physicochemical property of indigo is an expression of its low solubility in almost all solvents?

E. Draw reaction schemes for the two indigo syntheses described in the Background section in words.

F. Which class of synthetic dyestuffs also belongs to the class of vat dyes?

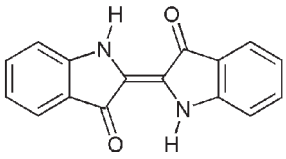
G. By what exact physicochemical value can a dyestuff be clearly distinguished from a compound which shows only a certain coloration? As an example, compare indigo with one of the nitrophenols.

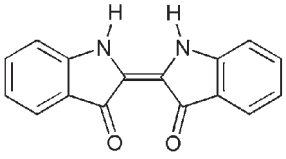
H. The assumption that indigo, purple and Akashin A are all biosynthetically derived from indole seems likely. Compare the sources of these natural products and make a comment on the occurrence and role of indole in evolution.

I. Why is the triplet of H-5/5' more shielded and the triplet of H-6/6' more deshielded?

J. The HSQC spectrum reveals that the two proton doublets have the same relative chemical shift order as their corresponding carbon atoms. The same is true for the two proton triplets. However, comparing the chemical shift order of the doublets and triplets, the ^{13}C chemical shift order is reversed. Is there an explanation?

K. All the spectra shown would also be consistent with the cisoid structure of indigo as shown below. Can you suggest an NMR spectroscopic experiment which would confirm that the indigo structure is indeed transoid?





Scheme 3.4-8

L. Explain the fragments with m/z = 104 and 76 in the mass spectrum.

6. Own Observations

3.5 Capsanthin

(3*R*,3'*S*,5'*R*)-3,3'-Dihydroxy-β,κ-caroten-6'-one

From sweet pepper powder

Capsicum annuum L. (Solanaceae)

$C_{40}H_{56}O_3$, MW 584.87

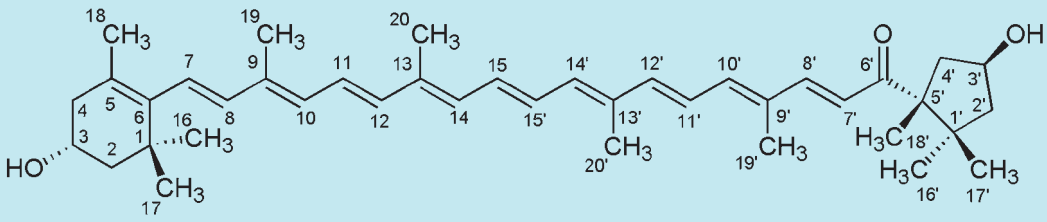
CAS RN 465-42-9, BRN 2068634, 2635416, 9603217

Deep orange crystals, mp 174−175 °C
$[\alpha]_D^{22}$ −68° (*c* 0.006 g/mL, $CHCl_3$)

Capsanthin is commercially available.

Synonymous names:
(All-*E*)-19-(4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)-1-(4-hydroxy-1,2,2-trimethylcyclopentyl)-4,8,13,17-tetramethyl-2,4,6,8,10,12,14,16,18-nonadecanonaen-1-one, All-*trans*-capsanthin

Level: medium



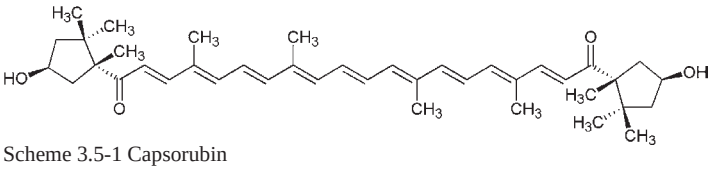
1. Background: June 3, Willstätter‘s dyestuff day

Fig. 3.5-1 Some sweet peppers are orange

The simultaneous occurrence of these yellow pigments, the carotenoids, with the green pigments, which seemed to indicate a special physiological role of the substances because of their great affinity to oxygen, gave rise to the preparation of the yellow substances in the pure state and their analysis. Two well-crystallized and nitrogen-free pigments occur in every green part of the plant and in many yellow parts. One of these, identical with the long-known carotene of carrots, is an unsaturated hydrocarbon of the formula $C_{40}H_{56}$. Its partner, xanthophyll, was still unknown in structure, although it predominates in leaves; according to composition and properties it is a carotene oxide ($C_{40}H_{56}O_2$).

Richard Willstätter (1872−1942),
On Plant Pigments
Nobel Lecture, 1920

Think about June 3: is that a special day for you? Maybe or not, it was an important day for a famous chemist. In 1920, Richard Willstätter was invited to deliver his Nobel Lecture *On Plant Pigments*, in Oslo on this day of the year [1]. Presenting his precise and sensitive work on the separation and structural elucidation of the chlorophylls a and b and the anthocyanins, he also mentioned yet another substance: the yellow xanthophyll, a third kind of compound which he had found always to be a companion of carotene in leaves. He already knew its molecular formula, $C_{40}H_{56}O_2$, and called it a carotene oxide (it was lutein). He had a feeling for the necessity that both kinds of compounds had to be present simultaneously to allow the leaf to live (see the citation in the margin). Meanwhile, their function as constituents of a green leaf is understood both as protective light filters for chlorophyll and as energy carriers (antenna pigments) within photosynthesis. When occurring in petals or fruits they attract insects or other animals which play a role in the plant’s life cycle. Still today we define xanthophylls as oxygen-containing carotenoids. The fruits of *Capsicum annuum* L. contain two main xanthophylls, capsanthin, $C_{40}H_{56}O_3$, and capsorubin, $C_{40}H_{56}O_4$.



Scheme 3.5-1 Capsorubin

However, the history of carotenoid isolation is much older. It begins in 1826 when Wackenroder, a really versatile chemist, isolated a natural dyestuff (it was β,β-carotene) from carrots in the form of red crystals and gave it the name carotene [2]. Often, this early work is forgotten and only the year 1831 is mentioned, and mostly this also without the full citation [3]. Unveiling of a completely unknown molecular structure occurs in several steps. First, the molecular formula has to be found, then the constitution and finally the configuration have to be determined. A lot of work on the constitution of tetraterpenoid *Capsicum* dyestuffs was done in the 1930s in Hungary by Zechmeister and von Cholnoky [4], at the source, Hungary, is a major producer of paprika in Europe. However, to establish the all-*trans* configuration of capsanthin and capsorubin within the chain of conjugated double bonds required new analytical techniques such as the application of ^{1}H NMR spectroscopy – just at the beginning in 1964 [5,6]. As is obvious, their biosynthetic origin as tetraterpenoids is the isoprenoid pathway combined with oxidation steps.

Although *Capsicum* fruits are an agricultural staple product (world production ca. 20 million tons/year), their denomination may be confusing. It is really not easy to give the fruit, from which the sweet pepper powder used in this isolation is made, a name which is understood all around the world. The fruits of *Capsicum* plants have various names depending on the variety within this species and the country concerned: just a few include chilli pepper, red or green pepper, capsicum, jalapeño;

chile (all these mean a spicy, hot pepper fruit) and paprika (as in Hungary; paprika also refers to the powdered spice made from the fruits). Closest to our plant source is the name bell pepper or sweet pepper for the large and mild form. In Hungary in the 1950s varieties were bred which are nearly free of capsaicin (8-methyl-*N*-vanillyl-*trans*-6-nonenamide), a colourless compound causing a sensation of burning in any tissue which comes in contact with it (i.e. hot in the mouth and hot on the skin).

Fig. 3.5-2 Flowering sweet pepper

The orange sweet pepper powder of such *Capsicum annuum* species contains up to 0.5% of dyestuff with only ca. 0.1% being capsanthin. It is used here for the extraction of capsanthin. Industrially, a paprika extract (Oleoresin Capsicum) containing both capsanthin and capsorubin is sold for colouring such different foodstuffs as meat, sausages, marzipan and mayonnaise − and for making cosmetics. Similarly, it is given as an animal feed additive for laying hens to obtain an orange egg yolk. In the European Union, the use of capsanthin as a natural food colour [7] is permitted and encoded E 160c. One may think that such a plant dye would not be very valuable, but the opposite is true. In 2006, the head of the Guangzhou Tianyang Foodstuffs Company in China was sentenced to 15 years in prison for adding the synthetic azo dye Sudan III (Sudan Red, CI Solvent Red 23) to chilli powder and chilli oil between 2002 and 2005. Any addition of this synthetic dye is not allowed for health safety reasons. Based upon this incident, the European Union decided that the import of *Capsicum* products such as chilli powder and paprika powder into the EU would only be allowed after it has been ascertained by chemical analyses that such products do not contain any synthetic dyes. However, it is not only the natural dye that makes *Capsicum* a valuable fruit. It is interesting, that it was also paprika from which the Hungarian chemist Szent-Györgyi isolated vitamin C, a merit which was rewarded with the Nobel Prize for medicine in 1937.

Fig. 3.5-3 A yellow variety

As often in this book, the natural origin of this plant is also South America, where domesticated forms propagated in the north (Mexico) and the west (Peru) already around 7000 BC. The first knowledge of *Capsicum* fruits in Europe resulted from the second voyage of Columbus (1493−1494), where the fruits initially had been called *pimienta* in Spanish, i.e. the same word as used for the real black pepper already known from India. Later, the difference became obvious, but still today *Capsicum* is occasionally called Spanish pepper. The botanical name *Capsicum annuum* was imparted in 1753 by the famous Swedish botanist Carl von Linné, the founder of the modern binominal nomenclature in taxonomy. It is the L. from Linné which follows many of the plant names cited in this book. *Capsicum* was drawn from the Greek word κάψα (kapsa),which means capsule, and is intended as a description of the shape of the fruits. The second part, *annuum*, might be throught to be derived from the fact that *Capsicum* is an annual plant; however, this would be wrong: the plant is perennial when growing in an appropriate mild climate. However, it is able to bear fruits even in the first year.

Carotinum denominari potest principium, quod in hoc succo detexi, tingens, purpureum, pulchras crystallos praehens, oleis aethereis et pinguibus tantum solubile et ad indolem resinarum durarum vel myricini accedens. Olem autem aethereum una cum saccharo ipso principium efficax esse censeo.

H. G. F. Wackenroder (1898−1854), Ref. [2]

As with many natural products, people have also considered the possibility of using the pure xanthophyllic compounds mentioned above, extracts containing them or just the *Capsicum* fruits for maintaining

"Dr. Munro, sir," said he, "I am a walking museum. You could fit what ISN'T the matter with me on to the back of a – visiting card. If there's any complaint you want to make a special study of, just you come to me, sir, and see what I can do for you. It's not every one that can say that he has had cholera three times, and cured himself by living on red pepper and brandy."

Sir Arthur Conan Doyle (1859–1930)
The Stark Munro Letters

Fig. 3.5-4 Red pepper to be stuffed with meat

Fig. 3.5-5 One of the many *Capsicum* varities

health or treating diseases. Generally, such carotenoids can act as antioxidants, i.e. they are able to quench singlet oxygen and to inhibit lipid peroxidation induced by free radicals. They are also able to suppress the generation of nitric oxide and superoxide. Furthermore, capsanthin and capsorubin showed in vitro activity against several tumour cell lines. Therefore, *Capsicum* fruits containing them are regarded as a valuable food [8] containing chemopreventive agents in respect of chemical carcinogenesis. Today, the compounds themselves seem to be at the beginning of a more detailed study of their molecular and physiological mechanism of action and possible further applicability [9].

2. Literature

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3. Isolation

3.1 Principle

Capsanthin shows an orange colour due to the absorption of blue light at about 480 nm (compare the UV/vis spectrum below) due to its extensively conjugated system of double bonds. It should be kept in mind that uptake of light energy may lead to *cis*−*trans* isomerizations similar to that taking place with the isomers of retinal in the retina of the eye during seeing. However, such isomerizations have to be excluded to avoid any difficult separation problems between diastereomers. Therefore, all steps of this isolation have to be done in a dark environment with complete exclusion of light. Any lamps necessary should be heavily dimmed. During extractions and operations with a rotary evaporator, flasks need to be wrapped in aluminium foil.

Capsanthin as a highly hydrophobic compound is extracted from sweet pepper powder by means of petroleum ether. However, by this procedure any other hydrophobic compounds, e.g. fats will be captured at the same time. Also, capsanthin and capsorubin as alcohols are known to occur in the plant as esters, at least to some extent. Therefore, a saponification procedure is connected with the extraction step. Carboxylates and glycerol thus obtained are removed by aqueous washing of the organic phase. Crude capsanthin is crystallized from the concentrated petroleum ether phase, purified in a first step by column chromatography and recrystallized from a small amount of carbon disulfide. The material thus obtained is still not completely pure and has finally to be purified by preparative HPLC.

Fig. 3.5-6
CD from the Red Hot Chili Peppers

I'm a Red Hot Chili Pepper!
Call me Dredd Scott Willy Schlepper!
How 'bout gred plot zilly fleffer?
Kiss it now!
Bling a ling a dingding yow!
Gimme that fork! Eat some pork!
Groucho Harpo Zeppo Chico
Fiji Guam Puerto Rico
Suck my feet!
Wocka wocka robble robble
Watchutalkinbout, Willis?
I know why the caged bird will whistle
Poopsie floopsie this steak is just gristle!
Zibba zabba jibba jabba
How now brown cow
Zei gezunt
My lyrics make no sense
But I sing songs in pluperfect tense
Look at all my participles dangling
I knew a Chinagirl named Ang Ling
Aeroplane roller coaster
By the way I broke your toaster
Rhododendron!
Tippi Hedren!
Plop plop
Fizz fizz
Gnip Gnop
Gee whiz!
New Hampshurbation!
Flapjacks hot cakes Slip 'n' Slide
Inky Blinky Pinky Clyde
Heidi Klum's got a great body
When I'm thirsty I drink a hot toddy
Zigga doodoo jam!
I need more smack.

Anthony Kiedis (1962−)
Co-founder of the
rock band Red Hot Chili Peppers

Fig. 3.5-7 The leaves of a Ginkgo tree turn pure yellow in autumn.

Stuffed Peppers with Ground Beef and Rice

Place peppers in a large pot; cover with salted water. Bring to the boil; reduce the heat, cover, and simmer for 5 minutes. Drain the peppers and set aside.
Heat olive oil and butter in a large skillet over medium heat until hot. Sauté chopped green pepper (from tops), chopped onion, and chopped celery for about 5 minutes, or until the vegetables are tender. Add tomatoes, tomato sauce, crushed garlic, oregano, basil, 1 tea spoon of salt, and 1/4 teaspoon of pepper. Simmer for about 10 minutes.
In a large mixing bowl, combine egg with remaining 1 teaspoon salt and 1/4 teaspoon pepper, and Worcestershire sauce. Gently stir to blend; add ground beef, cooked rice, and 1 cup of the tomato mixture. Mix well. Stuff the peppers with meat mixture and place in a 3-quart baking dish. Pour the remaining tomato mixture over the stuffed peppers. Bake at 180 °C for 55 to 65 minutes. If desired, top the stuffed peppers with a little shredded Cheddar cheese just before the peppers are done; bake until the cheese is melted.

3.2 Method

Sweet pepper powder (*Capsici fructus*) (100 g) and petroleum ether (250 mL, bp 30–80 °C) are placed in a 500 mL round-bottomed flask and mechanically stirred without heating for 4 h. The suspension is filtered through a Buchner funnel and the filter cake is washed with petroleum ether (100 mL). The red filtrate is diluted with diethyl ether (600 mL) and a solution of 30% (w/v) methanolic KOH (100 mL) is added. The mixture is stirred for 8 h at ambient temperature. The solution is then transferred to a separation funnel and washed with water (3 × 100 mL). All lower alkaline aqueous methanolic phases are discarded. The bright orange–red organic phase is dried over $MgSO_4$ and reduced in vacuo to a volume of 20 mL. To this extract petroleum ether (as above) is added (50 mL) and the solution is allowed to stand overnight in a refrigerator at 4 °C. Crude capsanthin crystallizes and is filtered off using a glass filter funnel; 69 mg are obtained.

3.3 Purification

The crude material is subjected to column chromatography.

Conditions: column, 60 × 1.5 cm; stationary phase, silica gel 60 (45 g, 0.040–0.063 mm); eluent, dichloromethane–ethyl acetate (2:1, v/v), 750 mL; crude capsanthin is added in 5 mL of eluent; fractions of 10 mL size are taken. The fractions obtained are analysed by TLC on silica gel 60_{F254} plates in the same eluent. In this system, the R_f value of capsanthin is 0.27. All fractions containing mainly capsanthin with only a tiny amount of a more polar side component (R_f 0.19) are collected and reduced to dryness in vacuo. The remaining orange residue is dissolved in carbon disulfide (15 mL) under reflux and allowed to stand overnight in a refrigerator at 4 °C. The deep orange crystals obtained are filtered off and dried in vacuo to yield 23 mg of capsanthin, mp 174–175 °C, $[\alpha]_D^{22}$ −68° (*c* 0.006 g/mL, $CHCl_3$).

However, both TLC and a ^1H NMR spectrum showed the product not to be completely pure, which prompted a final purification step by preparative HPLC using an RP18 column and methanol as solvent. The method described here was adapted from [10].

Fig. 3.5-8 Pepper spray for self-defence with capsicum as the active ingredient

4. Spectra and Comments

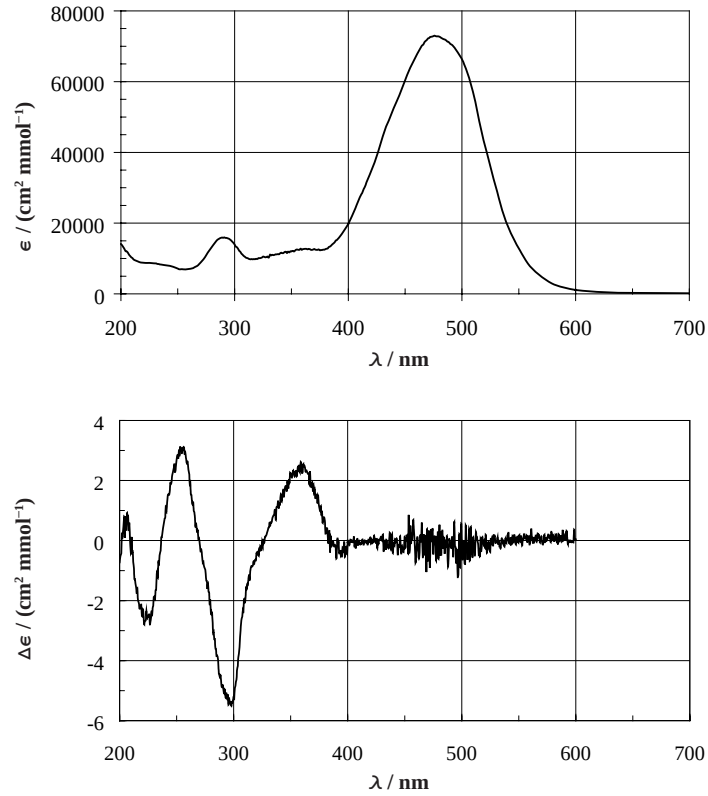
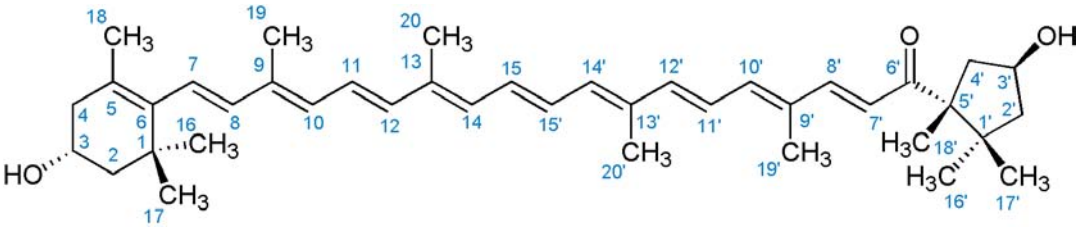


Fig. 3.5-9 Sweet pepper powder used for the isolation of capsanthin

Fig. 3.5-10 UV and CD spectra in ethanol

The dyestuff capsanthin gives a deep red impression to the human eye, since, as the UV/vis spectrum shows, the main absorption is at 480 nm with a very high ε value of about 75 000 cm^2 $mmol^{-1}$. Thus, the absorption band is in the blue to green region, leaving us the red part of the spectrum. Ten conjugated double bonds form the chromophore of the molecule, whereas the keto group of C-6' can be understood as an additional strong chromophore group. The CD spectrum does not show a Cotton effect at the centre of the main band, probably because the centre of the polyene chain is too far away from the stereogenic carbon atoms. In contrast, the weak absorption band at 290 nm, which may be related to the n–π* transition of the keto group, gives rise to a strong negative CD signal with a Δε value of –6 cm^2 $mmol^{-1}$.



Scheme 3.5-2

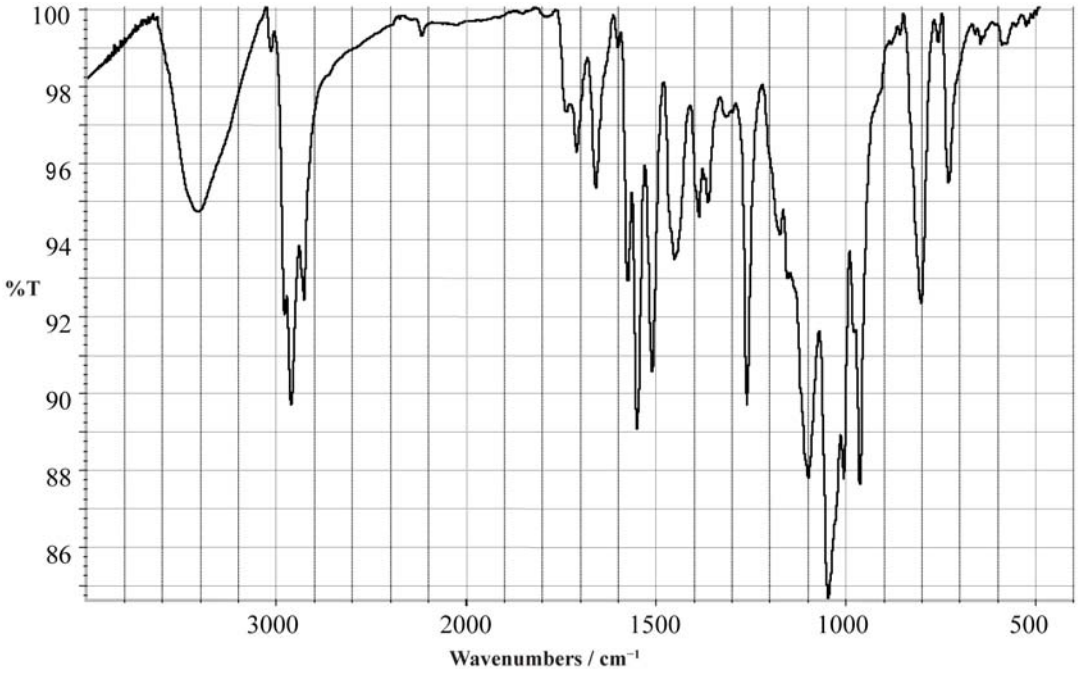
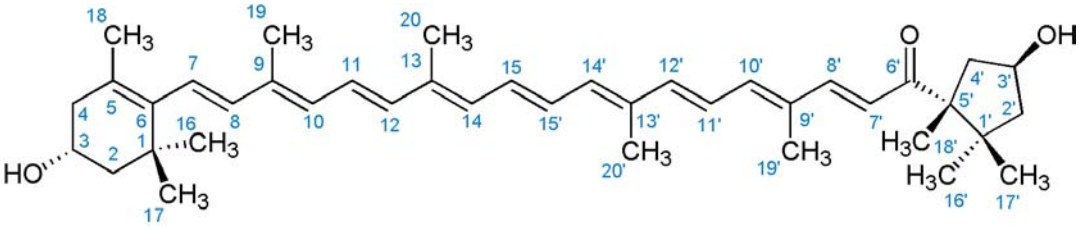


Fig. 3.5-11 IR spectrum in KBr

The IR spectrum is rather untypical. The OH band at 3400 cm^{-1} is followed by only a very minor band for sp^2 hybridized CH valence vibrations over 3000 cm^{-1}, and the main CH valence band lies below 3000 cm^{-1} typical for sp^3 hybridized carbon atoms. The carbonyl band at 1700 cm^{-1} is split as the C=C region at 1600 cm^{-1} into three sharp bands, probably indicating the long conjugated chain.



Scheme 3.5-3

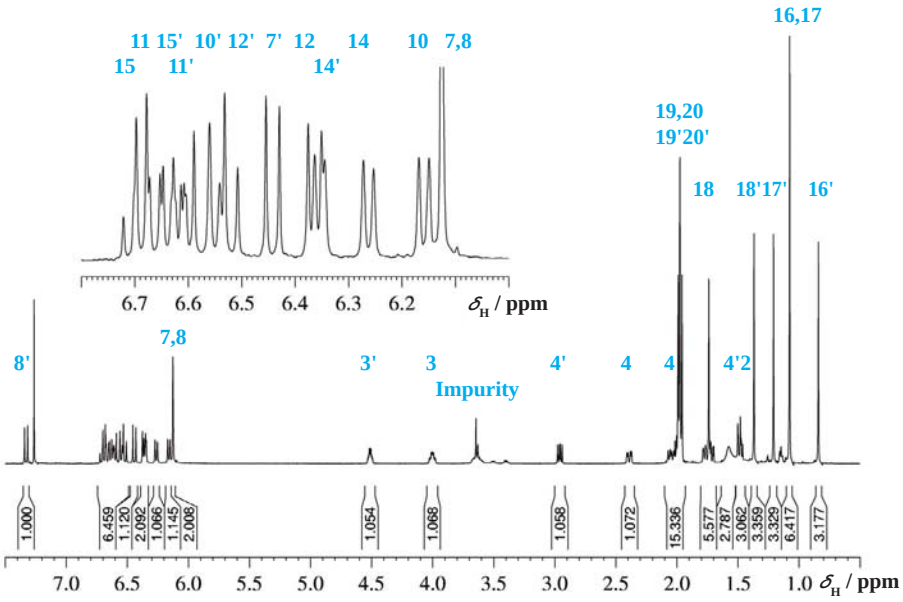


Fig. 3.5-12 ^{1}H NMR spectrum at 600 MHz in $CDCl_3$

Looking at the ^{1}H NMR spectrum and its different expansions, one first finds the signals of the 10 different methyl groups of the molecule. An unfortunate residual impurity gives rise to some signals at about 3.6 ppm. The two signals at 4.0 and 4,5 ppm must belong to the CH(OH) groups C-3 and C-3'. A singlet integrating for two protons in the olefinic region at 6.126 ppm may be assigned to H-7 and H-8. Several multiplets in the olefinic region between 6 and 6.7 ppm are from the main conjugated chain, but the doublet at 7.35 ppm can be safely assigned to H-8', revealing the typical situation of the β-proton of an α/β unsaturated ketone. Further assignments have to await the discussion of the COSY and NOESY spectra.

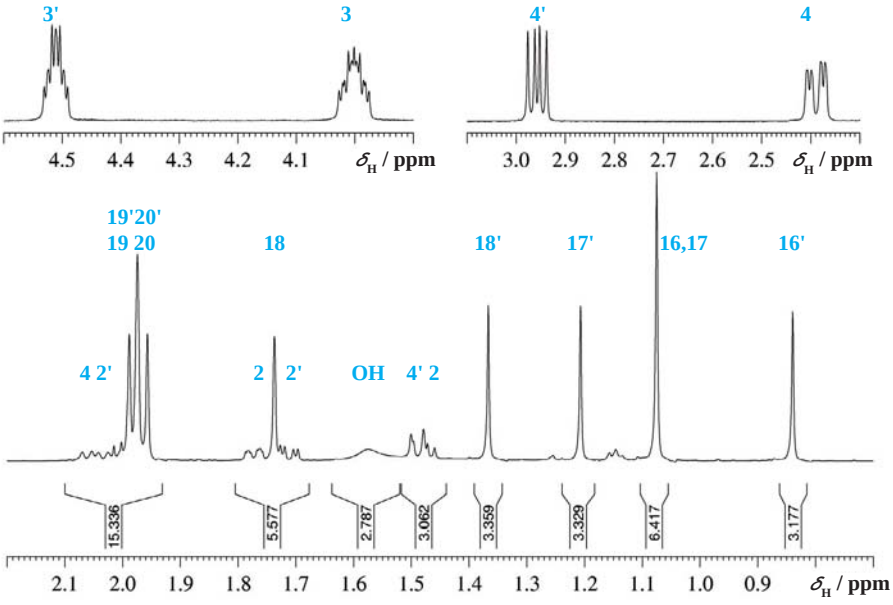
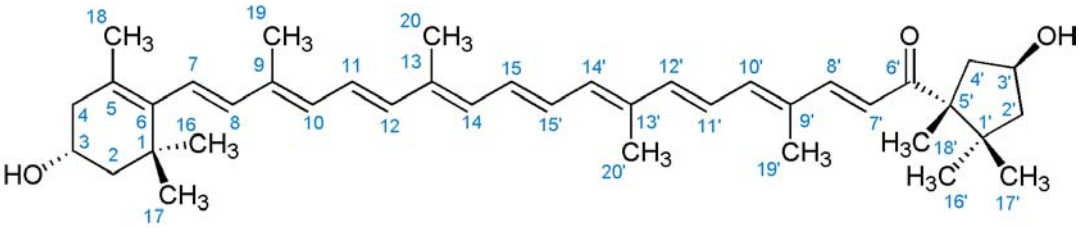


Fig. 3.5-13 Expansions of the ^{1}H NMR spectrum



Scheme 3.5-4

Fig. 3.5-14 Bell pepper

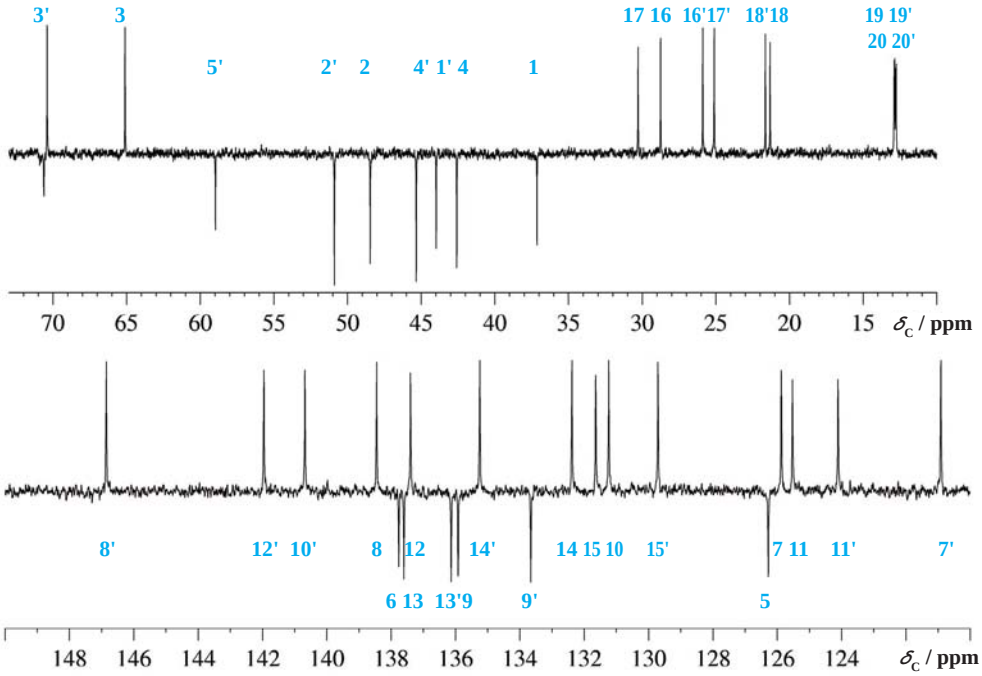


Fig. 3.5-15 APT ^{13}C NMR spectrum at 150 MHz in $CDCl_3$

From the 40 carbon signals of the molecule there is at this stage only a single one which can assigned with safety, namely the signal (not shown) at 202.9 ppm belonging to C-6'. The methyl group region ranges from 10 to 30 ppm, followed by the methylene group signals of the two rings systems from 35 to 60 ppm. An impressive pattern is given by the 20 olefinic signals, where the APT spectrum nicely distinguishes between the C_H and the C_q situations.

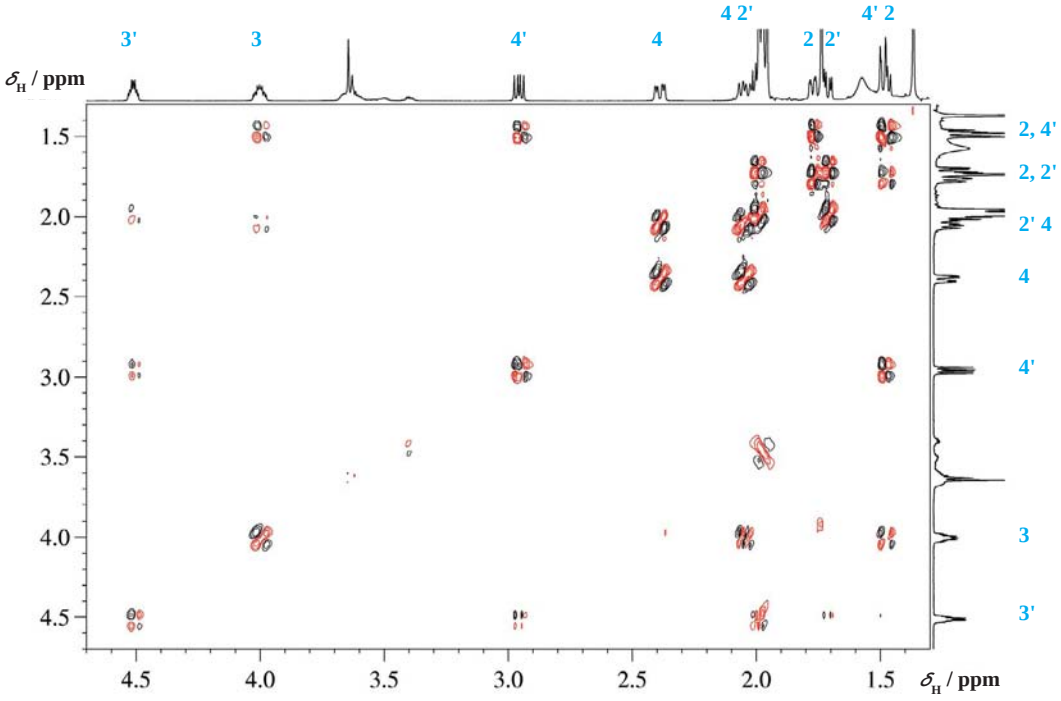


Fig. 3.5-16 Double quantum filtered COSY spectrum

The distinction between two very similar situations of the protons H-3 and H-3' at 4.5 and 4.0 ppm can be achieved by looking at the COSY spectrum. Both CH groups are connected with two diastereotopic methylene protons H-2 and H-4, and H-2' and H-4', respectively. Due to the anisotropy of the carbonyl group C-6', it is expected that the largest diastereotopicity will be found for the methylene protons of C-4' and, with a 1.5 ppm chemical shift difference for the signals at 2.958/1.485 ppm, this is indeed the case. Therefore, the signal at 4.51 ppm belongs to C-3' and the methylene group signals at 1.995/1.706 to C-2'. Similarly to H-3', H-3 is connected to the signals of H-4 at 2.385 and 2.047 ppm and to those of H-2 at 1.786/1.465 ppm.

Fig. 3.5-17 Green paprika fruits on turning orange

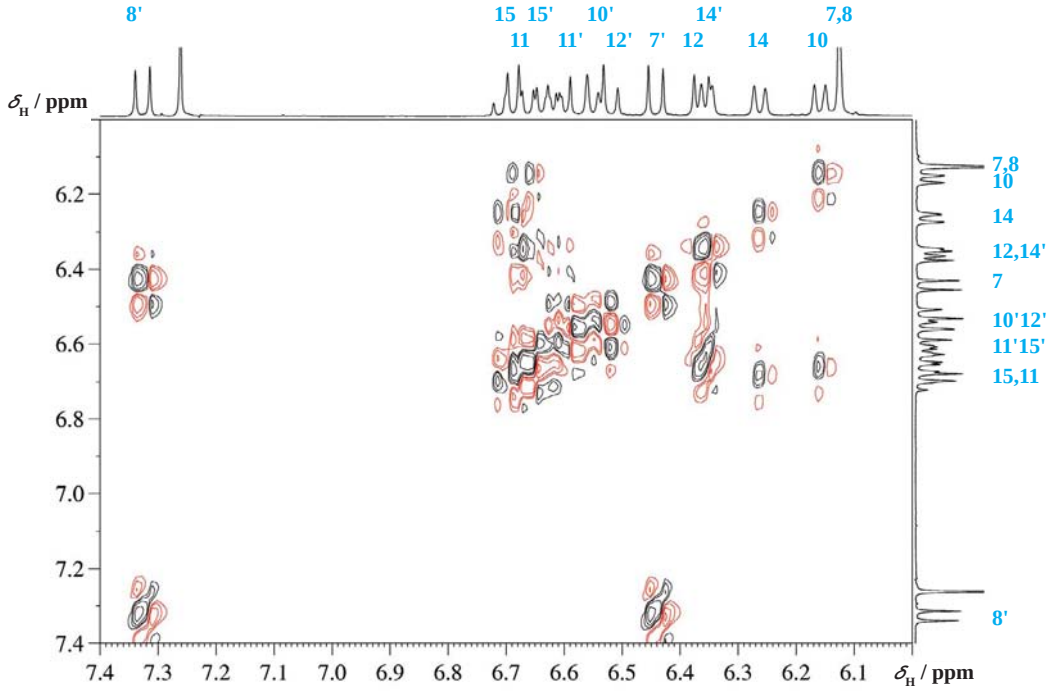
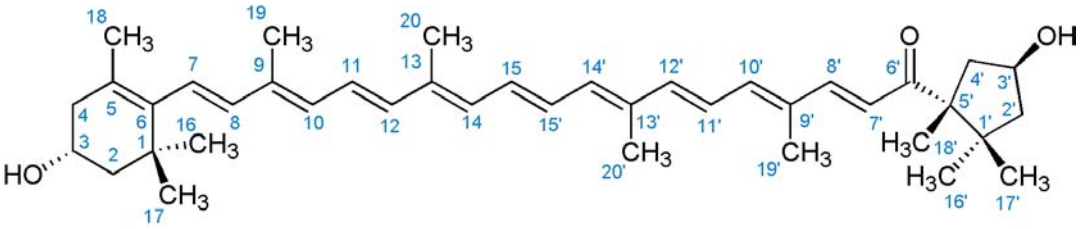


Fig. 3.5-18 Expansion of the COSY spectrum

Having thus fully assigned the protons of the six- and five-membered ring systems, one tries to elucidate the rather long *trans*-olefinic main chain. All spin coupling constants observed for the olefinic chain are in the order of 11−15 Hz, indicating the complete *trans* configuration of the molecule. Very clear is the assignment of H-7' and H-8', displaying in the ^{1}H NMR and COSY spectra the typical pattern of a *trans*-α/β-unsaturated ketone.

Fig. 3.5-19 Chilli peppers



Scheme 3.5-5

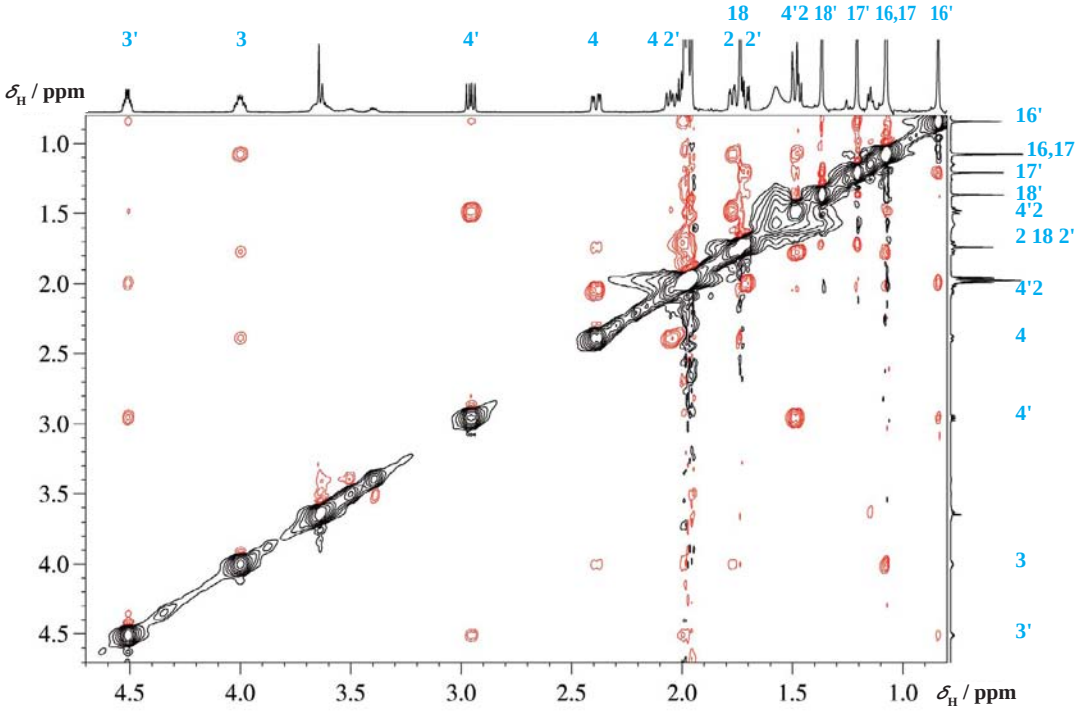


Fig. 3.5-20 Expansions of the NOESY spectrum in the aliphatic region

Capsanthin is a very good example to show the power of NOESY spectra for assignment purposes, since all methyl groups are attached to quaternary carbon atoms and thus spin coupling to the methyl groups is not present. As a consequence, the assignment of the 10 different methyl groups is mainly based on the NOESY spectrum with additional help from the HMBC spectrum discussed later. The signal at 1.075 ppm belonging to six protons shows NOE contacts to the signals of H-3 and H-2 and therefore can be assigned to H-16/17. The two methyl group signals at 0.84 and 1.207 ppm have an NOE contact to each other and furthermore to H-2', hence they can be assigned to C-16' and C-17'. Since the signal at 0.84 ppm further displays an NOE contact to H-3', this must be at the same side of the five-membered ring and therefore is assigned to H-16'. There is another NOE contact from H-17' to the methyl group at 1.367 ppm and this assigns the signal at 1.367 to H-18'.

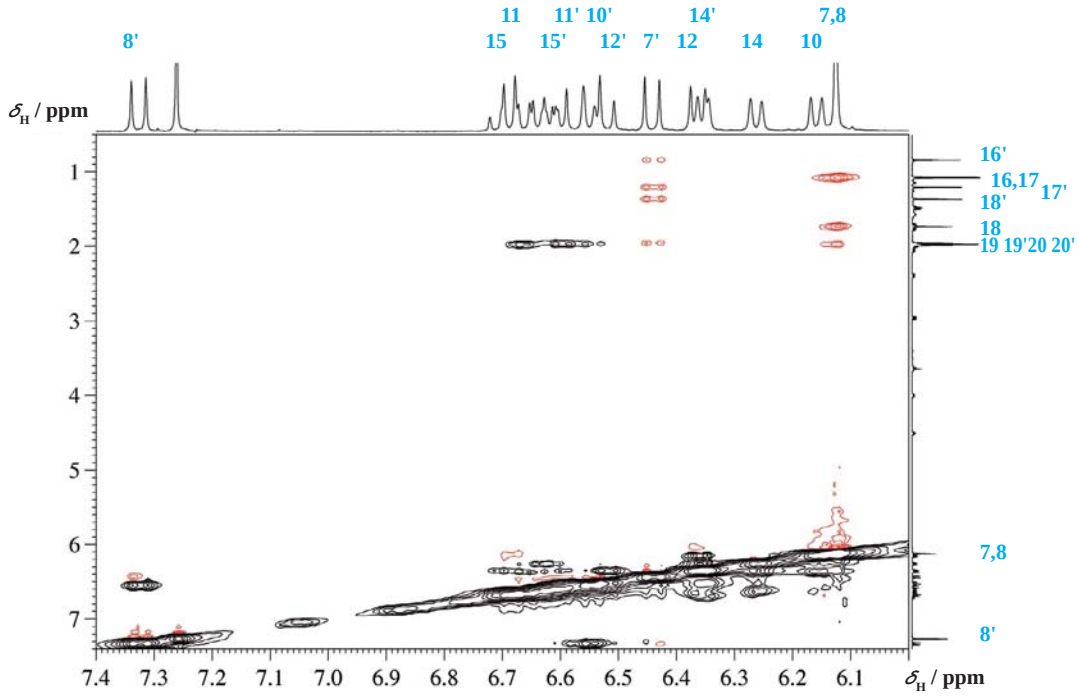


Fig. 3.5-21 Expansion of the NOESYspectrum connecting the olefinic with the aliphatic region

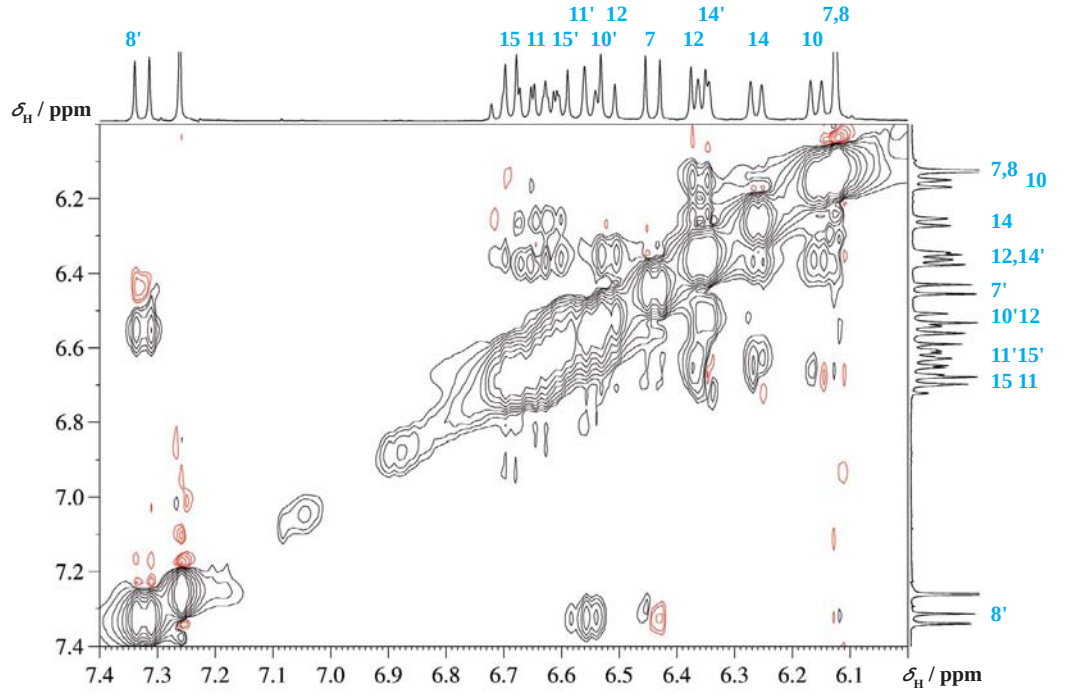
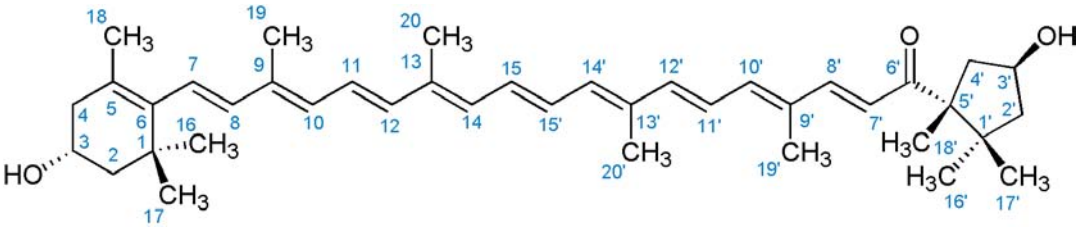


Fig. 3.5-22 Expansion of the NOESYspectrum in the olefinic region

Fig. 3.5-23 Red maple leaves − the chemical principle of the Indian summer

On the other side of the molecule, NOE contacts from the methyl groups H-16/17 and H-18 at 1.732 ppm can be seen for the olefinic proton singlet at 6.126 ppm integrating for two protons. Therefore, these protons can be assigned to H-7 and H-8. This is further corroborated by the NOE of a methyl group H-19. In both the carbon and proton NMR spectra the methyl groups H-19', H-20', H-19 and H-20 resonate very closely together and can only be tentatively distinguished.



Scheme 3.5-6

The connectivity along the conjugated olefinic chain is provided by COSY correlations between vicinal protons and via NOESY contacts between protons four bonds apart, and in addition via HMBC correlations over three bonds. Although the spectrum is rather crowded in this region, it can be resolved if one works with the spectra directly on a computer display. Rather obvious is the NOE contact from H-8' to the triplet of H-10' at 6.544 ppm. Astonishing is the sign of the NOESY cross peaks. Whereas the majority of the cross peaks are positive (opposite to the diagonal and red in our figures), as expected for a molecule of this size in $CDCl_3$ solution, there is a remarkable sign change within the conjugated olefinic chain. H-8' displays a negative NOE effect to H-10'. Similar is true for the NOE contacts between the inner protons of the main chain. Methyl group H-19' displays a positive NOE to H-7', but a negative NOE to H-11'. The NOE effects for the methyl groups H-20 and H-20' to the olefinic protons of the main chain are all negative. This indicates that the molecule tumbles at the borderline for the sign change of the NOE effect, and indeed, a slight change in temperature influences the sign of these NOE effects.

^{13}C Signals δ / ppm	Lit.	Type of Carbon	Assign-ment	^{1}H Signals δ / ppm, J / Hz	Observed connectivities
202.90	202.88	C_q	C-6'		HMBC from H-4' and H-18'
146.85	146.87	CH	C-8'	7.326	HMBC to C-6', C-7', C-10', C-19' and C-9'
141.96	141.97	CH	C-12'	6.523	HMBC to C-10', C-11' and C-14'
140.68	140.67	CH	C-10'	6.544	HMBC to C-8', C-19', NOE zu H-8'
138.45	138.46	CH	C-8	6.126	NOE to H-16, H-17, H-18, HMBC to C-10
137.76	137.79	C_q	C-6		HMBC from H-4, H-16, H-17 and H-18
137.60	137.59	C_q	C-13		HMBC from H-12 and H-14
137.40	137.42	CH	C-12	6.363, J = 15.2	HMBC to C-13 and C-14
136.13	136.12	C_q	C-13'		HMBC from H-12' and H-15'
135.92	135.91	C_q	C-9		HMBC from H-7, H-10 and H-11
135.25	135.25	CH	C-14'	6.35, J = 11.4	HMBC to C-12' and C-15'
133.66	133.65	C_q	C-9'		HMBC from H-7' and H-19'
132.38	132.39	CH	C-14	6.26, J = 11.4	HMBC to C-12 and C-15
131.64	131.66	CH	C-15	6.69	HMBC to C-14 and C-14', NOE to 14'
131.23	131.25	CH	C-10	6.16, J = 11.6	HMBC to C-8 and C-12, NOE to H-12, COSY to H-11
129.71	129.73	CH	C-15'	6.62	HMBC to C-14, NOE to H-14
126.28	126.31	C_q	C-5		HMBC from H-4 and H-18
125.87	125.91	CH	C-7	6.126	NOE to H-16, H-17, H-18
125.52	125.54	CH	C-11	6.67	HMBC from H-9 and H-13
124.11	124.11	CH	C-11'	6.608	HMBC to H-9' and H-12'
120.92	120.97	CH	C-7'	6.44, J = 15.2	NOE to H-16', H-17', H-18', H-19' HMBC to C-6' and C-9'
70.38	70.35	CHOH	3'	4.5	COSY to H-4' and H-2'
65.09	65.07	CHOH	3	4.0	COSY to H-4 and H-2
58.95	58.96	C_q	C-5'		HMBC from H-2', H-4', H-16', H-17' and H-18'

Table 3.5-1 NMR data for capsanthin

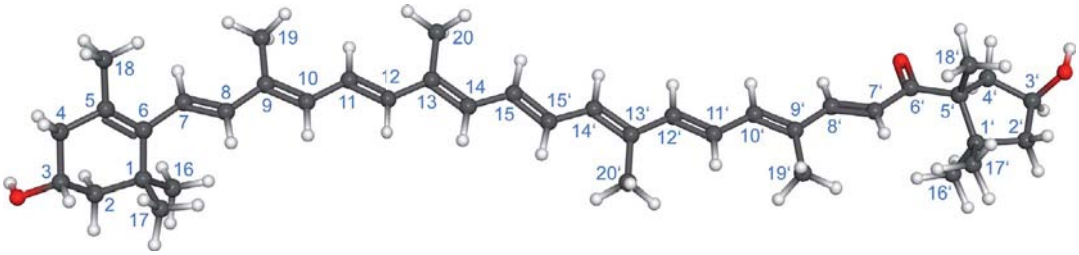


Fig. 3.5-24 Molecular model of capsanthin

^{13}C Signals δ / ppm	Lit.	Type of Carbon	Assign-ment	^{1}H Signals δ / ppm, J / Hz	Observed connectivities
50.90	50.96	CH_2	C-2'	1.995/1.706, J = 4.6; 13.6	HMBC to C-1'; C-4', C-5', C-16' and C-17'
48.46	48.52	CH_2	C-2	1.786/1.465, J = 11.7	HMBC to C-1, C-2, C-4, C-16 and C-17
45.34	45.39	CH_2	C-4'	2.958/ 1.485, J = 8.4, 14.3	HMBC to C-1', C-2', C-5' and C-18'
43.98	43.98	C_q	C-1'		HMBC from H-2', H-4', H-16' and H-17'
42.59	42.63	CH_2	C-4	2.385/ 2.047, J = 5.1, 16.6	HMBC to C-2 and C-3
37.14	37.14	C_q	C-1		HMBC from 16/17 and H-2
30.27	30.27	CH_3	C-17	1.075	HMBC to C-6
28.75	28.76	CH_3	C-16	1.075	NOE to H-3 and both H-2
25.88	25.91	CH_3	C-16'	0.840	NOE to H-3'
25.10	25.12	CH_3	C-17'	1.207	NOE to H-16', NOE to H-2'
21.63	21.62	CH_3	C-18'	1.367	NOE to H-17' and to H-2'
21.31	21.35	CH_3	C-18	1.732	HMBC to C-5 and C-6
12.89	12.87	CH_3	C-20	1.989	HMBC to C-12, C-13 and C-14
12.85	12.83	CH_3	C-20'	1.975	HMBC to C-12', C-13' and C-14'
12.79	12.78	CH_3	C-19	1.975	HMBC to C-8, C-9 and C-10
12.75	12.73	CH_3	C-19'	1.956	HMBC to C-8', C-9' and C-10'

Table 3.5-1 continued

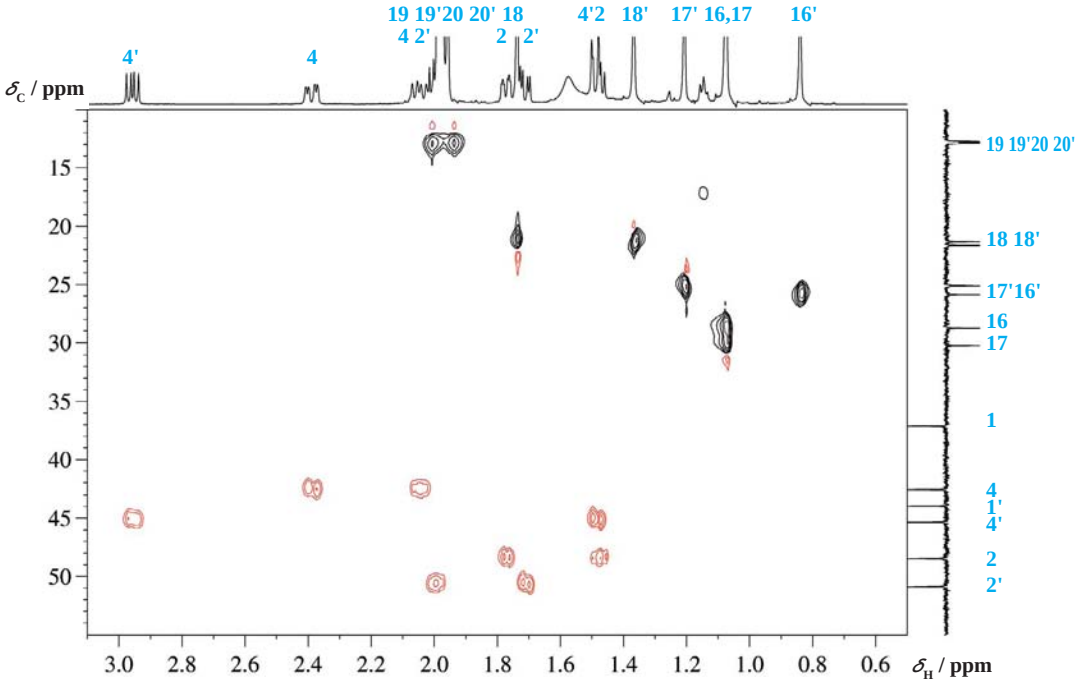


Fig. 3.5-25 Expansion of the ^{13}C edited HSQC spectrum

So far, we have only used arguments from ^{1}H NMR to assign the spectra. The expansion of the HSQC spectrum in the aliphatic region helps to find the pairs of signals of the diastereotopic methylene groups and corroborates their assignment. The ^{13}C assignment of the methyl group and of the olefinic signals follows the proton assignment.

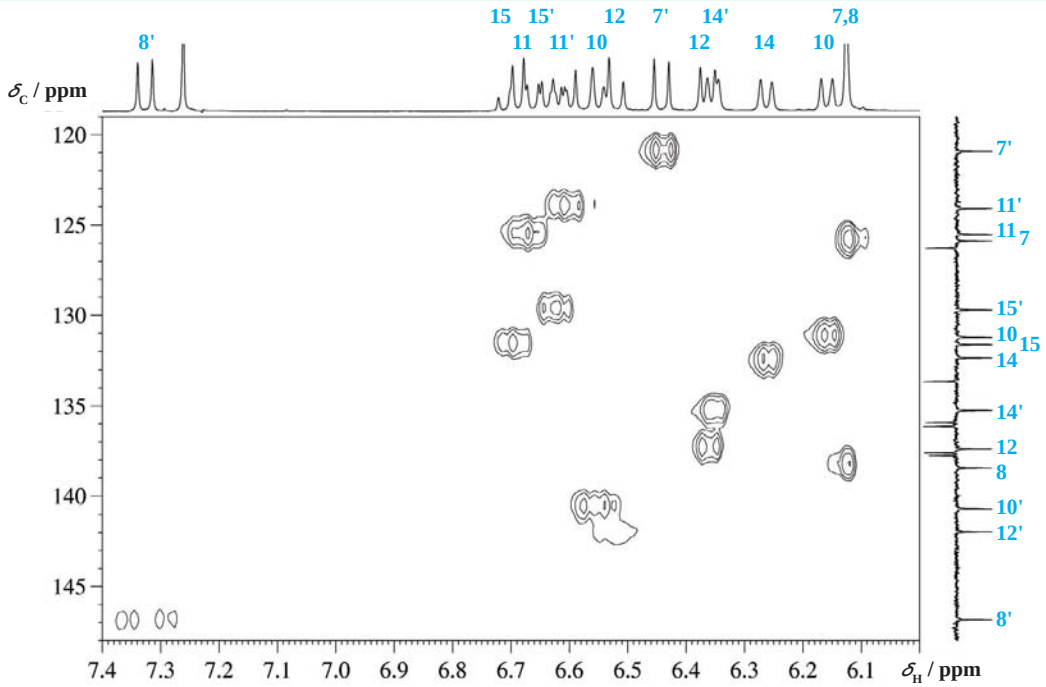


Fig. 3.5-26 Expansion of the HSQC spectrum in the olefinic region

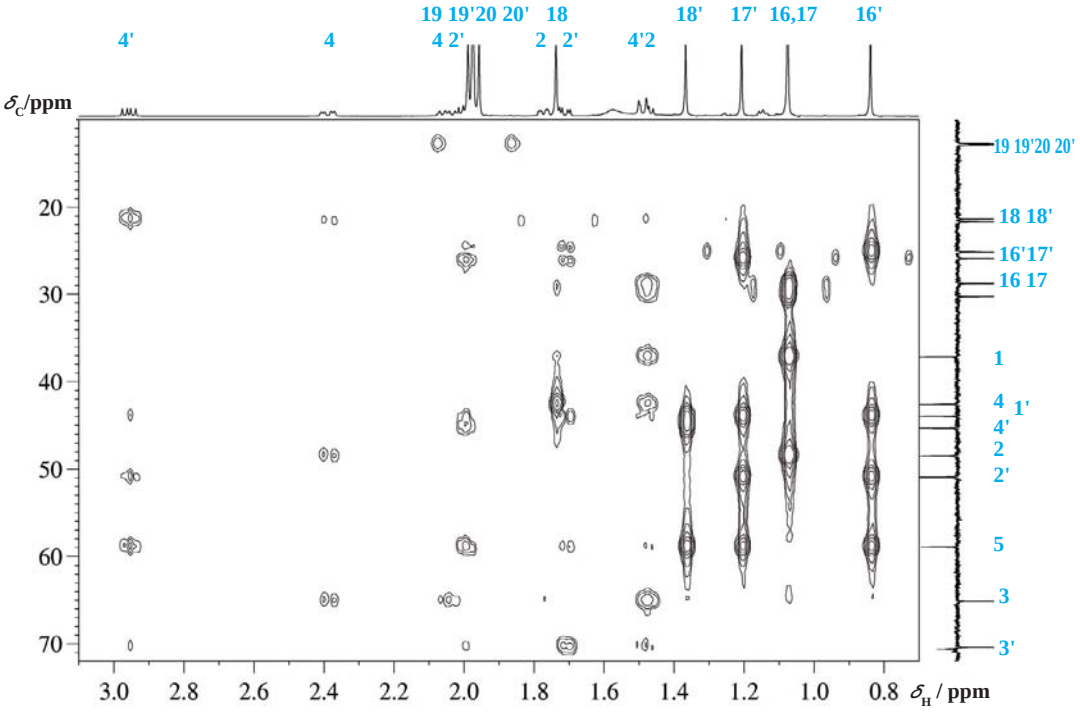
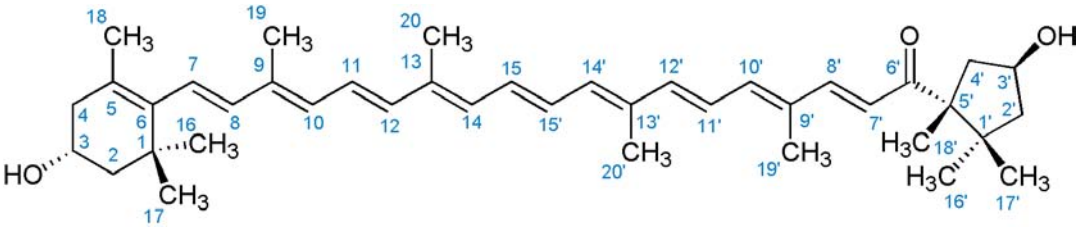


Fig. 3.5-27 Expansion of the HMBC spectrum in the aliphatic region



Scheme 3.5-7

Fig. 3.5-28 Red peppers in a super market

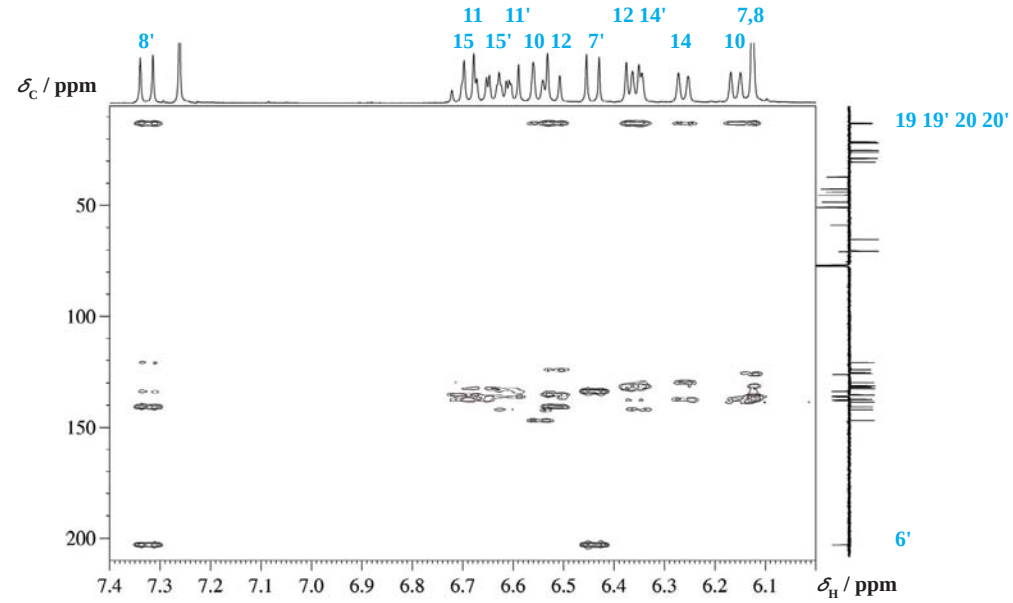


Fig. 3.5-29 Expansion of the HMBC spectrum in the olefinic region

The HMBC spectrum is especially helpful in the case of the methyl groups. The proton signals of the methyl groups 16', 17' and 18' are all connected to the carbon signal of C-1' at 58.95 ppm. Furthermore, these proton signals are coupled to C-2' at 50.9 and C-5' at 43.98 ppm. The methyl protons of H-18' are also spin coupled to C-4'. The geminal methyl groups H-16' and H-17', and also H-16 and H-17, see each other in the HMBC spectrum. Methyl group H-18 has a connection to C-4, as expected. The methyl group signals of H-19, H-19', H-20 and H-20' are very close together, as are their respective carbon signals. They could be distinguished by a high-resolution HMBC spectrum due to their connection to the transoid olefinic chain. Arguments for these assignments are given in the table. Furthermore, the HMBC spectrum is decisive for assigning the quaternary carbons of the olefinic chain, and these arguments are also given in the table.

Fig. 3.5-30 Autumn in the forest: leaves turn yellow. Chlorophyll is decomposed and xanthophylls, which are always present in the leaf, become visible

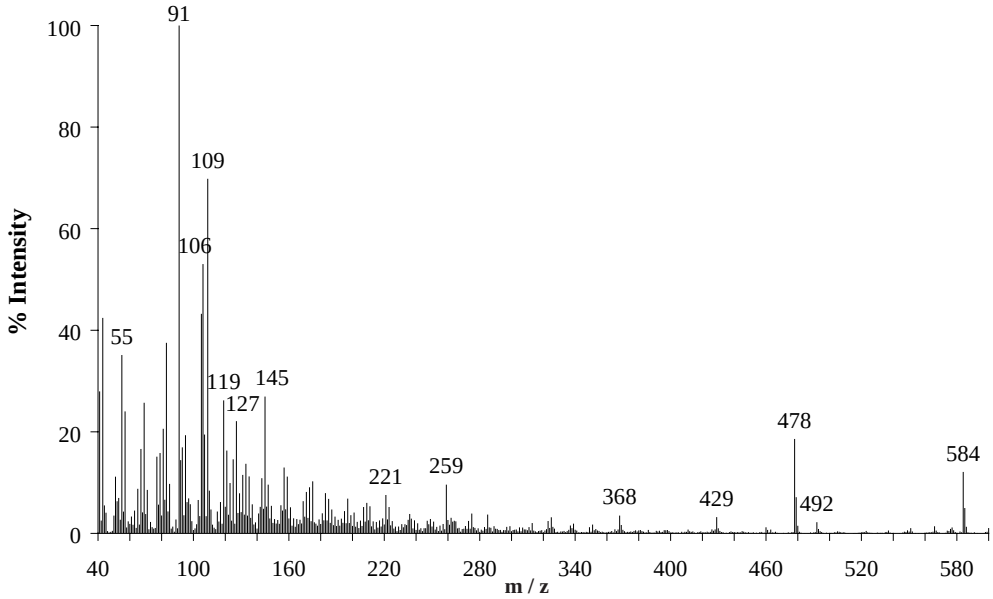
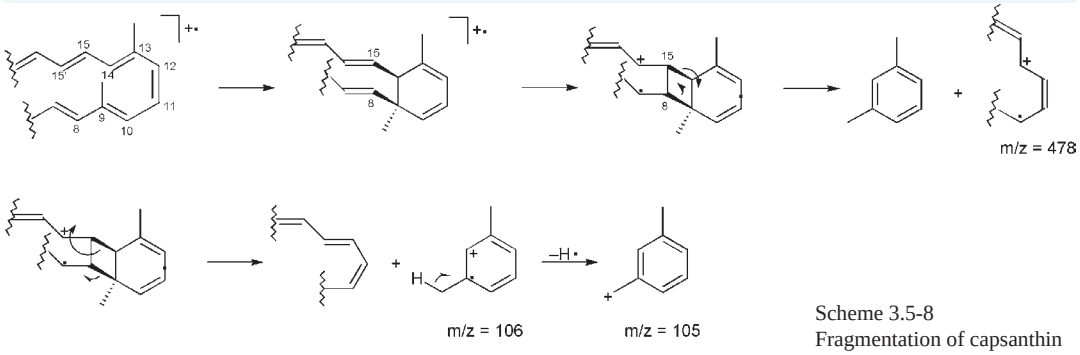


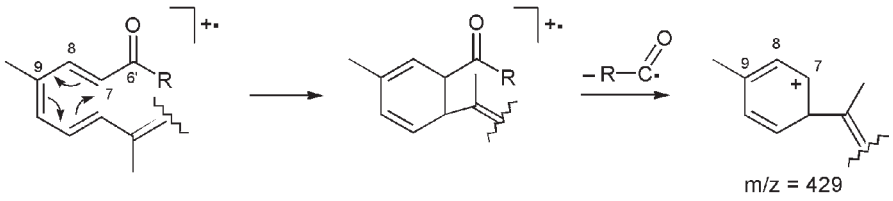
Fig. 3.5-32 Mass spectrum (EI)

A common feature of the mass spectra of carotenoids is the loss of a fragment with mass 106. This is usually explained by neutral loss of xylene from the molecular ion. This will first isomerize around the C-11−C-12 double bond followed by a disrotatory electrocyclization. Opening of the cyclobutane ring formed will produce xylene and an ion with m/z = 478. The cyclobutane ring can also open towards the other side and form ions with m/z = 105 and 106.



Scheme 3.5-8
Fragmentation of capsanthin

The small peak at m/z = 429 can be explained by α-cleavage at the keto group, where the charge is retained by the non-carbonyl fragment. A proposal for the mechanism is given below:



Scheme 3.5-9 Further fragmentation

5. Questions

A. Draw the formula of capsanthin with identification of the eight isoprene units.

B. Capsanthin crystals are deep orange whereas capsorubin crystals are purple. This qualitative test just by looking is confirmed by UV/vis spectroscopy, which show absorption maxima for capsanthin at 483 nm and for capsorubin at 468, 503 and 541 nm. What is such an effect called in terms of colour chemistry? Compare the structures of capsanthin and capsorubin given in this section and explain the structural reason for the effect.

C. A known effect in carotenoid chemistry is that all-*trans*-carotenoids have higher melting points than isomers with *cis*-double bonds. What does this mean in terms of energetic stability?

D. What do the Greek characters β and κ in the CA index name (3*R*,3'*S*,5'*R*)-3,3'-dihydroxy-β,κ-caroten-6'-one for capsanthin mean? And in the same manner, why has β-carotene in fact to be called β,β-carotene and which carotenoid compound can be called ψ,ψ-carotene?

E. Astaxanthin (3,3'-dihydroxy-β,β-caroten-4,4'-dione) is a red xanthophyll responsible for the red colour which becomes visible when a lobster is boiled, whereas fresh lobsters from the sea have a dark blue−black appeareance. Find out by an internet search what has been determined at the molecular level as the origin for this effect.

F. The two multiplets of H-3' and H-3 have different widths, although the chemical situation is very similar. Explain.

G. Which of the protons 4' (2.958/1.485 ppm) is on the same stereochemical site as proton H-3'?

H. Which of the protons 4 (2.385/2.047 ppm) is on the same stereochemical site as proton H-3?

I. Interpret all visible HMBC connections of H-4'.

J. Interpret the base peak of the mass spectrum.

6. Own Observations

Chapter 4 Carbohydrates

Sweet orange from Southeast Asia

In the field of carbohydrates and their derivatives, two scientists made very important contributions which won them the Nobel Prize:

1902

Hermann Emil Fischer
(Germany, 1852−1919)

Germany, Berlin University

"in recognition of the extraordinary services he has rendered by his work on sugar and purine syntheses".

1937

Sir Walter Norman Haworth
(Great Britain, 1883−1950)

Great Britain, Birmingham University

"for his investigations on carbohydrates and vitamin C".

4.1 Glucosamine hydrochloride

2-Amino-2-deoxy-D-glucose hydrochloride

From the shells of common shrimps

Crangon crangon L. (Crangonidae)

$C_6H_{13}NO_5 \cdot HCl$, MW 179,17 + 36.46

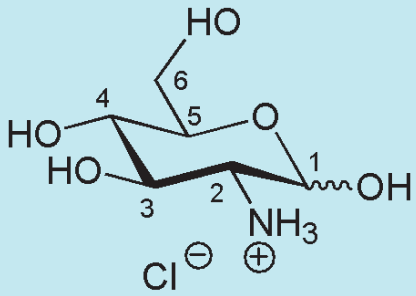
CAS RN 66-84-2 for the hydrochloride,
CAS RN 3416-24-8 for the free base,
no BRN for the hydrochloride,
BRN for the free base as α-form: 1423214,
as β-form: 1281602

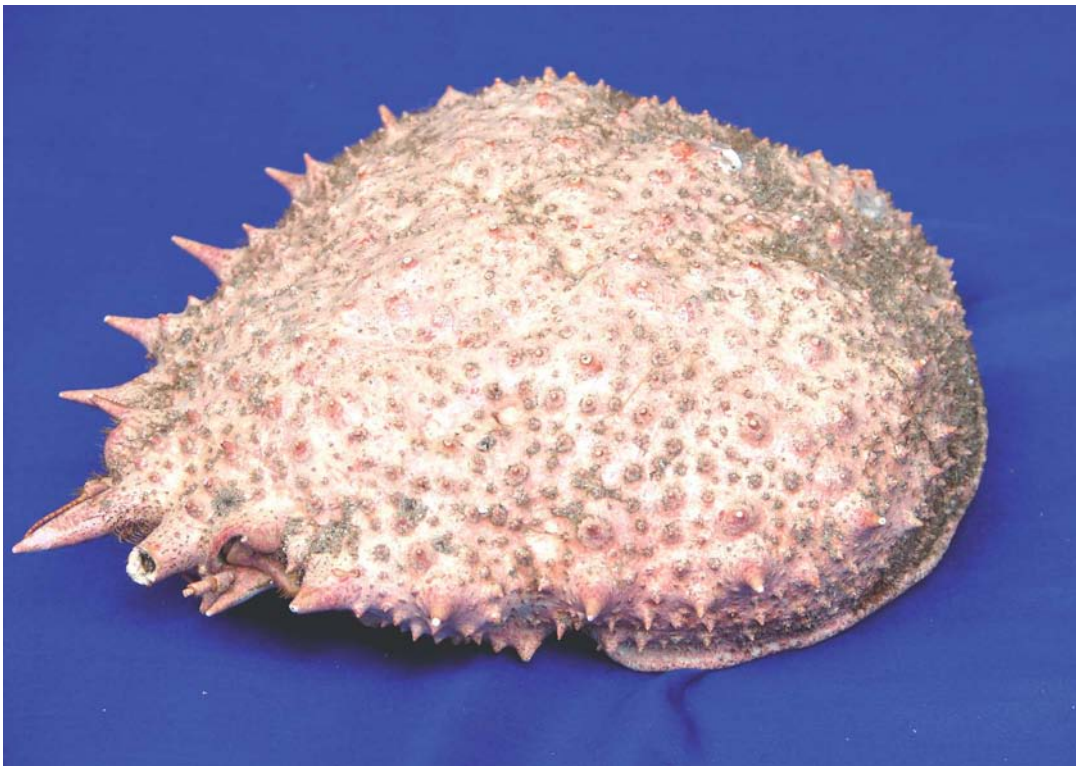
Off-white crystals, mp 198 °C (decomposition)
$[\alpha]_D^{24}$ +70° (*c* 0.01 g/mL, after mutarotation in H_2O)

Glucosamine is commercially available.

Synonymous names:
Chitosamine hydrochloride,
D-Glucosamine hydrochloride, Cosamin, D-GlcN

Level: easy





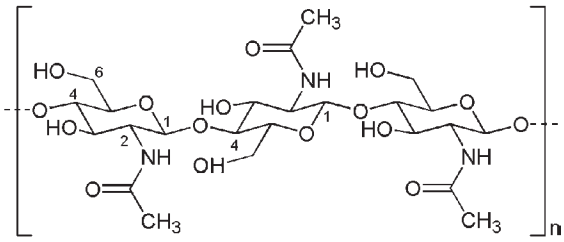
1. Background: Lobster, eye and knee

Als Gregor Samsa eines Morgens aus unruhigen Träumen erwachte, fand er sich in seinem Bett zu einem ungeheueren Ungeziefer verwandelt. Er lag auf seinem panzerartig harten Rücken und sah, wenn er den Kopf ein wenig hob, seinen gewölbten, braunen, von bogenförmigen Versteifungen geteilten Bauch, auf dessen Höhe sich die Bettdecke, zum gänzlichen Niedergleiten bereit, kaum noch erhalten konnte. Seine vielen, im Vergleich zu seinem sonstigen Umfang kläglich dünnen Beine flimmerten ihm hilflos vor den Augen.

Franz Kafka (1883−1924)
Die Verwandlung

Fig. 41-1 Display in a Normandy fish market

Some plants and animals have been regarded as completely utilizable by humans since ancient times. To name just two, local tribes have learned to use all of a coconut, and all of a bison. Do you think that all of a crab is useful? For some people it is. However, this does not mean that you should eat more from a lobster or an edible crab than their meat in the future. Crab shells contain chitin, a polysaccharide of the formula $(C_8H_{13}NO_5)_n$ the chain of which is made up from *N*-acetyl-2-amino-2-deoxy-D-glucose units linked via a β-1,4-glycosidic bond:



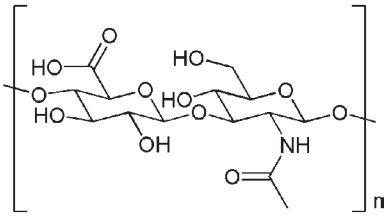
Scheme 4.1-1 Chitin

For short, the monomer is often simply called *N*-acetyl-D-glucosamine (D-GlcNAc). Chitin can exist in three polymorphic forms (α, β and χ forms) which are responsible for the hardness, toughness and flexibility of the natural material but of no meaning for the chemical degradation described here. The word chitin is based on the Latin word “chiton” for a mollusc. Structurally, chitin can be described as a close relative of cellulose, with a 2-acetylamino group instead of a hydroxyl group. One of the outcomes in the change of physicochemical properties is that the multiple 2-acetylamino substitution − due to the carbonyl group present − allows for increased hydrogen bonding between adjacent strands of this biopolymer. This makes chitin macroscopically a stronger material than cellulose fibres. Therefore, it is used e.g. as a material and for flexible and strong surgical threads that are biodegradable and thus disappear on wound healing. Chitin with a degree of more than 50% deacetylation up to complete deacetylation is called chitosan (poly-2-amino-2-deoxy- β-1,4-D-glucose) and is used for several industrial and medical purposes. E.g. it is able to clot blood rapidly when it is part of bandages. In all blood groups, monomeric D-GlcNAc is part of the blood determinant which is anchored in the glycocalix of the erythrocytes.

From the viewpoint of evolution, chitin is a successful invention for all animal organisms that want to move and to do this with a lightweight protection, such as lobsters, crabs, shrimps, ants and beetles. Other animals that do not move fast, such as snails and mussels, can afford a heavier shield made from calcium carbonate. In detail, the exoskeleton of the arthropods is a composite material of a polysaccharide (chitin) and a structure protein (sclerotin). In contrast to the assumption of many, it is not chitin, but sclerotin, that gives the hybrid material its strength, whereas chitin makes it flexible to the necessary degree. However, this section is not intended to look in detail at chitin and its many other applications, but at the medical application of its deacetylated monomer D-glucosamine (2-amino-2-deoxy-D-glucose). An early report on glucosamine hydrochloride appeared in 1876 [1]. The configuration

was assigned only in 1939 by Haworth by a sequence of chemical transformations that excluded the possibility that *chitosamine* (as he called it) may be a derivative of the epimeric mannose [2]. Today, glucosamine hydrochloride is made commercially by the acidic hydrolysis of crustacean shells.

In the animal and human body, D-glucosamine is part of the biosynthesis of a most important polysaccharide with fascinating properties: hyaluronic acid (see formula). In this context, it is believed and also experienced by many people that oral uptake of a D-glucosamine salt can directly have a positive influence on painful diseases such as osteoarthritis of the knee. This will be discussed again at the end of this background.



Scheme 4.1-2 Hyaluronic acid

Hyaluronic acid (synonym: hyaluronan) belongs to the glycosaminoglycans. It is distributed in the connective, neural and epithelial tissues and is a component of the extracellular matrix. The body of an adult contains ca. 15 g of it. Similarly to cellulose and amylose, hyaluronic acid can be regarded as a polymer that arises from the repeat of a disaccharide unit shown in the formula. This disaccharide consists of a D-glucuronic acid and an *N*-acetyl-2-amino-2-deoxy-D-glucose (GlcNAc) unit. The D-glucuronic acid has a β(1→3) glycosidic link to the GlcNAc, which in turn has a β(1→4) glycosidic link to the next D-glucuronic acid. There are about 25 000 repeats in the length, leading to a molecular mass of around 3–4 million Da, e.g. in the human synovial fluid. The structure of hyaluronic acid was determined in the 1950s [3]. However, molecules of this size are not yet the end of the story – hyaluronan itself is the precursor for even larger compounds, the proteoglycans. These represent heavily glycosylated glycoproteins in which a core protein is covalently linked with glycosaminoglycan chains mentioned above.

As always, the possible functions are all based on the chemical structure. Formation of intra- and intermolecular hydrogen bonds in and between hyaluronan molecules is also possible in addition to association and inclusion of large amounts of water by a swelling process due to the hydrophilic and in one case acidic substituents (COOH). In this respect, the biopolymer resembles a synthetic superabsorber of the polyacrylate type. Hence hyaluronic acid is able to bind really astonishing amounts of water in comparison with its own mass (up to 6 L of water per gram). To mention only a few, a biological construction of this kind is part of the human eye, it is the so-called *vitreous humour*, which is made up of 98% water, ca. 2% hyaluronic acid and some collagen. From this organ hyaluronan was first isolated. Another irreplaceable part of the body is the intervertebral discs, with their jelly-like shock absorber units

Er seufzte tief auf, kippte noch einen Wodka hinab und lachte hysterisch: "Ich habe in dem halben Jahre Entomologie gelernt, sage ich Ihnen. Ich weiß heute noch, wie der Marienkäfer auf lateinisch heißt, und der Pappelblattkäfer, und der Erlenkäfer, von dem es eine blaue und eine grüne Form gibt. An einem einzigen Tage, an einem Freitag kamen fünfzig, nein zweiundfünfzig Briefe mit zerquetschten Marienkäfern, dreißig mit Pappelblattkäfern und mindestens zwanzig mit Erlenkäfern. Die Schreiber bekamen beinahe Wadenkrämpfe in die rechten Arme, der Sekretär verriet Anzeichen von entomologischem Verfolgungswahnsinn, und ich hatte das Gefühl, als wenn alle Tische und Stühle sechs Beine und vier Flügel, zwei aus Chitin und zwei häutige, hätten."

Herrmann Löns (1866−1914)
Der zweckmäßige Meyer und andere Geschichten, Kapitel 21

Fig. 4.1-2 Deep-frozen cooked lobster. Frozen in salt water, caught wild in the northwest Atlantic

(*nucleus pulposus*) containing a hyaluronic acid gel. How does cartilage work? How is this shock-absorbing function possible? And how does the lubricating component called synovial fluid that is contained in any joint capsule act?

These fascinating properties of a hyaluronic acid gel are based on a combination of the flexibility of a gel and the incompressibility of water leading to the effect of thixotropy. This means that as a non-Newtonian pseudoplastic fluid, such a gel is able to time-dependently change its viscosity: the longer it undergoes shear, the lower becomes its viscosity. A well-known everyday example of this behaviour is ketchup, which one often can only pour out of a bottle after shaking it well. The only difference between ketchup and your knees is that you do not really need the former one, but you cannot do without the latter.

Die Gazkar stammten von Insekten ab. Ihr Chitinpanzer war schwarz, und ihre Facettenaugen schimmerten in Regenbogenfarben. Sie erreichten eine Körpergröße von etwa 1,60 m. Auf dem Kopf besaßen die Gazkar von Geburt an 17 Chitinstacheln. Wenn ein Gazkar nun befördert wurde, so wurde ihm ein Stachel abgebrannt. Wenn etwas passierte, das zu einer Degradierung geführt hätte, dann wurde der ehrenvolle Selbstmord des Betroffenen erwartet. Als Kleidung besaßen die Gazkar nur verschiedene Gürtel, mit denen sie ihre Strahlenwaffen transportierten. Außerdem besaßen sie noch eine selbstentfaltende Rettungskapsel, falls sie ihr Raumschiff schnell verlassen mussten.

Perry Rhodan (1996)
Krieger der Gazkar

To come back to our natural product of interest, D-glucosamine, a fundamental compound, is made naturally in the form of D-glucosamine-6-phosphate, which is the precursor of all N-containing sugars. Already a few examples of the above have shown the importance of hyaluronan, for example. Therefore, it is no surprise that the suggestion arose to isolate D-glucosamine from chitin as a readily available natural source and to administer it orally (in the form of a salt) in cases of some diseases. Doing so means supporting the body with a dose of a natural product that may help to make urgently needed hyaluronan-containing products such as synovial fluid produced naturally in the body, and others [4].

Finally, it is interesting to look at the practical and scientific opinions about the effect and hence the value of this “reasonable idea”. Practically, both authors of this book know a person in their environment for whom taking glucosamine hydrochloride tablets was immediately helpful against pain in the knee joint. To experience this personally is formidable! The scientific answer should be the objective and requires clinical studies with a multitude of patients and a statistical analysis. Surprisingly, the outcome of this is not of such clarity, on the contrary it is still undecided [5].

In principle, glucosamine salts are available for the consumer in both the USA and Europe, and it is a popular alternative medicine for the treatment of osteoarthritis. A typical dosage of a glucosamine salt is 1500 mg per day, with the amount of the free base varying depending on the anion present. Similarly, it is used as a supplement in veterinary medicine. However, in the USA it does not have approval by the FDA for medical use in humans and is classified as a dietary supplement. This means that evidence on safety and efficacy is not necessary unless it is not advertised as a treatment for a medical condition. In the EU, it is approved as a medical drug and sold in the form of its sulfate or hydrochloride (see photograph). Clinical studies have shown that it appears safe. Surprisingly, the regulation of the hexosamine biosynthesis pathway in which glucosamine is included is not yet fully understood, so there are some concerns that it may interfere with diabetes and obesity. Clinical studies on the question of whether glucosamine can help prevent the destruction of cartilage in the joint, a question which is

regarded as the hallmark of osteoarthritis, have led to both positive and negative results – a disenchanting situation! How should one behave, keeping in mind the above statements?

For an individual who suffers from osteoarthritis and seeks relief, the advice in an old rock song may turn out to be helpful: Have a try – (and consult your physician).

2. Literature

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3. Isolation

3.1 Principle

Chitin from crab shells (Fluka) was used for this preparation. For depolymerization and deacetylation, chitin is refluxed in concentrated hydrochloric acid, leading to a solution of 2-amino-2-deoxy-D-glucose hydrochloride, from which dark colorations are removed by means of activated charcoal and Celite®. Removal of water and addition of ethanol reduce the solubility of the hydrochloride and cause crystallization of the crude product, which is purified by crystallization from water−ethanol. Exclusively the α-anomer crystallizes. By a mutarotation experiment, it can be shown how an equilibrium of α- and β-anomers comes about

Fig. 4.1-3 For sale in a German chemist's shop: capsules with nutrients for joints, bones and cartilage

As the chelae of Crustaceans resemble in some degree the avicularia of Polyzoa, both serving as pincers, it may be worth while to show that with the former a long series of serviceable gradations still exists. In the first and simplest stage, the terminal segment of a limb shuts down either on the square summit of the broad penultimate segment, or against one whole side, and is thus enabled to catch hold of an object, but the limb still serves as an organ of locomotion. We next find one corner of the broad penultimate segment slightly prominent, sometimes furnished with irregular teeth, and against these the terminal segment shuts down. By an increase in the size of this projection, with its shape, as well as that of the terminal segment, slightly modified and improved, the pincers are rendered more and more perfect, until we have at last an instrument as efficient as the chelae of a lobster. And all these gradations can be actually traced.

Charles Darwin (1809−1882)
The Origin of Species, Chap. 7

after dissolving the crystalline product in water. This equilibration starts immediately after dissolution and is complete within 30 min, a short time when compared with D-glucose (8 h). The ratio of α:β-anomers is 62% : 38% in the equilibrium, as shown by the integrations for both anomeric protons in the ^{1}H NMR spectrum.

As typical for many saccharides, this product does not melt on heating but decomposes at a definite temperature.

3.2 Method

This isolation is based on a method reported in the literature [6]. In a 1 L round-bottomed flask chitin from crab shells (Fluka) is mixed with concentrated hydrochloric acid (530 mL), stirred rigorously and refluxed for 2.5 h in a silicone oil bath. Initially, a dark gelatinuous mass is formed which undergoes dissolution nearly completely, with some dark brown insoluble material remaining. The mixture is cooled to room temperature and filtered through a pad of 3 cm Celite® on a sintered glass funnel covered with a filter paper. The dark brown residue remains on the filter paper. To the brown filtrate, charcoal (65 g, grains) is added (caution: foaming) and the mixture is stirred at 60 °C for 30 min. The mixture is again cooled to room temperature and filtered through a pad of 5 cm Celite® on a sintered glass funnel. This operation is done twice. The filtrate is clear and pale brown. Water is removed on a rotary evaporator at 50 °C/40 mbar. Just after removal of a small amount of water, the crude solid product starts to crystallize. After removal of 400 mL of water, a slurry remains which mainly consists of a beige solid covered by a brown mother liquor. Ethanol (600 mL) is stirred into the slurry and the mixture is allowed to stand in a refrigerator at 4 °C overnight. The precipitate is filtered through Buchner funnel and washed with ethanol and diethyl ether (70 mL of each). The product forms small, glittering, beige crystals, which are dried in vacuo to yield 24.6 g of crude 2-amino-2-deoxy-D-glucose hydrochloride (ref. [6], 26 g).

3.3 Purification

For recrystallization, part of the crude 2-amino-2-deoxy-α-D-glucose hydrochloride (19.6 g; 90.9 mmol) is dissolved in boiling water (40 mL). On cooling, crystallization starts rapidly. To the solution, ethanol (160 mL) is added slowly with stirring and the mixture is allowed to stand in a refrigerator at 4 °C overnight. The product is filtered through a Buchner funnel, washed with ethanol and diethyl ether (50 mL of each) and dried in vacuo to yield off-white crystals (16.5 g, 76.5 mmol) of 2-amino-2-deoxy-α-D-glucose hydrochloride with a decomposition point of 198 °C.

Mutarotation experiment: $[\alpha]_D^{24}$ +87° (measured after 2 min, necessary for the dissolution of all material) → +70° (at equilibrium; *c* 0.01 g/mL, water). This corresponds to the result of [6]: $[\alpha]_D^{25}$ +100° → +72°.

Lobster Quadrille

The Mock Turtle sighed deeply, and drew the back of one flapper across his eyes. He looked at Alice, and tried to speak, but for a minute or two sobs choked his voice. "Same as if he had a bone in his throat," said the Gryphon: and it set to work shaking him and punching him in the back. At last the Mock Turtle recovered his voice, and, with tears running down his cheeks, he went on again:
"You may not have lived much under the sea" ("I haven't," said Alice)"and perhaps you were never even introduced to a lobster" (Alice began to say "I once tasted" but checked herself hastily, and said "No, never") "so you can have no idea what a delightful thing a Lobster Quadrille is!"
"No, indeed," said Alice. "What sort of a dance is it?"
"Why," said the Gryphon, "you first form into a line along the sea-shore"
"Two lines!" cried the Mock Turtle. "Seals, turtles, salmon, and so on; then, when you've cleared all the jelly-fish out of the way–"
"THAT generally takes some time," interrupted the Gryphon.
"–you advance twice"
"Each with a lobster as a partner!"cried the Gryphon.

Lewis Carroll (1832–1898)
Alice's Adventures in Wonderland,
Chapter 10

Note on attempts to prepare the free base:

A method reported by Stacey and Webber [6] to liberate the free base 2-amino-2-deoxy-D-glucose (chitosamine) by means of reaction with triethylamine in ethanol could not be repeated. Instead, use of the ion exchanger Amberlyst A-46 (OH^- form) in methanol for the same purpose gave rise to the free base, as could be shown by the absence of chloride ions and coincidence of the optical rotation value with literature values; however, crystallization could not be achieved. This seems to be a typical difficulty with the compound, considering the widely differing melting point values reported in databases.

4. Spectra and Comments

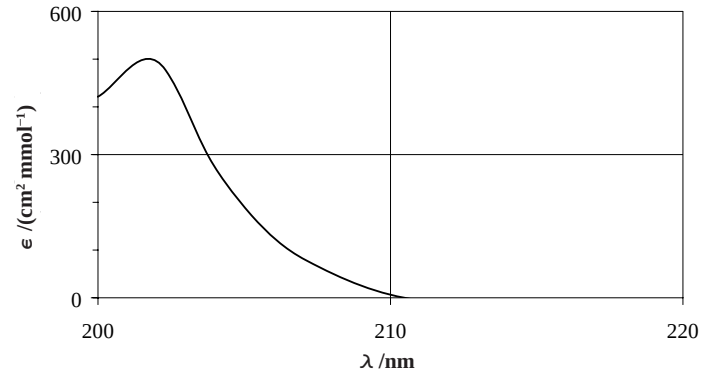
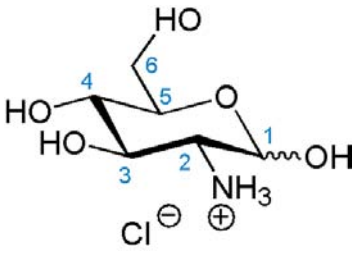


Fig. 4.1-4 UV spectrum in ethanol



Scheme 4.1-3

Typically for a sugar derivative, the UV spectrum does not show any relevant absorption above 210 nm. As a consequence, we do also not obtain a CD spectrum, although the compound is chiral.

fig. 4.1-5 Advertisement for lobsters and spider crabs in France

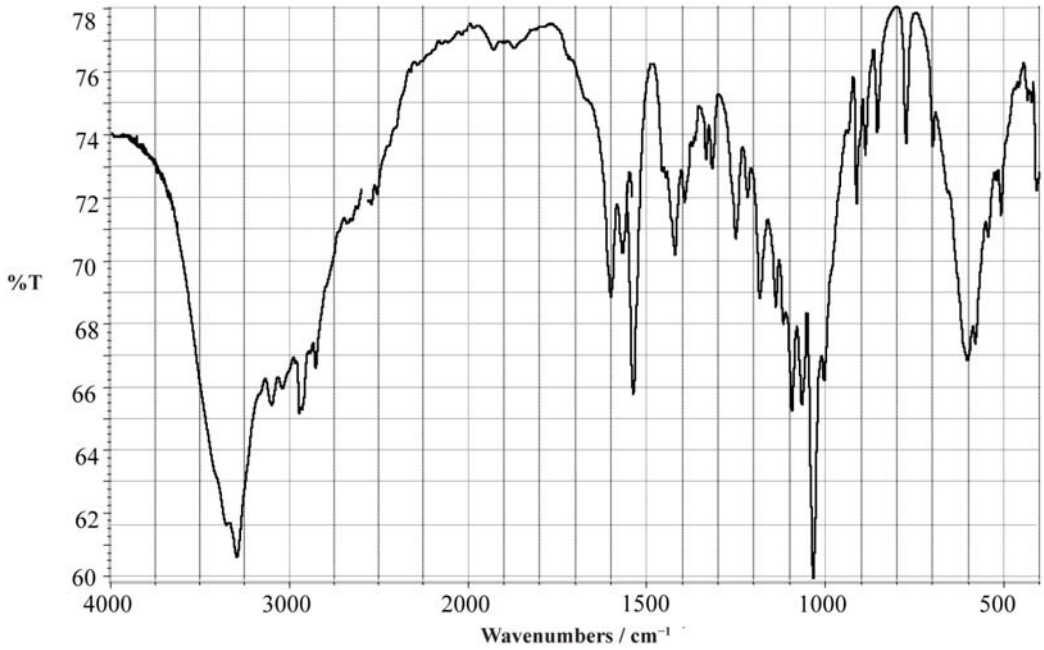
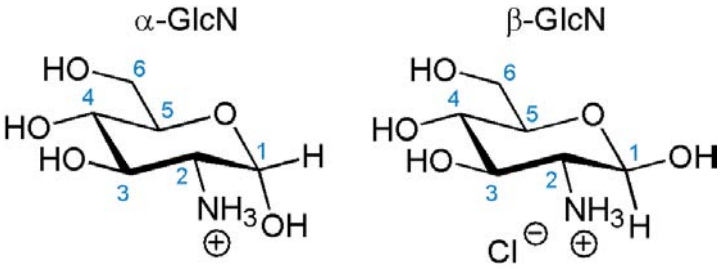


Fig. 4.1-6 IR spectrum in KBr

The IR spectrum reveals the presence of many OH groups, but otherwise no significant structural features.

Fig. 4.1-7 The shell of this sea urchin consists not of chitin but of calcium carbonate, whereas the shell of the spider crab shown at the front page of this section is a composite material of chitin and sclerotin



Scheme 4.1-4

La jeune fille ne disait rien, un peu rouge, un peu timide, gênée par le voisinage de cet homme dont elle soupçonnait les pensées.
Quand le homard apparut, César déclara: "Voilà un personnage avec qui je ferais volontiers connaissance."Lesable, souriant, raconta qu'un écrivain avait appelé le homard "le cardinal des mers", ne sachant pas qu'avant d'être cuit cet animal était noir. Cachelin se mit à rire de toute sa force en répétant: "Ah! ah! ah! elle est bien drôle."

Guy de Maupassant (1850−1893)
L'Heritage

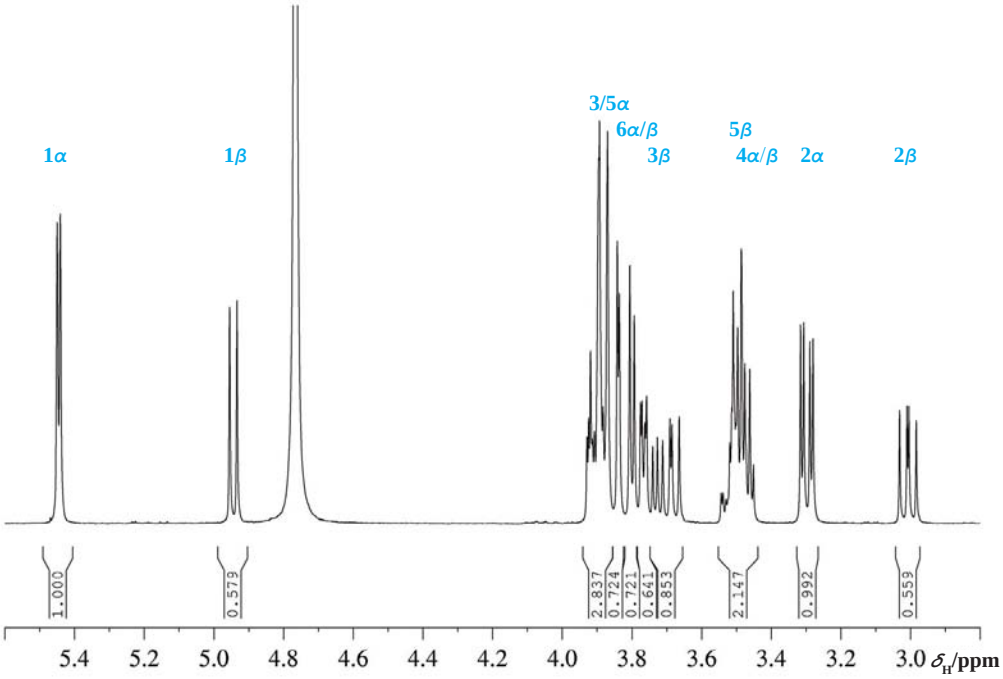


Fig. 4.1-10 ^{1}H NMR spectrum at 400 MHz in D_2O

In the ^{1}H NMR spectrum, signals of both the anomeric protons of the α- and the β-form appear to the left of the water signal at 5.45 and 4.95 ppm. Their integretion reveals an α:β ratio of about 1:0.6. Their relative assignment is obvious due to the larger axial axial ^{3}J spin coupling constant in the β-form. Looking at the spin splittings of the anomeric signals, one can directly identify the neighbouring protons H-2β and H-2α at 3.0 and 3.3 ppm, which have the same intensity ratio as the anomeric signals and display the corresponding spin coupling constants. The rest of the proton spectrum between 4 and 3.5 ppm shows considerable spectral overlap, which will be disentangled mainly with the help of the selective TOCSY spectra.

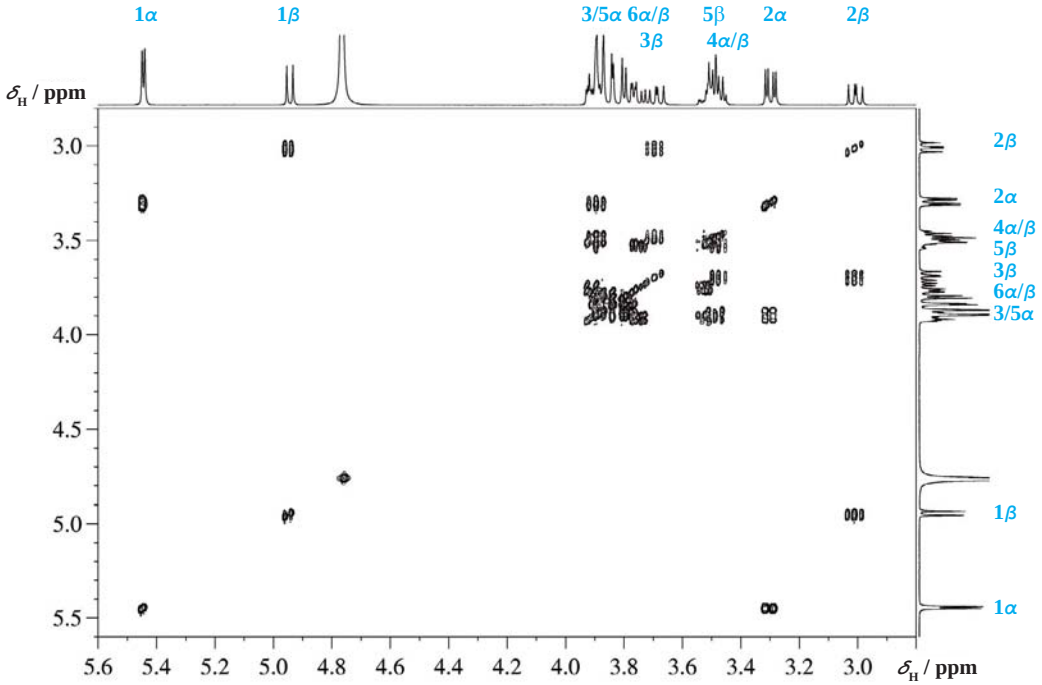


Fig. 4.1-9 COSY spectrum

In the COSY spectrum, we easily find the cross peaks leading from the anomeric protons to the respective protons at C-2 for both the α- and β-forms at 3.3 and 3.0 ppm. The COSY spectrum, which in this case is very helpful, leads us further to both H-3 at 3.89 and 3.69 ppm. From these signals we find the overlapping resonances of H-4α and H4-β at 3.5 ppm.

Fig. 4.1-10 Crab shell powder as used for isolation

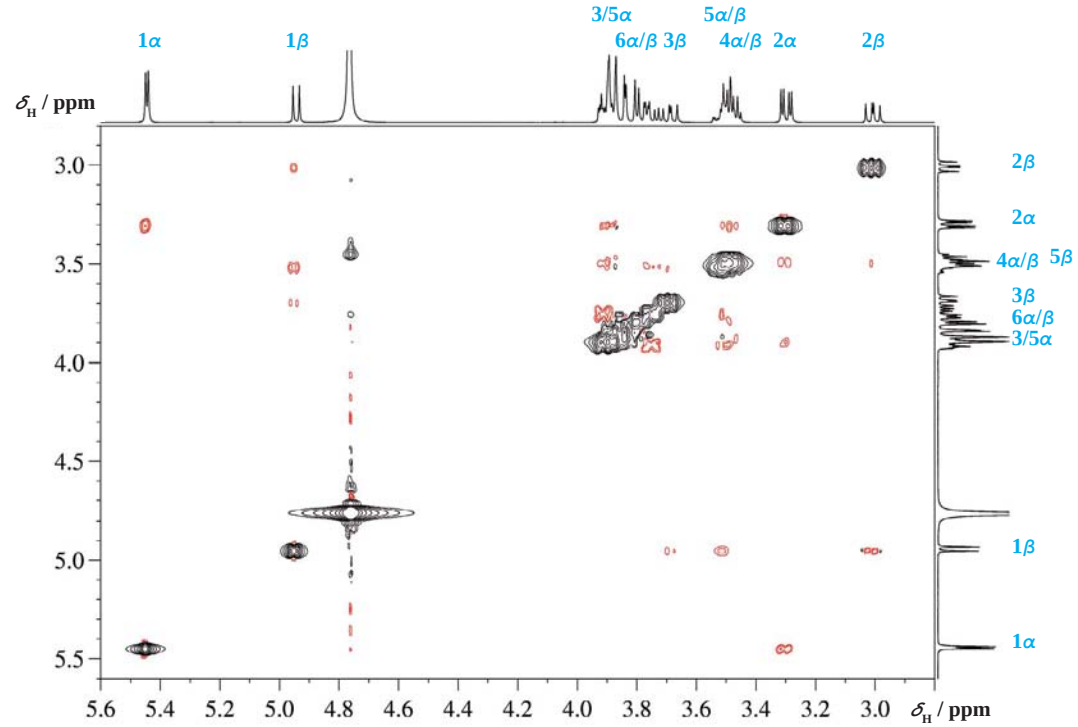


Fig. 4.1-11 NOESY spectrum

As is to be expected, the integral of the NOESY cross peak between the anomeric proton H-1α to H-2α is much larger than the corresponding integral between H-1β and H-2β, as can be judged by visual inspection. In contrast to H-1α, H-1β displays two additional cross peaks to H-3β and H-5β caused by 1,3-axial interactions. 1,3-Axial interactions also are responsible for the NOE cross peaks between H-2α/β and H-4α/β.

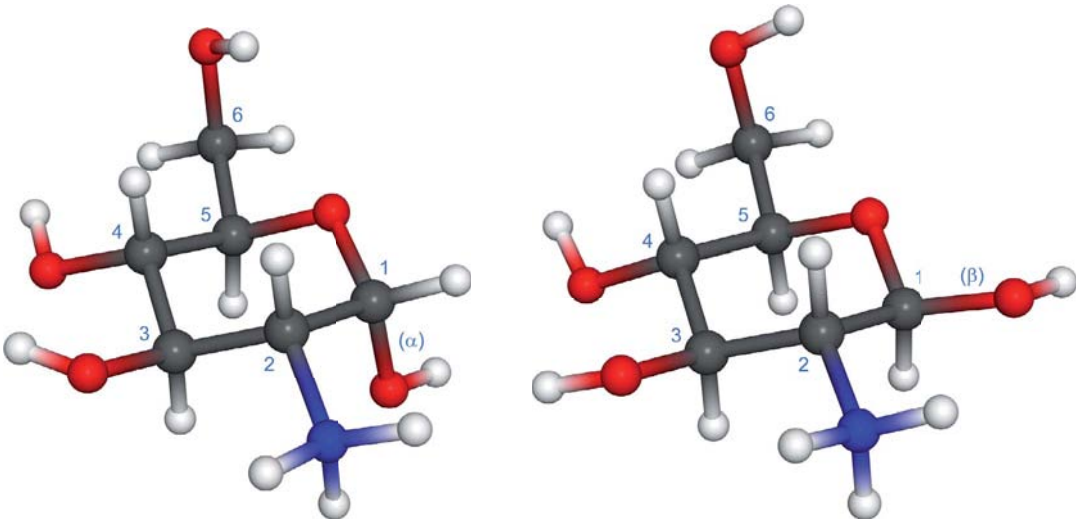


Fig. 4.1-12 Molecular models of glucosamine

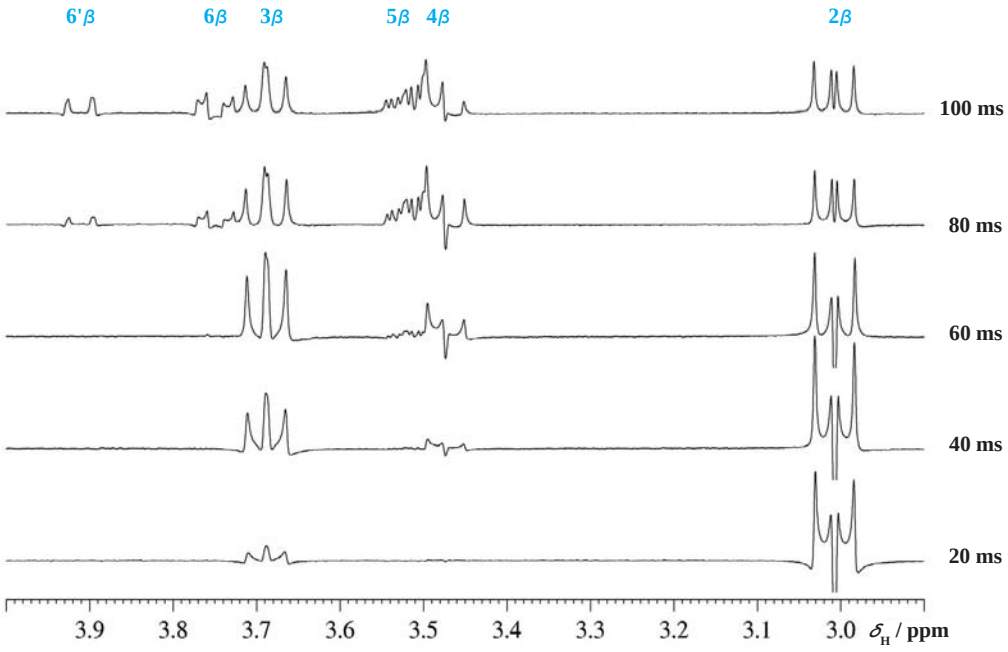


Fig. 4.1-13 Selective TOCSY spectra by irradiation of H-1β

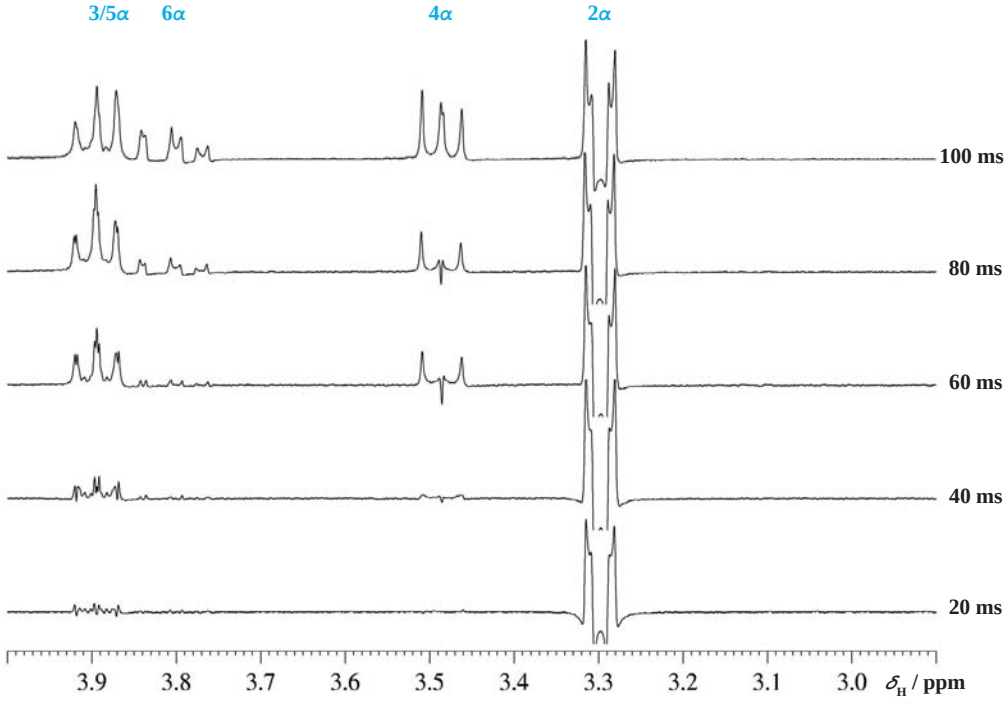


Fig. 4.1-14 Selective TOCSY spectra by irradiation of H-1α

As for the other carbohydrates described in this book, the method of choice for spectral assignment is selective TOCSY spectroscopy. In the first series shown, H-1β was irradiated with a 50 ms Gaussian pulse and the TOCSY mixing time was varied from 20 to 100 ms. As can be seen, the signals of the β-glucosamine show up subsequently. In the second series, H-1α was irradiated. Again, the corresponding proton signals of the α-form subsequently emerge. Without this technique, the correct assignment in this α/β mixture would cause considerable difficulties. Below are shown the 100 ms partial spectra of the α- and β-forms in comparison with the full spectrum.

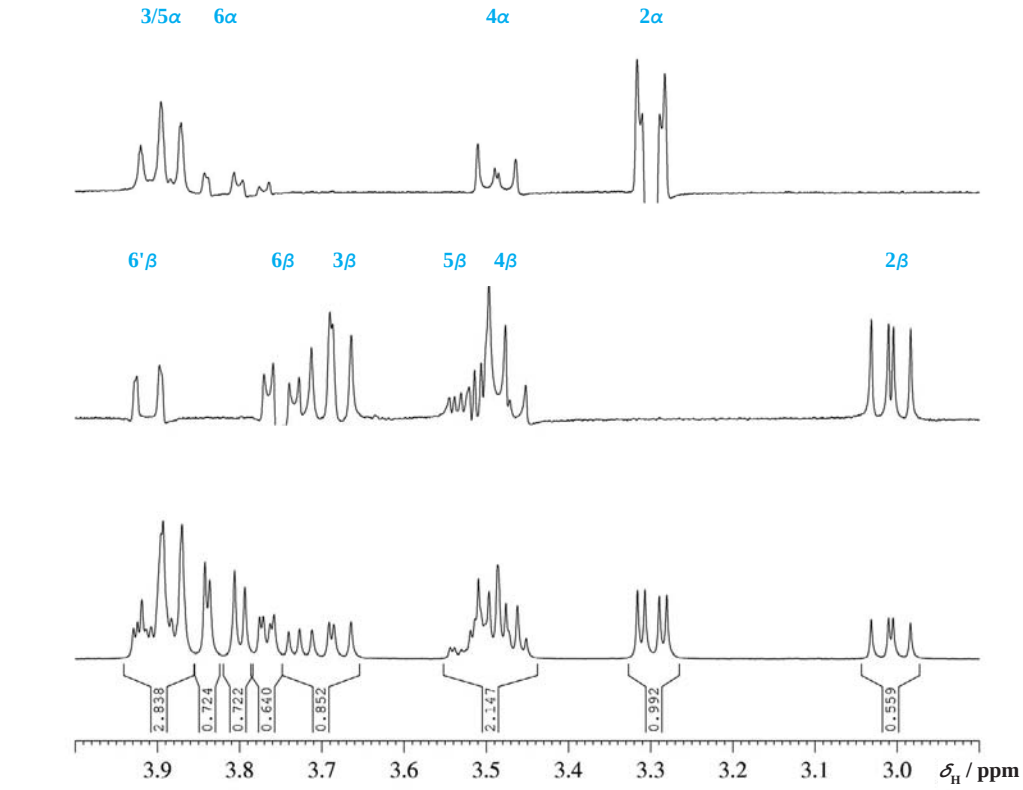
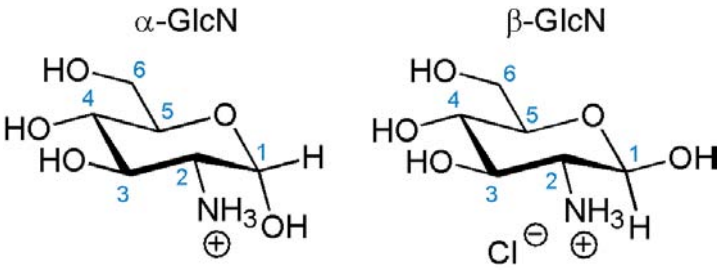


Fig. 4.1-15 TOCSY and ^{1}H NMR spectra



Scheme 4.1-5

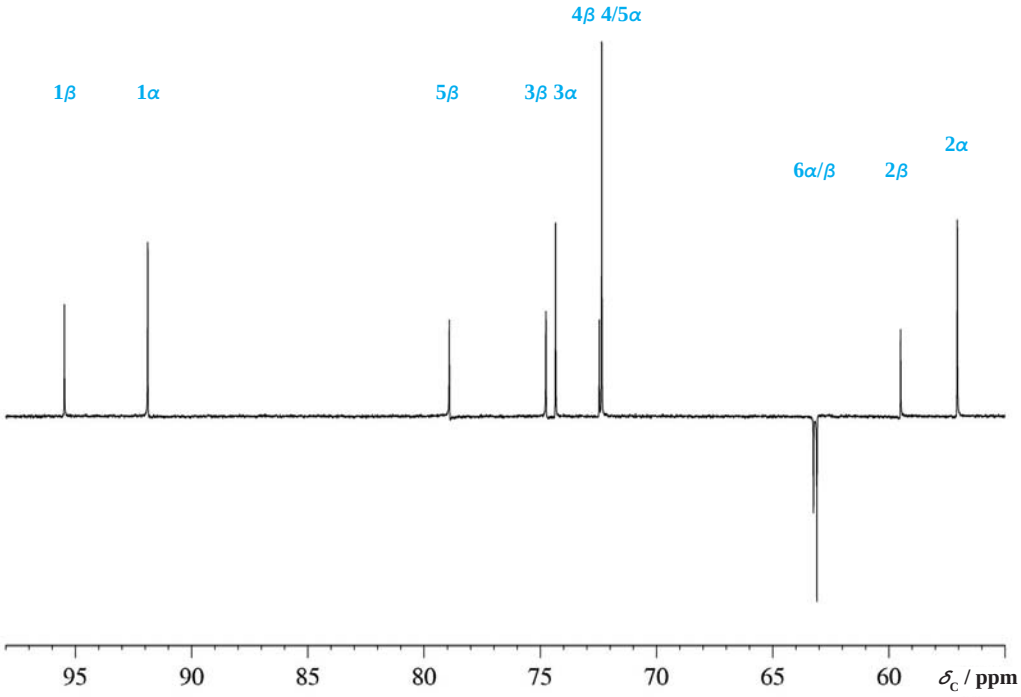
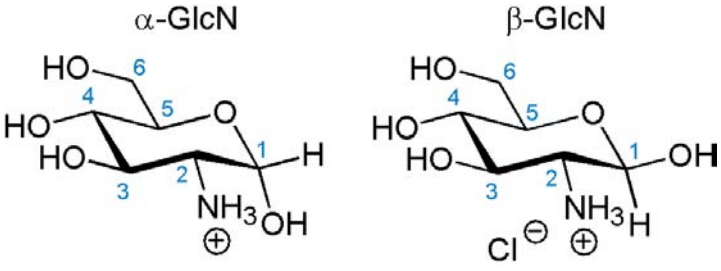


Fig. 4.1-16 ^{13}C NMR spectrum at 100 MHz in D_2O

The ^{13}C NMR spectrum consists of two sets of signals roughly corresponding to the integral ratio for the α/β anomers detected in the proton spectrum. The signals of the anomeric carbon atoms can be directly identified due to their typical chemical shift region and also the two methylene carbon atoms can be directly seen in the APT-edited spectrum. The assignment of the other signals is best left to the discussion of the HSQC spectrum, since we have already fully assigned the proton signals.



Scheme 4.1-6

Fig. 4.1-17 A crayfish in threatening gesture, found in a creek at San Miguel island, Azores. The crayfish is protected by a chitin armour like a lobster.

"Vous devriez partir pour les bains de mer, cher monsieur… Oui, c'est excellent. Et surtout mangez beaucoup de coquillages, ne mangez que des coquillages."
M. Chabre, repris d'espérance, demanda vivement:
"Des coquillages, docteur?... Vous croyez que des coquillages…?"
"Parfaitement! On a vu le traitement réussir. Entendez-vous, tous les jours des huîtres, des moules, des clovisses, des oursins, des arapèdes, même des homards et des langoustes.
Puis, comme il se retirait, il ajouta négligemment, sur le seuil de la porte:
Ne vous enterrez pas. Mme Chabre est jeune et a besoin de distractions…Allez à Trouville. L'air y est très bon."

Emile Zola (1840−1902)
Les Coquillages de M. Chabre

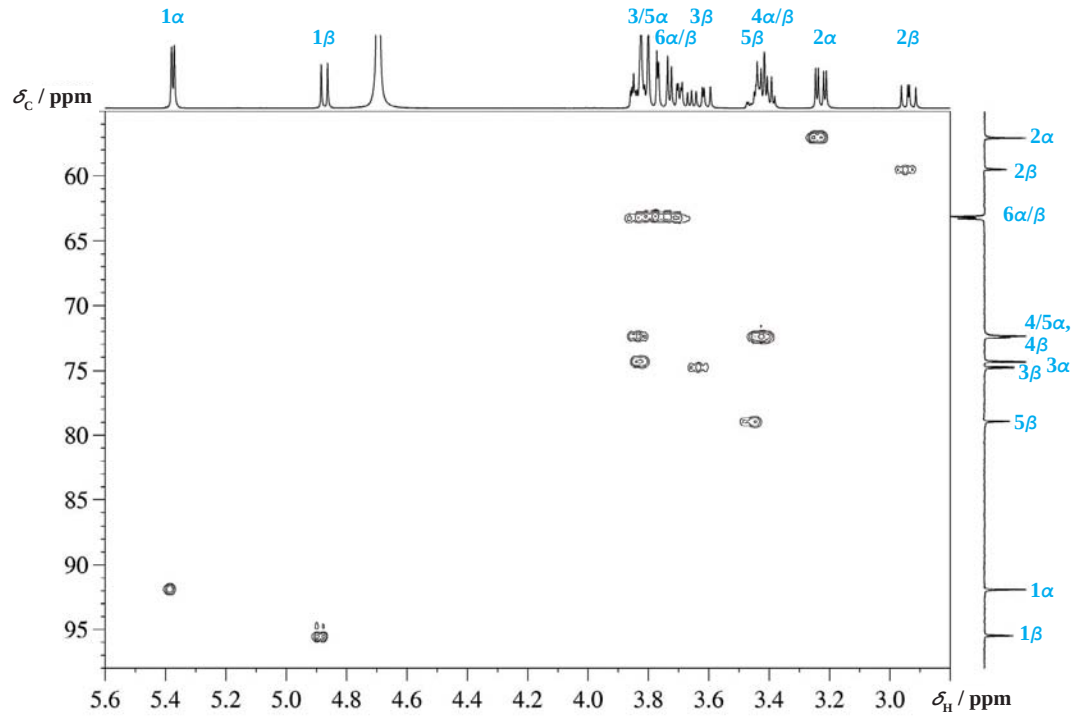


Fig. 4.1-18 HSQC spectrum

This spectrum is especially helpful in disentangling the closely resonating proton signals of H-4α/β and H-5α/β. It also demonstrates the relative large diasterotopicity of the methylene protons at the carbon atoms C-6. It is of interest that the relative order of the chemical shifts for the carbon atoms C-1 and C-2 is reversed with respect to the order of their proton chemical shifts.

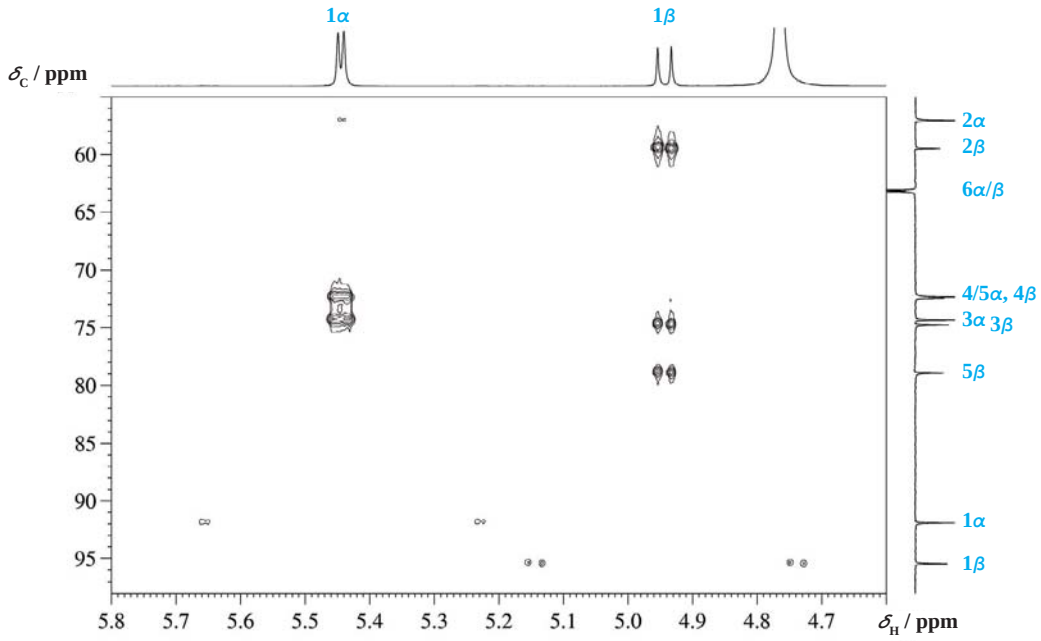


Fig. 4.1-19 Expansion of the HMBC spectrum in the anomeric region

The anomeric proton H-1α sees in the HMBC spectrum C-3α and C-5α via a three-bond CH spin coupling. The cross peak to C-2 is very weak. In contrast, the anomeric proton H-1β shows strong cross peaks to all three C-2β, C-3β and C-4β. One also observes some signal breakthrough of the large ^{1}J(CH) for both anomeric protons. H-2β is connected to C-1β, C-3β and C-4β, and similarly H-2α to C-1α, and to C-4α. There is much more information in the HMBC spectrum which corroborates the above assignments.

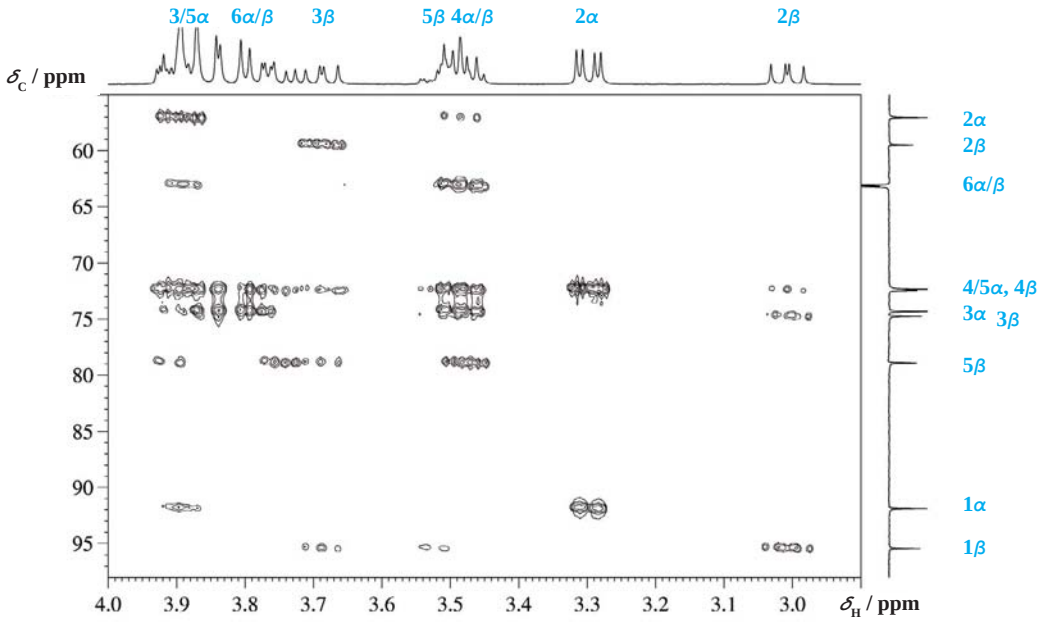


Fig. 4.1-20 Expansion of the HMBC spectrum between 4 and 3 ppm

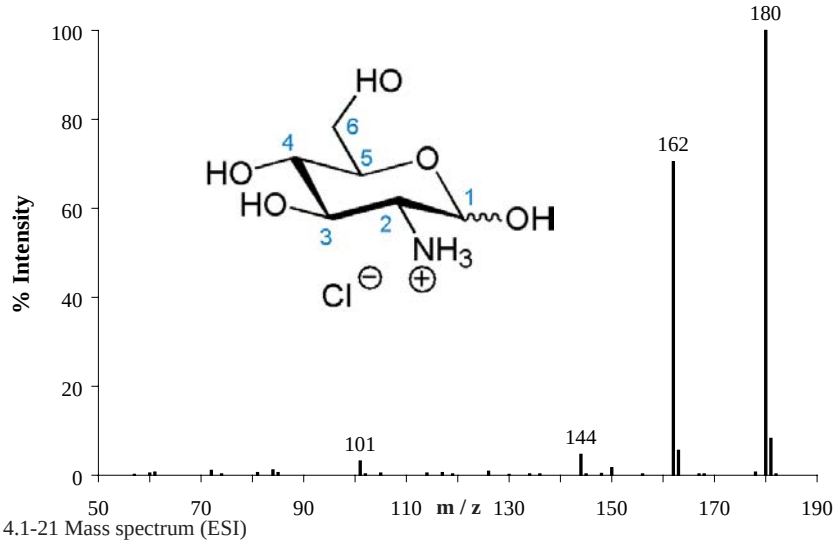
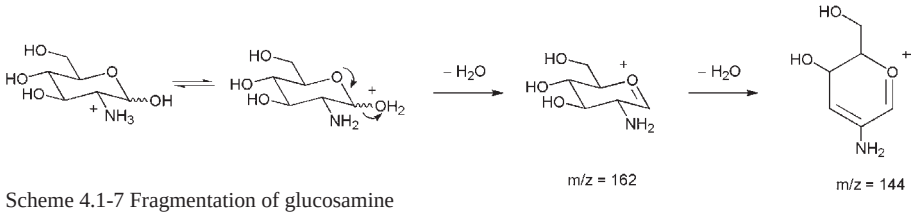


Fig. 4.1-21 Mass spectrum (ESI)

Glucosamine cannot be ionized by the classical EI technique and therefore the ESI mass spectrum is shown here. The substance displays the $[M + H]^+$ signal at m/z = 180 and this ion loses two water molecules as indicated below:



Scheme 4.1-7 Fragmentation of glucosamine

^{13}C Signals δ/ ppm	Type of Carbon	Assignment	Proton Signals δ/ ppm, J / Hz
95.5	CH	C-1β	4.94, J = 8.5
91.9	CH	C-1α	5.45, J = 3.5
78.9	CH	C-5β	3.52
74.8	CH	C-3β	3.69
74.3	CH	C-3α	3.89
72.5	CH	C-4β	3.48
72.4	CH	C-4α	3.49
72.4	CH	C-5α	3.87
63.2	CH_2	C-6β	3.91/3.75
63.1	CH_2	C-6α	3.82/3.79
59.5	CH	C-2β	3.01 J =10.6, 8.5
57.1	CH	C-2α	3.30 J =10.7, 3.7

Table 4.1-1 NMR data for glucosamine

5. Questions

A. Suggest a reason why databases such as SciFinder® may have decided to describe aldoses such as D-glucose and D-glucosamine with an open-chain formula showing an aldehyde group although this formula does not represent the real − i.e. cyclic − structure of the aldose in the crystalline state and is also present to an extent of only a few ppm in aqueous solution.

B. What is the structure of the polysaccharide pectin, which occurs e.g. in citrus peel? What is its main use? Based on the information in this section, make a suggestion, as to which groups in the pectin structure will be most responsible for the strong swelling effect.

C. Agar (agar) is used as a culture medium in microbiology. Structurally, agar (agar) is a heteropolysaccharidic mixture from the cell wall of dulses, consisting of agarose (jellying part) and agaropectin (non-jellying component). The structural subunits are D-galactose, 3,6-anhydro-L-galactose and D-galacturonic acid. The structure of agarose is regular, whereas that of agaropectin is rather complex. Even an only 1% hot solution forms a solid gel on cooling. Our question is: in principle, also these polysaccharides are full of energy if digested. Why is it nevertheless possible that a huge amount of microorganisms can be grown on an agar (agar) culture medium without the danger of decomposing the medium?

D. Suggest a reason why the rate constant for mutarotation in glucosamine is much larger than in glucose.

E. Why does H-1α give much stronger cross peaks in the HMBC spectrum to C-3α and C-5α than H-1β to C-3β and C-5α?

F. Suggest a possible explanation for the different order in chemical shielding for the anomeric protons versus their corresponding carbon atoms.

6. Own Observations

4.2 Lactose

4-*O*-β-D-Galactopyranosyl-D-glucose

From milk

$C_{12}H_{22}O_{11}$, MW 342.30

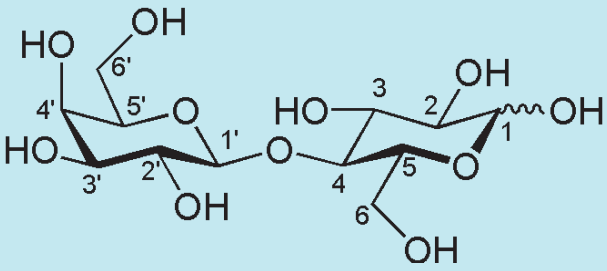
CAS RN 63-42-3,
BRN 93796, 1292745, 3768231

Colourless crystals
mp 201–202 °C (anhydrous),
mp 215–219 °C (as monohydrate)
$[\alpha]_D^{24}$ +55.8° (*c* 0.0378 g/mL, H_2O)
after 6 h and complete mutarotation

Lactose is commercially available.

Synonymous names:
(+)-Lactose, D-(+)-Lactose,
Aletobiose, Lactobiose,
Lactose anhydrous,
Milk sugar, Tablettose

Level: easy



1. Background

De lacte. Sed et cum mulier semen de viro concipit, ita quod in eo crescere incipit, tunc etiam de eadem naturali vi sanguis mulieris sursum ad ubera trahitur, et quod de esca et potu sanguis esse debebat, in lac vertitur, ut ex hoc infans nutriatur, qui in ventre illius crescit. Et ut infans in utero matris suae crescit, sic et lac in uberibus eius augmentatur, ut ex hoc infans nutriatur.

Hildegard Bingensis, (1098−1178)
Causae et Curae, Lib. II.

Fig. 4.2-1 Doctors say that there is no real equivalent to breastfeeding, especially due to the characteristic antibodies that are transferred to the next generation in this way

Lactose, an ingredient of milk, is a disaccharide consisting of a molecule of β-D-galactose which is connected througha 1→4 glycosidic bond to a molecule of D-glucose. A possible short notation is [β-Gal (1→4)-Glc]. It is noteworthy that this expression does not explain the structure completely. The reason is that lactose belongs to the reducing disaccharides. This is based on the fact that the cyclic hemiacetal of the glucose can open the ring and form an intermediate hydroxyaldehyde form, responsible for the reducing properties. The lifetime of this intermediate is short because it tends to undergo ring closure, forming both an α- and a β-like glucose moiety, which gives rise to the names α-D-lactose and β-D-lactose for the molecule as a whole.

Because the enthalpies of formation of the two so-called anomers are slightly different, on standing in aqueous solution an equilibrium comes about with the β-isomer favoured (analogously to D-glucose). This equilibration process is called mutarotation, because a solution of either the α- or the β-anomer as chiral compounds in a polarimeter shows a continuous change in the rotation angle for plane polarized light until the composition of the equilibrium is reached. Of course, an NMR experiment is also able to follow the equilibration by observation of the signal intensities of the anomeric protons. The formula illustrated shows intentionally a wavy bond for the anomeric OH group to express this structural variability in solution. However, for databases this is a problem. Interestingly, therefore, the Scifinder® database decided to draw the ring-opened hydroxyaldehyde form as the structure for lactose (!). Just to estimate what this means: this structure is definite; however, it cannot be isolated and in solution only exists far beyond the 1% level. As with D-glucose, there are tricks to crystallize from solution either the pure α- or β-anomer. α-D-Lactose, which is also the commercially available form, e.g. at a pharmacy, crystallizes from an aqueous solution below 93 °C and the β-anomer above this temperature.

Lactose belongs to the organic compounds with which *organic chemistry* was in existence already before this term was coined in 1807 by Berzelius. It was Scheele who, as early as in 1780, isolated lactic acid from sour milk as a product of the fermentation of lactose by *Streptococcus lactis*. For a certain time, there was a debate whether arabinose (from gum arabic) and lactose were be identical or not [1]. This assumption was eventually negated [2]. If the reader were to assume that lactose is only available as an animal product, this would also be wrong, as it has been found as a phytochemical in Forsythia pollen [3].

The Lord said, “I have indeed seen the misery of my people in Egypt. I have heard them crying out because of their slave drivers, and I am concerned about their suffering. So I have come down to rescue them from the hand of the Egyptians and to bring them up out of that land into a good and spacious land, a land flowing with milk and honey”.

Old Testament, Exodus 3, 7-10

Milk, in this experiment cow’s milk, is an emulsion produced by the mammary glands of female mammals. This feature gave the whole class its latin name *Mammalia*. Cow’s milk contains 4.5% lactose, human mother’s milk even more, ca. 6.7%. There is no real equivalent to breastfeeding, especially due to the characteristic antibodies that are transferred to the next generation in this way, and breastfeeding cannot be replaced by an artificial milk substitute. In the European Union, the term milk is synonymous with cow’s milk, and any other

kind of milk has to be declared with the animal species (goat, sheep, horse, buffalo etc.). Cow's milk contains, besides 87.5% water, also carbohydrates (4.8%), milk fat (4.2%), proteins (3.5%), minerals and trace elements (0.7%), several vitamins such as A, B_1, B_2, B_{12}, D, H and K and pantothenic acid.

Lactose is used in many tablet formulations as a carrier (see synonymous names). Lactose is distinctly less sweet than ordinary sugar (saccharose): it has only 25% of its sweetness. A well-known use is its application as a mild laxative for a constipated baby. An expected effect is that it supports the development of healthy gut flora in the digestive tract.

To digest lactose requires that it can be split into the two monosaccharides of which it is composed. This cleavage is made by β-galactosidase (formerly lactase), an enzyme secreted by the intestinal villi. All infants have enough of this enzyme. A problem arises for many people when they grow up. With maturity, the production of lactase gradually ceases. Between the age of three and five years in the majority of humans lactase production is stopped or much decreased. Chinese and Japanese people lose up to 90% of their ability to digest lactose within their first four years. Eventually, they are unable to digest lactose. Hippocrates recognized lactose intolerance, in the 4th century BC, (see the text in the margin).

Such a loss of lactase during maturation is the default pattern in most adult humans when viewed worldwide. The result is called lactose intolerance. It may be a problem but should not be regarded as a disease, it is just the expression of a different biochemical equipment between ca. one half of mankind and the other. It is characterized by the possibility of only being able to consume none or a only small amount of milk (250 mL) per day. Milk-borne products such as cheese and yoghurt may then also have to be avoided, otherwise stomach ache, excess gas production and diarrhoea occur, because gut bacteria adapt to undigested lactose and metabolize it similarly to other sugars with the production of large amounts of gases during fermentation [4].

So, what is it that from the enzymatic equipment divides mankind into two groups? Those who are able to digest lactose as adults belong to a group which has a certain mutation on chromosome 2 which allows them to bypass the usual scale-down of lactase production and to consume milk and milk products lifelong. The areas in which milk consumption is possible for the majority of the population is Northern Europe, India, the Middle East and part of East Africa, with the well-known Maasai tribe as stockfarmers [5]. It has been found that this genetic change by mutation must be a relatively recent one, from around 4000 BC but here is not the place to discuss this in detail. Possibly, Darwin is the most famous person known to not have been equipped with this mutation.

As a means to help lactose-intolerant people drink milk as healthy product in many respects, lactose-free milk has been developed. However, the term lactose-free does not explain its basis. Has lactose been removed from the milk? And, if so, how? Or what has happened? If you test this milk you can guess it: the first impression on the tongue

Τυρός γὰρ, ἐπειδὴ τουτέῳ σημείῳ ἐχρησάμην, οὐ πάντας ἀνθρώπους ὁμοίως λυμαίνεται, ἀλλ᾽ εἰσὶν οἵτινες αὐτέου πλερεύμενοι οὐδ᾽ ὁτιοῦν βλάπτονται ἀλλὰ καὶ ἰσχύν, οἵσιν ἄν ξυμφέρῃ, θαυμασίως παρέχεται εἰσὶ δὲ οἳ χαλεπῶς ἀπαλλάσσουσι διαφέρουσι δὲ τουτέων αἱ φύσιες διαφέρουσι δὲ κατὰ τοῦτο ὅπερ ἐν τῳ σώματι ἔνεστι πολέμιον τυρῷ ὑπὸ τοιουτέου ἐγειρεταί τε καὶ κινέεται οἷσιν ὁ τοιοῦτος χυμὸς τυγχάνει πλέον ἐνεὼν καὶ μᾶλλον ἐνδυναστεύων ἐν τῳ σώματι, τουτέους μᾶλλον καὶ κακοπαθέειν εἰκός. Εἰ δὲ πάσῃ τῃ ἀνθρωπίνῃ φύσει ἦν κακὸν, πάντας ἄν ἐλυμαίνετο. Ταῦτα δὲ εἴ τις εἰδοίη, οὐκ ἄν πάσχοι.

Hippocrates (460−370 BC)
Ancient Medicine, 20

Fig. 4.2-2 Lactose-free milk is not free of "sugars". It even tastes slightly sweeter than untreated milk due to the content of twice as many "sugar" molecules in the form of D-glucose and D-galactose obtained by lactose cleavage

is that it tastes sweeter than normal milk, and this gives a hint about what has been done. Lactose has been split enzymatically into the two monosaccharides by passing the milk over an immobilized lactase. In other words, it is predigested milk in one respect. As glucose and galactose are sweeter than the weakly sweet lactose, the whole milk is felt to be sweeter than normal cow's milk.

Upon my secure hour thy uncle stole,
With juice of cursed hebenon in a vial,
And in the porches of my ears did pour
The leperous distilment; whose effect
Holds such an enmity with blood of man
That swift as quicksilver it courses through
The natural gates and alleys of the body,
And with a sudden vigour doth posset
And curd, like eager droppings into milk,
The thin and wholesome blood: so did it mine;
And a most instant tetter bark'd about,
Most lazar-like, with vile and loath-some crust,
All my smooth body.
Thus was I, sleeping, by a brother's hand
Of life, of crown, of queen, at once dispatch'd.

William Shakespeare (1564–1616)
Hamlet, Prince of Denmark, I, 5

2. Literature

[1] H. Kiliani, "Ueber die Identität von Arabinose und Lactose" [On the identity of arabinose and lactose] *Ber. Dtsch. Chem. Ges.* **1880**, *13*, 2304–2306.

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[8] R. E. March, C. J. Stadey, "A tandem mass spectrometry study of saccharides at high mass resolution" *Rapid Commun. Mass Spectrom.* **2005**, *19*, 805–812.

3. Isolation

3.1 Principle

Starting material: raw milk, pasteurized milk, homogenized milk, curdled milk or directly whey are suitable sources for the isolation of lactose. Milk from the viewpoint of chemistry has to be regarded as a complex mixture of nutrients, such as proteins, fats, carbohydrates, vitamins, salts and others, in the form of an emulsion. To gain access to lactose as a hydrophilic disaccharide requires removal of the less hydrophilic macromolecular proteins and also the hydrophobic fats. To achieve this, as a prerequisite, any kind of milk has to be prompted to curdle. Curdling can best be brought about by addition of a pill of rennet to the warm milk. Rennin is an enzyme from rennet. Thereby, the protein casein is precipitated as curd cheese, including the fatty constituents that are present. The whey which can be drained off still contains, in addition to lactose, lactalbumin, a colloidal soluble protein,

which can be removed by heating, causing precipitation that allows filtration, and riboflavin (vitamin B_2), which is responsible for the pale yellow colour of milk but does not disturb the isolation of lactose, which crystallizes from the concentrated whey on longer standing and is purified by recrystallization from aqueous ethanol.

3.2 Method

Pasteurized milk (500 mL) is curdled with stirring at 40 °C by addition of a pill of rennet. After 30 min, the curdled milk is filtered through cheesecloth and the turbid yellow filtrate (ca. 300 mL) of whey is used for the next step, in which it is heated to boiling in a beaker. Lactalbumin coagulates and can be removed by filtration through a Buchner funnel. The whey now shows only slight turbidity. In a large round-bottomed flask (use of a 1 L or even 2 L vessel is recommended so as to be able to keep the initial foaming effect under control), the whey is now reduced in vacuo to a volume of 50 mL. The turbid concentrate (turbid due to colloids − cannot be cleared by filtration) is put into a 100 mL beaker and allowed to stand open for crystallization for several days. Eventually, lactose forms a hard crystalline layer at the bottom of the beaker. Any liquid is decanted, and the wet layer of lactose containing some slimy constituents is scratched off on to a plate of clay for drying. A mass of 12 g of crude lactose is thus obtained.

3.3 Purification

It is dissolved in a small amount of boiling water (30 mL). A curd-like side component is removed by filtration. The filtrate is poured into an Erlenmeyer flask. Ethanol (125 mL) is slowly poured into the aqueous lactose solution until turbidity occurs. The sealed flask is allowed to stand at –18 °C overnight. Lactose crystallizes and is filtered off, washed with a few mL of ice-cold ethanol and air dried. An amount of 8 g of a D-(+)-lactose monohydrate is obtained as colourless crystals of mp 215−219 °C (dec.), $[\alpha]_D^{24}$ +55.8° (*c* 0.0378 g/mL, H_2O) after 6 h and complete mutarotation.

Ich lebte still und harmlos − Das Geschoß
War auf des Waldes Tiere nur gerichtet,
Meine Gedanken waren rein von Mord
Du hast aus meinem Frieden mich heraus
Geschreckt, in gärend Drachengift hast du
Die Milch der frommen Denkungsart mir verwandelt,
Zum Ungeheuren hast du mich gewöhnt − Wer sich des Kindes Haupt zum Ziele setzte,
Der kann auch treffen in das Herz des Feinds.

Friedrich Schiller (1759−1805)
Wilhelm Tell, IV, 3

Fig. 4.2-4 Rumination with outlook! A herd of cows producing milk while overlooking the Atlantic Ocean from an island of the Azores

4. Spectra and Comments

Fig. 42-8 Lactose crystals as isolated

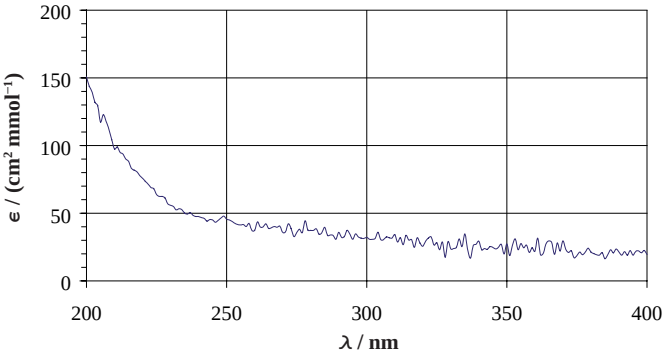
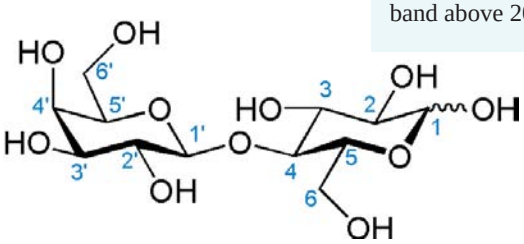


Fig. 4.2-5 UV spectrum in water

As for all carbohydrates, the UV/vis spectrum shows no absorption band above 200 nm due to the lack of any chromophore.

Scheme 4.2-1



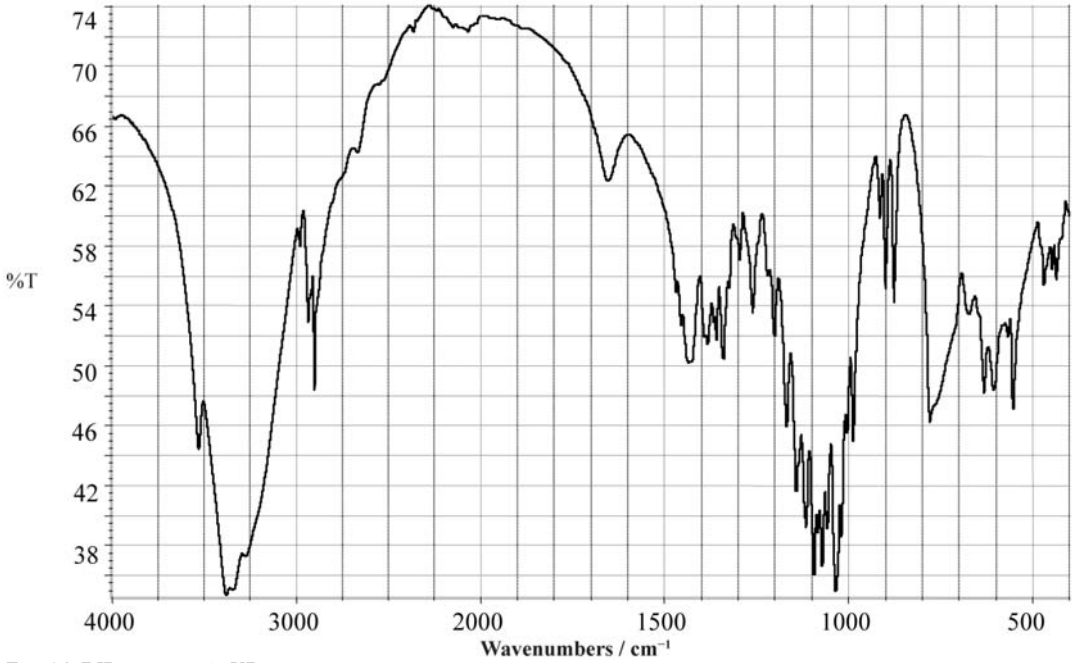


Fig. 4.2-7 IR spectrum in KBr

According to the structure, we find in the IR spectrum a huge band for the OH valence vibration, with small sharp signals on its flank due to the sp^3 CH valence band at 2900 cm^{-1}. In the fingerprint region the area of the C–O single bond vibration from 1200 to 1000 cm^{-1} is occupied by many bands.

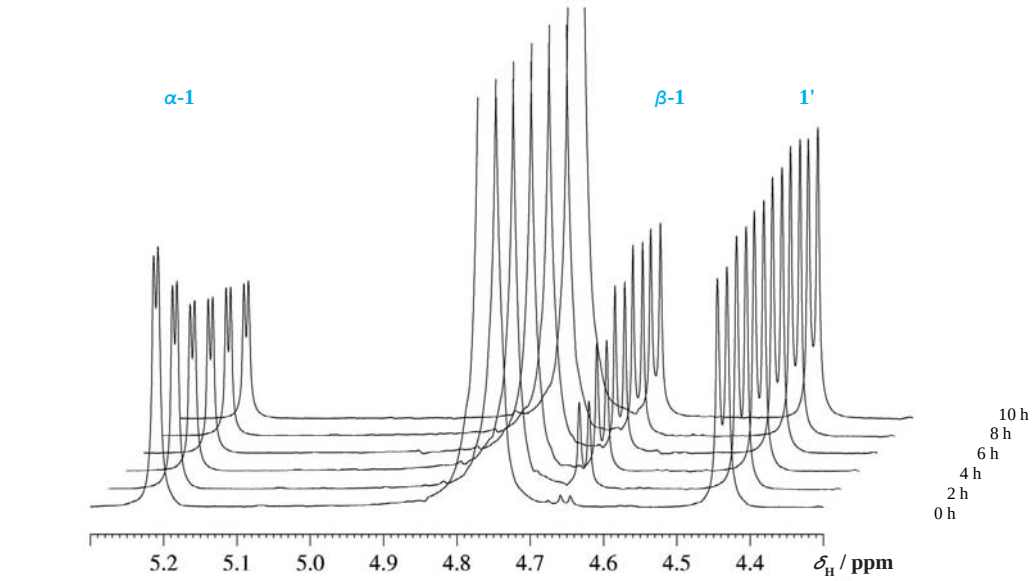


Fig. 4.2-8 Mutarotation at 600 MHz

All NMR spectra shown below are equilibrium spectra, recorded about 24 h after dissolution, since lactose mutarotates towards an equilibrium of about 60% β-glucose. This mutarotation can be followed by polarimetry or NMR spectroscopy, observing the three anomeric protons. The signal of the anomeric proton of the galactose unit at δ_H = 4.43 ppm is not able to indicate this change and is identical for both forms. The kinetic NMR spectra which have been recorded every hour display the decaying signal of the α-anomeric glucose proton at δ_H = 5.2 ppm and the increasing anomeric signal of the β-form at δ_H = 4.65 ppm. The experimental points were fitted using the model of a reversible first-order reaction, which yielded for the forward rate constant $k_1 = 5.27 \times 10^{-5}\ s^{-1}$ and for the backward rate constant $k_{-1} = 3.27 \times 10^{-5}\ s^{-1}$ in D_2O at 298 K.

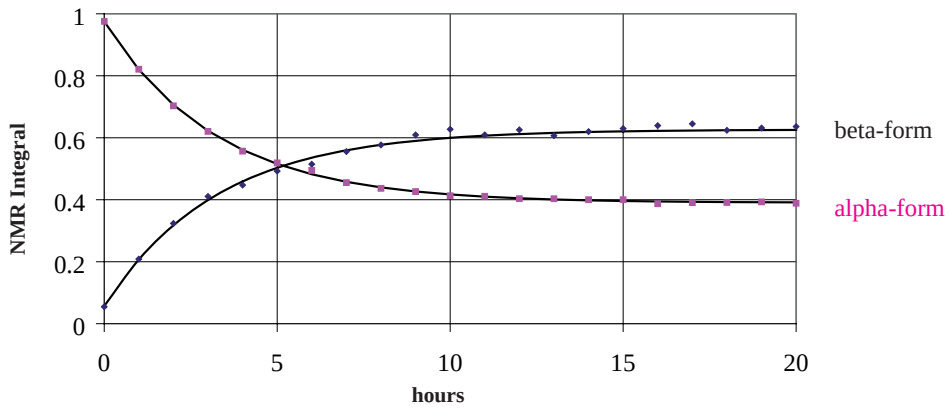


Fig. 4.2-9 Reaction kinetics of the mutarotation

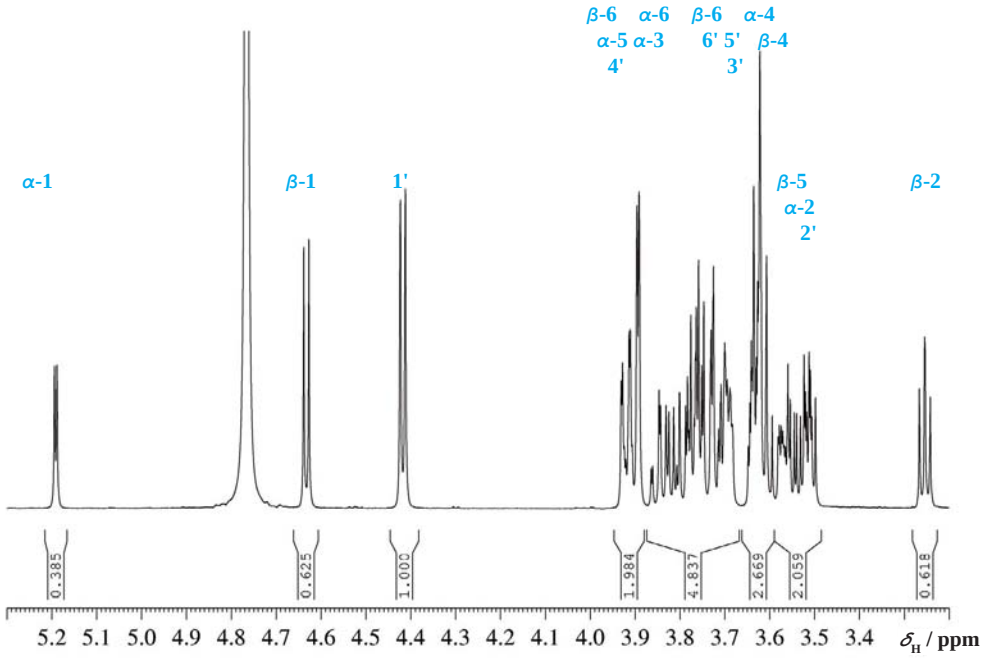


Fig. 4.2-10 ^{1}H NMR spectrum at 700 MHz in D_2O

From the kinetic NMR experiment shown above it was deduced that the anomeric protons of the galactose unit both appear at at δ_H = 4.43 ppm, hence their combined integral was set to 1.0. The anomeric signals of α- and β-glucose show their typical splitting of 3.8 and 8.1 Hz, whereas the galactose unit displays 7.8 Hz for its anomeric proton. The remaining 18 proton signals of lactose absorb in a region of less than 1 ppm with only one triplet somewhat separated at δ_H = 3.26 ppm. Hence an assignment will clearly be very difficult.

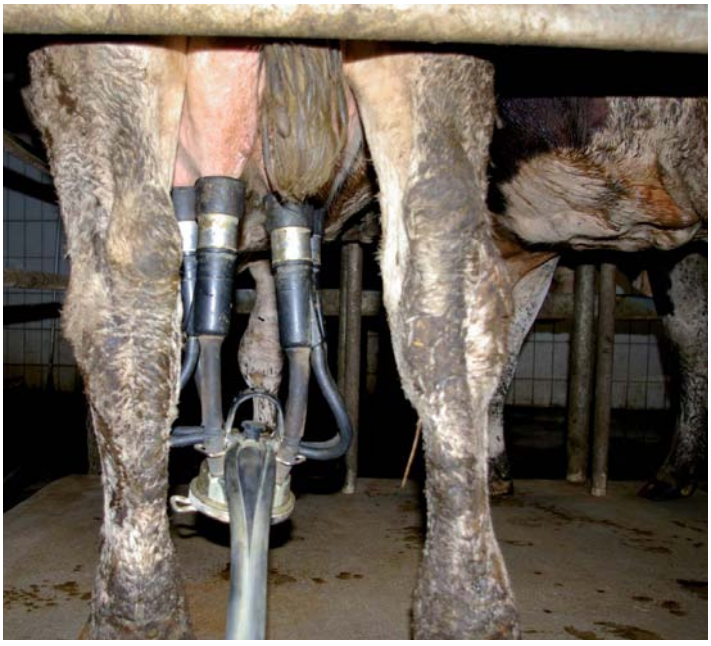
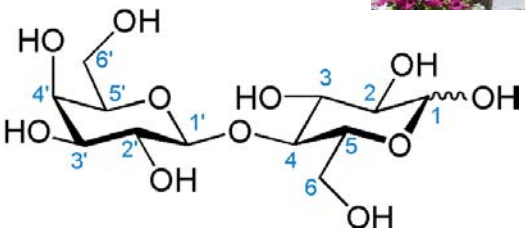


Fig. 4.2-11 Mechanical milking

Fig. 4.2-12 An iron statue of a cow in a village in the Pyrenees expresses the esteem of stock farming



Scheme 4.2-2

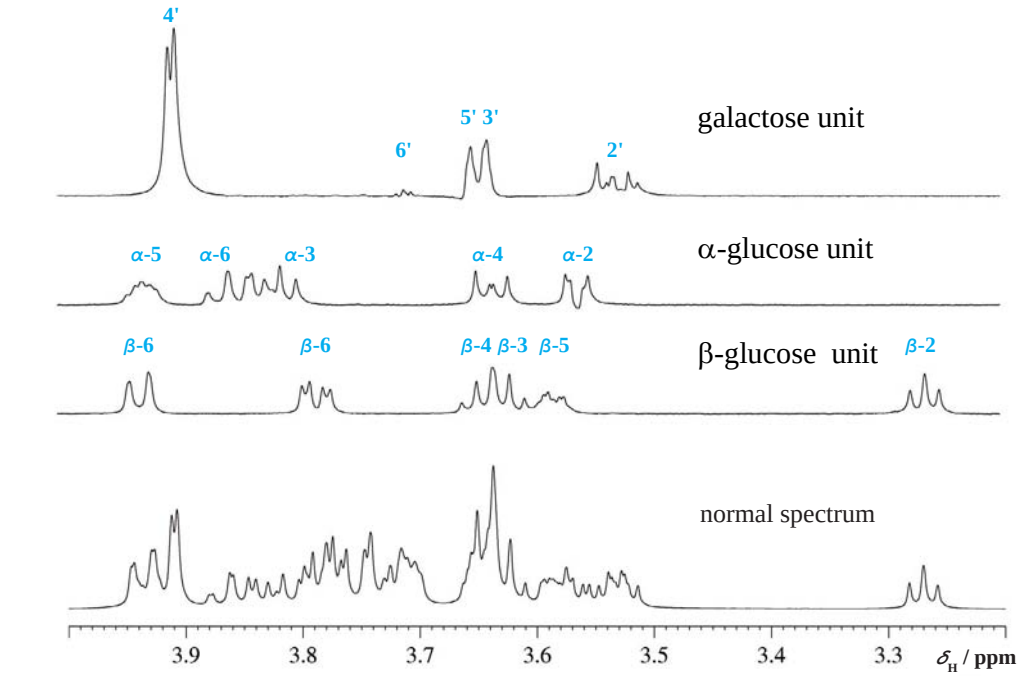


Fig. 4.2-13 Selective TOCSY spectra at 700 MHz

The best spectroscopic technique to disentangle such a spectrum is selective TOCSY, starting from the three anomeric signals which have been safely assigned. Shown are the normal equilibrium spectrum and the three subspectra recorded with a TOCSY mixing time of 200 ms, which ensures the observation of all protons within one sugar unit.

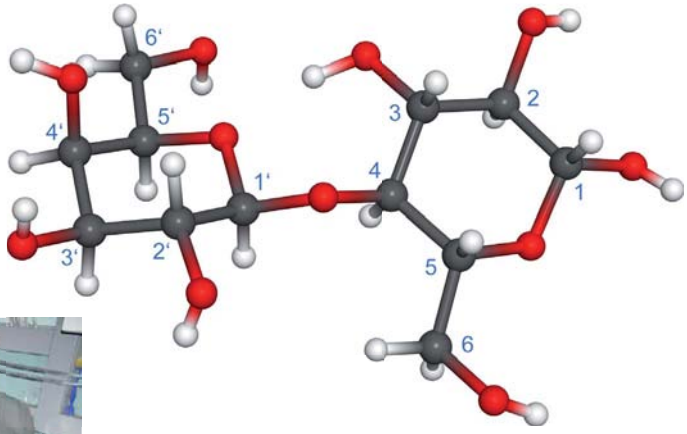


Fig. 4.2-15 Molecular model of lactose

Fig. 4.2-14 Stainless-steel vessel for the storage of up to 2000 L of raw milk at a farm in the Swabian Mountains

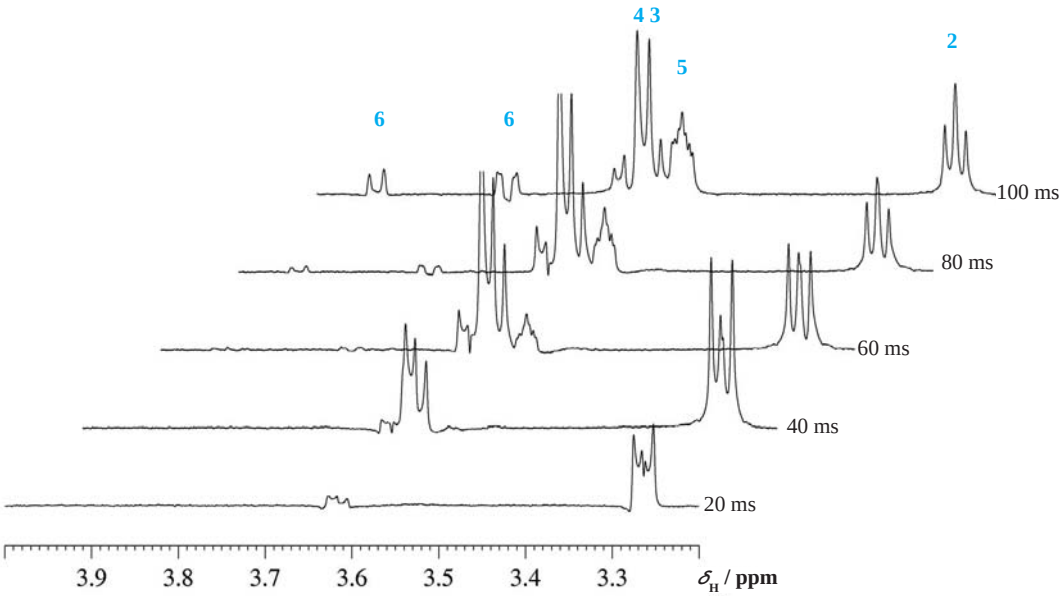


Fig. 4.2-16 Irradiation of the anomeric proton signal of the β-glucose unit

By varying the mixing time one achieves a signal assignment within the different units and this is shown here for the β-glucose form, as an example. As can be seen, at a 20 ms mixing time, only the β-glucose proton H-2 at 3.26 ppm gives a signal, followed by H-3 and H-4 at 3.62−3.65 ppm appearing after 40 and 60 ms mixing times. Finally, the signal of proton H-5 emerges at 3.58 ppm and this is followed by the diastereotopic proton signals H-6 of the methylene group.

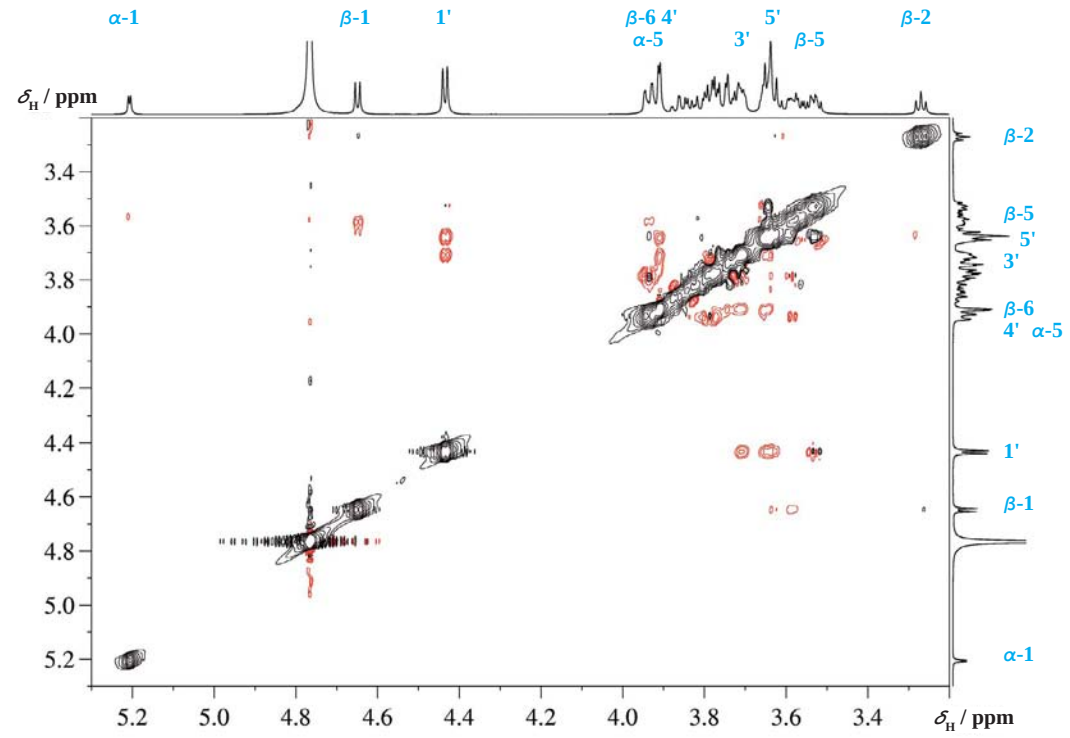


Fig. 4.2-17 NOESY spectrum

In the NOESY spectrum, the anomeric proton of the β-glucose has an NOE contact to H-5, and the anomeric proton of the galactose nicely displays NOE contacts to H-3' and H-5'. These, in turn, display NOE contacts to H-4', although H-4' is in an equatiorial position. The NOESY spectrum also helps to distinguish the signals of β-H-3 and β-H-5 due to a cross peak from β-H-6 to β-H-5.

Fig. 4.2-18 Caution, cattle!

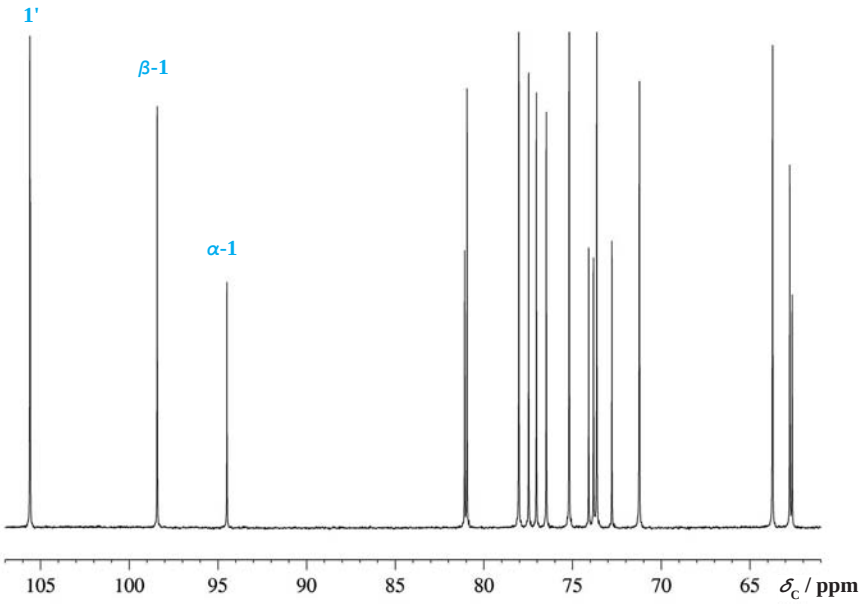


Fig. 4.2-19 ^{13}C NMR spectrum at 176 MHz

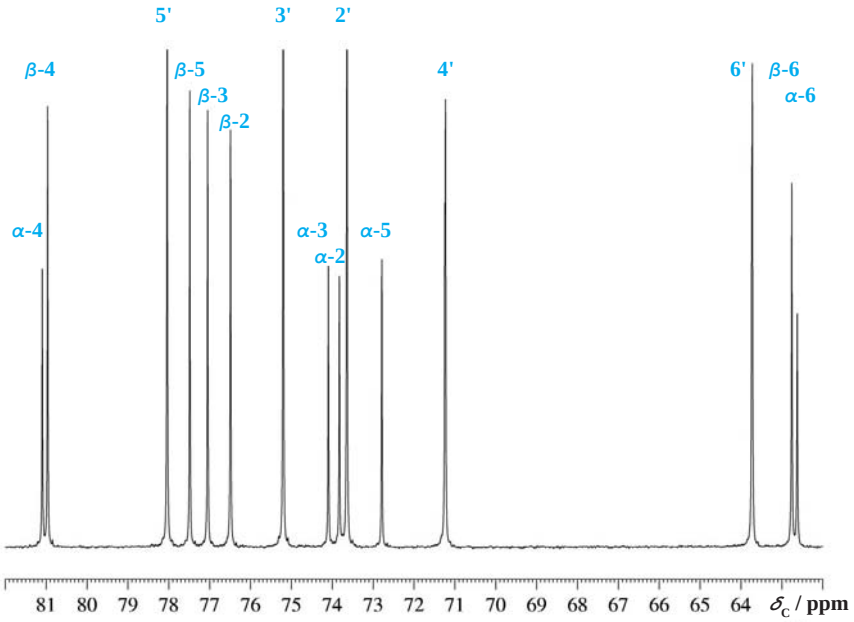


Fig. 4.2-20 Expansion of the ^{13}C NMR spectrum

Inspecting the ^{13}C NMR spectra, we find only a tiny splitting of the anomeric galactose carbon signal C-1' and for C-4' which is not seen at the overview spectrum given here. Additional assignment confirmation in the ^{13}C NMR spectrum comes from the relative intensities, since one observes small, medium and large signals resulting from the α-glucose, β-glucose and galactose units. The detailed assignment uses the proton information and the CH correlation given in the HSQC spectrum.

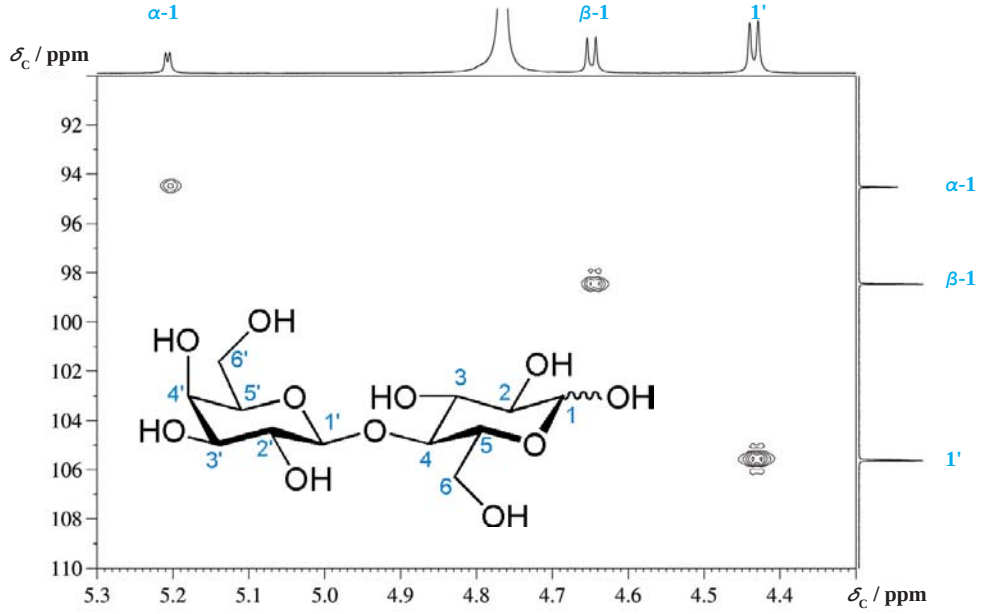


Fig. 4.2-21 CH-edited HSQC spectrum in the anomeric region

The first expansion of the HSQC spectrum in the anomeric region of the spectra reveals the interesting fact that the relative sequence of the anomeric carbon signals is just the opposite of those of the protons.

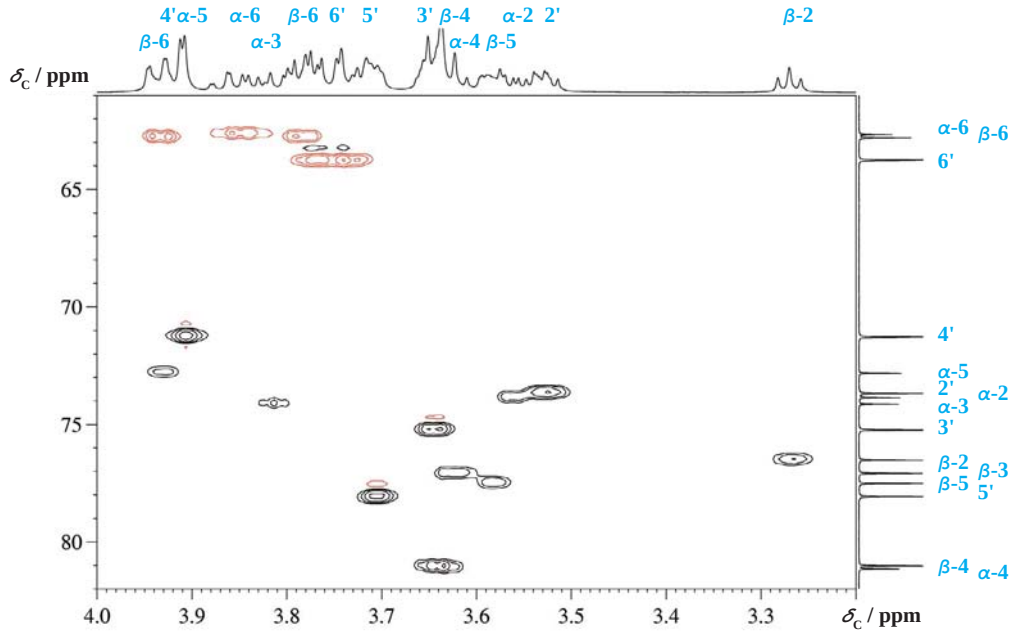


Fig. 4.2-22 CH-edited HSQC spectrum between 4 and 3 ppm

In the second expansion of the edited HSQC spectrum we see in red the signals of the three CH_2 groups. The proton positions can be obtained from the TOCSY spectra.

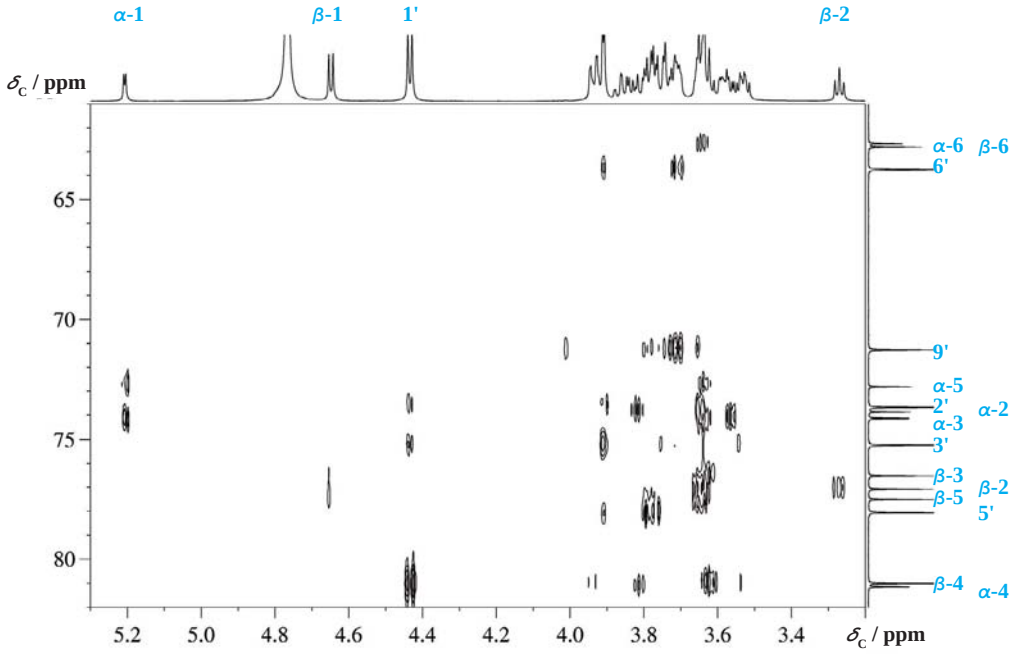


Fig. 4.2-23 Expansion of the HMBC spectrum

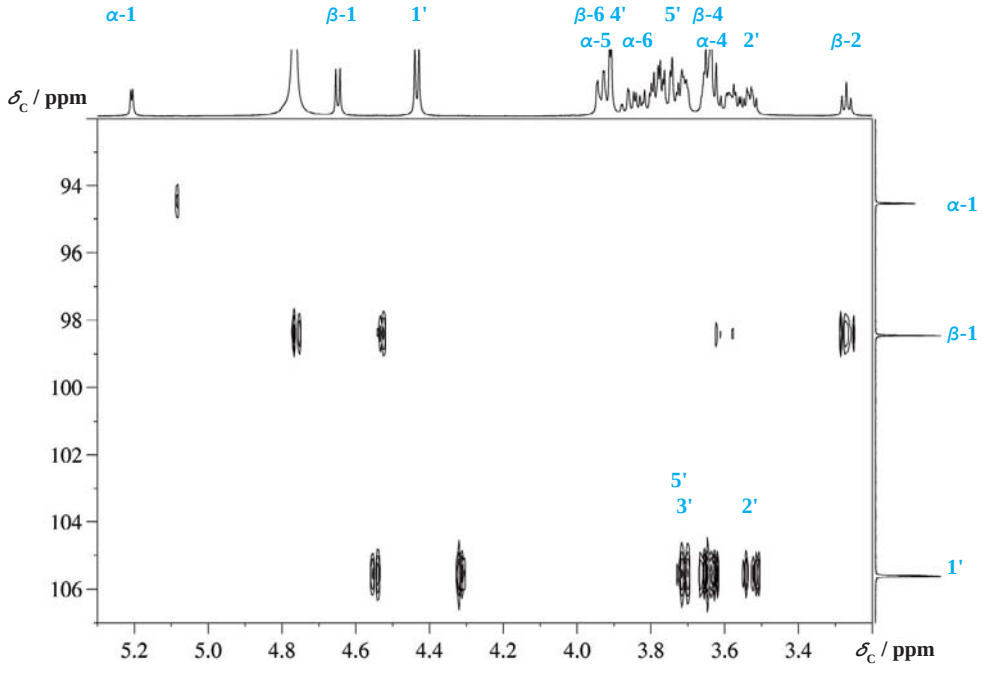


Fig. 4.2-24 Expansion of the HMB spectrum in the anomeric region

Although the HMBC spectrum would not be needed for the assignments discussed above, it confirms nicely the data in the table. For example, carbon C-1' is reached from both α- and β-H-4, and also from H-5', H-3' and H-2'. The anomeric protons are connected to the carbon atoms 2 and 5 of the sugar rings via two and three bonds. Clearly to be seen are the three bond couplings from β-H-1 to β-C-3 and β-C-5 and indicative for the galactose-1'−glucose-4 junction is the intense correlation signal from H-1' to α/β-C-4.

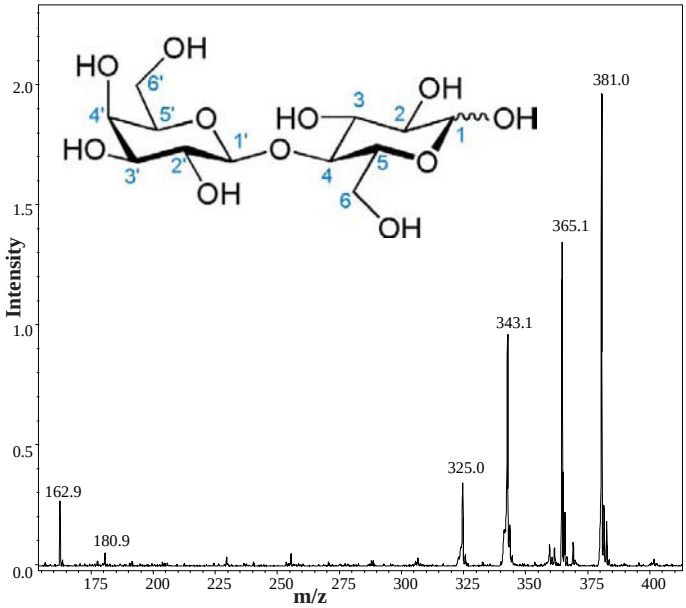


Fig. 4.2-25 Mass spectrum (ESI-FT/ICR)

Since a carbohydrate such as lactose cannot be ionized with the traditional EI technique, the mass spectrum was recorded in MeOH using electrospray ionization. The spectrum reveals signals at m/z = 343 $[lactose + H]^+$, 365 $[lactose + Na]^+$ and 381 $[lactose + K]^+$. Furthermore, we find a signal indicating loss of water at m/z = 325.

^{13}C Signals δ / ppm	Type of Carbon	Assignment	Proton Signals δ / ppm, J / Hz
105.62	CH	α-C-1'	4.43 J = 7.8
105.60	CH	β-C-1'	4.43 J =7.8
98.46	CH	β-C-1	4.64 J = 8.1
94.52	CH	α-C-1	5.19 J = 3.8
81.14	CH	α-C-4	3.64
81.01	CH	β-C-4	3.64
78.09	CH	C-5'	3.70
77.51	CH	β-C-5	3.58
77.07	CH	β-C-3	3.62
76.52	CH	β-C-2	3.26
75.23	CH	C-3'	3.65
74.12	CH	α-C-3	3.81
73.86	CH	α-C-2	3.56
73.66	CH	C-2'	3.53
72.81	CH	α-C-5	3.93
71.27	CH	β-C-4'	3.91
71.26	CH	α-C-4'	3.91
63.74	CH_2	C-6'	3.77/3.74
62.79	CH_2	β-C-6	3.93/3.78
62.66	CH_2	α-C-6	3.85

Table 4.2-1 NMR data for lactose

5. Questions

A. Although the receptors for smell and taste are not known at the structural level yet, a chemist can make a guess as to what feature may be responsible among the natural products for a sweet taste. Go through the world of organic compounds consisting of C, H, and O and make one.

B. D-Glucose, D-mannose and D-galactose are the three main aldohexoses occurring in Nature. There are in principle 29 other possibilities because they have five chiral centres in their cyclic structure. Explain why these have been favoured in biosynthesis during evolution over many other sugars such as allose, gulose, altrose, idose and talose.

C. Whey is pale yellow in colour due to the riboflavin (vitamin B_2) contained. Draw a formula of riboflavin and explain why it is yellow in solution (the colour as a solid in bulk being even orange) and why it is a water-soluble vitamin.

D. Would you predict that the IR spectra for the α- and β-forms of lactose are distinguishable?

E. What would you predict for the rate constants of mutarotation, if they were measured at 308 K?

F. Which form of lactose would be stabilized by methylation at C-1?

G. Do you expect a CD spectrum of lactose?

H. Inspecting the selective TOCSY spectra, one observes that for the galactose unit H-5' and the methylene group H-6' do not answer, whereas at the same mixing time both forms of glucose reveal the complete proton spectrum. Suggest a reason.

I. Find an explanation for the two positive NOE cross signals in the NOESY spectrum.

J. In the expansion of the HMBC spectrum the cross peaks for the anomeric protons seem to be out of place. Explain.

K. Explain the two signals in the mass spectrum at m/z = 163 and 181.

6. Own Observations

4.3 Amygdalin

(2*R*)-[(6-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)oxy]benzeneacetonitrile

From bitter almonds

Prunus dulcis var. *amara* (Rosaceae)

$C_{20}H_{27}NO_{11}$, MW 457.43

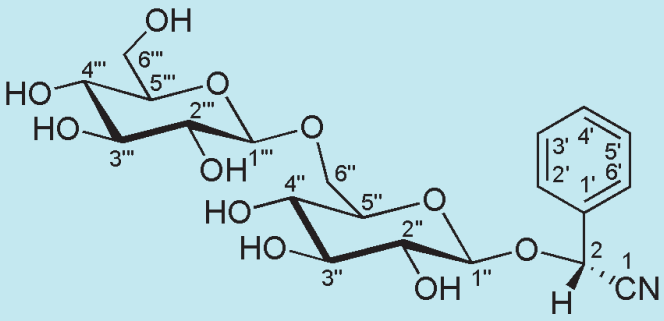
CAS RN 29883-15-6, BRN 66856

Colourless crystals,
mp 220−221°C, $[\alpha]_D^{20} = -41°$
(*c* 0.01 g/mL, H_2O)

Amygdalin is commercially available.

Synonymous names:
Mandelonitrile-β-gentiobioside,
(−)-Amygdalin, (*R*)-Amygdalin

Level: easy



1. Background: No apple trees in the jailhouse garden!

Amygdalin got its name from the Greek word αμυγδαλη for *almond* in English (in French: *amande*). Interestingly, the beginning of the word was lost in some languages to give *mandorla* in Italian and *Mandel* in German. Amygdalin is a bitter cyanogenic glycoside occurring in the variety of the almond tree *Prunus dulcis* belonging to the family Rosaceae, that produces bitter almonds. Amygdalin is a widespread compound that can also be found in the kernels of apricots, plums, peaches, nectarines, papaya, cherries and apples.

And it came to pass, that on the morrow Moses went into the Tabernacle of witness and, behold, the rod of Aaron for the house of Levi was budded, and brought forth buds, and bloomed blossoms, and yielded almonds.

Old Testament, IV. Moses 17: 8

As a chemical, amygdalin is classified as a hazardous material (classification Xn Harmful), but why? Amygdalin, as other cyanogenic glycosides which occur in ca. 3000 plant species, is a kind of shrouded poison because on cleavage it is able to deliver hydrocyanic acid, a severely toxic compound. Therefore, it has to be assumed that this property has a biological meaning: to protect the next generation from pests. The tool for a possibly necessary activation is also part of the kernel's equipment: it is a set of enzymes called emulsin, which consists of a β-glucosidase (amygdalase) and a hydroxynitrilase (mandelonitrillyase). These enzymes are capable of cleaving the disaccharidic gentiobioside amygdalin stepwise to give first the monosaccharidic glucoside prunasin, and from this the cyanohydrin of benzaldehyde, which in aqueous solution forms benzaldehyde and hydrogen cyanide. The lethal dose of cyanide is 1 mg/kg, only. This is due to blocking of the ferric ion of cytochromoxidase and the respiratory chain and finally preventing oxygen transfer from haemoglobin into the tissue. The toxicity of cyanide is remarkable because only small amounts of it can be transformed enzymatically into less toxic thiocyanate by the liver and excreted with the urine. Therefore, care has to be taken and bitter almonds should not be eaten in the same manner as sweet almonds, especially not by children, because intoxications have occurred already with as few as five such almonds.

In 1803, a Dr. Schrader in Berlin detected that crushed bitter almonds released "prussic acid" (HCN). The first to discover the glycoside in substance were the French chemists Robiquet and Boutron-Charlard in 1830. In 1837, Liebig and Wöhler investigated the structure of the compound and found that it covered benzaldehyde, hydrocyanic acid and a sugar [1]. In ancient Egypt, seeds of peaches were even used to execute the death penalty. Nevertheless, still nowadays on baking the traditional German Christmas fruit loaf "Stollen", a few bitter almonds have to be added to the dough to get the right flavour. However, they are usually not sold in a supermarket, but in a pharmacy in small amounts together with appropriate advice. Whereas seeds such as bitter almonds may be regarded as a specialty, cyanogenic glycosides are a serious problem in several everyday foods in tropical countries such as manioc (cassava) roots, yams, bamboo shoots and Lima beans [2]. About 400 million people depend on manioc as a source of starch, but in the root the toxic cyanogenic glycoside linamarin is also present. Hence manioc can only serve as food after a special detoxifying preparation: thorough grinding of the raw roots leads to contact between the cyanogenic

glycoside and enzymes capable of cleaving it. Hydrocyanic acid is then removed from the crushed mass by evaporation on standing, roasting or boiling in water, which has to be discarded. Thus, acute intoxications can be avoided but chronic ones by uptake of sublethal amounts of cyanide remain a serious problem. In the light of this background it may seem odd that amygdalin was included in the list of vitamins by the biochemist Krebs in the 1920s as vitamin B_{17}.

Synonymously, an amygdalin extract isolated from apricot seeds was given the name "Laetrile", drawn from shortening of the words laevorotatory mandelonitrile. Many investigations have been conducted to determine whether "Laetrile" has anti-cancer activity or not. Still today, believers of alternative curative treatments keep faith with "Laetrile". One of the nutritional additives they recommend is apricot seeds. However, the US National Institutes of Health have reported that clinical trials on the performance of "Laetrile" led to the conclusion that it had no therapeutic effect. In 1974, the American Cancer Society dismissed the product as quackery. Finally, the US FDA did not approve it as a preventive or cure for cancer and the production and distribution of "Laetrile" was prohibited in the USA in 2000.

Some things are clear from a chemist's point of view. Amygdalin is definitely no vitamin – it is not essential for life and if you do not take it at all you will not suffer from a dietary deficiency disease, as would be the case with a real vitamin. Also, it is not true that a lack in amygdalin results in a break out of cancer. Additionally, eating natural amygdalin in the form of apricot seeds will induce your body to decontaminate the cyanide formed up to the level that can be accomplished by the body anyway. Uptake of more will result in symptoms of cyanide poisoning – there is no other biochemical possibility.

Maybe an unbelievable end is the best one for this story: it has been rumoured that during the reign of the Imperial Germany, the cultivation of apple trees in jailhouse gardens was prohibited to prevent suicides of prisoners provoked by collecting and eating vast amounts of apple kernels.

2. Literature

[1] F. Wöhler, "Ueber die Wirkung der Säuren auf das Amygdalin" [On the effect of the acids on amygdalin] *Ann. Chem. Pharm.* **1848**, *66*, 238−241.

[2] D. Selmar, "Cyanide in foods: biology of cyanogenic glucosides and related nutritional problems" *Recent Adv. Phytochem.* **1999**, *33* (Phytochemicals in Human Health Protection, Nutrition and Plant Defense), 369−392.

[3] A. A. Ribeiro, "Proton and carbon-13 NMR analysis of D-amygdalin: oligosaccharide assignment and sequencing" *Magn. Reson. Chem.* **1990**, *28*, 765−773.

[4] E. Stahl, W. Schild, In: "Isolierung und Charakterisierung von Naturstoffen", G. Fischer Verlag, Stuttgart, **1986**, pp. 51−54.

Would I could meet that rogue Diomed! I would croak like a raven; I would bode, I would bode. Patroclus will give me any thing for the intelligence of this whore: the parrot will not do more for an almond than he for a commodious drab. Lechery, lechery; still, wars and lechery; nothing else holds fashion: a burning devil take them!

William Shakespeare (1564−1616)
Troilus and Cassida V, 2

Fig. 4.3-1 A piece of the famous "Stollen" from the city of Dresden

"Once upon a time," said he, "there lay on my counter two gingerbread cakes, one in the shape of a man wearing a hat, the other of a maiden without a bonnet. Their faces were on the side that was uppermost, for on the other side they looked very different. Most people have a best side to their characters, which they take care to show to the world. On the left, just where the heart is, the gingerbread man had an almond stuck in to represent it, but the maiden was honey cake all over. They were placed on the counter as samples, and after lying there a long time they at last fell in love with each other; but neither of them spoke of it to the other, as they should have done if they expected anything to follow.

Hans Christian Andersen (1805−1875)
Under the Willow-Tree (1853)

3. Isolation

3.1 Principle

Fig. 4.3-2 Collected samples of amygdalin from students' exercises

Amygdalin is a glycoside of a cyanohydrin. The first measure essential for its isolation is to denature the enzyme set emulsin present in the bitter almonds to prevent cleavage of the glycoside. This is achieved by heating the almonds at 105 °C for a certain time. Due to its disaccharidic moiety which is attached to a comparatively small nonpolar aglycone, amygdalin is rather hydrophilic and therefore soluble in polar organic solvents such as methanol and ethanol but not in nonpolar solvents such as diethyl ether, dichloromethane, chloroform and hydrocarbons. The solubility in hot water is 0.1 g/mL. Therefore, fatty almond constituents are first removed by dichloromethane extraction. From the degreased residue, the remaining amygdalin is dissolved with boiling ethanol.

3.2 Method

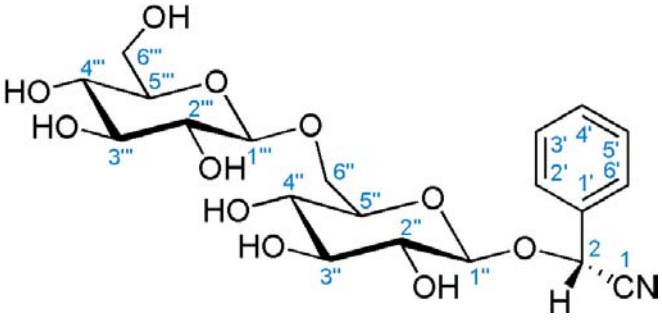
Bitter almonds (100 g) are heated in an oven for 3 h at 105 °C. After cooling, the almonds are crushed in a blender to a fine powder; 50 g of this almond powder are placed in the thimble of a Soxhlet apparatus and extracted with dichloromethane for 8 h to remove the fatty constituents. The degreased almond powder is air dried. Its mass is now ca. 20 g. This powder is then heated under reflux with 250 mL of ethanol (96%) in a 500 mL round-bottomed flask. The hot mixture is filtered and the filtrate is concentrated in vacuo to a volume of 10 mL. After cooling, diethyl ether (10 mL) is slowly added, causing precipitation of crude amygdalin as off-colourless crystals: 2 g, mp 216−220 °C.

Era inevitable: el olor de las almendras amargas le recordaba siempre el destino de los amores contrariados. El doctor Juvenal Urbino lo percibió desde que entró en la casa todavía en penumbras, adonde había acudido de urgencia a ocuparse de un caso que para él había dejado de ser urgente desde hacía muchos años. El refugiado antillano Jeremiah de Saint-Amour, inválido de guerra, fotógrafo de niños y su adversario de ajedrez más compasivo, se había puesto a salvo de los tormentos de la memoria con un sahumerio de cianuro de oro.

Gabriel Garcia Márquez (1928−)
El Amor en los Tiempos del Cólera

3.3 Purification

Most of the 1.0 g of crude amygdalin is dissolved by refluxing in 75 mL of ethanol. A small amount of undissolved matter (ca. 10%) is removed by filtration and the filtrate is kept into a deep freezer (18 °C) overnight in a sealed Erlenmeyer flask to initiate crystallization. Colourless microcrystalline amygdalin is obtained and filtered off. Concentration of the mother liquor on standing in a beaker yields a second crop. Amount: 300 mg, mp 220−221 °C, $[\alpha]_D^{20} = -41°$ (*c* 0.01 g/mL, H_2O).



Scheme 4.3-1

4. Spectra and Comments

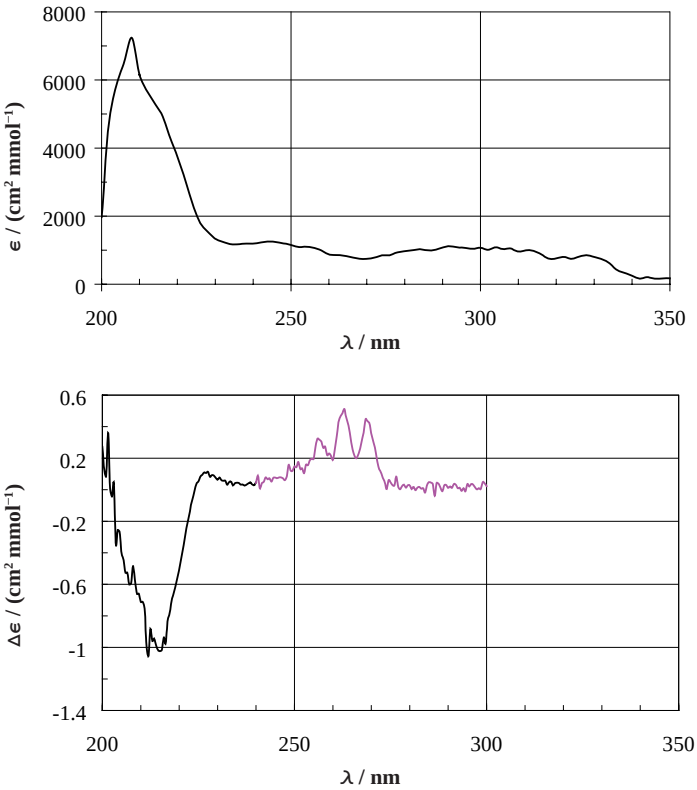


Fig. 4.3-3 UV and CD spectra in ethanol

There is a broad shoulder in the UV spectrum after the main signal reaching until 350 nm. Since the molecule contains chiral centres and a chromophore, this gives rise to circular dichroism and therefore the CD spectrum is shown. The main absorption band at 215 nm shows a negative Cotton effect ($\Delta\varepsilon = -1$ cm^2 mmol^{-1}) whereas at 260 nm a very weak positive effect (enlarged by a factor of 10) can be found.

Fig. 4.3-4 A sample of purified amygdalin

"Noyeau?" said the coroner, with interest.
„I see, that means something to you, doctor", said Mr. Egg.
"It does indeed", said the coroner. "Noyeau is a liquor flavoured with oil of bitter almonds, or peach-stones – correct me if I'm wrong, Mr. Egg – and contains, therefore a small proportion of hydrocyanic acid."
"That's it" said Monty. "Of course, in the ordinary way, there isn't enough of it to hurt anybody in a single glassful, or even two. But if you let a bottle stand long enough, the oil will rise to the top, and the first glass out of an old bottle of Noyeau has been known to cause death."

Dorothy L. Sayers (1893−1957)
Bitter Almonds (1939)

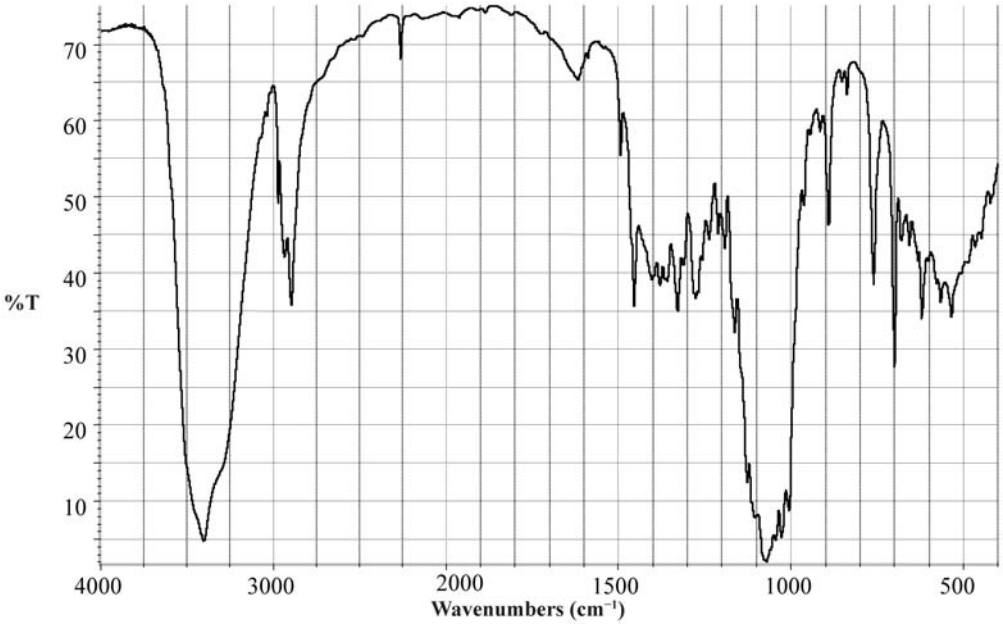
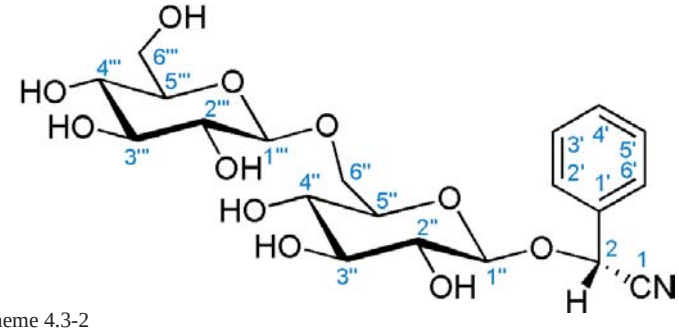


Fig. 4.3-5 IR spectrum in KBr

Two very strong bands at 3400 and 1100 cm^{-1} dominate the IR spectrum, indicating the carbohydrate moiety with OH and C–O valence vibrations. Often overlooked in similar cases, but very important in this spectrum, is the small and sharp signal at 2200 cm^{-1} indicating the cyano group. The monosubstitution of the benzene ring can be derived by the two CH deformation bands at 760 and 700 cm^{-1}.

Fig. 4.3-6 Almond pins used in home bakery



Scheme 4.3-2

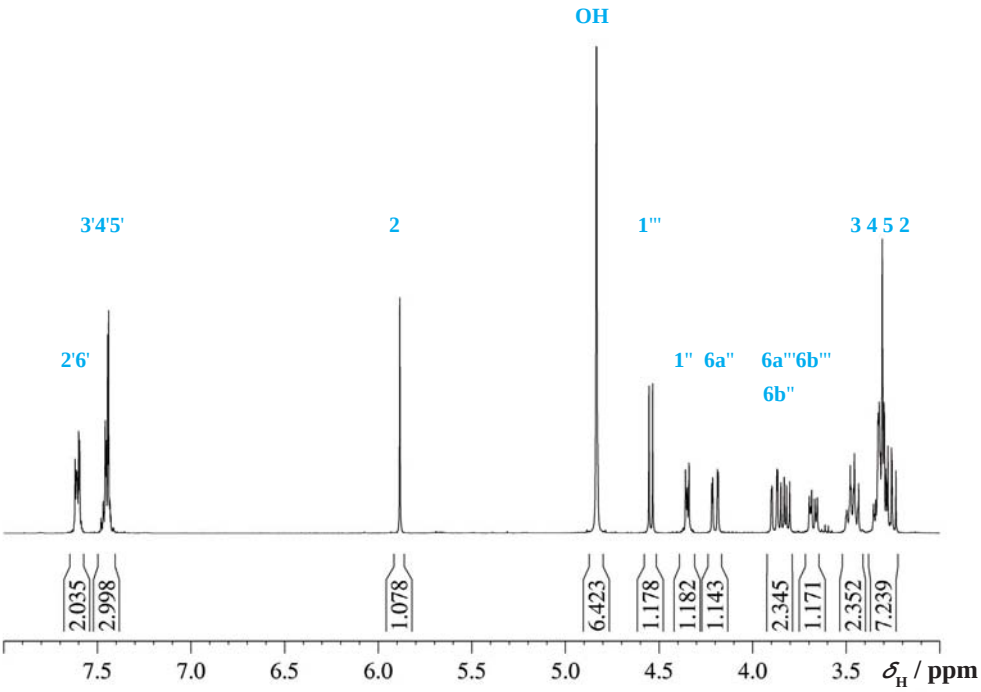


Fig. 4.3-7 ^{1}H NMR spectrum at 400 MHz in CD_3OD

The ^{1}H NMR spectrum recorded in CD_3OD inspected from left to right first reveals the typical AA'BB'C pattern of a monosubstituted benzene ring, then a singlet at δ = 5.88 ppm which stands for the OCH−CN group and the singlet at δ = 4.82 which accounts for the exchangable OH protons. The integral of the right-most signal group is somewhat enhanced due to the residual solvent protons which absorb underneath. In addition, in the 1D ^{1}H NMR spectrum only large axial−axial spin couplings can be observed, hence we have glucose units in both cases.

The relative assignment of the two anomeric proton signals at δ = 4.54 and 4.35 ppm can either be obtained by observing that the signal at 4.54 ppm is a clear doublet with no other coupling and therefore must belong to H-1''', or by looking at the NOESY spectrum which clearly gives the connection between the CHOCN signal and H-1''. Having secured these two entry points into the carbohydrate part of the molecule, one has several possibilities for further assignment.

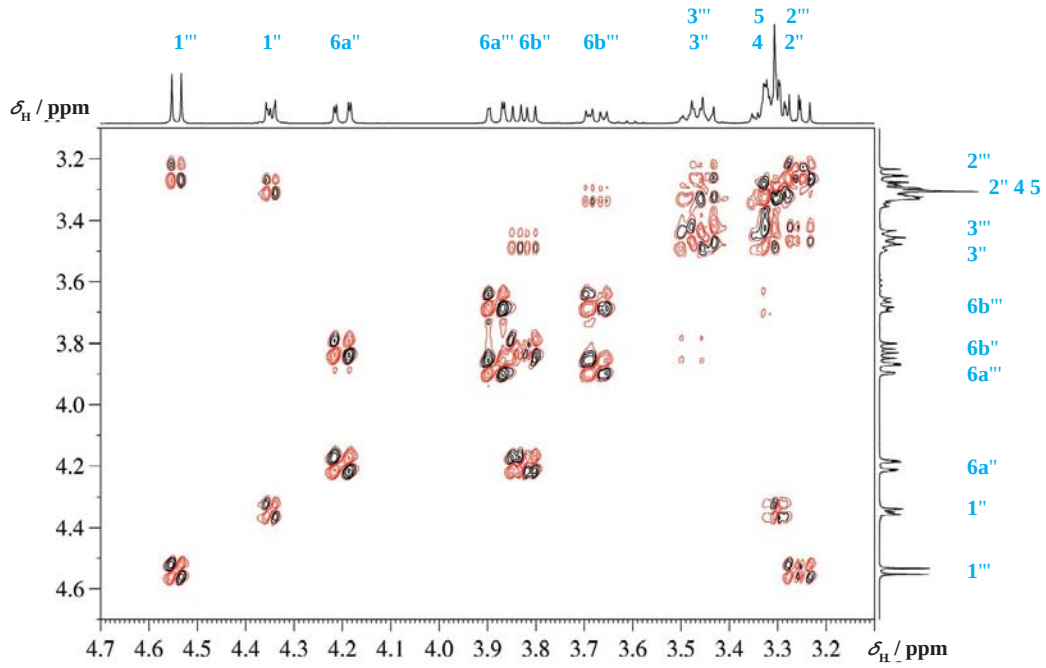
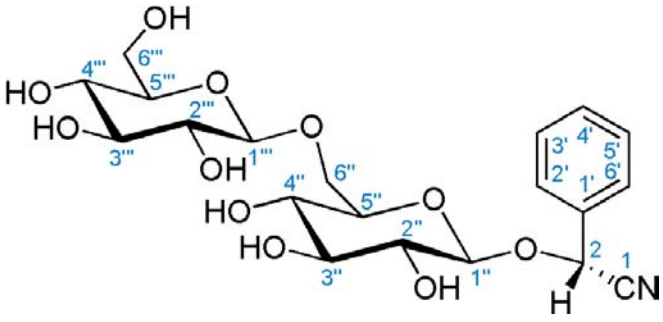


Fig. 4.3-8 Double quantum filtered COSY spectrum

The COSY spectrum reveals in a straightforward way the assignment for the protons 2'' and 2''' starting from the corresponding anomeric protons. Then it becomes difficult using the COSY spectrum alone, since protons 4 and 5 of both sugar moieties fall together and protons 3 can also hardly be differentiated between the two sugar rings. Only the diastereotopic protons at both 6 positions are clearly separated from each other.

Fig. 4.3-9 Seven bitter almonds may be sufficient to kill a person



Scheme 4.3-3

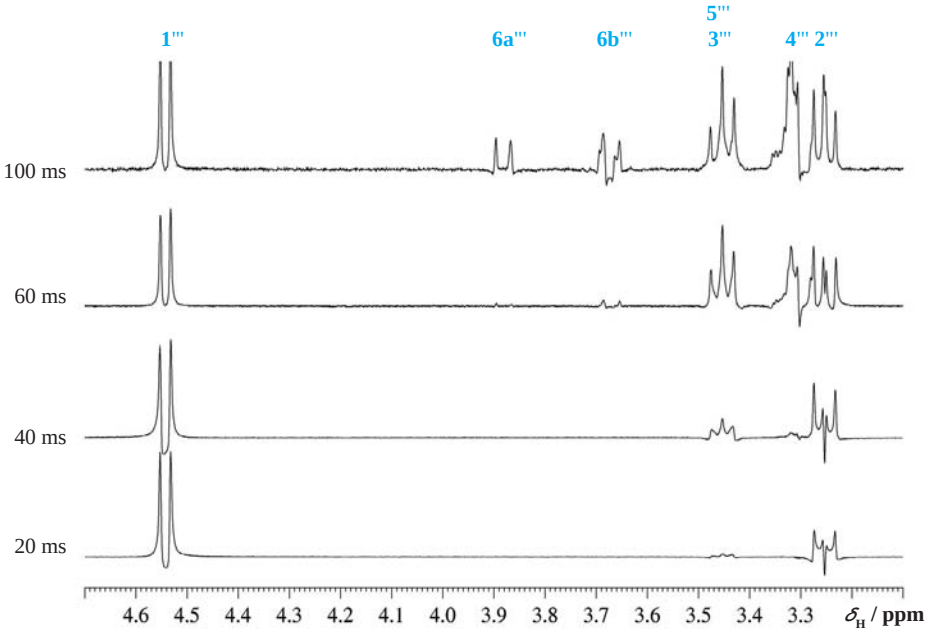


Fig. 4.3-10 Selective TOCSY spectra, H-1''' irradiated, different mixing times

A very important tool in carbohydrate NMR spectroscopy is the selective TOCSY technique, depicted in two figures here. By selecting one anomeric signal with a selective radiofrequency pulse and choosing different TOCSY mixing times, one can create spectra where the hydrogens along one carbohydrate residue appear subsequently. Whereas the spectrum with a 20 ms mixing time behaves like a selective COSY, the spectrum with a 100 ms mixing time reveals all hydrogen atoms of one glucose ring.

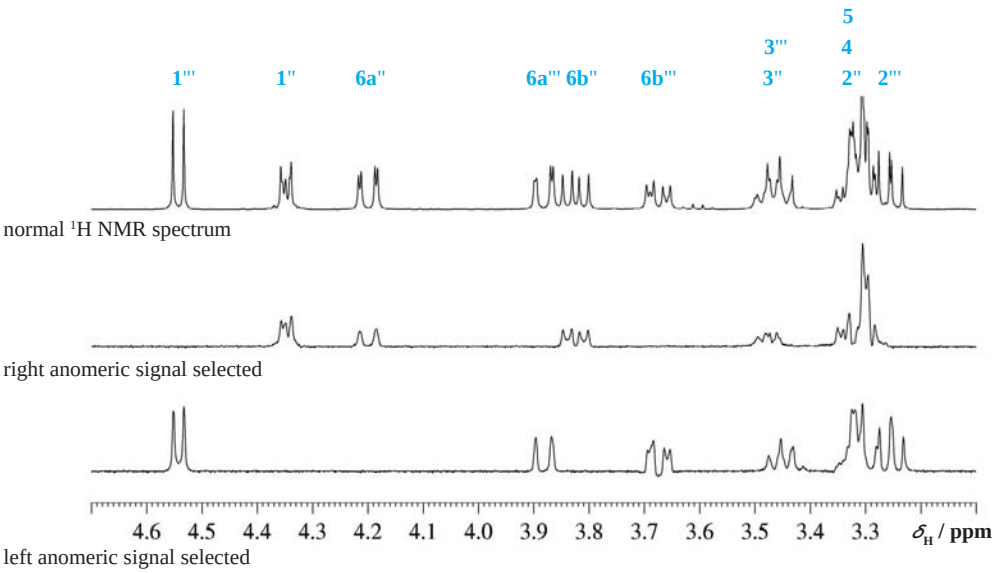


Fig. 4.3-11 Selective TOCSY spectra, 150 ms mixing time

As seen from the second TOCSY figure, the magnetization does not spill over into the other glucose ring, the two glucose spin systems remain separated, when their corresponding anomeric protons are irradiated.

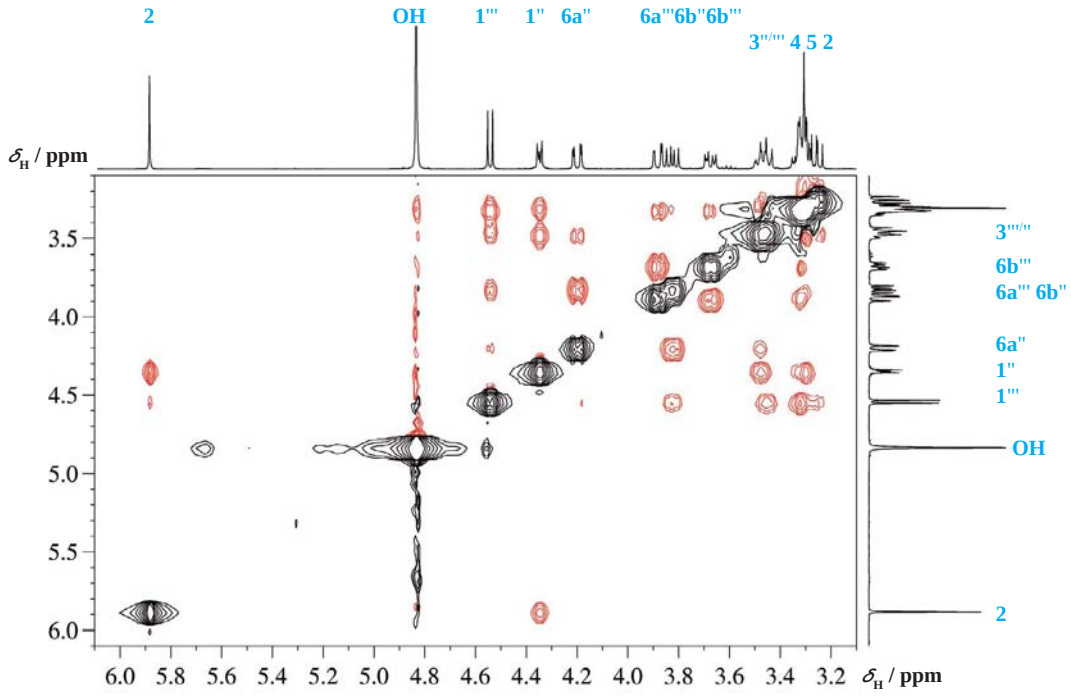
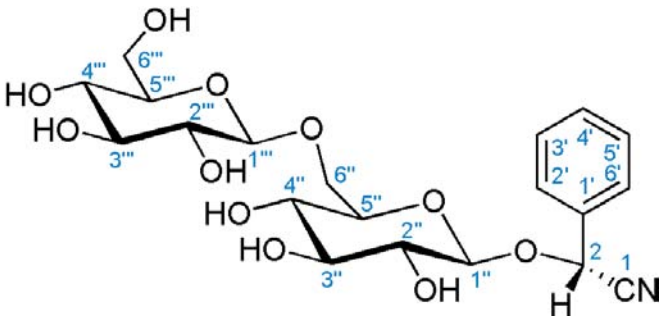


Fig. 4.3-14 NOESY spectrum

Of vital importance in structure elucidation of carbohydrates is the type of connectivity between sugar residues. The 1'''−6'' linkage in this case is most easily revealed by the strong NOESY cross signals between H-1''' and H-6''. The NOESY spectrum, in comparison with the DQF-COSY spectrum shown, also reveals the stereochemistry within the carbohydrate residues. As an example, H-1''' cross relaxes with H-3''' and H-5''', therefore both must also be axially situated. The same is true for H-1'' with respect to H-3'' and H-5''.



Scheme 4.3-4

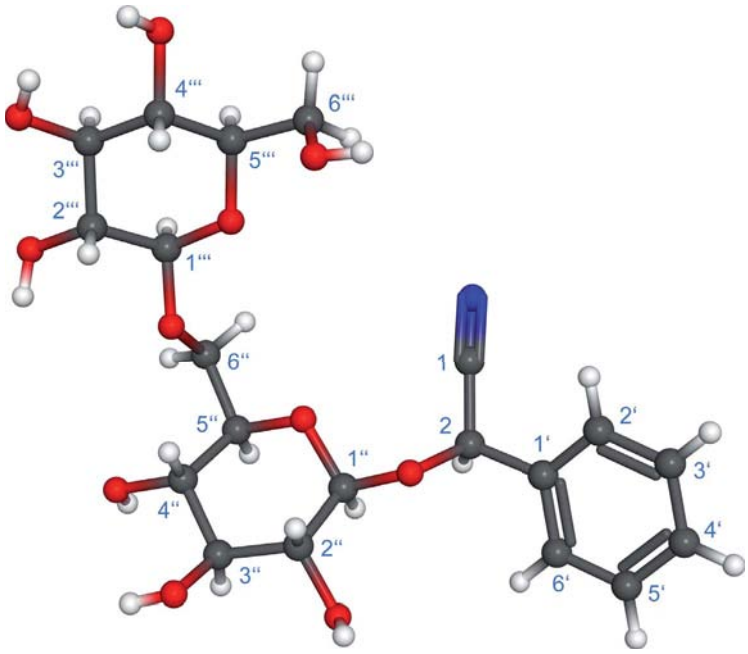


Fig. 4.3-13 Molecular model of amygdalin

Fig. 4.3-14 Such an amount of bitter almonds can only be purchased in a pharmacy and not a supermarket, in Germany.

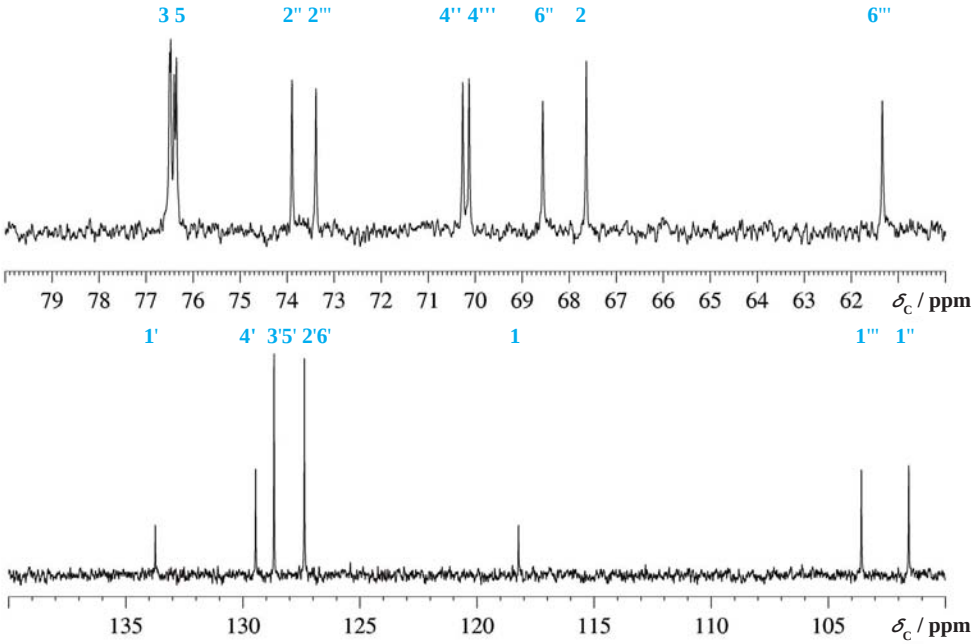
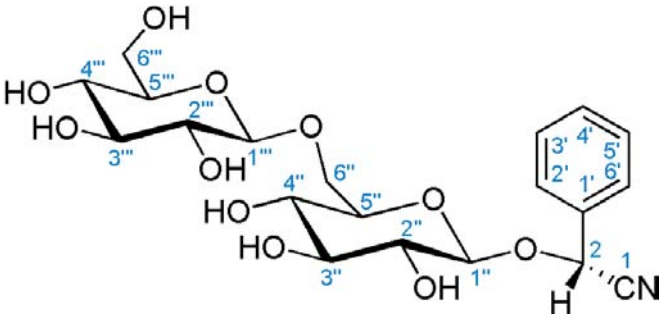


Fig. 4.3-15 ^{13}C NMR spectrum at 100 MHz in CD_3OD

The ^{13}C NMR spectrum shows the signal of the cyano group as expected at 118.2 ppm and the two anomeric carbon signals at 103.6 and 101.6 ppm. All the other carbohydrate signals appear in the typical narrow chemical shift range between 60 and 80 ppm with four very closely resonating signals between 76 and 77 ppm.

^{13}C Signals δ / ppm	Type of Carbon	Assignment	Proton Signals δ / ppm, J / Hz
133.7	C_q	C-1'	
129.4	CH	C-4'	7.7–7.4
128.7	CH	C-3',C-5'	7.7–7.4
127.4	CH	C-2', C-6'	7.62–7.60
118.2	C_q	C-1	
103.6	CH	C-1"'	4.54, J = 7.82
101.6	CH	C-1"	4.35
76.47–76.35	CH	C-3", C-3"', C-5", C-5"'	3.47, 3.45 3.36–3.28
73.9	CH	C-2"'	3.25, J = 9.3, 7.7
73.4	CH	C-2"	3.36–3.28
70.3–70.1	CH	C-4", C-4"'	3.36–3.28
68.6	CH_2	C-6"	6a": 4.20, J = 11,85, 1.92 6b": 3.82, J = 11.7, 6.8
67.6	CH	C-2	5.88,
61.3	CH_2	C-6"'	6a"': 3.88, J = 11.8, 1.93 6b"': 3.67, J = 11.9, 5.3

Table 4.3-1 NMR data for amygdalin



Scheme 4.3-5

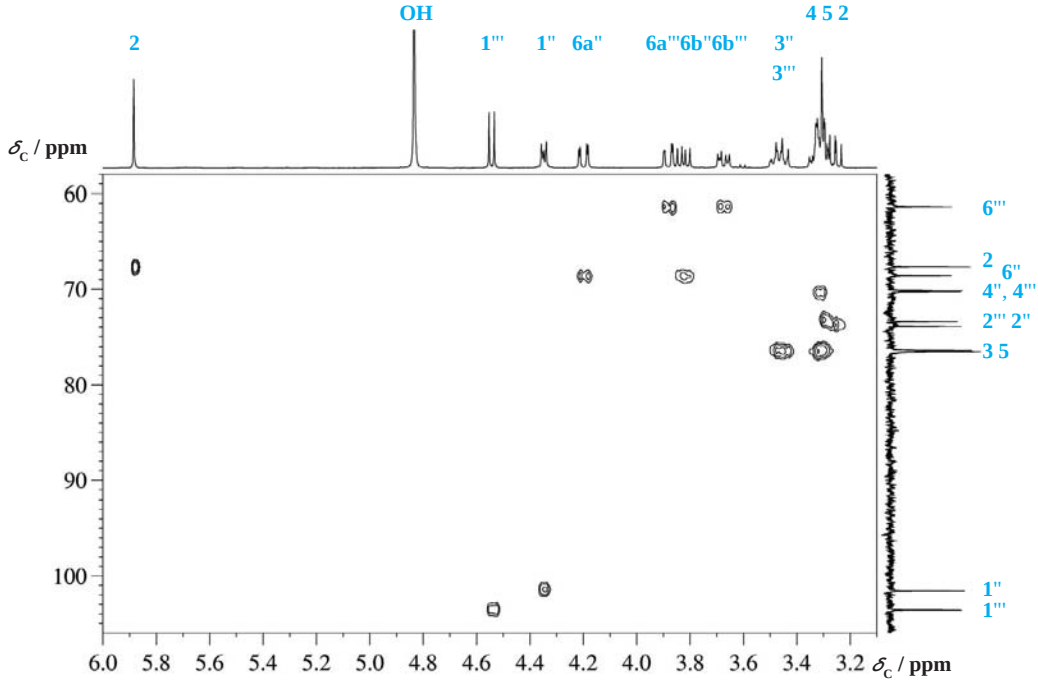


Fig. 4.3-16 Expansion of the HSQC spectrum

The residual ^{13}C NMR assignments start from the HSQC spectrum, since all protons have already been assigned. The relative assignment of the anomeric carbon signals can be made immediately and also the diastereotopicity of the protons H-6'' and H-6''' is nicely observed since they are connected to the same carbon atoms.

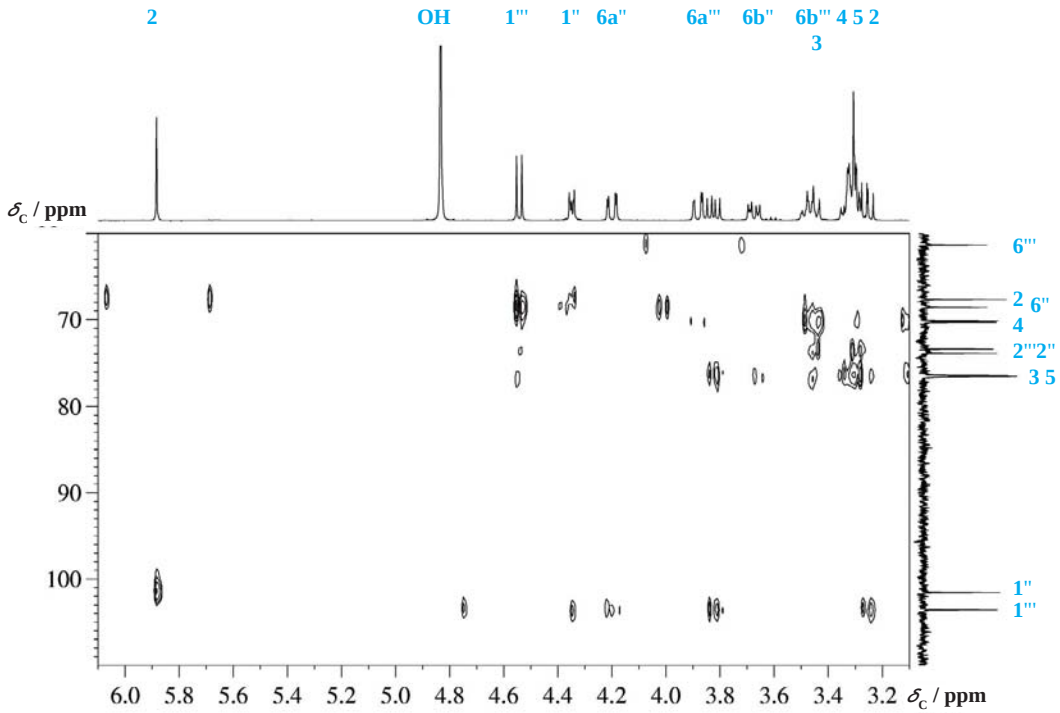
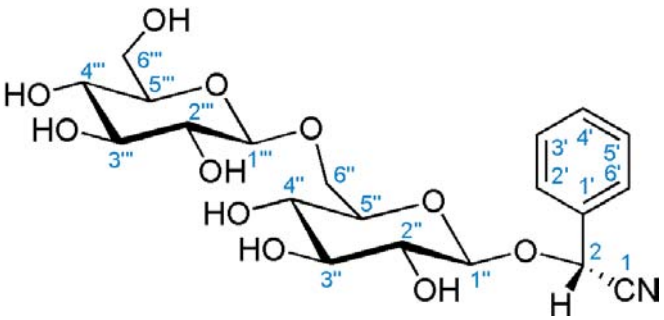


Fig. 4.3-17 Expansion of the HMBC spectrum

The linkages between the different parts of the molecule can also be observed in the HMBC spectrum, where there is a cross peak between H-2 and C-1'', confirming that the acetonitrile moiety is connected to the anomeric site of the double-dashed glucose. Furthermore, cross signals are observed between H-6'' and C-1''', again confirming the 1−6 linkage between the two glucose moieties.



Scheme 4.3-6

Das Verhalten des Amygdalins und des weissen, käseähnlichen Bestandtheils der Mandelkerne gewinnt ein noch höheres Interesse, wenn man sich erinnert, dass die Gegenwart von Amygdalin in den Kernen von dem zufälligen Standorte des Baumes abhängig ist. Zwischen zwei Bäumen, von denen der eine süsse, der andere bittere Mandeln trägt, haben die Botaniker keine wahrnehmbare Verschiedenheit gefunden. Es sind Fälle bekannt, wo das einfache Versetzen einen Baum süsse Mandeln tragen machte, der vorher bittere Mandeln lieferte; gewiss eines der interessantesten Beispiele des Einflusses, den gewisse Bestandtheile im Boden auf den Lebensprocess der Pflanzen ausüben.

Justus von Liebig **(**1803−1873)
Chemical Letters XVIII

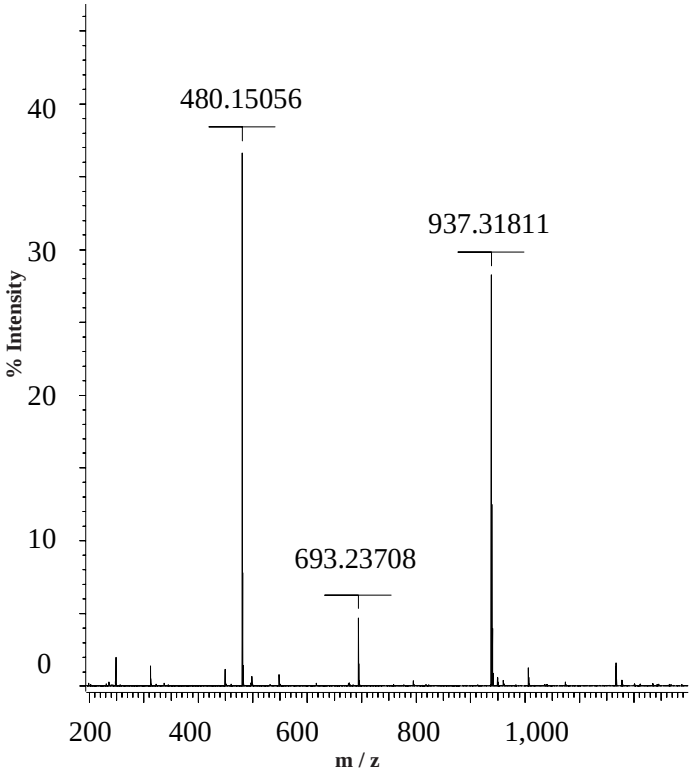


Fig. 4.3-18 Mass spectrum (ESI-FT/ICR)

Due to the large carbohydrate part of the molecule, it is hardly possible to obtain a classical mass spectrum with electron impact ionization which contains the molecular ion. By reducing the energy of the electron impact source, however, some fragmentation spectra are observed. Here, we show instead the high-resolution mass spectrum obtained via electrospray ionization (ESI) on an FT/ICR instrument. ESI is the method of choice for this class of compounds. Unfortunately, usually only the quasimolecular ions with no further fragmentation are produced in this way. Here one observes two major peaks at m/z = 480.14806 and 937.3071. These can be interpreted as $[M + Na]^+$ (theoretically 480.1476318 for $C_{20}H_{27}NO_{11}Na$) and $[2M + Na]^+$.

5. Questions

A. The biosynthetic origin of the cyano group in the aglycone is decarboxylation and dehydration of an aminocarboxylic acid. Which compound is it in the case of amygdalin?

B. Describe the action of the enzyme emulsin found in almonds.

C. The glucoside prunasin {=[(β-D-Glucopyranosyl)oxy]benzeneacetonitrile} is a monosaccharidic precursor of the disaccharidic amygdalin. However, prunasin would have a toxic effect if the plant were to try to transport it to its seeds. The reason is that normal β-glucosidases would be able to cleave it and thus would finally liberate toxic HCN at the wrong place and time. What is a reasonable assumption for the biochemical sense of the attachment of a second glucose unit linked to the -CH_2OH unit of the first one?

D. Which part of the molecule gives rise to the UV absorption?

E. Can one derive the absolute stereochemistry at C-2 from the CD spectrum?

F. Give examples of other structural elements which have absorptions at about 2200 cm^{-1} in the IR spectrum.

G. Why might the integral value of the signal at 4.8 ppm be less than expected for 7 OH groups?

H. The ^{13}C NMR signals of the two glucose units apparently come “in close pairs”, only the signals of C-6" and C-6"' are separated by about 7 ppm. Why?

I. The NOESY spectrum shows a cross peak between H-6a" and H-3". Build a molecular model and show the reason.

J. The $\Delta\delta$ value for the protons 6" is considerably larger than that for the protons 6"'. Suggest an explanation.

6. Own Observations

4.4 Hesperidin

(2*S*)-7-{[6-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy}-2,3-dihydro-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one

From the peel of mandarin oranges *Citrus reticulata* Blanco
or oranges *Citrus sinensis* L. Osbeck (Rutaceae)

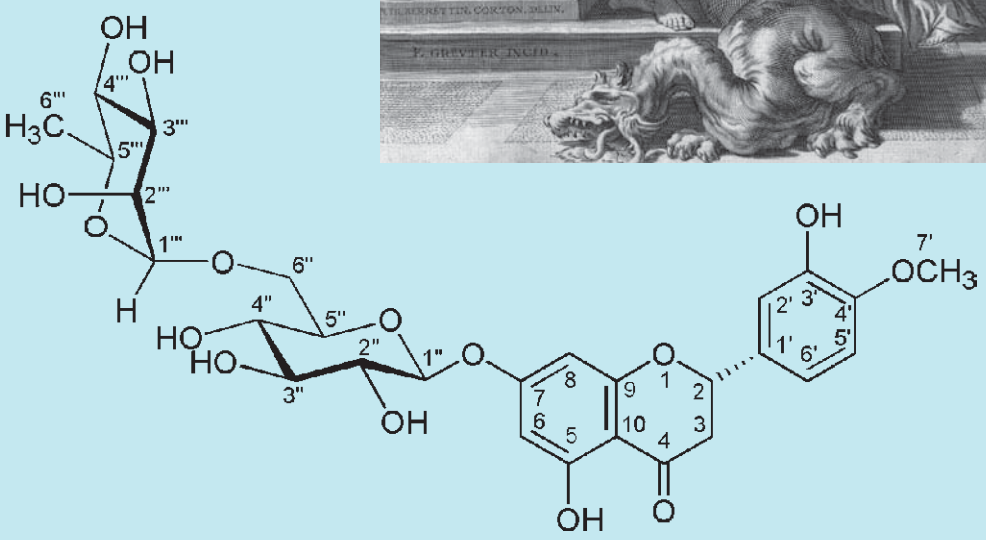
$C_{28}H_{34}O_{15}$, MW 610.56
CAS 520-26-3, BRN 1279620

Off-white crystals, mp 252−252.5 °C
$[a]_D^{23}$ −86.3° (*c* 0.0164 g/mL, pyridine)

Hesperidin is commercially available.

Synonymous names: 3',5,7-Trihydroxy-4'-methoxy-7-(6-*O*-α-L-rhamnosyl-D-glucoside) flavanone, Hesperetin 7-rhamnoglucoside, Hesperidoside, Cirantin

Level: easy



1. Background: The 11th labour of Heracles

Fig. 4.4-1 Golden shining mandarins on one of the author's balcony

The denomination of compounds with a suitable, if possible even meaningful, trivial name would constitute its own "chapter" of natural products chemistry. Hesperidin is a name which has no obvious link to properties of the natural product and at first glance no persuading link to the oranges from which it is isolated.

Hesperidin will remind the reader of the Greek myth of the Hesperides, a couple of nymphs. They were obliged to guard an orchard in which trees with golden apples grew, i.e. no citrus fruits. These apples were planted from branches of a tree which were a wedding gift of Gaia, the goddess of earth, when Hera, as her older sister, finally accepted Zeus, the king of gods, as her husband. Although the Hesperides had the task of guarding the trees, they plucked golden apples for themselves, occasionally. However, Hera predicting this weakness, placed Ladon, a never-sleeping dragon with 100 heads, as a mighty reinforcement in the grove. The task of stealing the golden apples of the Hesperides is called the eleventh labour of Heracles. Indeed, he brought this about by tricking the giant Atlas a few times in a very smart manner. However, Athena, the goddess of civilization, was unhappy with that thievery and returned the stolen golden apples to garden to keep all things in balance.

Already in the 17th century, the term "hesperides" was used for citrus fruits [1], presumably, to establish a link between the golden colour of oranges and the colour of the holy apples of the Hesperides. Although the peels contain hesperidin it is not responsible for their glaring orange coloration: pure hesperidin is a very pale yellow compound.

The term orange refers to the tree *Citrus sinensis* L. and its fruits. This fruit is a hybrid obtained from the pomelo (*Citrus maxima*) and the tangerine (*Citrus reticulata*), a mandarin orange. The word orange has its origin in the Sanskrit word *narang*. The root of it is retained in the Spanish name *naranja*, whereas in other languages the n at the beginning was cut off, as in the Italian *arancia*. Precisely, fruits of *Citrus sinensis* have to be called sweet oranges to distinguish them from bitter oranges from *Citrus aurantium*, which in contrast are the origin of the well-known marmalade, which was invented in 1779 in Dundee, Scotland.

In a number of languages oranges are called China's apples according to their geographic origin China, e.g. in Dutch as *sinaasappel* and in the northern and eastern parts of Germany as *Apfelsine*.

The sweet orange was brought from India to Europe in the 16th century by Portuguese traders. Already during his second voyage to the New World, Columbus brought citrus seeds to the Carribean. The fruits proved to be a good protection against the feared scurvy. The mandarin oranges came much later in the 19th century from China to Europe, and were first grown in Palermo, Sicily.

Brazil and the USA are the top producing countries for oranges with ca. 18 and 8 million tons, respectively, in 2005.

Here the orange tree is found.
Shedding beauty all around;
Living in this southern grove,
From its fate it will not move;
For as its roots lie fast and deep,
So its purpose it will keep.
With green leaves and blossoms white,
It brings beauty and delight.
Yet foliage and sharp thorns abound
To guard the fruit so ripe and round,
Golden clusters, clusters green,
Glimmer with a lovely sheen,
While all within is pure and clear
Like heart of a philosopher.

Qu Yuan (340−278)
Ode to the Orange Tree

Almost every part of oranges is used for different applications: they are not only a source of orange juice, but also of sweet orange oil, made by pressing the peel, which is used as a flavouring for beverages, a fragrance constituent of perfumes (see also section 5.1 on D-limonene) and even as an environmentally friendly cleaning agent for degreasing. Whereas all this seems to belong to general knowledge, only a few may know that the important polysaccharide pectin is extracted from the white part of the peel (called pericarp or albedo) on an industrial scale. Pectin is a mixture of three polysaccharides, homogalacturonan, and the rhamnogalacturonans I and II. In acidic solutions, as typical for making jams and jellies, pectin is able to form a gel. Of course, this property to behave as a thickening agent makes the outer sides of oranges as good a business as the inner parts.

Fig. 4.4-2 A jealous complexion?

Precisely, the rhamnose which is a part of pectin is L-rhamnose, in the same way as the L-rhamnose in the disaccharide part of hesperidin.

Structurally, hesperidin is a flavanone glycoside and thus belongs to the flavanoids, an important class of plant secondary metabolites, all derived from the 2-phenylchromen-4-one moiety. Flavanoids contribute to the coloration of many flowers, leaves and fruits (yellow, red, blue), and some of them can also be used as natural dyestuffs for dyeing, especially after staining of the fibre. It is likely that hesperidin plays a role in plant defence.

The count is neither sad, nor sick, nor merry, nor well; but civil count, civil as an orange, and something of that jealous complexion.

William Shakespeare (1564−1616)
Much Ado About Nothing, II, 1

Two strongly related structural classes, the flavones and flavonols, have aglucones with a double bond between C-2 and C-3 and hence a conjugated system spread over the entire aglucone which is substituted by auxochromic OH or OCH_3 groups that enhance the colour. In hesperidin this double bond is lacking, a reason for its paleness.

Biogenetically, the flavanoid aglucone is made starting from the amino acid phenylalanine, which is converted into 4-cumaroyl-SCoA. Combination with malonyl-SCoA gives rise to a hydroxysubstituted open-chain chalcone which is cyclized to a corresponding heterocyclic flavone. Finally, further processing takes place, including glycosylation.

Flavonoids were discovered in the 1930s by the Hungarian physiologist Albert Szent-Györgyi von Nagyrapolt, who also isolated L-ascorbic acid (vitamin C) and received the Nobel Prize in Physiology in 1937. Although his initial classification of bioflavonoids as vitamin P was abandoned, their physiological performance is beyond any doubt.

Flavonoids show antimicrobial, anti-inflammatory, antiallergic and anticancer activity [4]. They are regarded as antioxidants, which may protect cells against free radicals and oxidative damage. Many of the beneficial effects that arise from consumption of fruit, vegetables, tea, and red wine (moderate) are attributed to bioflavonoids, thus attracting the attention of both consumers and food manufacturers. High expectations exist especially in their potential to prevent cardiovascular diseases and cancer, for example. In our nutrition, hesperidin is helpful for the integrity of blood vessels.

The aglycone of hesperidin is called hesperitine. The disaccharide attached is called rutinose, which is a trivial name for 6-*O*-L-rhamnosyl-D-glucose. Two close relatives will be mentioned. Naringin ((2*S*)-7-{[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy}-2,3-dihydro-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one) is responsible for the bitter taste of grapefruit juice. The grapefruit, to make botanic explanations still more complex, is regarded as a cross of pomelo (*Citrus maxima*) and sweet orange (*Citrus sinensis*) which arose from Barbados in the late 18th century. The aglycone naringenin occurs naturally in citrus fruits as well as hesperetin.

Kennst du das Land, wo die Zitronen blühn,
Im dunklen Laub die Goldorangen glühn,
Ein sanfter Wind vom blauen Himmel weht,
Die Myrte still und hoch der Lorbeer steht?
Kennst du es wohl?
Dahin, dahin
Möcht ich mit dir, o mein Geliebter, ziehn!

Johann Wolfgang Goethe (1749–1832)
Mignon

A third glycoside, neohesperidin, is of special interest for food chemistry beause it serves as a source for the production of a sweetener. Neohesperidin is a regioisomer of hesperidin, in particular (2*S*)-7-{[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy}-2,3-dihydro-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one, and has a bitter taste. However, catalytic hydrogenation leads to the open-chain neohesperidin dihydrochalcone form (abbreviation NHDC, listed as food additive E 959 in the EU). In the EU it was approved as a sweetener in 1994. In the USA, this is not the case; there it is considered as a flavour enhancer. The industrial production of NHDC is based on neohesperidin extracted from bitter oranges. One gram of NHDC is as sweet as ca. 500 g of sugar. However, the typical use is not in the pure form but as an additive to other artificial sweeteners to which it has a synergistic effect. Likewise, it is able to reduce or cover the bitter taste of certain compounds. Therefore, pharmacologists are fond of NHDC as a kind of chemical mask for bitterness of active ingredients in tablets. NHDC is free of calories. It does not undergo metabolism in the body and is eliminated by the urine.

2. Literature

[1] Giovanni Battista Ferrari, “Hesperides Sive de Malorum Aureorum Cultura et Usu Libri Quattor” [The Hesperides, or four books on the cultivation and use of the golden apples], Rome, **1646**.

Fig. 4.4-3 Such a bag of mandarins is more than enough for the experiment described here

[2] K.-F. Tseng, R.-D. Yu, "The studies of flavone derivatives in Chinese drugs. I. The isolation method of hesperidin from Chen-pi (citrus peel)" *J. Chinese Pharm. Assoc.* **1936**, *1*, 14−23.

[3] J. A. Pino, "Flavonoids present in citrus fruit" *Alimentaria* (*Madrid*) **1997**, *286*, 63−79.

[4] A. Garg, S. Garg, L. J. D. Zaneveld, A. K. Singla, "Chemistry and pharmacology of the *Citrus* bioflavonoid hesperidin", *Phytotherapy Research* **2001**, *15*, 655−669.

[5] J. J. Peterson,. J. T. Dwyer,. G. R. Beecher, S. A. Bhagwat, S. E. Gebhardt,. D. B. Haytowitz, J. M. Holden, "Flavanones in oranges, tangerines (mandarins), tangors, and tangelos: a compilation and review of the data from the analytical literature" *Journal of Food Composition and Analysis* **2006**, *19* (Suppl.), S66−S73.

[6] D.-H. Kim, M.-J. Song, E.-A. Bae, M. J. Han, "Inhibitory effect of herbal medicines on Rotavirus infectivity" *Biol. Pharm. Bull*. **2000**, *23*, 356−358.

[7] B. Domon, K. Hostettmann, "Mass spectrometric studies of underivatized polyphenolic glycosides" *Phytochemistry* **1985**, *24*, 575−580.

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Fig. 4.4-4 A drawing of Qu Yuan

3. Isolation

The isolation follows the method described in ref. [8].

3.1 Principle

Dried and milled peel of mandarin orange or oranges still contains some ethereal oils which are hydrophobic and have first to be carefully removed by a Soxhlet extraction with a nonpolar solvent such as dichloromethane. This solvent does not dissolve the glycoside hesperidin, which is too polar. From the residue, remaining hesperidin can be extracted with methanol as a rather polar organic solvent, giving a crude product. Recrystallization from a DMSO−water mixture yields hesperidin of better quality. Finally, recrystallization from glacial acetic acid gives pure hesperidin. Although this isolation is almost only described from orange peel in the literature (content 5–8%), we have found that using mandarin orange peel yields about twice the amount of hesperidin. Peels of lemons and grapefruits do not yield hesperidin.

3.2 Method

Dried and milled (use a kitchen blender) mandarin orange peel (70 g) is placed in the thimble of a Soxhlet apparatus and extracted with dichloromethane (750 mL) for 8 h to remove the ethereal oil constituents. The extract is discarded. The remaining peel from the thimble is air dried. A 50 g amount of such peel powder is again placed in a thimble of a Soxhlet apparatus and extracted with methanol (750 mL) for 3 h to dissolve the hesperidin. The methanolic extract is concentrated in

vacuo to a syrup, to which 6% (v/v) aqueous acetic acid (50 mL) is added with stirring. The precipitate obtained is filtered off by suction and dried at 60 °C or in vacuo on a rotary evaporator. The melting range of this pale yellow material is 235–245 °C and its mass is around 3 g. For a first recrystallization, a 5% (m/m) solution of this crude product in DMSO (weigh the DMSO on a balance) is made by panning both components in a 250 mL round-bottomed flask in a water bath at 70 °C. To this solution, the same volume of distilled water at 70 °C is added slowly with stirring. The mixture is allowed to cool slowly to room temperature, which gives rise to crystallization of hesperidin as tiny off-white needles. The glycoside is filtered off by suction through a sintered glass filter funnel, washed with water and dried in vacuo using an oil pump. The material thus obtained (mass = 2.0 g) has a mp of 262−263 °C (in agreement with the range of mp given in databases), but was shown not to be completely pure yet by NMR spectra.

3.3 Purification

A 2.0 g amount of the above hesperidin is dissolved in boiling glacial acetic acid and allowed to stand overnight for recrystallization. Filtration gives off-white microcrystalline hesperidin (0.7 g, mp 252−252.5 °C, very sharp melting range), $[a]_D^{23}$ −86.3° (*c* 0.0164 g/mL, pyridine), which can be shown to be pure by NMR. It should be noted that complete removal of all acetic acid from recrystallization requires 4 h in a flask heated at 80 °C in the vacuum of an oil pump (0.01 mbar).

Every Friday five crates of oranges and lemons arrived from a fruiterer in New York − every Monday these same oranges and lemons left his back door in a pyramid of pulpless halves. There was a machine in the kitchen which could extract the juice of two hundred oranges in half an hour if a little button was pressed two hundred times by a butler's thumb.

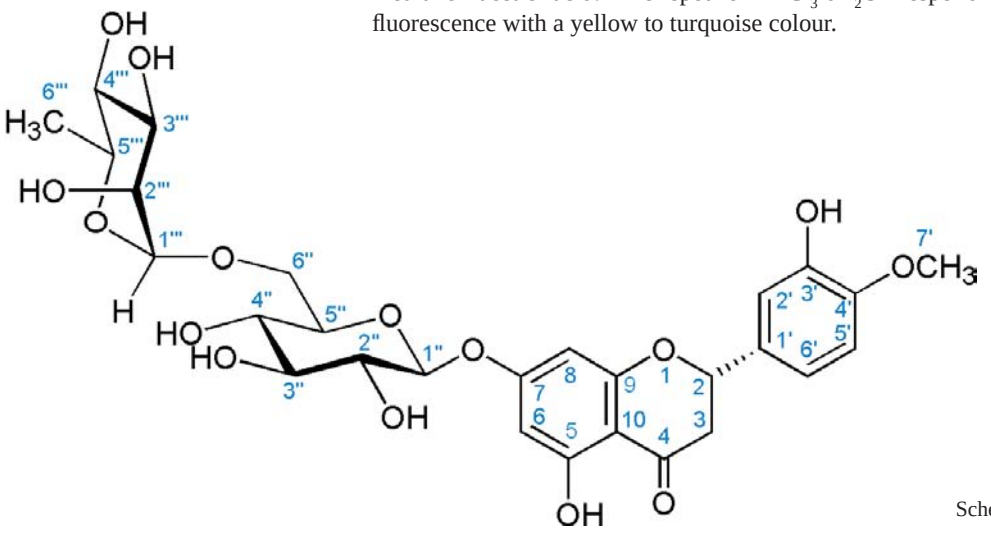
Francis Scott Key Fitzgerald
(1896−1940)
The Great Gatsby

Note for TLC analysis of hesperidin:

Silica gel 60_{F254} plates were used. As the eluent, the upper layer of *n*-butanol−acetic acid−water (4:1:5, v/v/v) was used.

Detection with a 254 nm UV lamp: the spot of hesperidin shows a fluorescence quenching.

Detection with a 365 nm UV lamp: the developed chromatogram is sprayed with a solution of 2.0 g $AlCl_3 \cdot 6H_2O$ in 100 mL of 5% (v/v) methanol−acetic acid. The spot of $AlCl_3 \cdot 6H_2O$ hesperidin shows fluorescence with a yellow to turquoise colour.



Scheme 4.4-1

4. Spectra and Comments

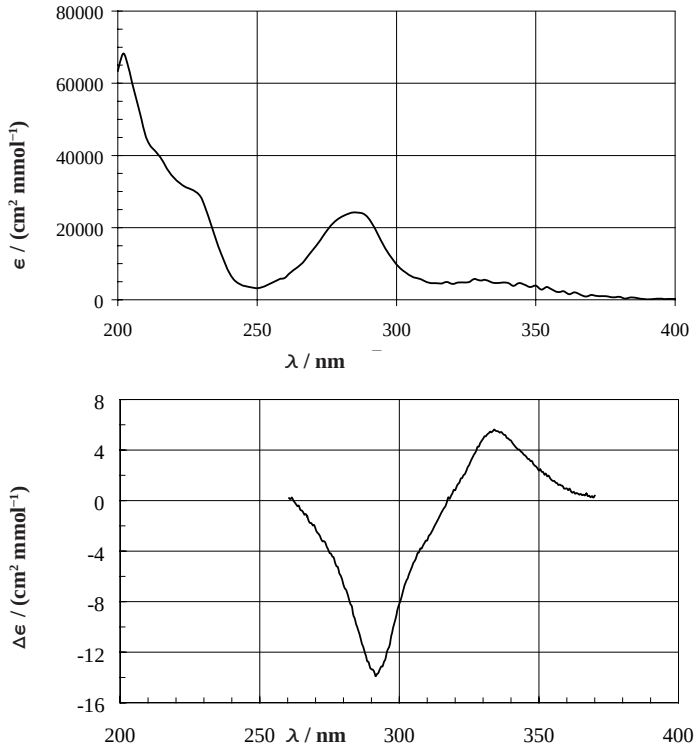


Fig. 4.4-7 UV and CD spectra in ethanol

Fig. 4.4-5 Fresh mandarin peel has first to be dried

Fig. 4.4-6 Pile of dried, milled and pre-extracted mandarin orange peel prior to extraction with methanol

The molecule consists of the flavanoid and the glycosidic parts and the discussion of the spectra best follows this partition. In addition to the usual absorption in the aromatic $\pi \rightarrow \pi^*$ region, the molecule displays a significant UV maximum at 286 nm which is caused by the conjugation of the keto group and the other oxygen atoms with the aromatic ring systems. As discussed above, due to the lack of a double bond between C-2 and C-3, the system is not fully conjugated and therefore there is no significant absorption above 350 nm. The CD spectrum, which was measured in DMF for sensitivity reasons, shows a positive Cotton effect for the shoulder at 330 nm and a very strong negative band for the absorption band at 290 nm, probably due to the vicinity of the keto group to the chiral centre at C-2. Below 260 nm the CD spectrum could not be measured due to the self-absorption of DMF.

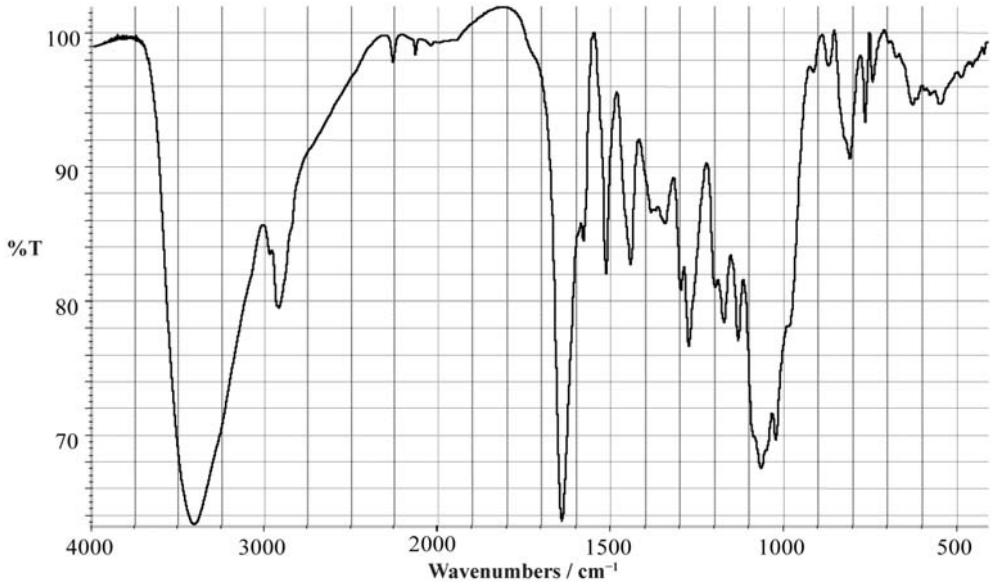
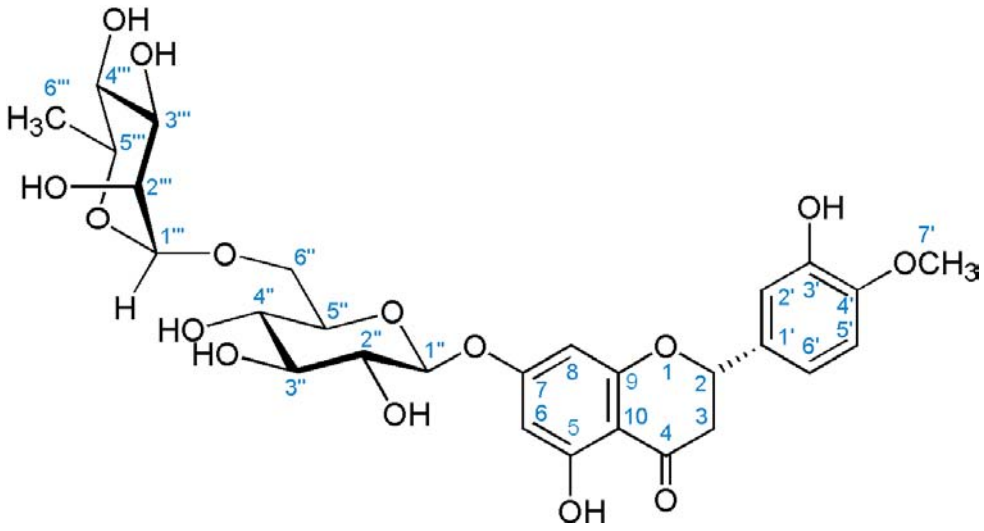


Fig. 4.4-8 IR spectrum in KBr

In the IR spectrum, of course, the OH band and the carbonyl stretching band are predominant. In addition, the aromatic C=C band at 1600 cm^{-1} and aromatic overtones at 2000 cm^{-1} can be detected.



Scheme 4.4-2

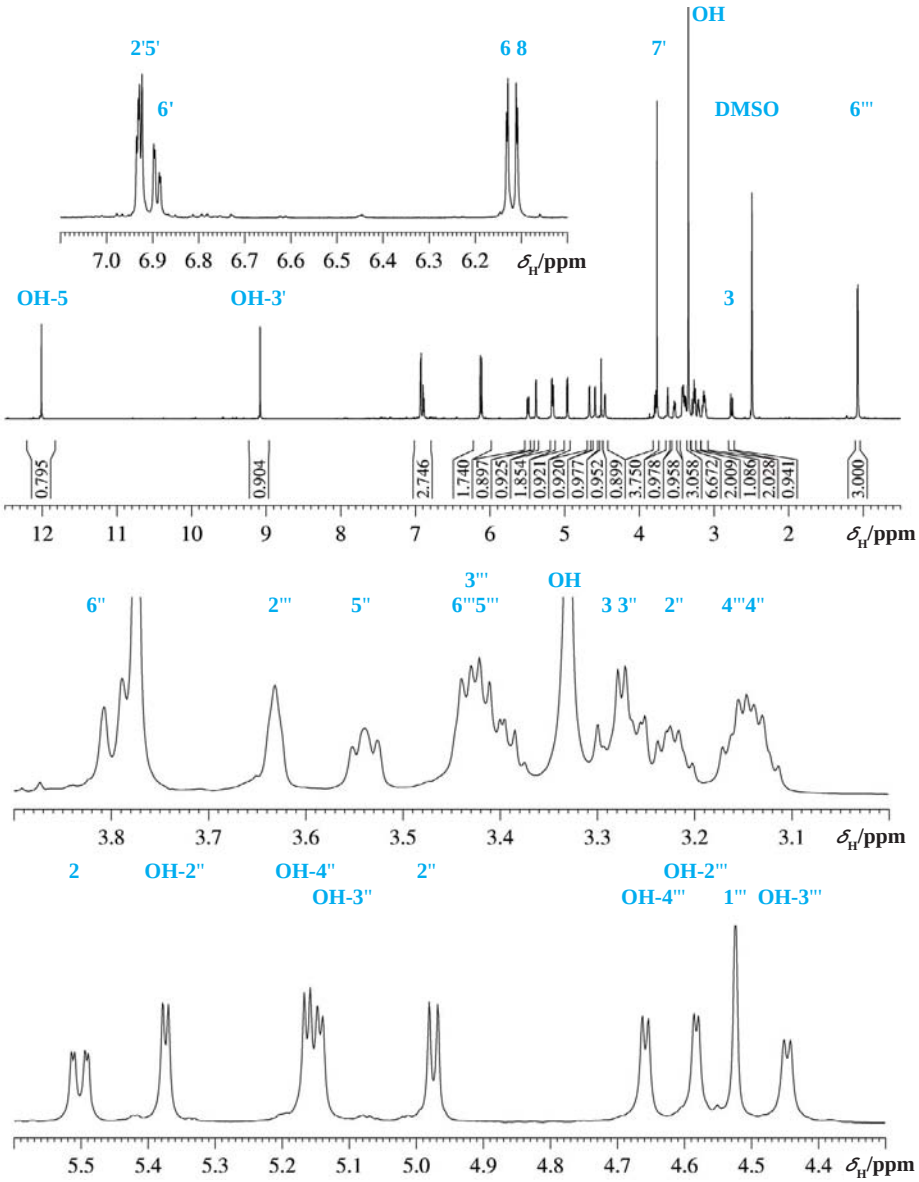
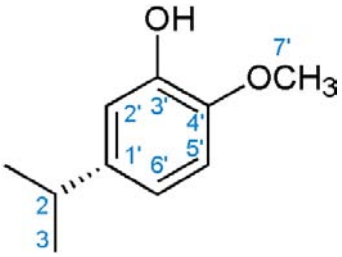


Fig. 4.4-9 ^{1}H NMR spectrum at 700 MHz in DMSO-d_6 with expansions

The proton NMR spectrum, recorded in DMSO, reveals two sharp OH resonances at 12 and 9 ppm. The first can be safely assigned to the OH group at C-5 due to the hydrogen bond to the carbonyl atom. The signal at 9 ppm must then belong to the phenolic OH group at C-3'. Three aromatic protons appear at about 7 ppm and two more shielded aromatic protons at 6.1 ppm. We start with the assignment of the ^{1}H NMR spectrum of the phenyl ring attached at C-2 of the benzopyranone moiety. The methoxy group H-7' at C-4' can be very easily identified. The three hydrogen atoms H-2', H-5' and H-6' form an ABC spin system, where, however, the signals of H-2' and H-5' overlap and only the doublet-type signal of H-6' is somewhat separated at 6.90 ppm. The remaining two aromatic protons at 6.1 ppm belong to H-6 and H-8 and are shielded due to their β-position with respect to the oxygen atoms. A multitude of small signals appears between 5.5 and 3 ppm, interrupted by the OCH_3 signal at 3.77 ppm, a strong OH signal at 3.75 ppm, and finally a methyl group signal at 1 ppm.



Scheme 4.4-3 Partical structure of hesperidin

Fig. 4.4-10 Ripe oranges

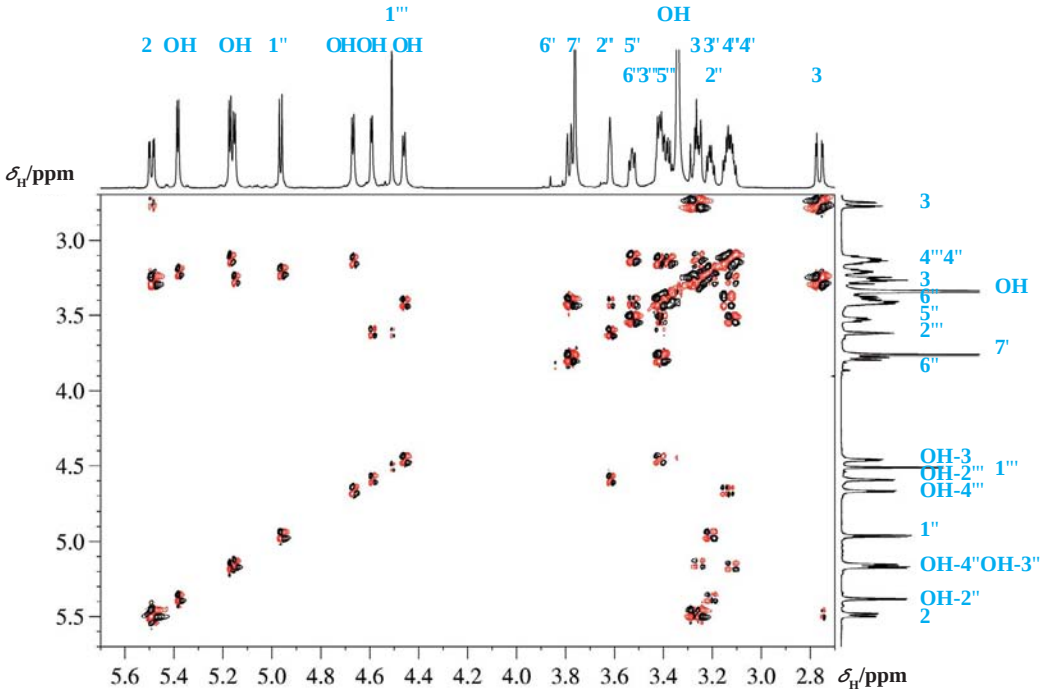


Fig. 4.4-11 Double quantum filtered COSY spectrum

The double quantum filtered COSY spectrum reveals that the double doublet signal at 5.5 ppm is coupled to two protons at 3.27 and 2.77 ppm, which are in turn strongly coupled to each other. Therefore, we can assume that the signal at 5.5 ppm belongs to H-2 and the other two signals form the diastereotopic methylene group of C-3. Another diastereotopic methylene group with similar strong coupling is detected in the COSY spectrum at 3.8 and 3.4 ppm and assigned to the protons of C-6". At this stage it is difficult to proceed with further analysis with proton information alone. Since the spectrum was taken in DMSO, all the OH groups give sharp signals and they have to be distinguished from CHO signals.

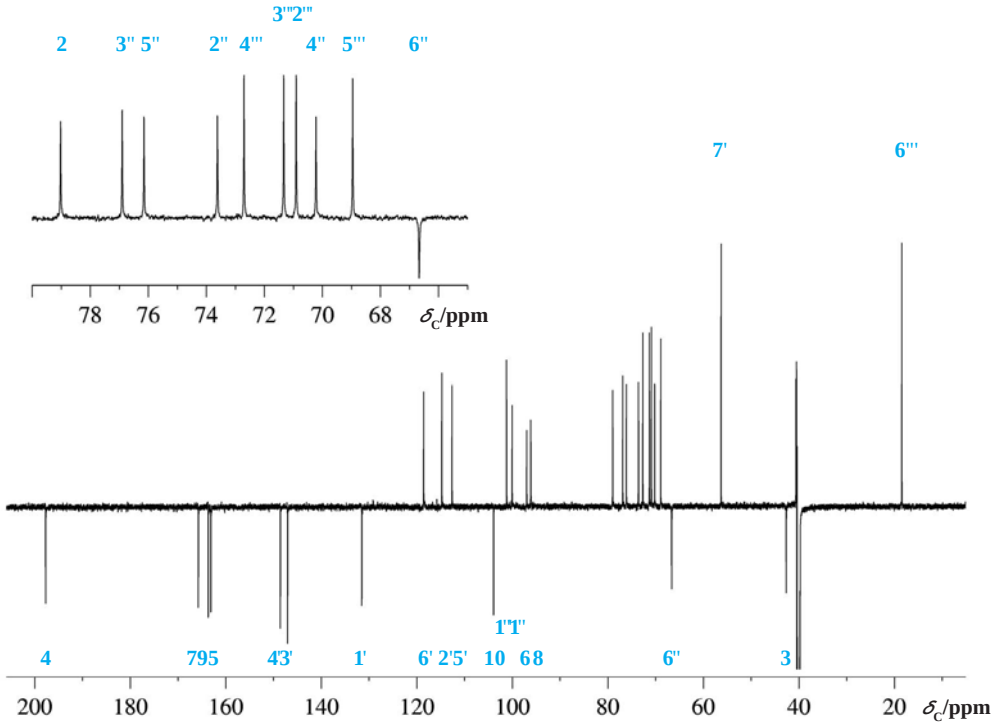
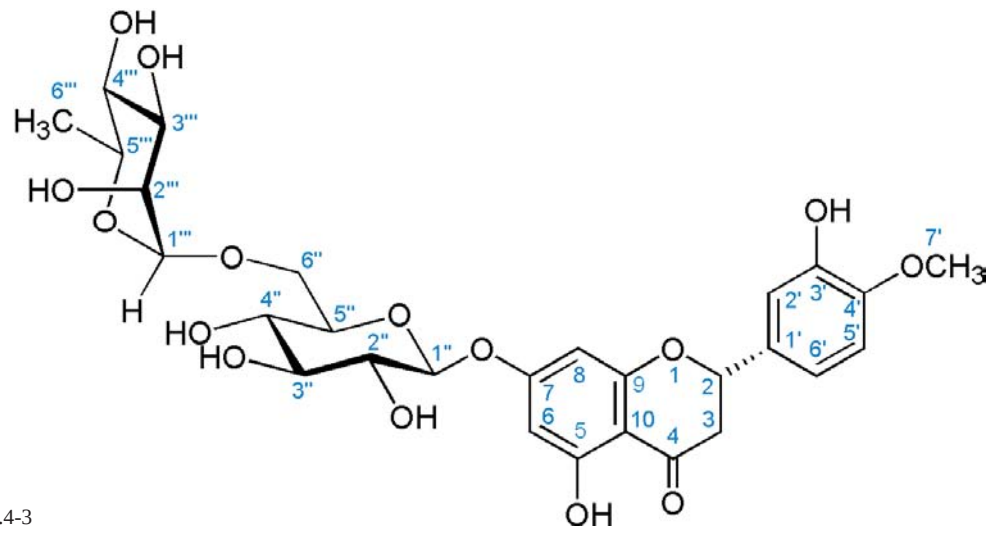


Fig. 4.4-12 APT ^{13}C NMR spectrum at 175 MHz in DMSO-d_6

In the carbon NMR spectrum, the carbonyl group, the methoxy group and the methyl group can be directly identified. We further find two negative signals at 42 and 66 ppm which belong to C-3 and C-6". In the region from 90 to 120 ppm we find seven positive signals which must belong to the five H-bearing aromatic carbon atoms and the two anomeric carbon atoms. Further assignments will rely on the HSQC and HMBC spectra.



Scheme 4.4-3

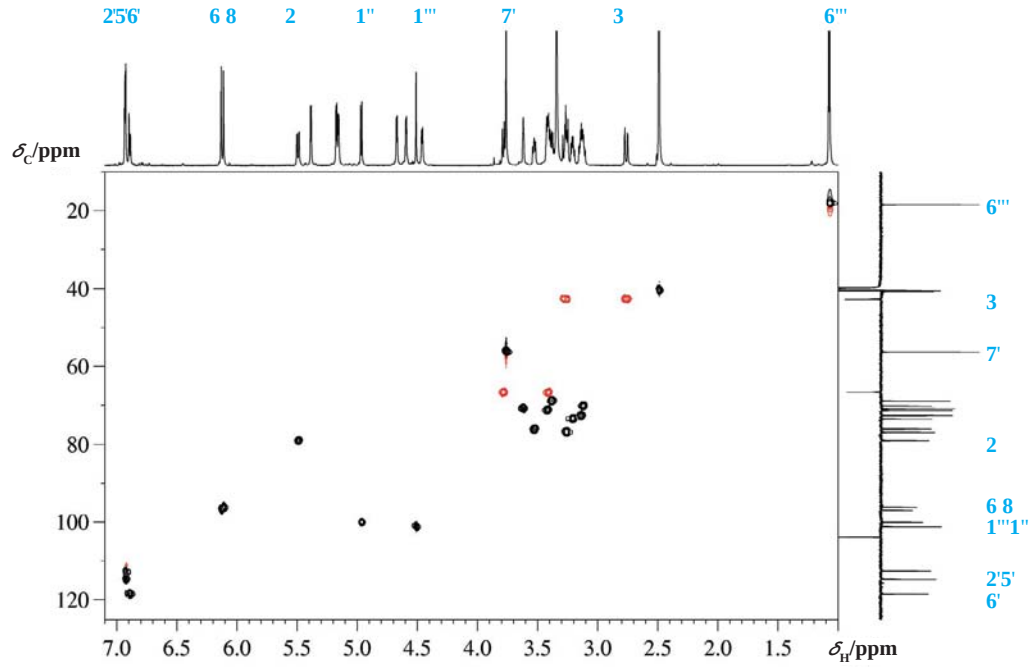


Fig. 4.4-13 Edited HSQC spectrum

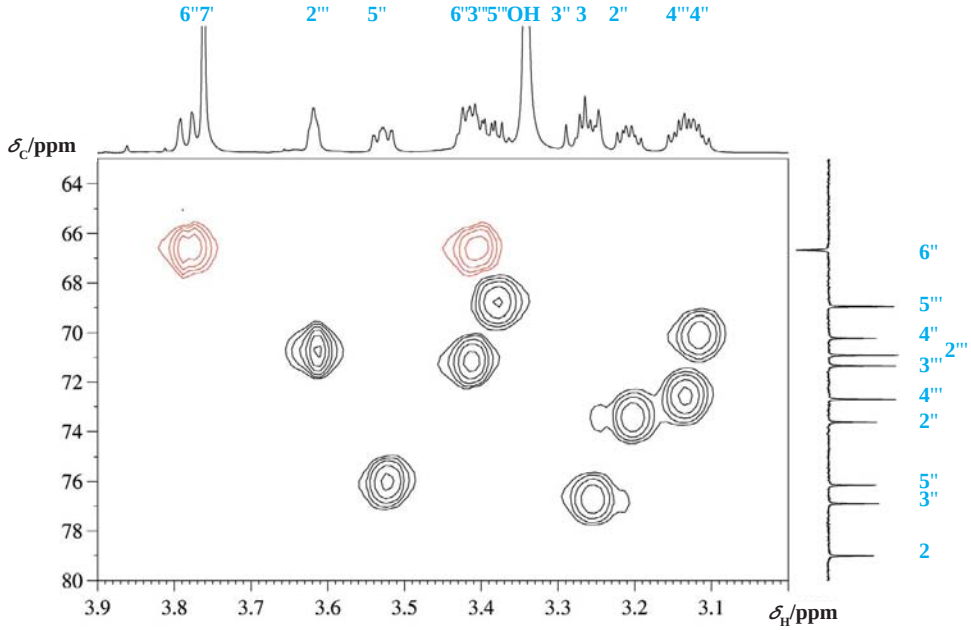


Fig. 4.4-14 Expansion of the HSQC spectrum in the sugar region

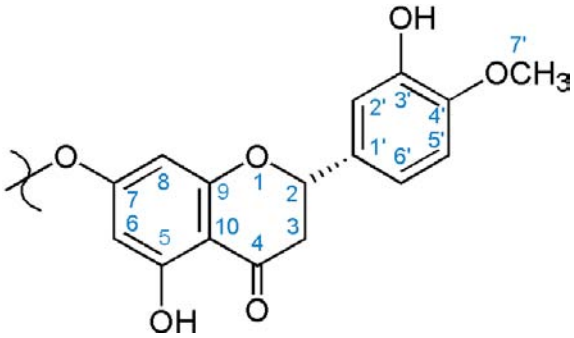
In this case, the strength of the HSQC spectrum is that it reveals which of the many sharp proton signals are from protons bound to a carbon atom.Therefore, the two anomeric protons with their respective carbon atoms can be identified. Since H-1" has an axial−axial coupling partner H-2", the proton signal with the large splitting at 4.97 ppm is assigned to H-1", whereas the signal at 4.52 ppm showing no distinct spin coupling must belong to H-1'''. The edited HSQC spectrum further corroborates the assignment of the diastereotopic methylene groups displayed in red.

Fig. 4.4-15 Green oranges

Fig. 4.4-16 Mandarin oranges from the supermarket

First time my father overheard me
listening to
this bit of music he asked me,
"what is it?"
"it's called Love For Three Oranges,"
I informed him.
"boy," he said, "that's getting it
cheap."
he meant sex.
listening to it
I always imagined three oranges
sitting there,
you know how orange they can
get,
so mightily orange.

Charles Bukowski (1920−1994)
Three Oranges



Scheme 4.4-5 Partial structure of hesperidin

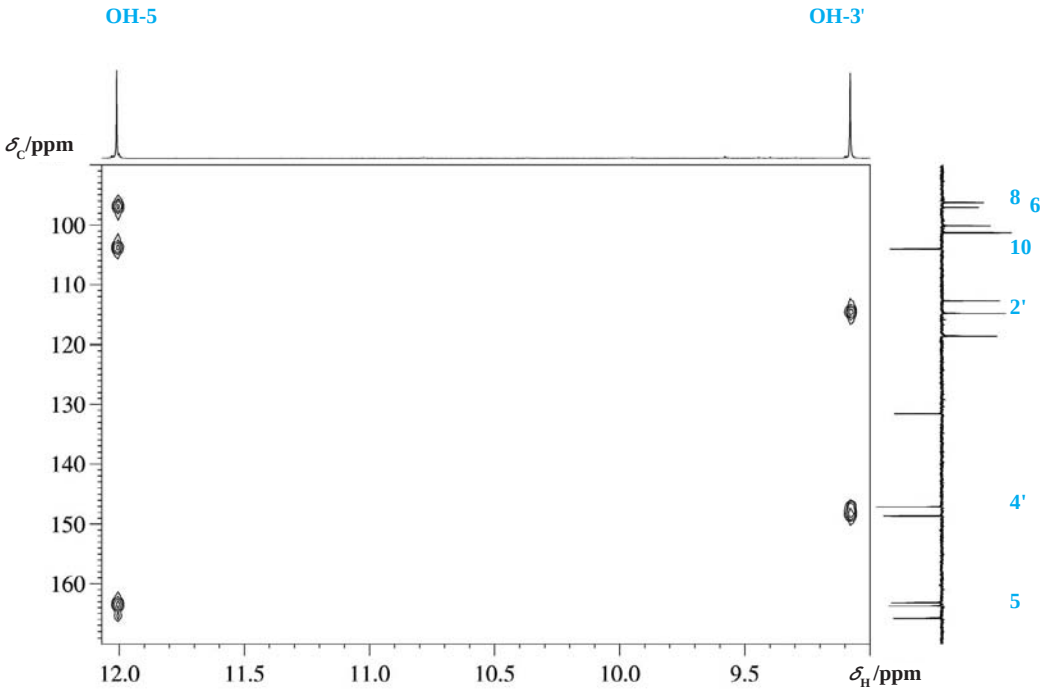


Fig. 4.4-17 Expansion of the HMBC spectrum for the OH groups

The molecule is a perfect example of the power of the HMBC spectra. The OH group at C-5 (12 ppm) which is strongly deshielded due to the hydrogen bond to the carbonyl oxygen displays three HMBC correlations, namely to C-6 (96.4 ppm), C-5 (163 ppm) and C-10 (103.3 ppm). C-6 can be distinguished from C-10 due to the sign in the APT ^{13}C NMR spectrum.

The HMBC spectrum (section not shown) also determines the assignment for C-4' at 148.0 ppm due to the spin coupling with the methoxy group H-7'. The phenolic hydroxyl group OH-3' resonates at 9.07 ppm; in the expansion of the HMBC spectrum given, it shows two correlation peaks over three bonds, one to C-2' at 114.2 ppm and one to C-4' already found at 148.0 ppm.

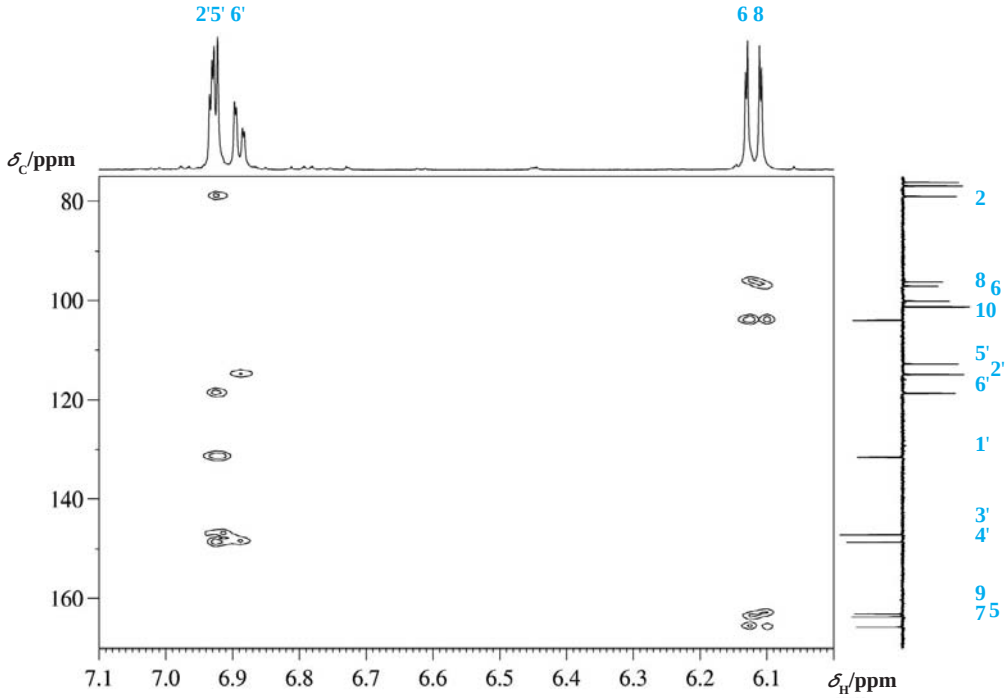


Fig. 4.4-18 Expansion of the HMBC spectrum for the aromatic protons

In the second HMBC expansion for the aromatic protons, H-6' displays correlations over three bonds to C-4' at 148 ppm and C-2' at 114.2 ppm. In turn, the overlapping signals of H-2' and H-5' show in the HMBC spectrum correlations to C-2 at 78 ppm, C-6' (117.9 ppm), C-4' (for H-2'), C-1' (130.9) and C-3' (146.5) for H-5'. For this aromatic ring the HMBC spectrum strictly displays only correlations over three bonds, which is very helpful for the assignment. The proton signals of H-6 and H-8 at 6.15 ppm both show HMBC correlations to C-8 and C-6, respectively, and also both to the quaternary carbon atom C-10 at 103.2 ppm. Both also show a two-bond correlation to C-7 at 165 ppm, and C-7 is also be verified via HMBC from the anomeric hydrogen H-1" (see below).

Fig. 4.4-19 Make a decision!
Travelling on Cuba's highways will take you to a rest house sooner or later where refreshment in the form of orange juice with or without Havana Club rum is offered (see bottles on the right). One of the authors decided in this situation to take the juice only and recommends this as a correct decision during the tropical heat of the day

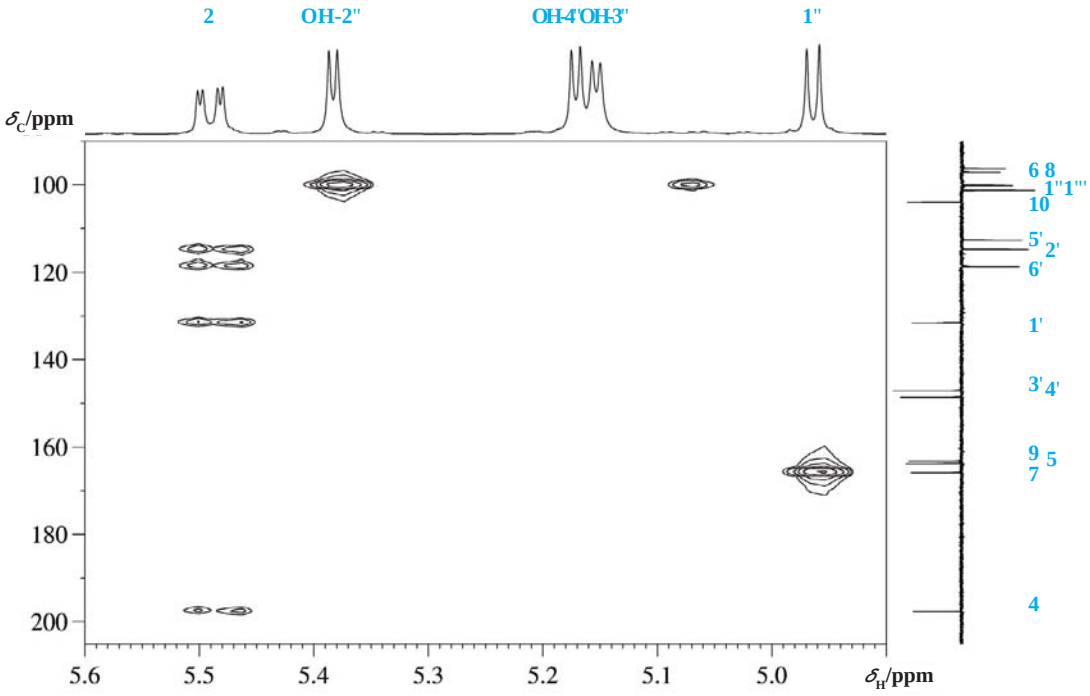
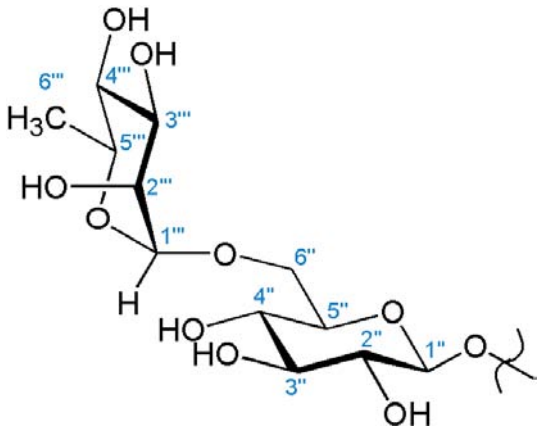


Fig. 4.4-19 Expansion of the HMBC spectrum between 5.6 and 4.9 ppm



Scheme 4.4-6 Partial structure of hesperidin

In the third HMBC expansion, the proton signal of H-2 at 5.5 ppm is most helpful. It shows a spin coupling with the carbonyl group C-4, and confirms the assignment of C-1', C-2' and C-6'. Finally, the signal of C-9 can be reached via three bonds from H-2. The OH group at 5.4 ppm is connected to the anomeric carbon atom C-1", hence this signal should be assigned to OH-2". The anomeric proton H-1" is connected to the signal of C-7 via three bonds.

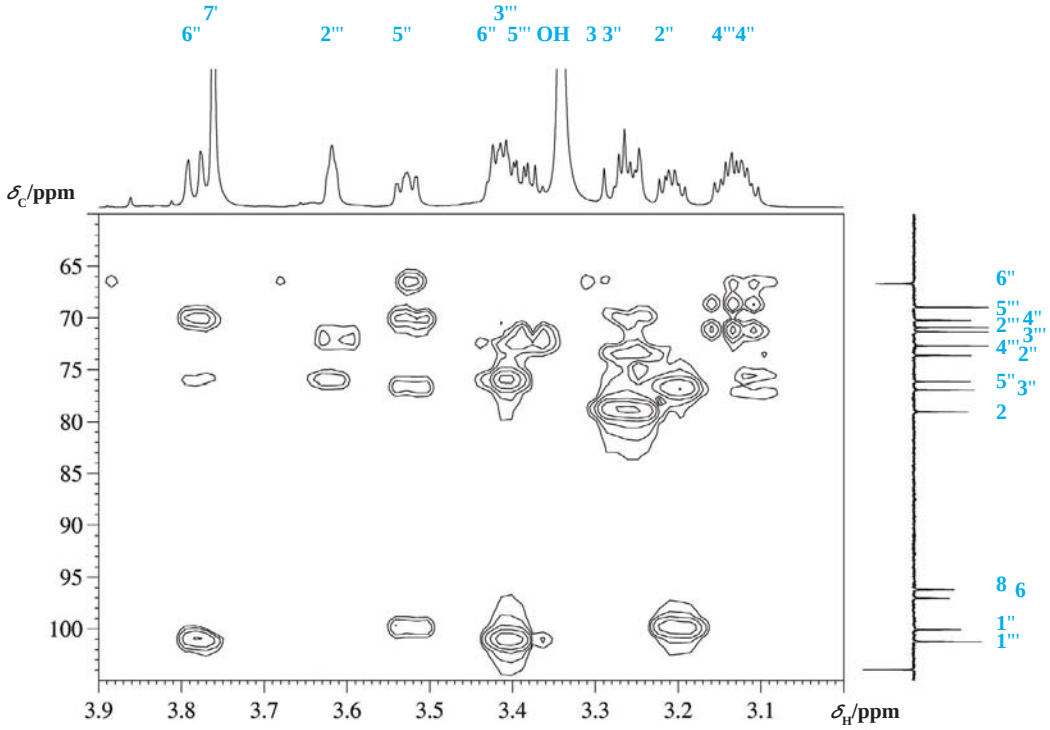


Fig. 4.4-20 Expansion of the HMBC spectrum between 3.9 and 3.0 ppm

In the final HMBC expansion, the carbohydrate part of the molecule is addressed. We observe that both protons H-6" have, as expected, a correlation to C-1''', whereas H-5" and H-2" correlate with C-1". The correlation of one of the protons at C-3 with C-2 can also be seen.

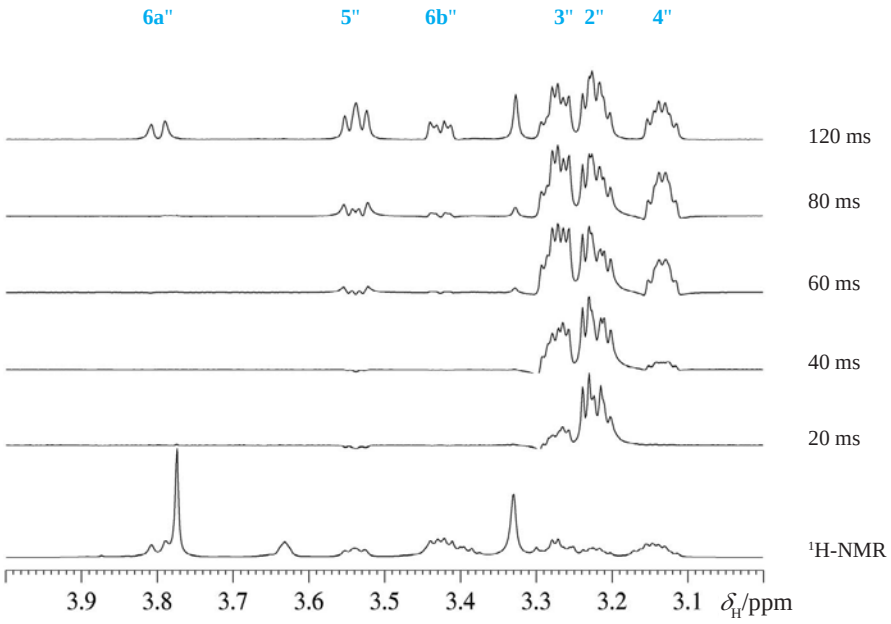


Fig. 4.4-21 Series of selective TOCSY spectra

The best help for the proton assignment in the carbohydrate part of the molecule is a series of selective TOCSY spectra as also demonstrated in the other section of this chapter. In the spectra shown, the signal of H-1" was irradiated. With increasing mixing time, the individual protons of the glucose ring emerge in the spectrum and can therefore safely be assigned.

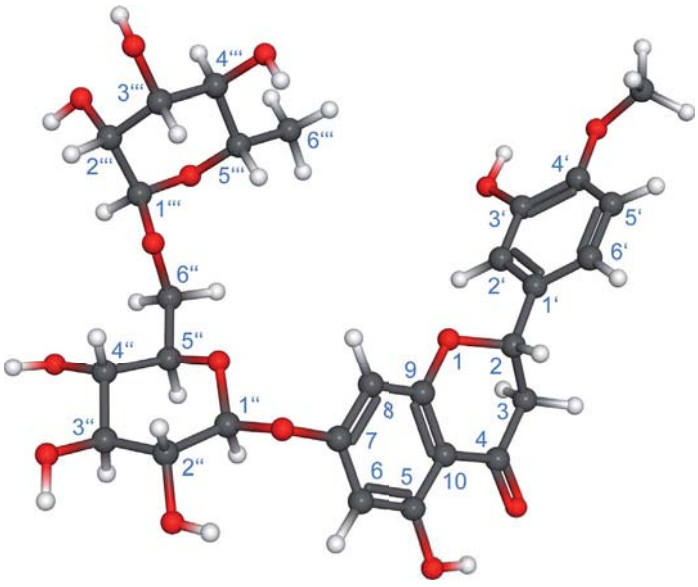


Fig. 4.4-22 Molecular model of hesperidin

^{13}C Signals δ / ppm	Type of Carbon	Assignment	Proton Signals δ / ppm, J / Hz
197.0	C_q	C-4	
165.1	C_q	C-7	
163.0	C_q	C-5	
162.5	C_q	C-9	
148.0	C_q	C-4'	
146.5	C_q	C-3'	
130.9	C_q	C-1'	
117.9	CH	C-6'	6.900
114.2	CH	C-2'	6.938
112.1	CH	C-5'	6.938
103.3	C_q	C-10	
100.6	CH	C-1'''	4.521
99.5	CH	C-1''	4.974
96.4	CH	C-6	6.141
95.5	CH	C-8	6.122
78.4	CH	C-2	5.5
76.3	CH	C-3''	3.268
75.5	CH	C-5''	3.537
73.0	CH	C-2''	3.22
72.1	CH	C-4'''	3.152
70.7	CH	C-3'''	3.432
70.3	CH	C-2'''	3.632
69.6	CH	C-4''	3.133
68.3	CH	C-5'''	3.4
66.0	CH_2	C-6''	3.799/3.426
55.7	CH_3	C-7'	3.774
42.1	CH_2	C-3	3.27/2.77
17.8	CH_3	C-6'''	1.085
		OH-5	12.01
		OH-3'	9.07
		OH-2''	5.39
		OH-4''	5.18
		OH-3''	5.16
		OH-4'''	4.66
		OH-2'''	4.58
		OH-3'''	4.45

Table 4.4-1 NMR data for hesperidin

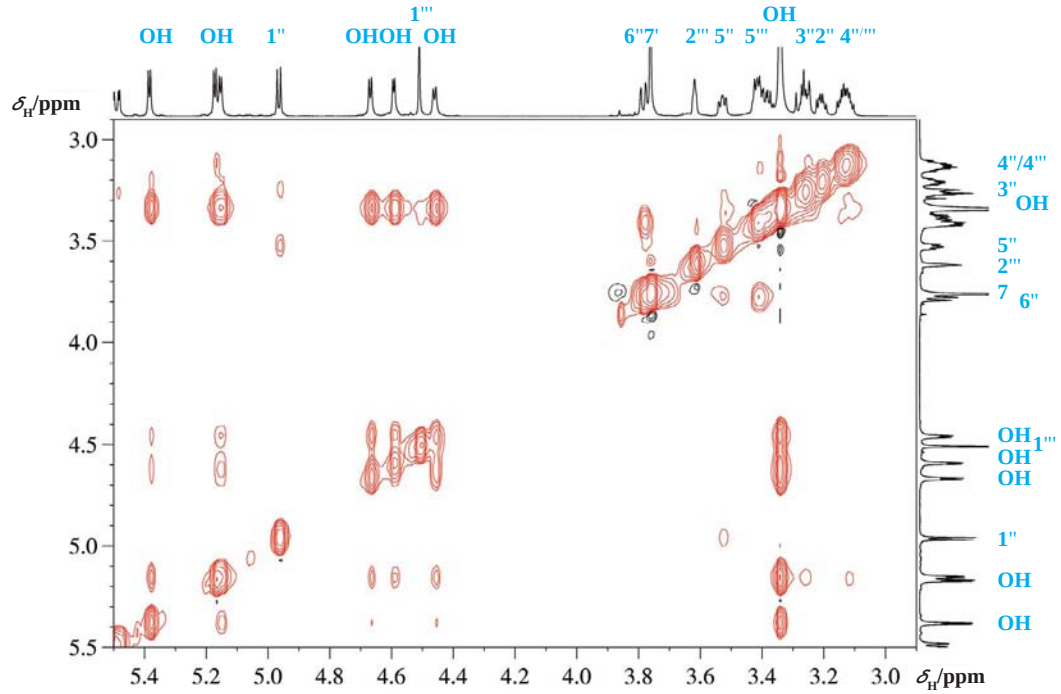


Fig. 4.4-23 NOESY spectrum

Since the spectrum was recorded in DMSO, all OH groups can be seen and identified via the COSY, the selective TOCSY and the NOESY spectra, which here behaves as an EXSY technique. In fact, one could construct an exchange matrix between all OH groups in addition with the residual water peak at 3.33 ppm. Note that due to the viscosity of the solvent, all NOESY correlations have the same sign as the diagonal. Of importance are the bis axial NOE connectivities between H-1" and H-3" or H-5".The diastereotopic methylene group C-6" has already been mentioned and, of course, the methyl group C-6"' is easily identified and helps to find H-5"'. The HMBC spectrum is also essential for carbohydrate assignments.

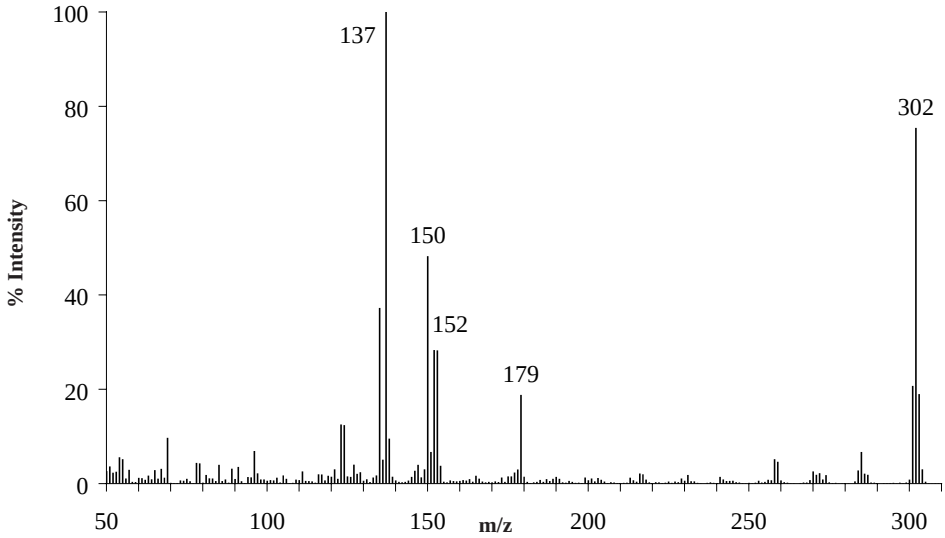
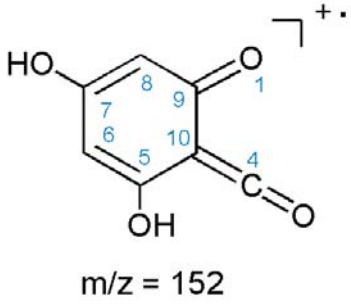


Fig. 4.4-24 Mass spectrum (EI)

Hesperidine is, due to its disaccharide component, a rather polar molecule and therefore it is difficult to obtain mass spectra with electron impact ionization. Nevertheless, since the aromatic part of the molecule is about the same size as the attached carbohydrate, one is able to obtain EI mass spectra displaying a cation of m/z = 302, which is the mass of the aglycone + 1 obtained after cleavage of the glycosidic bond with an additional hydrogen transfer. Flavonoid compounds often display a retro-Diels–Alder reaction eliminating a ketene fragment with m/z = 152, and this can be observed in the EI spectrum shown. However, it is much better to use electrospray ionization. This technique produces the quasi-molecular ion with an additional sodium ion $[M + Na]^+$ at m/z = 633.17940. As can be seen, the intensity of the isotope peak at m/z = 634.18157 corresponds to about 28 carbon atoms. Furthermore, the spectrum displays a signal at m/z = 655.16178, which is explained by $[M + 2Na^+ – H^+]$. The atomic composition of all these signals can be verified by computer analysis, since the spectra were recorded with a resolution of better than 2 ppm.



Scheme 4.4-7 Fragment of hesperidin after retro-Diels–Alder reaction

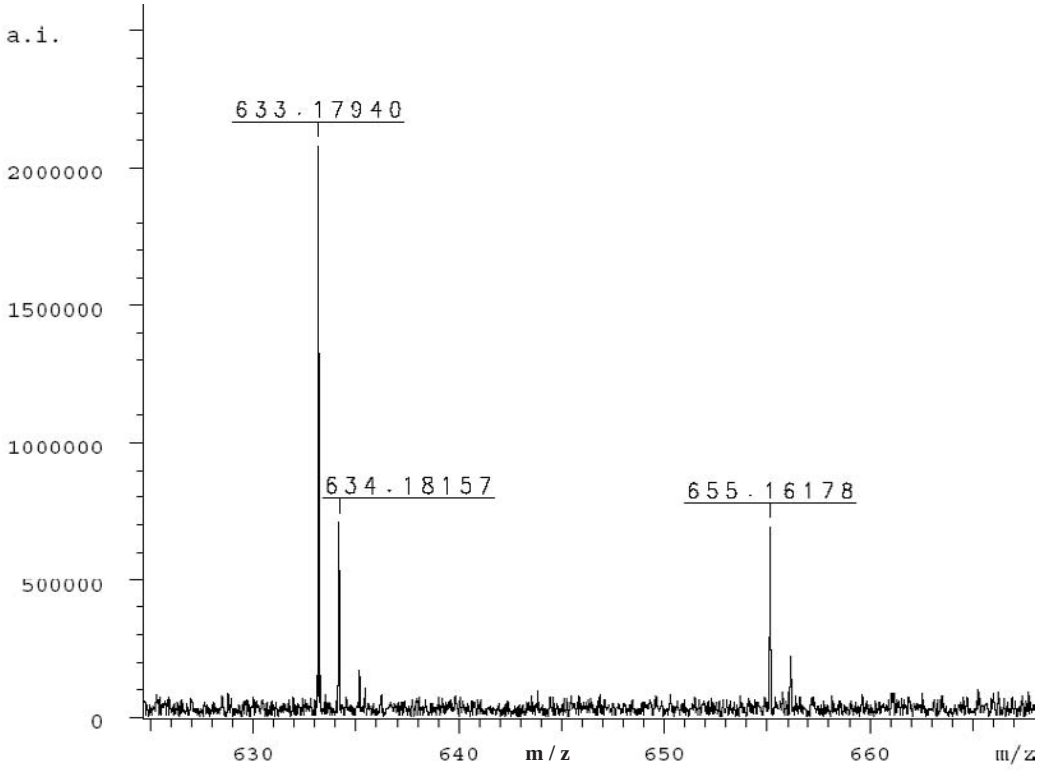


Fig. 4.4-25 Mass spectrum (ESI-FT/ICR)

5. Questions

A. Draw structures of naringine and neohesperidin with the help of their names given above and the formula of hesperidin.

B. The disaccharide rutinose mentioned above has the molecular formula $C_{12}H_{22}O_{10}$ and consists of the monosaccharides L-rhamnose und D-glucose. Is rutinose a reducing disaccharide or not?

C. Some related disaccharides have the molecular formula $C_{12}H_{22}O_{11}$, such as cellobiose, maltose, saccharose, lactose and trehalose. From which monosaccharides are these isomers made? How are they linked to each other?

D. Which of them are reducing disaccharides and which are not?

E. Find the two compounds that behave like diastereomers. Describe the structural difference between them.

F. Draw a reaction scheme for the ring-opening catalytic hydrogenation of neohesperidin to form NHDC. Explain why the heterocycle can be opened in this way.

G. How would one describe the UV transition at 286 nm?

H. In the APT ^{13}C NMR spectrum a small positive signal can be seen directly to the left of the negative DMSO signal. The signal does not belong to hesperidin. Explain.

I. How would one stereochemically assign the two protons H-6a" and H-6b"?

J. Aromatic carbon atoms bearing an oxygen typically resonate around 160 ppm; see, for example, C-7, C-9 and C-5. However, C-3' and C-4' show signals around 150 ppm. Explain.

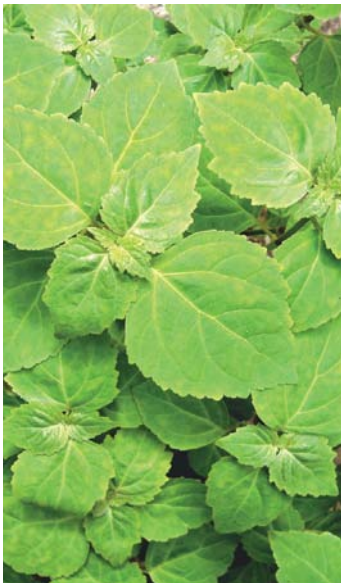
K. There is no COSY cross peak between H-1'" and H-2'" to be seen, but there is a weak NOESY cross peak. Explain.

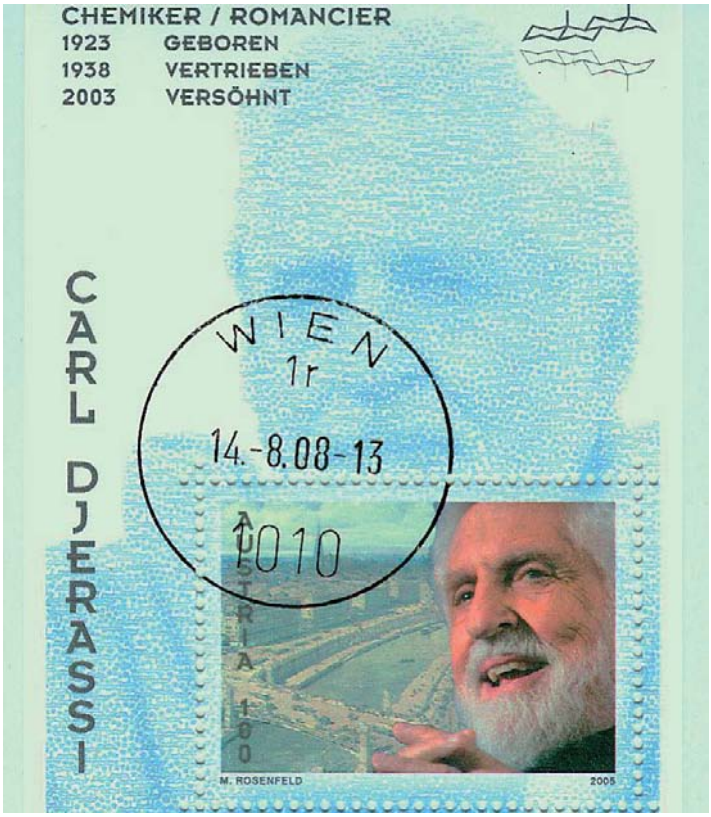
L. How would one assign the absolute stereochemistry at C-2?

M. Explain the signals in the EI mass spectrum at m/z = 179 and 150 and the base at peak m/z = 137.

6. Own Observations

Chapter 5 Terpenoids





Austrian stamp for Carl Djerassi, eminent terpenoid chemist

Terpenoids mark a rather wide field of chemistry. Many chemists have contributed to this field and four of them won the Nobel Prize:

1927

Heinrich Otto Wieland
(Germany, 1877 − 1957)

Germany, Munich University

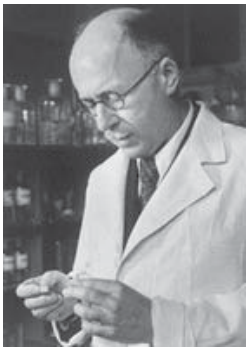
“for his investigations of the constitution of the bile acids and related substances”.

1928

Adolf Otto Reinhold Windaus
(Germany, 1876 − 1959)

Germany, Goettingen University

“for the services rendered through his research into the constitution of the sterols and their connection with the vitamins”.



1939

Leopold Ruzicka
(Vukovar, then Austria-Hungary, 1887 − 1976)

Switzerland, Eidgenössiche Technische Hochschule, (Federal Institute of Technology), Zürich

“for his work on polymethylenes and higher terpenes”.

1939

Adolf Friedrich Johann Butenandt
(Germany, 1903 − 1995)

Germany, Berlin University and Kaiser-Wilhelm-Institut (now Max-Planck-Institut) für Biochemie, Berlin-Dahlem

“for his work on sex hormones”.

5.1 Limonene

(4*R*)-(+)-1-Methyl-4-(1-methylethenyl)cyclohexene

from Brazilian sweet orange oil
Citrus sinensis L. (Rutaceae)

$C_{10}H_{16}$, MW 136.23

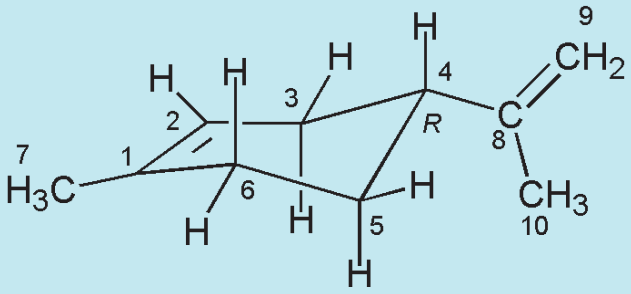
CAS RN 5989-27-5, BRN 2204754, 3587825, 4291143

Colourless liquid, bp 177−178°C, bp 42−43 °C (900 Pa)
$[\alpha]_D^{25}$ +114.0° (*c* 0.264 g/mL, ethanol)

Limonene is commercially available.

Synonymous names:
D-(+)-Limonene, (+)-α-Limonene, (+)-Dipentene, (*R*)-*p*-Mentha-1,8-diene

Level: easy



1. Background: A banal purpose engulfs most of it

Fig. 5.1-1 Sweet oranges, an attribute for sunslime during dark winter days

Essential oils are produced from both orange species (compare also Section 4.4 on hesperidin for botanic relations). However, the difference in the amounts isolated could not be greater. Whereas sweet orange oil from sweet oranges (*Citrus sinensis* L. Osbeck) is by far the most frequent essential oil manufactured (more than 10 000 t/year), bitter orange oil from bitter oranges (*Citrus aurantium* Risso) is a typical niche product (about 10 t/year only).

The essential oils arise in glands inside the peel of the oranges. (4*R*)-(+)-Limonene is formed by cyclization of geranyl diphosphate. It is the most frequent monoterpenoid made by plants. The essential oil can be obtained by cold pressing the peels of both sweet and bitter oranges. Alternatively, steam distillation is a possible way of isolation. However, as always in this procedure, the heat of the steam may cause chemical transformations of some special components. With a content of ca. 90% sweet orange oil is very rich in (4*R*)-(+)-limonene or D-(+)-limonene, respectively.

The monoterpene derivative (4*R*)-(+)-limonene described here is a cyclic alkadiene and responsible for the typical pleasant orange–lemon like citrus smell. In contrast, pure (4*S*)-(–)-limonene, the main constituent of the Silver Fir cone oil (*Abies alba*, syn. *A. pectinata* Mill.) has an odour more reminiscent of turpentine (compare also Section 5.2 on menthol for smell differences of enantiomers). Unusually, the racemic mixture of both enantiomers has its own name in this case: dipentene. This racemate can be obtained by heating one of the enantiomers, e.g. (4*R*)-(+)-limonene, to 300 °C. A natural source of dipentene is Siberian Fir needle oil. Dipentene is not only made on an industrial scale from the essential oil mentioned here but is also obtained from oil of turpentine and byproducts of camphor synthesis.

Media fert tristis sucos tardumque saporem
felicis mali, quo non praesentius ullum,
pocula si quando saevae infecere novercae,
miscueruntque herbas et non innoxia verba,
auxilium venit ac membris agit atra venena.
ipsa ingens arbos faciemque simillima lauro,
et, si non alium late iactaret odorem,
laurus erat: folia haud ullis labentia ventis,
flos ad prima tenax; animas et olentia Medi
ora fouent illo et senibus medicantur anhelis.

P. Vergilius Maro (70–19)
Georgica, II 126−131

(4*R*)-(+)-Limonene is used for the manufacture of food, beverages and cosmetics as a flavouring or fragrance additive. However, this is not the application that consumes most of the sweet orange oil. A rather banal purpose takes most of the limonene, whether as pure D-limonene in sweet orange oil or in the form of dipentene: the necessity to clean many technical and household goods from oil, fat, grease, tar or resin-like compounds.

Limonene has been found to be an exceptionally good solvent for all of these. Additionally, it is more biodegradable than cleaning agents based on mineral oil distillates such as cleaner's naphtha that consist mainly of alkanes. Furthermore, it is a biogenic solvent accessible from renewable sources. The dissolving power is high enough for it to act as a paint stripper on painted wood. It is also used as a diluter for varnishes. All such products have an acceptable smell for the consumer.

However, as always when using pure chemicals, their risks must be kept in mind. In Europe, pure D-limonene is classified as Xi (irritant) and N (dangerous for the environment) and so is dipentene. It may act as a skin irritant or even skin sensitizer, especially with permanent

application. Hence exposure of the unprotected skin to diluters or cleaning agents containing these compounds should be avoided e.g. by wearing protective gloves.

The chemical stability is limited. Limonene is susceptible both to acids and bases. The most common reaction when stored in an improper manner is autoxidation to the corresponding carvone enantiomer.

2. Literature

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[2] A. Chiralt, J. Martinez-Monzo, T. Chafer, P. Fito, "Limonene from citrus" *Functional Foods* **2002**, *2*, 169−187.

[3] M. Trytek, P. Kowalski, J. Fiedurek, "Presence, biological role and biotransformation of limonene" *Pol. Biotechnologia* **2003**, *2*, 206−217.

[4] A. F. Thomas, Y. Bessiere, "Limonene", *Natural Product Reports* **1989**, 291−309.

[5] R. Pétiaud, Y. B. Taärit, "An empirical determination of ^{13}C nuclear magnetic resonance spectra of terpene isomers generated by isoprene cyclodimerisation" *J. Chem. Soc., Perkin Trans. 2* **1980**, 1385−1390.

[6] M. Vincenti, S. R. Horning, R. G. Cooks "Energetics of retro-Diels−Alder fragmentation in limonene as characterized by surface-induced dissociation, and energy- and angle-resolved mass spectrometry" *Org. Mass Spectrom.* **1988**, *23*, 585−593.

Malus assyria, quam alii medicam vocant, venenis medetur. Folium eius est unedonis intercurrentibus spinis. Pomum ipsum alias non manditur, odore praecellit foliorum quoque, qui transit in vestes una conditus arcetque animalium noxia. Arbor ipsa omnibus horis pomifera est, aliis cadentibus, aliis maturescentibus, aliis vero subanscentibus. Temptavere gentes transferre ad sese propter remedii praestantiam fictilibus in vasis, dato per cavernas radicibus spiramento, qualiter omnia transitura longius seri aptissime transferrique meminisse conveniet, ut semel quaeque dicantur. Sed nisi apud medos et in perside nasci noluit. Haec est cuius grana parthorum preceres incoquere diximus esculentis commendandi halitus gratia. nec alia arbor laudatur in medis.

Plinius Maior (23−79)
Naturalis Historia Liber, XII

3. Isolation

3.1 Principle

A suitable source for the isolation of (*R*)-(+)-limonene is Brazilian sweet orange oil, obtained from squeezing sweet orange peel. It is a byproduct of making orange juice. (4*R*)-(+)-Limonene can be separated by two fractionated distillations in vacuo. The goal is to obtain a small fraction of nearly pure limonene rather than a complete separation of all limonene contained from other constituents. Furthermore, it was intended to use standard laboratory glassware skilfully in this case of an already highly enriched crude material instead of a sophisticated distillation equipment. Although limonene is thermally stable enough to be distilled at ambient pressure, it is recommended to do the fractionation in vacuo.

Fig. 5.1-2 Lemon tree as sold in a supermarket

3.2 Method

Brazilian sweet orange oil (36 g) containing 90% of (*R*)-(+)-limonene is subjected to fractional distillation in vacuo at 1000 Pa in the stream of an electronically regulated heat gun with an outlet temperature of 120 °C. A 10 cm Vigreux column is used and the distillation is run rather

Fig. 5.1-3 An assortment of five different citrus fruits all containing limonene

slowly. The refractive index of the orange coloured starting material is n_D 1.4714 at 24 °C. Two cuts are made the mass of which is 27 g in total (see table below). The distillation is then interrupted for safety reasons although no increase in the boiling point is observable. The remaining material has n_D 1.4745 at 24 °C and a mass of 9 g. The two fractions obtained are clear and colourless with an odour not as heavy in its citrus fruit note as that of the starting oil.

Fraction	bp at 1000 Pa (°C)	Amount (g)	n_D at 24 °C	Use of
I	25–44	4.0	1.4708	Discarded
II	40–44	23.0	1.4708	For redistillation

Table 51-1 Results of raw distillation

Estimation of the purity of fraction II according to ^{1}H NMR data: ca. 98.5%.

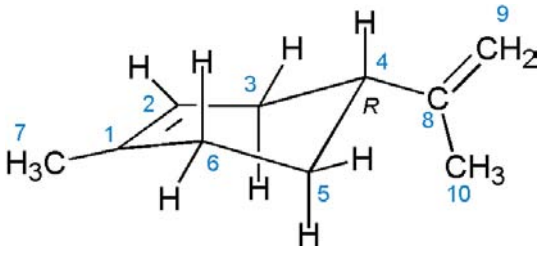
3.3 Purification

A 17.0 g amount of fraction II from above is subjected to a second very slow distillation with the same equipment except for a longer Vigreux column (30 cm).

Fraction	bp at 900 Pa (°C)	Amount (g)	n_D at 24 °C	Use of
I	42–43	1.4	1.4696	NMR analysis
II	42–43	14.8	1.4702	Stored
III	43–44	0.8	1.4706	NMR analysis

Table 51-2 Results of fine distillation

Estimation of the purity of all fractions by ^{1}H NMR shows that fraction II was the richest in (4*R*)-(+)-limonene: >99% $[\alpha]_D^{25}$ +114.0° (*c* 0.264 g/mL, ethanol).



Scheme 51-1

De las flores lanzó por el claro de luna,
de un aroma del amor exasperado,
empapado en la fragancia,
amarillez mandilada del árbol de limón,
y de sus limones del plantarium descendidos a la tierra
Producción blanda!
Las costas, los mercados brillaron intensamente
con la luz, con oro sin refinar;
abrimos dos mitades de un milagro,
ácido congelado goteado de los hemisferios de una estrella,
el licor más intenso de la naturaleza,
único, vivo, concentrado,
nacida del limón fresco,
fresco, de su casa fragante,
de su ácido, simetría secreta.

Cuchillos
rebanó una catedral pequeña en el limón,
el apse encubierto,
cristal manchado ácido abierto,
revelado, topaz exudado gotas,
altares, arquitectura fresca.

Así pues, cuando usted lleva a cabo el hemisferio
de un limón del corte sobre su placa,
usted derrama un universo del oro,
un cubilete amarillo de milagros,
una entrerrosca fragante del pecho de la tierra,
un rayo de la luz que fue hecha fruta,
el fuego minucioso de unplaneta.

Pablo Neruda (1904–1973)
Nobel Prize for Literature, 1971
Oda al Limón

Fig. 5.1-4
Essential oils, whether obtained from raw materials by cold pressing or steam distillation, belong to the basic ingredients of perfumes.
Photograph taken at *Habana 1791*, the oldest perfumery in Havana.

4. Spectra and Comments

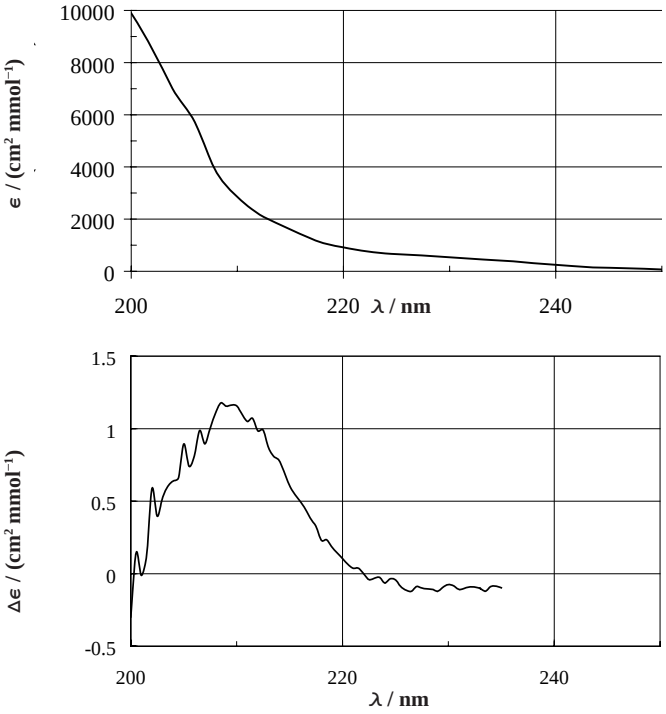


Fig. 5.1-5 UV and CD spectra in ethanol

The UV spectrum displays a single absorption band with a shoulder at about 207 nm due to the two isolated double bonds. No vibrational fine structure is to be seen. The CD band of this absorption is positive, but very weak.

Quand le soleil se couche, on respire au bord des golfes le parfum des citronniers; puis, le soir, sur la terrasse des villas, seuls et les doigts confondus, on regarde les étoiles en faisant des projets. Il lui semblait que certains lieux sur la terre devaient produire du bonheur, comme une plante particulière au sol et qui pousse mal tout autre part.

Gustave Flaubert (1821−1880)
Madame Bovary, Chapter 7.

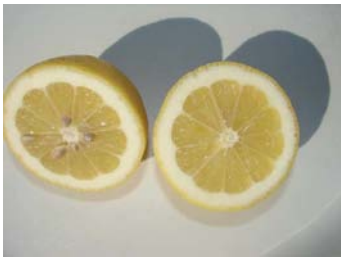


Fig. 5.1-6 Slices of lemon, ready for a cocktail

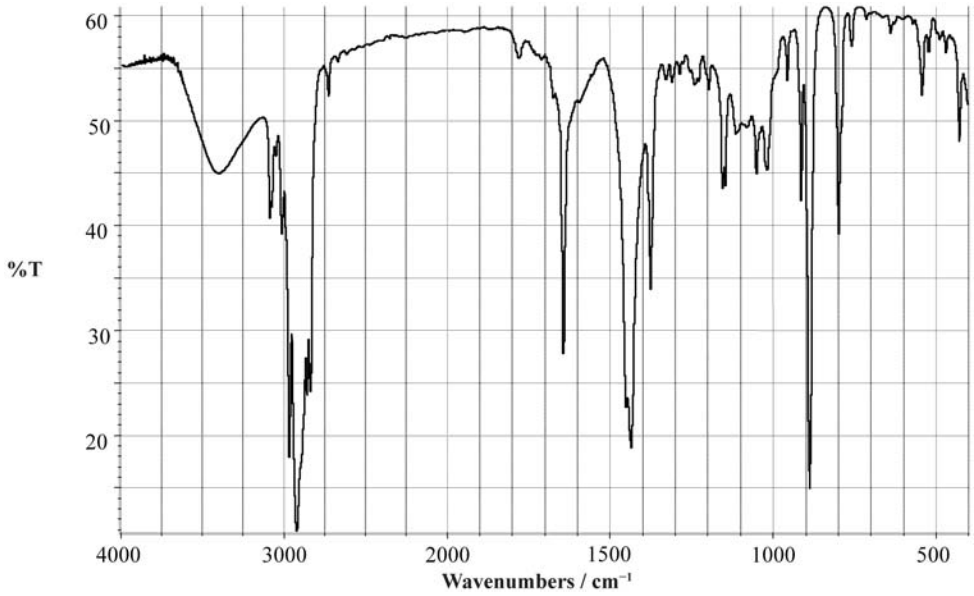


Fig. 5.1-7 IR spectrum as film

The IR spectrum shows CH vibration bands for both sp^2 and sp^3 hybridized CH fragments. The frequency of the small sharp peak at 3100 cm^{-1} is indicative of a terminal $=CH_2$ group. In addition, a sharp C=C vibration can be seen at 1630 cm^{-1}.

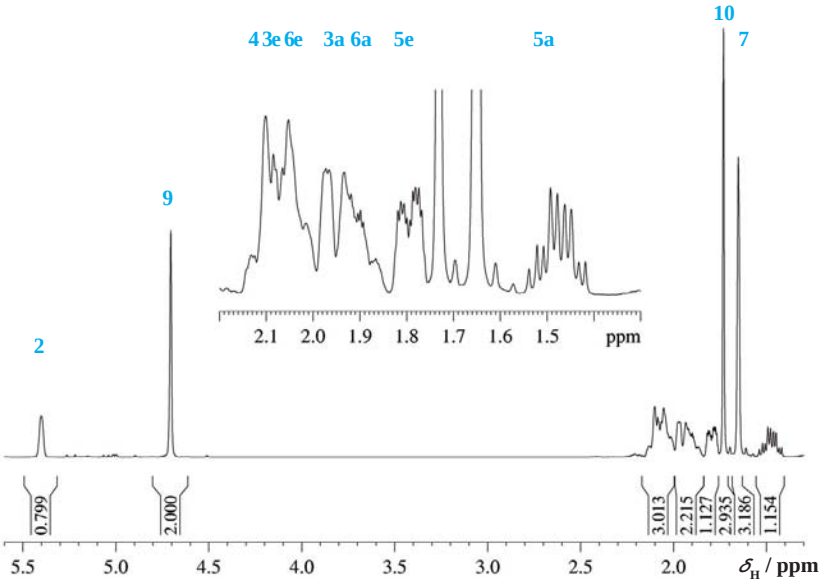


Fig. 5.1-10 ^1H NMR spectrum at 400 MHz in $CDCl_3$

The discussion of the ^1H NMR spectrum starts with the observation of the two olefinic signals with an intensity ratio of 1:2, indicating that even at 400 MHz the signals of the two olefinic protons at C-9 are not separated from each other. The two methyl group signals are clearly separated, although they are attached to a similar double bond. The other proton signals are difficult to assign using only the ^1H spectral information.

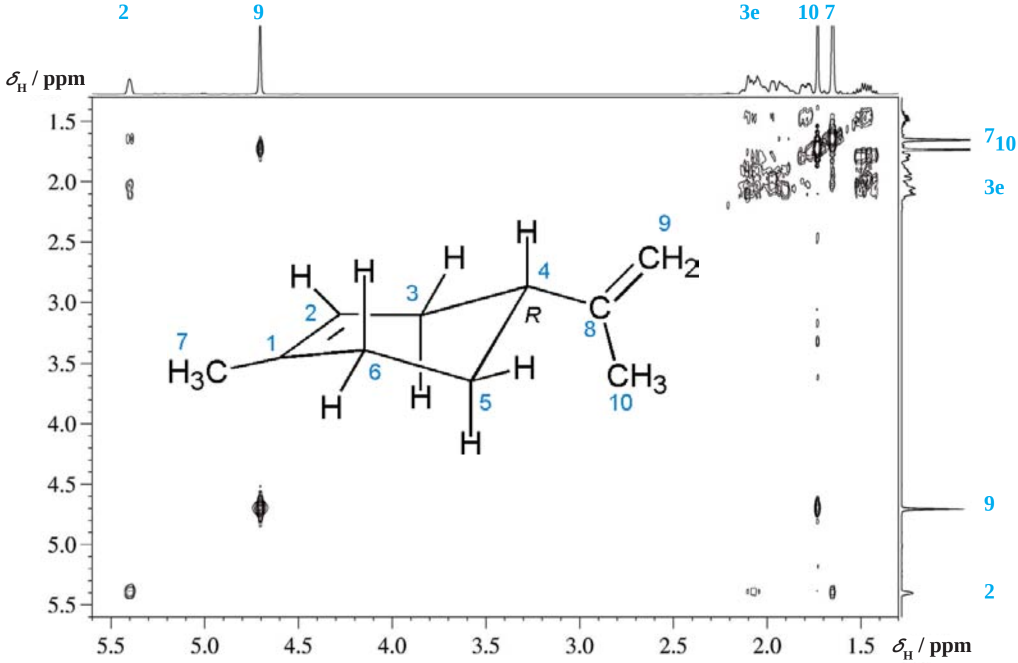


Fig. 5.1-9 COSY spectrum

The individual assignment of the two methyl groups can be easily extracted from the COSY spectrum, where both methyl group signals show cross peaks via an allylic coupling over four bonds to their respective olefinic protons H-2 and H-9. A cross peak between the olefinic proton H-2 and the allylic neighbours H-3 assigns their resonance position.

Fig. 5.1-10 Leaves of a sweet orange tree are in a continous change without shedding all foliage at once.

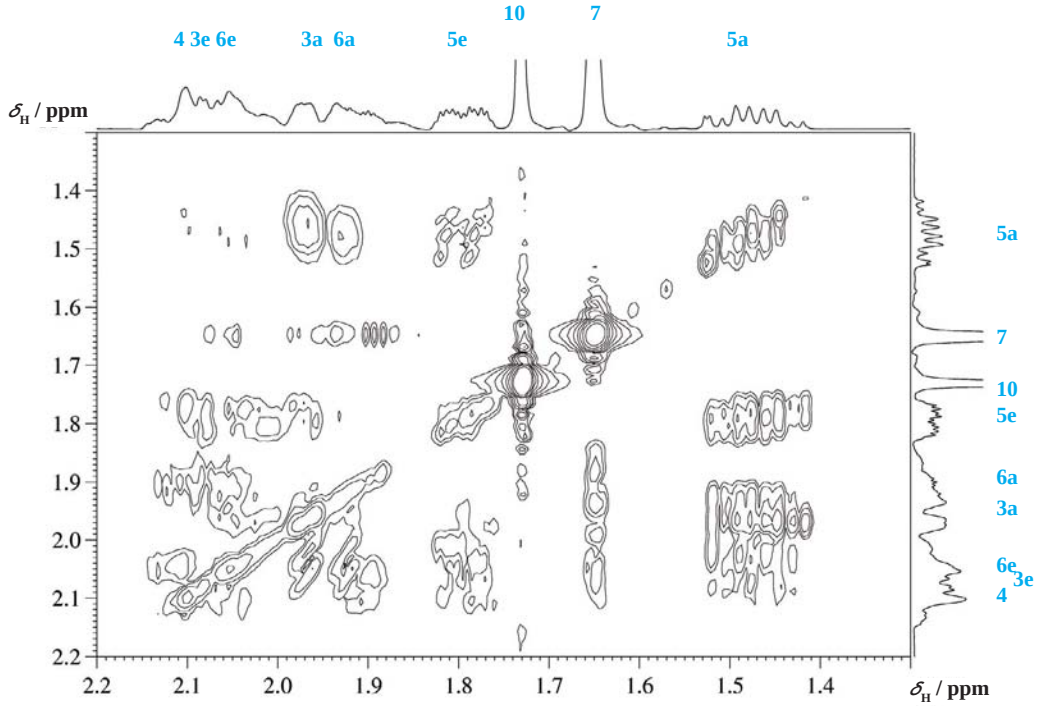
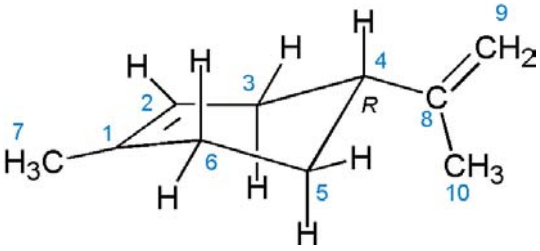


Fig. 5.1-11 Expansion of the COSY spectrum

The expansion of the COSY spectrum in the aliphatic part is at first sight discouraging and we will wait to use this information until further assignments from the HSQC and the NOESY spectra are available.



Scheme 5.1-2

Fig. 5.1-12 Plantation of sweet oranges (*Citrus sinensis*) in Valle de Vinales, Cuba

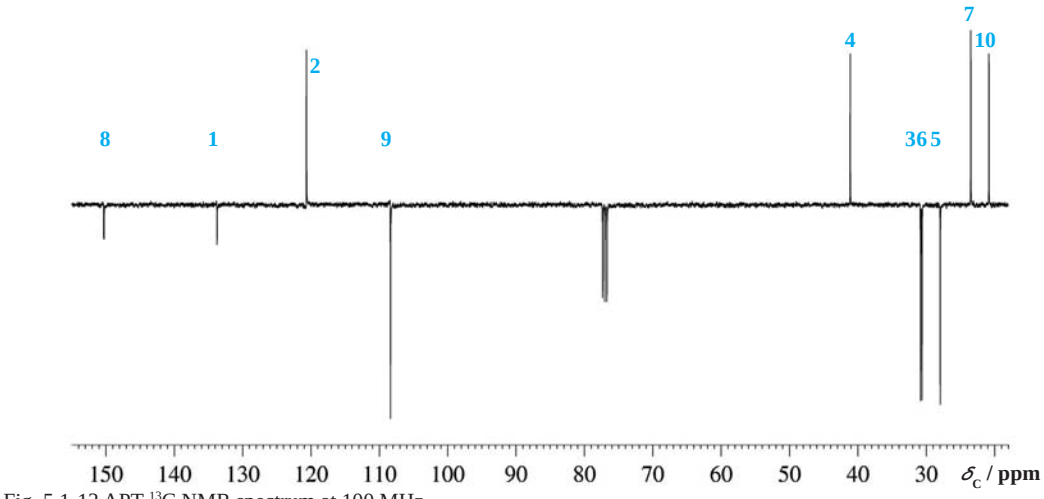


Fig. 5.1-13 APT ^{13}C NMR spectrum at 100 MHz

The signals of the two proton-bearing olefinic carbon atoms C-2 and C-9 are immediatedly assigned due to their sign in the APT spectrum. The safe assignment of the two corresponding quaternary olefinic carbon atoms C-1 and C-8 can be obtained by using shift increments or can be assured from the HMBC spectrum. Two of the methylene carbon atoms resonate very close together at 30.7 ppm; one further methylene carbon atom signal appears at 28 ppm. Finally, the positive signal in the APT spectrum at 41 ppm must be from C-4, being the only aliphatic methine carbon atom in the molecule. With the help of the HSQC spectrum, therefore, H-4 is easily identified.

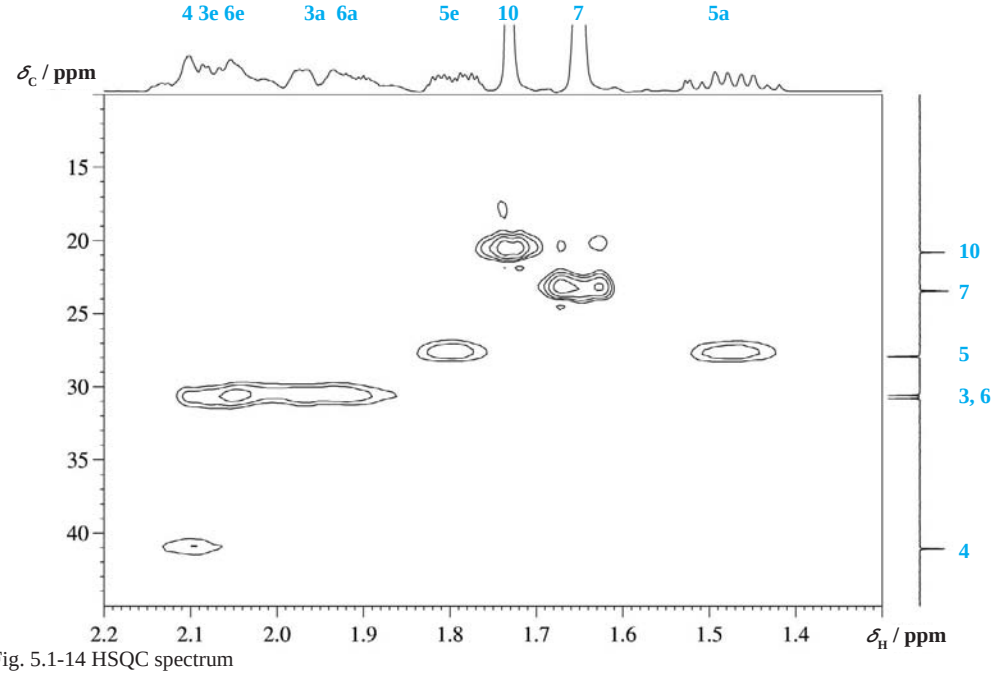


Fig. 5.1-14 HSQC spectrum

The two allylic methylene groups C-3 and C-6 show two very closely resonating ^{13}C signals and their proton signals also overlap. The most aliphatic methylene group C-5 at 28 ppm bears two diastereotopic protons at 1.8 and 1.47 ppm. The correlation peaks for the two methyl groups indicate that the ^{13}C chemical shift order in this molecule is reversed with respect to the proton chemical shifts.

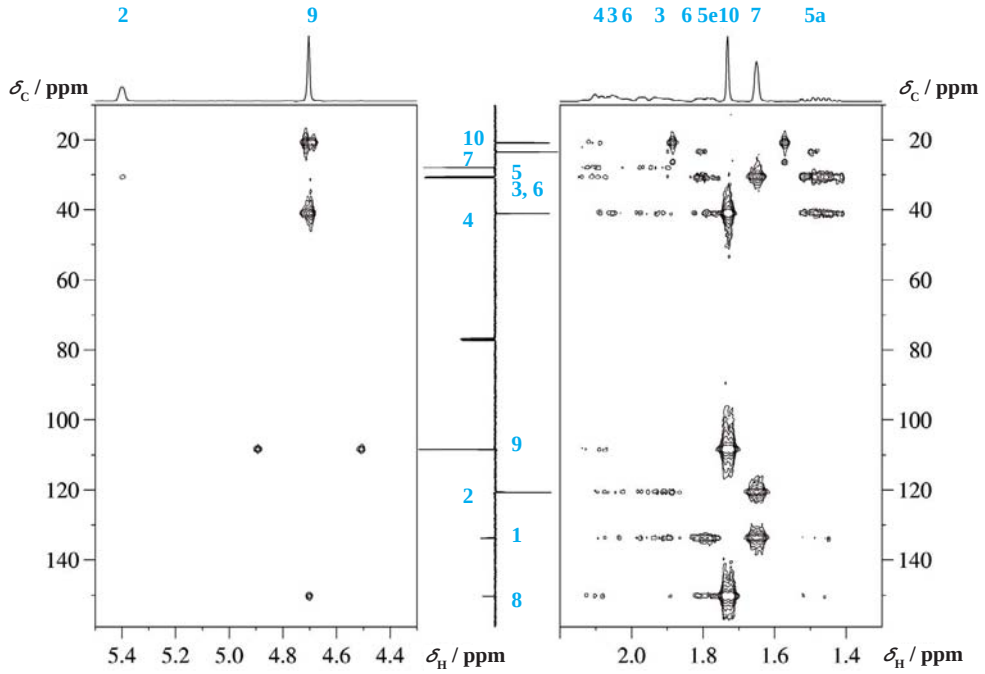


Fig. 5.1-15 HMBC spectrum

The two expansions of the HMBC spectrum first confirm the assignment of the quaternary olefinic carbon atoms C-1 and C-8, since H-9 shows a correlation signal to the carbon atom at 150.3 ppm, whereas H-7 shows a weak connection to the carbon signal at 133.7 ppm. H-9, of course, shows three bond correlations to C-4 and C-10. Both H-5 show a two-bond correlation to C-4; very important is the assignment's confirmation due to the correlation of one of the H-5 signals at 1.8 ppm with C-1.

I descended a little on the side of that delicious vale, surveying it with a secret kind of pleasure, though mixed with my other afflicting thoughts, to think that this was all my own; and I was king and lord of all this country indefeasibly, and had a right of possession; and, if I could convey it, I might have it in inheritance as completely as any lord of a manor in England. I saw here abundance of cocoa trees, orange, and lemon, and citron trees; but all wild, and very few bearing any fruit, at least not then. However, the green limes that I gathered were not only pleasant to eat, but very wholesome; and I mixed their juice afterwards with water, which made it very wholesome, and very cool and refreshing.

Daniel Defoe (1659−1731)
Robinson Crusoe, July 4th

Fig. 5.1-16 Mandarin tree in the Brooklyn Botanic Garden

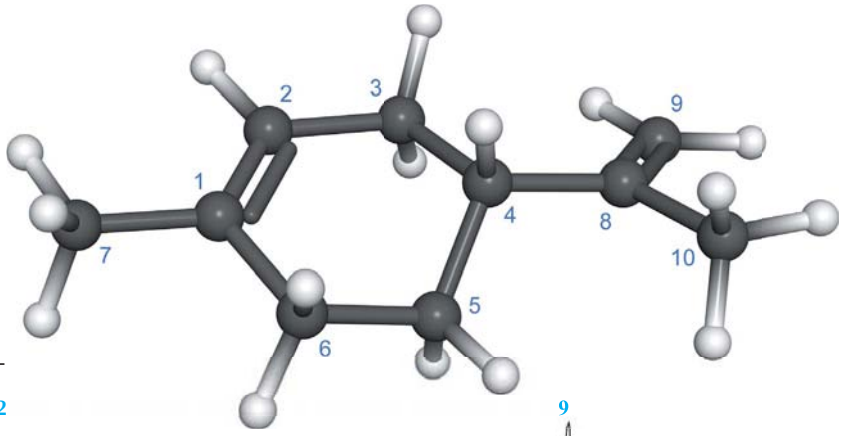


Fig. 5.1-17 Molecular model of limonene

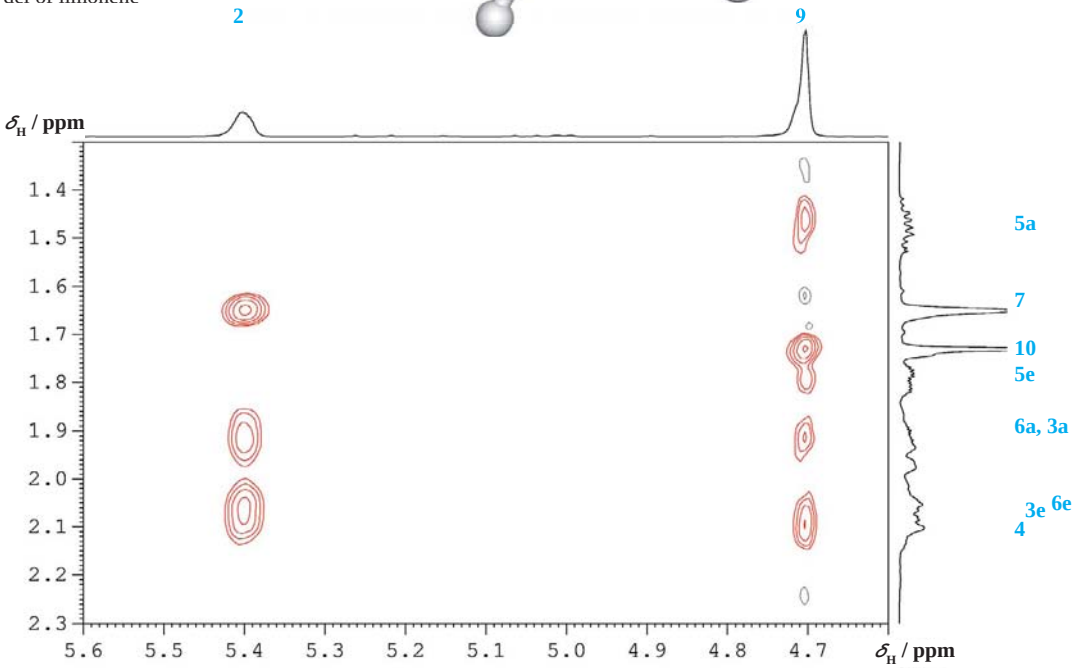


Fig. 5.1-18 NOESY spectrum

Having secured the positional assignment with the help of COSY, HSQC and HMBC, we can try a stereochemical assignment of the diastereotopic protons in the methylene groups. The first expansion of the NOESY spectrum corroborates the assignment for H-3 already made from the COSY spectrum, as the olefinic proton H-2 clearly shows NOE cross peaks to this diastereotopic methylene group. In addition, H-2 displays a cross peak to its vicinal methyl group H-7. The olefinic protons H-9 display five NOE cross peaks, one of which is, of course, to the methyl group H-10. One has the same frequency as the cross peak displayed by H-2 and this assigns the resonance at 1.9 ppm to one of the H-3 protons. There are a strong and a weaker cross peak to the signals at 1.47 and 1.8 ppm and this confirms the assignment of both H-5. Looking back at the COSY spectrum shows that these two signals are strongly coupled to each other. A relative assignment is reached if one looks at the large spin splitting of H-5 at 1.47 ppm. This must be the axial proton due to one geminal and two large axial−axial couplings. As this axial 5a proton shows in the two NOE expansions cross peaks to both H-9 and H-10, one can assume that the isopropene group is essentially freely rotating. An NOE cross peak from H-5a to the H-3 signal at 1.9 ppm can be seen, which assigns this one as axial also.

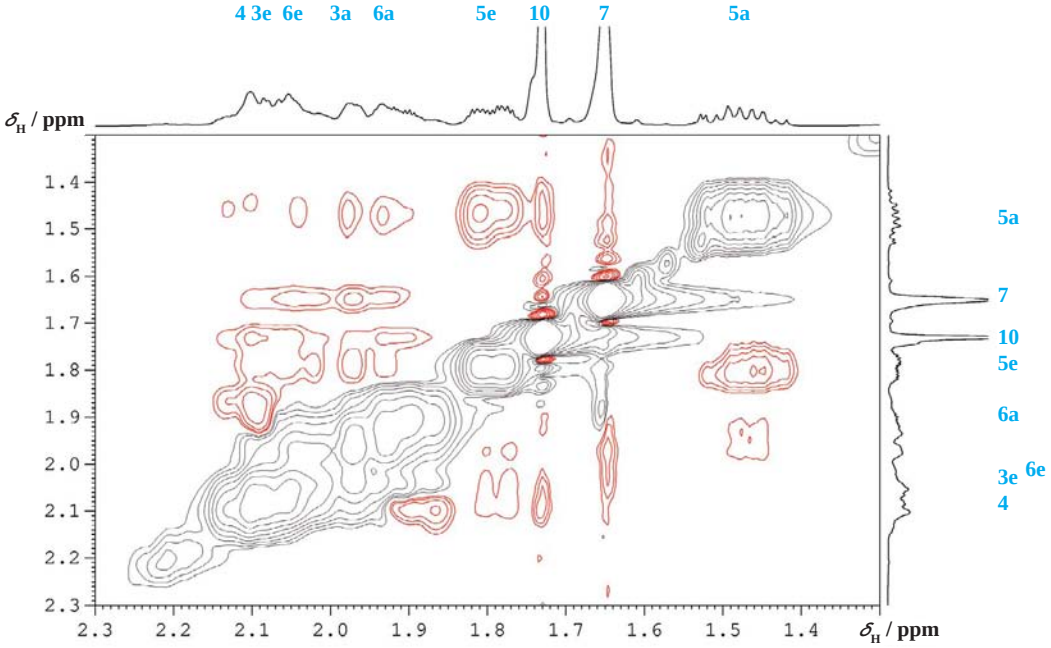
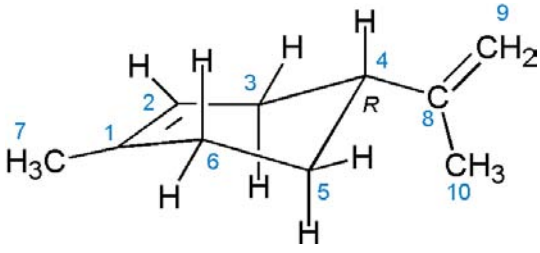


Fig. 5.1-19 Expansion of the NOESY spectrum in the aliphatic region

Fig. 5.1-20 Ripe lime fruits still have green peel



Scheme 5.1-3

Limey is an old American and Canadian slang nickname for the British, originally referring to British sailors. The term is believed to derive from lime-juicer, referring to the Royal Navy and Merchant Navy practice of supplying lime juice to British sailors to prevent scurvy in the 19th century. The term is derogatory in the sense that the British would be allegedly more preoccupied with the savings of limes over lemons which were traditionally used to prevent scurvy. The term is thought to have originated in the Caribbean in the 1880s.

Wikipedia

^{13}C Signals δ / ppm	Type of Carbon	Assign-ment	Proton signals δ / ppm
150.3	C_q	C-8	
133.7	C_q	C-1	
120.7	CH	C-2	5.4
108.4	CH_2	C-9	4.7
41.1	CH	C-4	2.1
30.8	CH_2	C-3	H-3e: 2.1; H-3a: 1.95
30.6	CH_2	C-6	H-6e: 2.1; H-6a: 1.95
27.9	CH_2	C-5	H-5e: 1.80; H-5a: 1.47
23.5	CH_3	C-7	1.65
20.8	CH_3	C-10	1.73

Table 5.1-3 NMR data for limonene

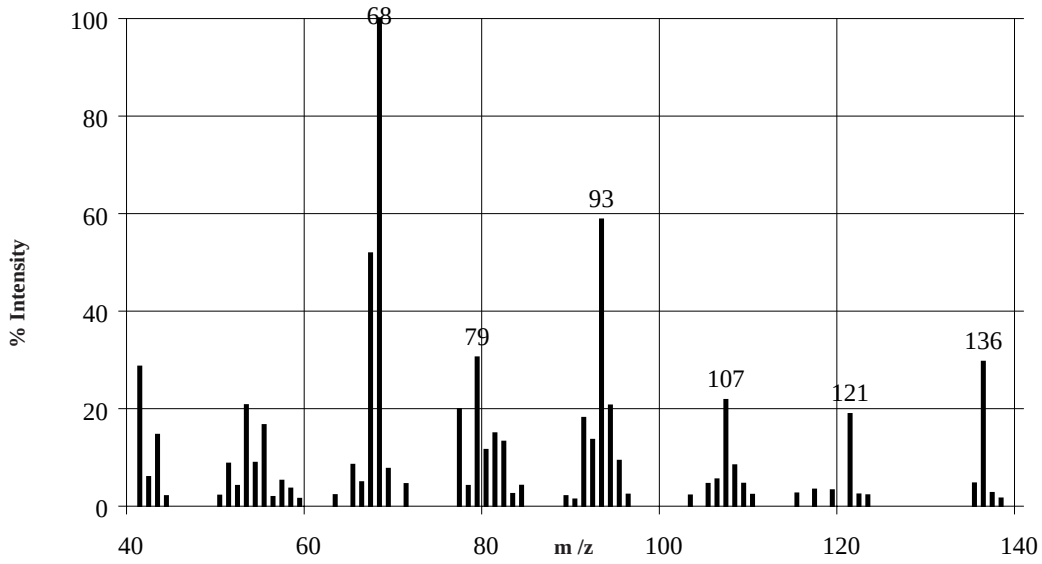
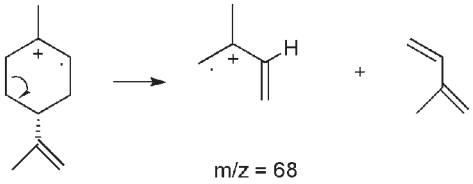


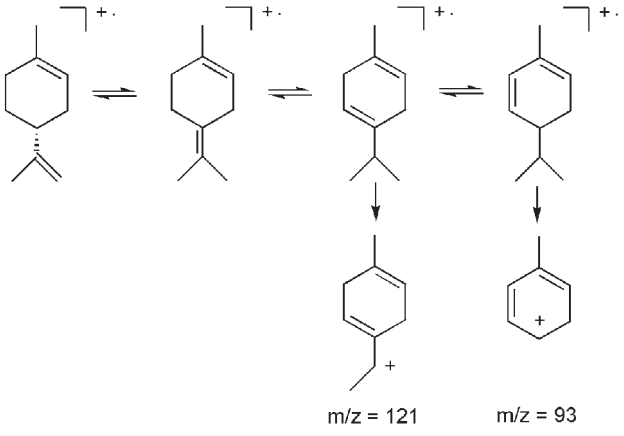
Fig. 5.1-21 Mass spectrum (EI)

The base peak of the electron ionization mass spectrum has exactly half of the molecular mass, indicating that the molecule is split into two parts with five carbon atoms each. A retro-Diels–Alder reaction can be assumed for this process:



Scheme 5.1-4 Fragmentation of limonene

The molecular ions of cycloalkenes can undergo several shifts of the ionized double bond as indicated below. From the last two structures, fragmentation of a methyl group or an isopropyl group becomes feasible:



Scheme 5.1-5 Further fragmentation of limonene

5. Questions

A. Limonene is a terpene consisting of two isoprene units. Mark these two building blocks in the limonene formula.

B. Suggest a reason for the good solubility properties of the racemic limonene dipentene. Compare the dissolution properties of alkanes, cycloalkanes, alkenes and aromatic compounds such as benzene and toluene.

C. Limonene, as a monoterpene, is in principle made up of two isoprene units. Give the names of two natural isoprene polymers.

D. Make a suggestion why dipentene used for cleaning purposes is more easily biodegradable than mineral oil distillates.

E. Both pure limonenes and the racemate are listed in pure form as irritant (Xi). Why is it wise to wear protective gloves when working with dipentene-containing cleaning agents? Make an assumption of what could happen on contact of dipentene with the human skin.

F. Both limonenes belong to the *p*-menthadiene class of monoterpene derivatives. Among them, further isomeric natural products are known. In addition to the chiral α-phellandrenes and β-phellandrenes, both existing as pairs of enantiomers which are not the subject of this question, there are four achiral isomers α-terpinene, β-terpinene, γ-terpinene and terpinolene. Draw the structures of these four isomers, make a suggestion which of them would be the thermodynamically most stable one and back up your opinion.

G. To which vibration would one assign the IR band at 1450 cm^{-1}?

H. The proton chemical shifts of the methyl groups C-7 and C-10 decrease in the order C-10 > C-7, but the corresponding carbon chemical shifts in the order C-7 > C-10. Suggest a reason.

I. Using a molecular mechanics program, calculate the lowest energy position for the isopropene group.

J. Suggest structures for the ions at *m*/*z* = 107 and 79.

6. Own Observations

5.2 Menthol

(1*R*,2*S*,5*R*)-5-Methyl-2-(1-methylethyl)cyclohexanol

From the essential oil of Japanese peppermint
Mentha arvensis var. *piperascens* L. (Lamiaceae)

$C_{10}H_{20}O$, MW 156.26

CAS RN 2216-51-5, BRN 1902293

Colourless crystals,
mp 40–42 °C, $[\alpha]_D^{25}$ –50.7°
(*c* 0.050 g/mL, ethanol)

Menthol is commercially available.

Synonymous names:
Menthyl alcohol, *p*-Menthan-3-ol

Level: easy

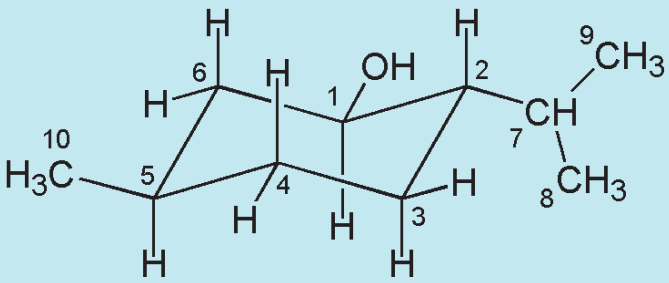


Fig. 5.2-1 Peppermint tea

Fig. 5.2-2 Peppermint liquor

Accubuere dei, mensam succincta tremensque
ponit anus, mensae sed erat pes tertius inpar:
testa parem fecit; quae postquam subdita clivum
sustulit, aequatam mentae tersere virentes.

P. Ovidius Naso (43–17)
Metamorphoses VIII, 660
(Philemon and Baucis)

1. Background: 12 000 tons per year – and enantiopure

The name *Mentha* for the genus descends from a Greek myth. According to this, the nymph *Minthe*, the daughter of Kokytes, was bewitched into a plant by Proserpina, a goddess of underworld.

Likely, you will have the association of peppermint (*Mentha piperita* L.) when reading of menthol and may regard this plant as a suitable material for the isolation procedure. However, peppermint oil is used in industry in its entirety due to its marvellous flavour and it is not as rich in menthol content as other oils. Instead, Japanese peppermint oil (obtained in our case from a supplier in Vietnam) has a higher menthol content and is the typical source for (–)-menthol isolation on an industrial scale of more than 8000 tons/year, mainly done in India and China. Another 4000 tons are made synthetically as the pure (–)-enantiomer, as will be discussed below. With this amount, (–)-menthol is at position 2 in the market of flavourings. Natural menthol as a crystalline compound has been known in Japan since ancient times, 2000 years ago. In Europe, it was first isolated in 1771 by H. D. Gaudius using steam distillation of peppermint, and it took him a lot of effort to manifest and accept that what he found was only similar to camphor and a novel compound.

Menthol, a monocyclic monoterpene secondary alcohol, is biosynthesized from limonene by stepwise oxidation and two hydrogenations. Hence, the intermediates of this biosynthetic pathway, i.e. piperitenone, piperitone, its isomer pulegone, and menthone, are always companions in any (–)-menthol-producing plants. The essential oil may be obtained from above-ground plant material by classical steam distillation or by a modern destraction process with supercritical carbon dioxide. It is a rather viscous, destraction colourless liquid with a rich (–) menthol content (up to 90%).

As the consumption of menthol exceeded the natural resources, a stimulus for a suitable synthetic approach on a technical scale arose in the 20th century. The first demand for such an approach was that it had to supply only the “natural” (–)-enantiomer of menthol because its organoleptic properties (cool, fresh, minty, sweet) are vastly superior to those of (+)-menthol (weaker cool and minty, but musty, phenolic and bitter). The odour threshold for (–)-menthol is 1 ppm. The solubility in water is slight (below 1 g/L), but this is sufficient for all applications. The different odour of the two enantiomers is very noticeable and occurs with many enantiomers [2]. The molecular reason is that on contact with the chiral smell receptors of the body, diastereomorphic associations are formed which may cause different rather than the same sensations.

Today, the (–)-enantiomer is produced on the thousands of tons scale according to two methods, which use completely different starting materials and different ways to obtain the natural enantiomer [3].

The first process, called the Haarmann and Reimer process, was established in 1973 in Germany. Ultimately, (–)-menthol is gained by means of a resolution of racemic (±)-menthol. Starting from *m*-cresol, first thymol is made by alkylation with propene. Catalytic hydrogenation

leads to a mixture of all four possible pairs of diastereomers with the constitution of 5-methyl-2-(1-methylethyl)cyclohexanol, i.e. each to a pair of enantiomeric menthols, isomenthols, neomenthols and neoisomenthols. Astonishingly, although their molecular mass is equal, the boiling points are different enough to allow a separation of (±)-menthol isomers by distillation. The crucial step of removing the (–)-enantiomer from the racemate is then resolved by esterification with benzoic acid and fractional crystallization of the (–)-menthyl benzoate after adding the corresponding seedling crystals. This means that resolution is done still on the level of enantiomeric esters, without using diastereomeric differentiation by a chiral auxiliary. Finally, (–)-menthyl benzoate is hydrolysed to supply the natural menthol isomer.

The second process is called the Takasago process and was established in 1984 in Japan, and marks a milestone of an industrial process which makes use of an enantioselective catalysis. This allows the resolution of a racemate to be avoided. The process is based on isoprene from steam crackers, which is dimerized under alkaline metal catalysis to yield myrcene as an open-chain monoterpene. It is then transformed with diethylamine into achiral *N*,*N*-diethylnerylamine. In the next and key step, the first chiral centre is introduced. It consists in an enantioselective double bond shift catalysed by a chiral rhodium catalyst carrying a Noyori (*S*)-BINAP ligand. This isomerization may appear as a comparatively small structural change. However, the opposite is true. First, it gives rise to (*R*)-citronellal enamine, enantioselectively. Then, it contains in a hidden manner all prerequisites which are necessary for the correct installation of the second and third chiral centres required for the final (–)-menthol. The enamine enantiomer can be hydrolysed to (*R*)-citronellal, which by a carbonyl ene reaction is diastereoselectively cyclized to form one isopulegone enantiomer as a direct precursor for (–)-menthol accessible by a final catalytic hydrogenation of the remaining double bond. If a masterpiece for enantioselective catalysis is wanted, this is one, and at what a scale! In organic chemistry, pure menthol enantiomers can be used as chiral auxiliaries for stereoselective synthesis and also as a means for the resolution of racemic carboxylic acids.

In addition to its attractive smell, natural menthol has numerous external and internal medical applications, some of which everbody has already made use of.

A typical feature of menthol is, that on rubbing it on the skin in the form of a cream, it causes a pleasant sensation of cold. This is used for local anaesthetic and analgesic effects, e.g. to relieve pain from muscle cramps or headaches, even from migraine headache. Interestingly, such cooling sensations arise only as a feeling, and not as a real physical effect. Menthol, e.g. when used in a sunburn spray, does not really reduce the temperature of skin, but only causes the feeling. It is able to chemically trigger receptors in the skin that produce the impression “it is cold”. An analogous form of the hot type of sensations is capsaicin, the pungent factor of chilli peppers. It stimulates heat sensors in the tongue or skin without a real rise in temperature. Furthermore, an

Here’s flowers for you;
Hot lavender, mints, savoury, marjoram:
The marigold, that goes to bed wi’ the sun
And with him rises weeping: these are flowers
Of middle summer, and I think they are given
To men of middle age. You’re very welcome.

William Shakespeare (1564–1616)
The Winter’s Tale, IV, 3

Fig. 5.2-3 Medical creams containing menthol

Fig. 5.2-4 Cigarettes with added menthol

Fig. 5.2-5 Blooming peppermint plants (*Mentha piperita* L.)

Idque in Mentha Piperitide ipso experimento didici. Recentem plantam, anno superiore, distillatione subjeci: aqua stillatitia erat valde aromatica; olei aetherei multum; concretae Camphorae nihil. Separatum utcunque ab aqua oleum, & aquam primam saturiorem, seorsim singula, suis condita vitris, loco frigido reposita, toto anno servavi sperans, Camphorae quid in alterutro esse concreturum. At spes fefellit: nihil crystalline apparuit. Cuius quidem rei vix aliam invenio rationem, nisi quod viridem plantam adhibuerim. Ex quo fortassis etiam factum est, ut nemo Chemicorum, quod sciam, Campforae meminerit ex hac Menthae specie a se destillando acquisitae; quamvis & aqua illius & spiritus, usu Medicinali, multis locis, inde ab aliquot annis valde frequentetur.

H. D. Gaudius
Adversariorum varii argumenti
Liber unus, Caput VII, Leiden 1771
(Original found in the University Library, Leipzig)

ethanolic solution of menthol has an antiseptic effect, and it acts also as an insect repellent. Menthol preparations with up to 1% content act as an antipruritic and reduce itching, whereas at higher concentrations just the counter-irritant effect sets in.

Menthol effects the relief of minor sore throat and is therefore a constituent of cough pills and balms. Decongestants for chest and nasal sinuses contain menthol (see photograph of Vick VapoRub).

In internal application, menthol preparations act against spasms of the stomach and intestines and stimulate the production of bile. Additionally, the blood pressure is raised.

However, most of the menthol production is used against a trivial but frequent nuisance for many people, the bad-breath problem (halitosis). Therefore, oral hygiene products such as toothpaste and mouthwash consume about half of the menthol production, and – believe it or not – one-third (4000 tons) is on the market as an ingredient of chewing gum.

2. Literature

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[3] B. Schäfer, In: "Naturstoffe der chemischen Industrie" Elsevier Spektrum Akademischer Verlag, 1st edition, Munich, **2007**, pp. 96−104.

[4] Atta-ur-Rahman, M. Yaqoob, A. Farooq, S. Anjum, F. Asif, M. I. Choudhary "Fungal transformation of (1*R*,2*S*,5*R*)-(–)-menthol by *Cephalosporium aphidicola*" *J. Nat. Prod.* **1998**, *61*, 1340−1342.

[5] T. J. Belloc, F. Sánchez-Ferrando "Measurement of the sign and the magnitude of heteronuclear coupling constants from spin-state-edited *J*-cross-polarization NMR experiments" *Magn. Reson. Chem.* **2004**, *42*, 852−862.

Fig. 5.2-6 Peppermint sweets

3. Isolation

3.1 Principle

The source for the isolation of (–)-menthol is not peppermint oil but a steam distillate called Japanese peppermint oil of a related plant, named *Mentha arvensis* var. *piperascens* L., which has a 70% (–)-menthol content. The method used for separation is simple and it is the historically long known one: deep cooling of the essential oil causes crystallization of (–)-menthol. On standing in a normal refrigerator, crystallization does not occur and only turbidity can be observed. However, when cooled to –18 °C (–)-menthol crystallizes in the form of a felted mass. Crystallization is possible both due to the high content of (–)-menthol in the oil and the fact that (–)-menthol as an alcohol has the ability to form hydrogen bonds to other menthol molecules. This is impossible for other terpenoid constituents of the alkene type which do not have this structural feature and therefore remain liquid at this temperature. Separation is thus possible by simple filtration and recrystallization. Technically, menthol is separated by centrifugation and removal of the supernatant oil. However, Japanese peppermint oil dementholized in this way still contains ca. 50% of menthol.

Fig. 5.2-7 Mint plants in a garden

3.2 Method

A flask containing 110 g of colourless 70% Japanese peppermint oil (supplied from a Vietnamese source) is stored overnight in a deep freezer at –18 °C. (–)-Menthol crystallizes and the impression arises that all of the material has solidified. This is proven to be untrue when a spatula is pressed on the surface of the material, showing a flexible mass with liquid parts still in the flask. Therefore, the flask is allowed to stand for another day in a normal refrigerator at +4 °C. The mass in the flask is now a mixture of solid (–)-menthol and a liquid which is separated by suction filtration through a sintered glass filter funnel. The portion of colourless crude (–)-menthol has a mass of 8.3 g and the filtrate a mass of 101.7 g. The filtration requires some time due to the high viscosity of the liquid portion.

ἀλλὰ οὐαὶ ὑμῖν τοῖς Φαρισαίοις, ὅτι ἀποδεκατοῦτε τὸ ἡδύοσμον καὶ τὸ πήγανον καὶ πᾶν λάχανον, καὶ παρέρχεσθε τὴν κρίσιν καὶ τὴν ἀγάπην τοῦ θεοῦ: ταῦτα δὲ ἔδει ποιῆσαι κἀκεῖνα μὴ παρεῖναι.

New Testament, Luke, 11, 42

3.3 Purification

The crude (–)-menthol is dissolved in a flask at room temperature by shaking it with *n*-hexane (25 mL). The flask is stoppered and allowed to stand in a deep freezer overnight, causing crystallization of (–)- menthol as colourless needles. Filtration gives the pure compound (mass 3.2 g) with mp 40−42 °C, which is in accordance with reference data, and an optical rotatory power of $[\alpha]_D^{25}$ –50.7° (*c* 0.050 g/mL, ethanol).

Fig. 5.2-8 An apple mint plant. This species *Mentha suaveolens* belongs to the genus *Mentha* in the Lamiaceae family. It is possible to make apple mint jelly or to use the leaves as a spice

4. Spectra and Comments

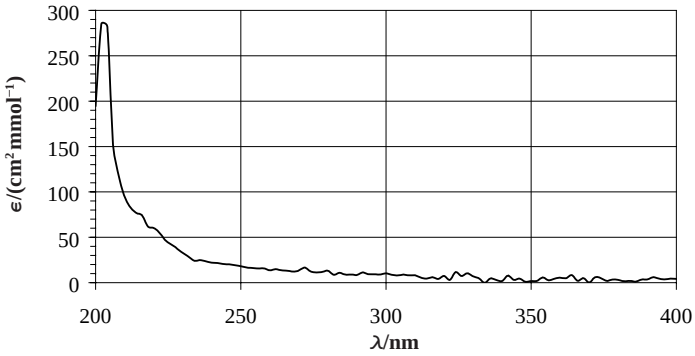


Fig. 5.2-9 UV spectrum in ethanol

As is to be expected, the UV spectrum essentially shows no absorption due to the lack of any chromophore and is therefore not characteristic.

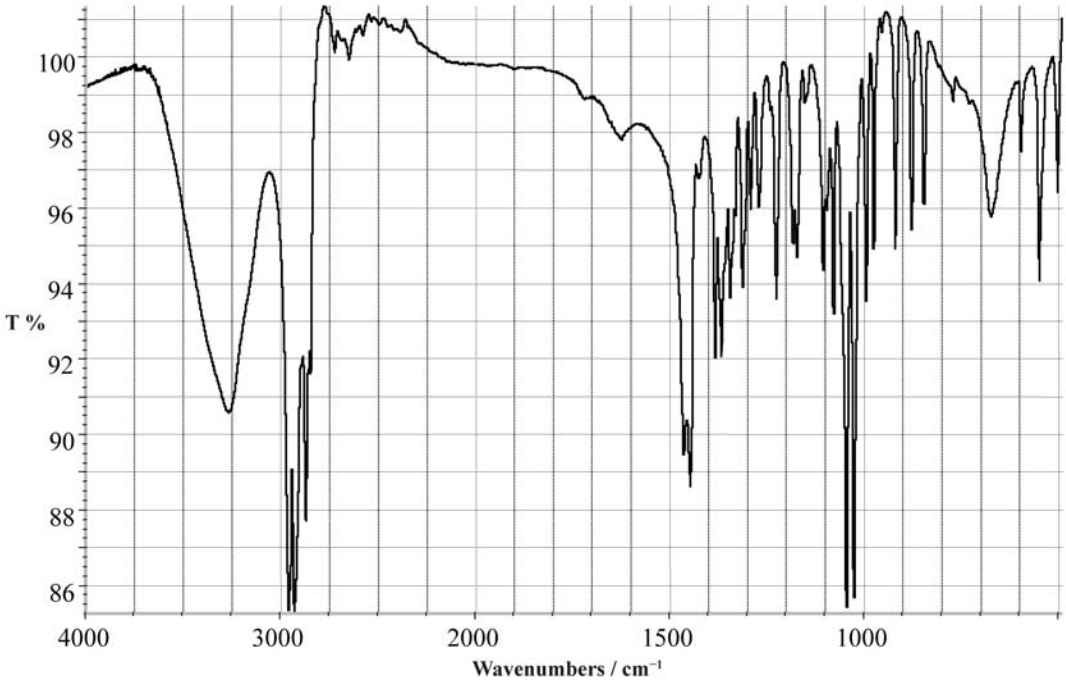


Fig. 5.2-10 IR spectrum in KBr

The IR spectrum is dominated by the strong OH valence vibration and the CH valence vibrations of the sp^3 hybridized carbon atoms.

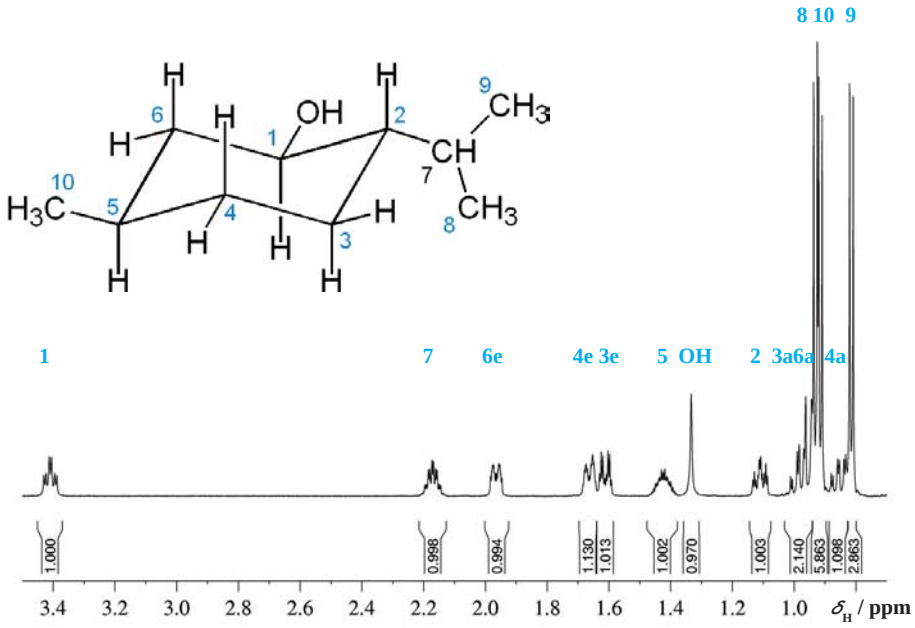


Fig. 5.2-11 ^{1}H NMR spectrum at 600 MHz in $CDCl_3$

The proton spectrum reveals distinct multiplets, most of them with an integration of 1H, and in addition one singlet from the OH group and three doublets from the methyl groups. The linewidth and position of the OH signal are very dependent on concentration and residual humidity. The assignment of the proton signals is straightforward using the COSY and HSQC spectra.

Mrs. Corney twice essayed to speak: and twice failed. At length summoning up courage, she threw her arms around Mr. Bumble's neck, and said, it might be as soon as ever he pleased, and that he was "a irresistible duck."
Matters being thus amicably and satisfactorily arranged, the contract was solemnly ratified in another teacupful of the peppermint mixture; which was rendered the more necessary, by the flutter and agitation of the lady's spirits. While it was being disposed of, she acquainted Mr. Bumble with the old woman's decease.
"Very good," said that gentleman, sipping his peppermint; "I'll call at Sowerberry's as I go home, and tell him to send tomorrow morning. Was it that as frightened you, love?"

Charles Dickens (1812−1870)
Oliver Twist XXVII

Fig. 5.2-12
Mojito is a traditional Cuban cocktail made of the following ingredients: pale Cuban rum, muddled lime wedges (lime juice is only a poor substitute), fresh spearmint leaves, sugar (however, traditionally fresh sugar cane juice), carbonated water and ice. It was invented in Cuba after 1910. After the cocktail Daiquiri, the Mojito was the second favorite drink of Ernest Hemingway, who lived directly in La Habana in a small room of the hotel Ambos Mundos between 1932 and 1939. His most favourite bars were the La Floridita and the Bodeguita del Medio in La Habana Vieja, the central part of the city. It is rumored that soon a quarrel broke out between these bars because each of them claimed to be the favourite one of the famous writer. Eventually, this confliction was settled by Hemingway with a sentence in a style worthy of Solomon: "My Daiquiri in La Floridita, my Mojito in the Bodeguita". *Salud!*

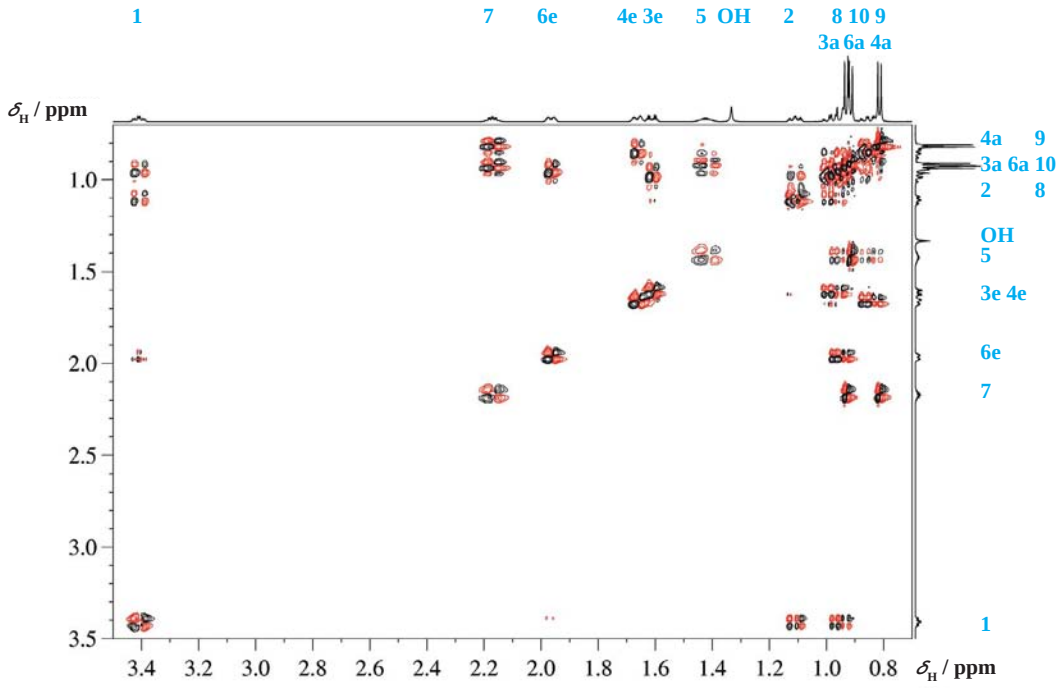
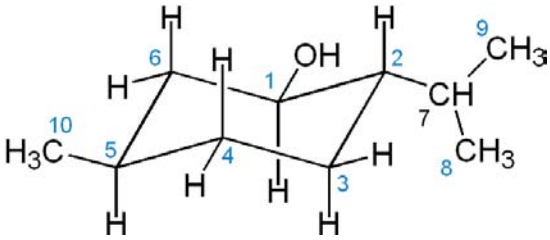


Fig. 5.2-13 Double quantum filtered COSY spectrum

Clearly H-1 is the most deshielded signal and shows two strong and one less intense cross peaks in the COSY spectrum. The two strong peaks must stem from the large axial−axial spin coupling to H-2 at 1.11 ppm and H-6a at 0.95 ppm, whereas the small coupling constant would lead to H-6e (1.97 ppm) considering the Karplus equation. H-7 can be recognized due to its septet by the spin coupling to the methyl protons of the isopropyl group. Inspection of the COSY cross peaks for H-7 shows that the two methyl group signals of the isopropyl group embrace the methyl group signal of H-10 at 0.914 ppm. This methyl group is connected to the signal at 1.43 ppm, being the most complicated multiplet of the molecule and safely assigned to H-5. A cross peak starting from H-2 leads obviously to one of the methylene protons at C-3 at 0.96 ppm.



Scheme 5.2-1

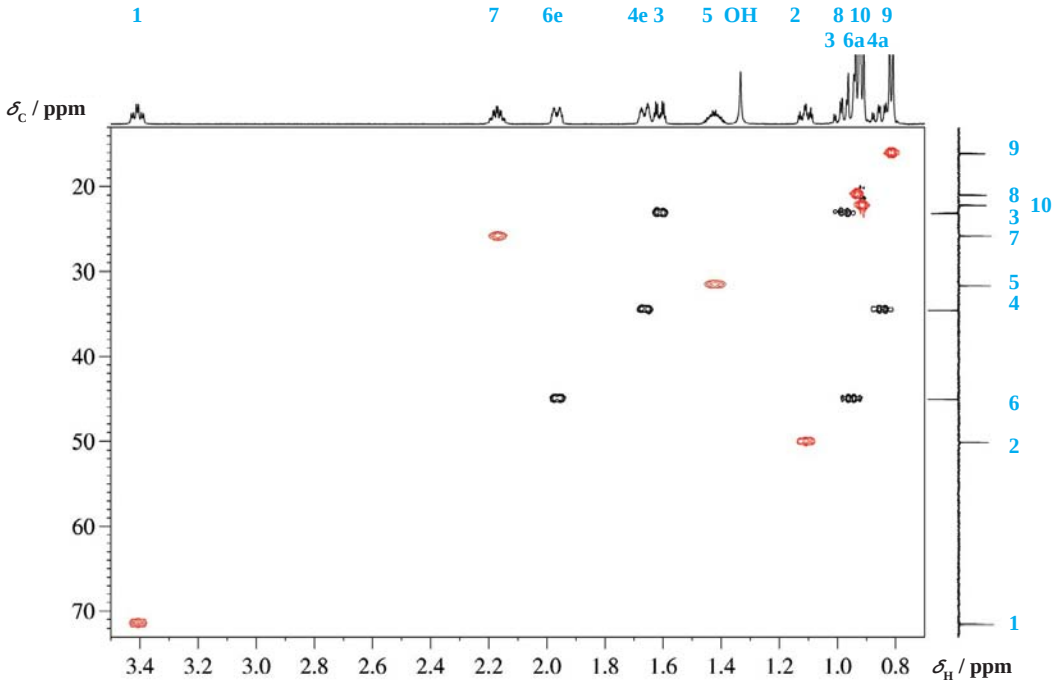


Fig. 5.2-14 HSQC spectrum

The distinction between H-2 and H-6a can be obtained from the HSQC spectrum, which reveals the diastereotopic methylene group of C-6 displayed in black. Looking further into the HSQC spectrum, one finds two more diastereotopic methylene groups. Since we already know from the proton spectrum the signal of one H-3 at 0.96 ppm, we can use the HSQC information to assign the residual signals for C-3 and C-4.

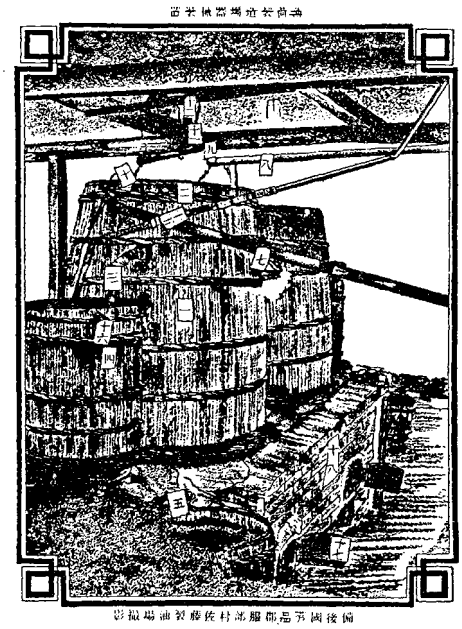


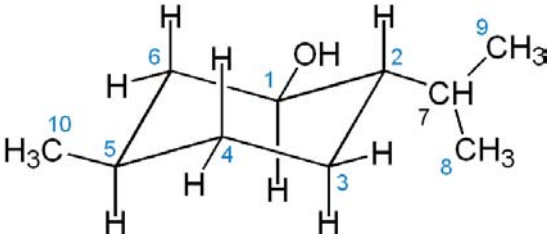
Fig. 5.2-15 Apparatus for the steam distillation of Japanese peppermint oil in Sato's factory in Hiroshima at the end of the 19th century. Taken from: E. Gildemeister, F. Hoffmann *Die ätherischen Öle*, 4th edition, Akademie Verlag, Berlin, vol. VII, 1961, p. 357

Fig. 5.2-16 That a Daiquiri, as a mint-free cocktail, is shown here in the section on menthol has no other reason than to complete the Mojito story above. The name of this cocktail, made of pale Cuban rum, sugar cane syrup, lime juice and a lot of finely crushed ice, is due to a village, a beach and an iron mine with the same name near Santiago de Cuba. The drink was invented in 1905 by a group of American mining engineers, obviously to get along better with the tropical heat. Whereas due to its large volume a Mojito is a long drink, a Daiquiri belongs to the six most famous short drinks, so called because it contains at most 120 mL. It is said that Daiquiri was also a favourite drink of President J. F. Kennedy

The best smell

Some years ago, the warehouseman of our chemical store retired after a life of more than 40 years among thousands of different chemicals he had to handle each working day. At the farewell party I said to him: "Well, certainly, you will be fed up now with all those pungent odours from most of these bottles around us…"
He answered: "Oh no, that has not been so difficult as you believe. I always had my refresher nearby. Come on, I'll show you my best bottle."
He took me around the corner to an impressive big brown bottle with a nice old label from the 1920s, and pulled out the ground stopper, carefully:
"Try this − that's really the best smell!"
I put my nose over the open bottle, inhaled slowly and got a powerful fresh sensation of peppermint. It was a pound of pure L-menthol which had kept him going all the years.

Story experienced by one of the authors



Scheme 5.2-2

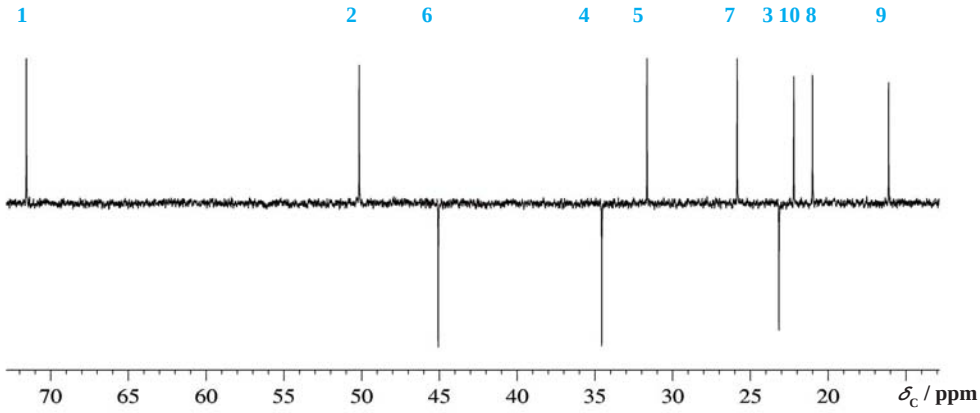


Fig. 5.2-17 APT ^{13}C NMR spectrum at 150 MHz in $CDCl_3$

Since all protons could be assigned using the COSY and HSQC spectra, the ^{13}C assignments are taken from the HSQC spectrum and are given in the table below. Of course, they are in agreement with the relative phases in the APT spectrum.

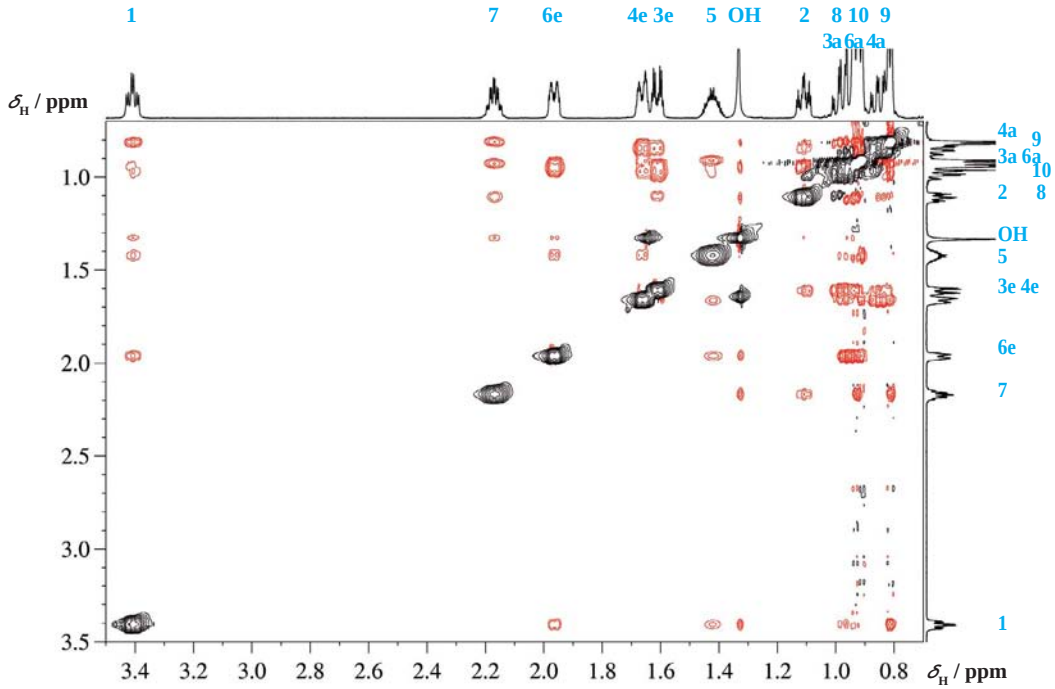
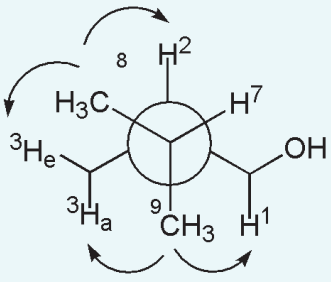


Fig. 5.2-18 NOESY spectrum

After the positional assignment of proton and carbon atoms, the molecules require a stereochemical assignment. This concerns the protons within the three methylene groups with respect to their axial or equatorial position and the preferred conformation around the C-2–C-7 bond. Methylene groups of a cyclohexane ring display a large geminal spin coupling and in principle two characteristic vicinal spin couplings, the larger axial–axial and the smaller axial–equatorial values. It is difficult, however, to disentangle these values in some cases and therefore, for the stereochemical assignment, the NOESY spectrum is the decisive tool. The NOESY spectrum of menthol was recorded with a mixing time of 3 s to provide intense cross peaks in this small molecule.

Starting from the axial proton H-1, cross signals can be seen to H-3 at 0.96 ppm, H-5 at 1.43 ppm, H-6 at 1.97 ppm and the methyl group H-9 at 0.81 ppm. H-5 is also axial as H-1 and this cross peak resembles the typical 1,3 interaction in six-membered rings. The same is true for the signal of H-3 at 0.96 ppm and therefore this is assigned to H-3a. The signal of H-6 at 1.97 ppm must be assigned to H-6e since the axial H-6 proton stays on the other side of the cyclohexane ring and therefore cannot show an NOE interaction to H-1. This is confirmed by the missing NOE signal from this proton to H-2. Looking at the axial proton H-5, one finds cross peaks to protons 6e at 1.97 ppm and H-4 at 1.66 ppm, which must therefore also be the equatorial ones. All methylene protons show strong but trivial NOE cross signals to their geminal partners. The assignments, here are corroborated further by the NOE cross signals with the methyl groups.

The spin coupling between H-7 and H-2 is 2.6 Hz; according to the Karplus equation, this corresponds to a dihedral angle for H-7–C-7–C-2–H-2 of about 70°. The protons of methyl group 9 have NOE contacts to H-1 and H-3a, whereas the protons of methyl group 8 at 0.93 ppm show only NOE contact to H-2 and H-3e. From this result, a conformation as given here can be derived and the two methyl groups are individually assigned.



Scheme 5.2-3

Author's peppermint sauce

Ingredients:
5 tablespoons of fresh peppermint leaves chopped into fine pieces
250 mL water
75 mL vinegar
1 tablespoon sugar
1 pinch of salt

Method:
Bring water, vinegar and sugar to simmer in a saucepan for 5 min. Add 4 tablespoons of peppermint leaves, stir well and allow the sauce to cool to room temperature, slowly. Cover the pan. Add the last tablespoon of mint not before you serve the sauce and season it with a pinch of salt.

Fig. 5.2-19 Mint chocolates

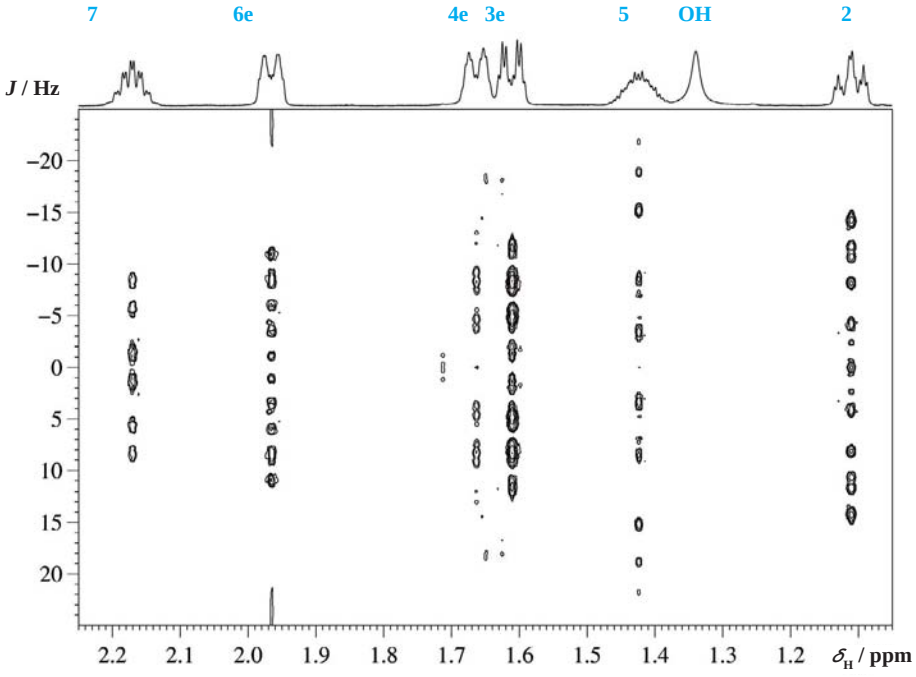


Fig. 5.2-20 2D *J*-resolved NMR spectrum

Shown here is a *J*-resolved 2D NMR spectrum which is very valuable in this case and easily allows one to extract the spin coupling constants of the individual complicated multiplets.

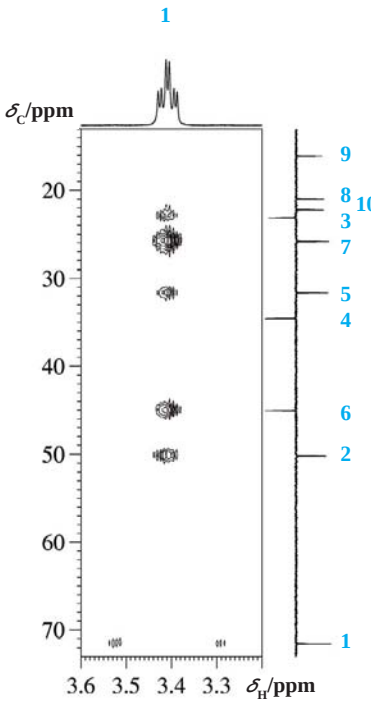


Fig. 5.2-21 Expansion of the HMBC spectrum for H-1

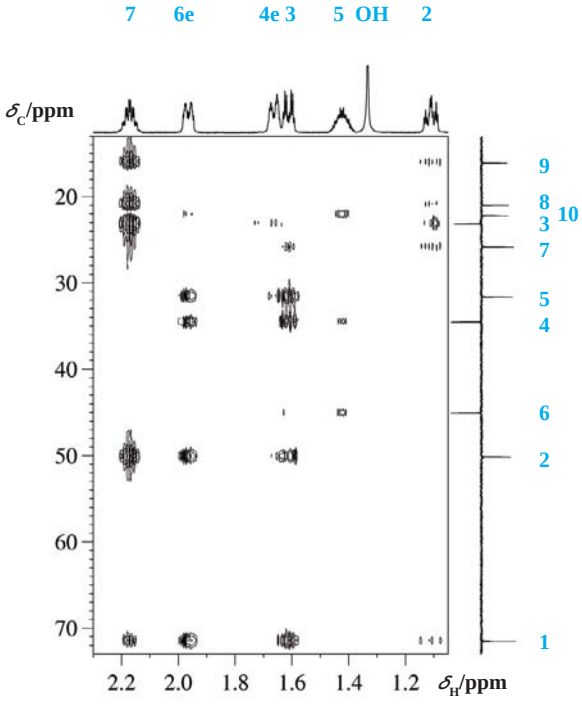


Fig. 5.2-23 Expansion of the HMBC spectrum for the ring protons

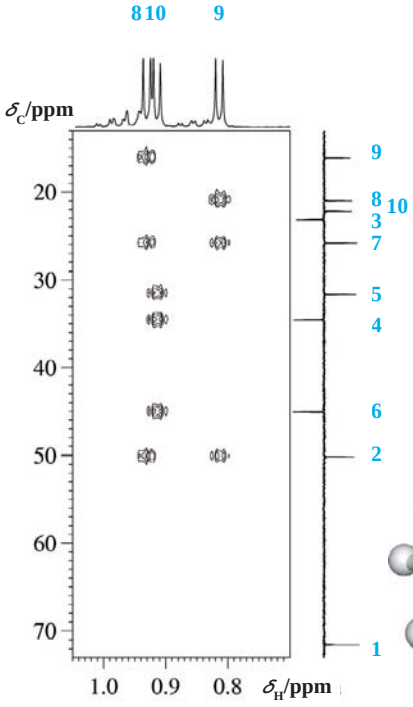


Fig. 5.2-22 Expansion of the HMBC spectrum for the methyl groups

The HMBC spectrum displays all signals with two- and three-bond relationships in this molecule. It is especially instructive for the assignment of the methyl groups. The protons of methyl group 8 show cross peaks to carbon atoms 9, 7 and 2; similarly, the protons of methyl group 9 show cross peaks to carbon atoms 8, 7 and 2, whereas the protons of methyl group 10 are connected to C-5, C-4 and C-6.

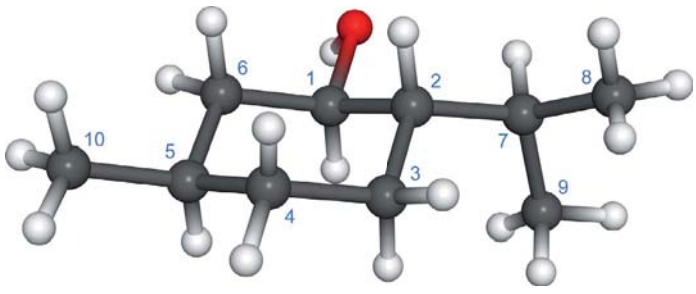


Fig. Fig. 5.2-24 Molecular model of menthol

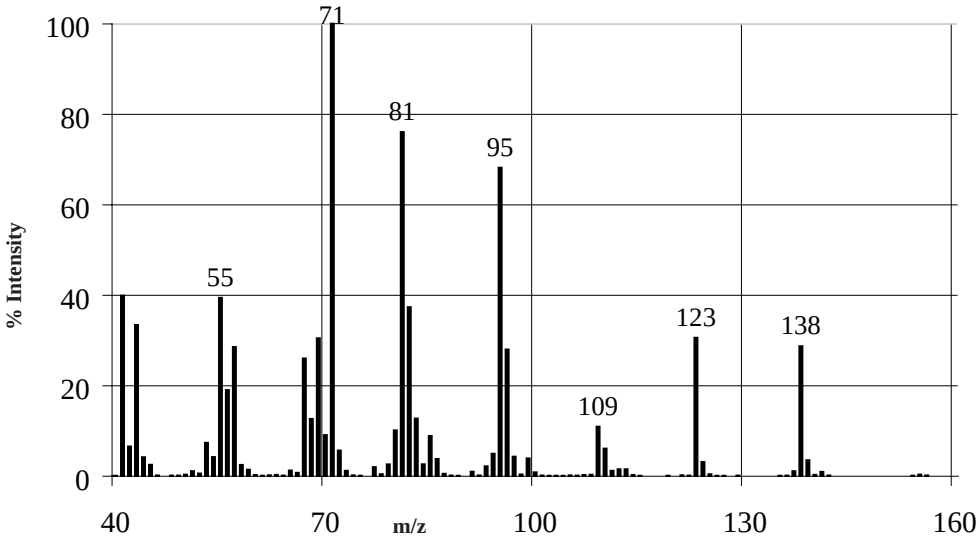
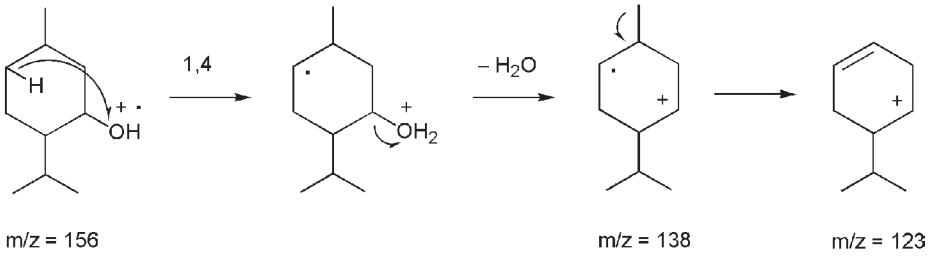
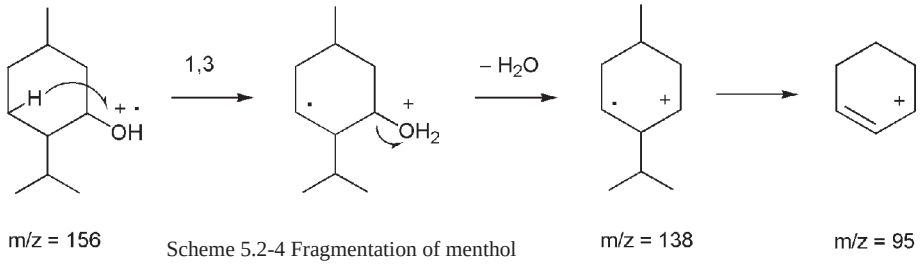


Fig. 5.2-25 Mass spectrum (EI)

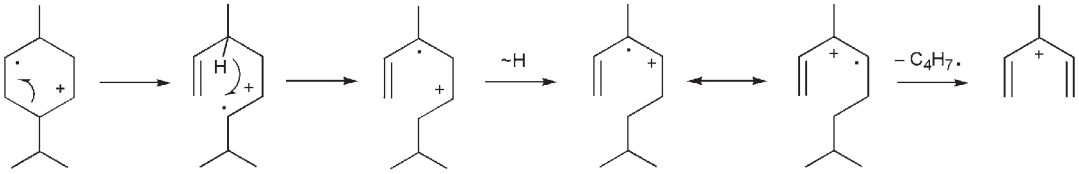
The EI mass spectrum does not give the molecular ion, but after a 1,4- or a 1,3-hydrogen shift a loss of one water molecule to form a species with m/z = 138. This loses either a methyl group to form an ion with m/z = 123 or the isopropyl group to form the ion with m/z =95.



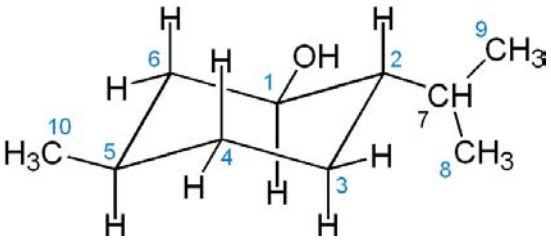


Scheme 5.2-4 Fragmentation of menthol

The ion with m/z = 81 can be explained by a series of hydrogen shifts as given below:



Scheme 5.2-5 Hydrogen shifts in menthol



Scheme 5.2-6

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H signals δ / ppm, J / Hz
71.5	CH	C-1	3.41 J = 10.4, 4.3
50.2	CH	C-2	1.11 J = 11.2, 3.05
45.1	CH_2	C-6	e: 1.97 J = 12.2 a: 0.95
34.6	CH_2	C-4	e: 1.663 J = 13.0, 3.0 a: 0.845 J = 13.0, 3.4
31.6	CH	C-5	1.43
25.8	CH	C-7	2.17 J = 7.0, 2.6
23.2	CH_2	C-3	e: 1.61 J = 13.0, 3.0 a: 0.963 J = 12.7, 3.5
22.2	CH_3	C-10	0.914 J = 6.6
21.0	CH_3	C-8	0.93 J = 7.0
16.1	CH_3	C-9	0.81 J = 7.0

Table 5.2-1 NMR data for menthol

Fig. 5.2-27 A catmint plant. This species *Nepeta cataria* belongs to the genus *Nepeta* within the family Lamiaceae. The name results from the fact that cats are attracted by the smell of this plant, that stimulates their pheromonic receptor

5. Questions

A. Draw a synthetic scheme with reasonable reagents for the Haarmann and Reimer process for (–)-menthol starting from *m*-cresol.

B. Draw a synthetic scheme with reasonable reagents for the Takasago process for (–)-menthol starting from isoprene.

C. (–)-Menthol is an example of a most stable stereoisomer. Draw the structures of all eight possible stereoisomers with the constitution of 5-methyl-2-(1-methylethyl)cyclohexanol in their chair conformation (perspective view) and explain the reason why (–)-menthol is the most stable.

D. Ascertain the complete description of the configuration for all isomers according to the *R*/*S*-nomenclature. If you want, you can use the full description given on the first page of this chapter for (–)-menthol to accomplish this easily. Correlate the name menthol and the names of the other isomers isomenthol, neomenthol, and neoisomenthol with your drawings.

E. How can one determine whether (+) or (–) has to be assigned to a given structure?

F. The flavour profile of the two menthol enantiomers is different and evaluated by flavourists in terms of sensations of the following kind: cool, fresh, minty, sweet, hay, carrot, herbaceous, woody, phenolic, earthy, musty, bitter. Make a suggestion that explains the reason for this on the molecular level of the flavour and odour receptors.

G. Why is there no cross peak in the COSY spectrum between H-7 and H-2?

H. Inspect the HMBC spectrum for all ^{2}J and ^{3}J connectivities.

I. Would you expect a CD spectrum from menthol?

J. Explain the base peak in the mass spectrum at m/z = 71.

6. Own Observations

5.3 Thujones

α-Thujone and β-Thujone

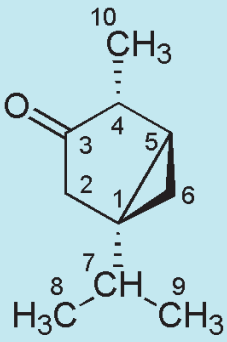
$C_{10}H_{16}O$, MW 152,24

Colourless, viscous liquid

Note: both bps correspond to a small range from to 198 to 203 °C at ambient pressure

Caution! Both thujones are EU classified as Xn (Harmful) and are toxic in the pure state.

Level: difficult



from Common sage (S)
Salvia officinalis L. (Lamiaceae)

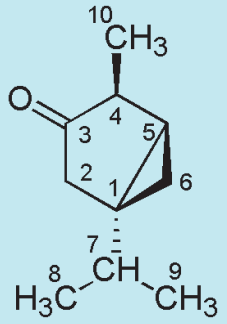
CAS RN 546-80-5,
BRN 2206060, 4660369, 7473419

$[\alpha]_D^{20}$ –19.2° (neat data base)

Synonymous names:
(1*S*,4*R*,5*R*)- 4-methyl-1-(1-methylethyl)-bicyclo[3.1.0]hexan-3-one,
(–)-Thujone, (–)-α-Thujone

bp 83.8−84.1 °C (17 hPa)

Commercially available.



from Wormwood (Absinth wormwood) (W)
Artemisia absinthium L. (Asteraceae)

CAS RN 471-15-8,
BRN 2041871, 2500355, 4660368, 10152962

$[\alpha]_D^{20}$ +72.5° (neat, database)

Synonymous names:
(1*S*,4*S*,5*R*)- 4-methyl-1-(1-methylethyl)-bicyclo[3.1.0]hexan-3-one,
(+)-Isothujone, (+)-β-Thujone

bp 85.7−86.2 °C (17 hPa)

Commercially not available.

1. Background: Mice death and the Green Fairy

Καὶ ὁ τρίτος ἄγγελος ἐσάλπισεν: καὶ ἔπεσεν ἐκ τοῦ οὐρανοῦ ἀστὴρ μέγας καιόμενος ὡς λαμπάς, καὶ ἔπεσεν ἐπὶ τὸ τρίτον τῶν ποταμῶν καὶ ἐπὶ τὰς πηγὰς τῶν ὑδάτων.
καὶ τὸ ὄνομα τοῦ ἀστέρος λέγεται ὁ Ἄψινθος. καὶ ἐγένετο τὸ τρίτον τῶν ὑδάτων εἰς ἄψινθον, καὶ πολλοὶ τῶν ἀνθρώπων ἀπέθανον ἐκ τῶν ὑδάτων, ὅτι ἐπικράνθησαν.

New Testament
Book of Revelation, 8, 10−11

This section differs from all others because it describes the isolation of two monoterpene ketone diastereomers from two different sources. Both thujones belong to the group of volatile naturally occurring restricted compounds from flavorings such as β-asarone, coumarin, hydrocyanic acid and safrole, the oral uptake of which should be avoided. The name thujone is derived from the thuja plants, a genus of evergreen coniferous trees in the cypress family from which thujones were first isolated. The correct constitution was described by the German chemist F. W. Semmler in 1900 under the term tanaceton [1].

Oil of wormwood (*Absinthii aetheroleum*), used here for the isolation of β-thujone, is an essential oil obtained by steam distillation of the leaves of wormwood, a herbaceous perennial plant descending from Europe, Asia and Northern Africa, which is prevalent on the edge of fields, footpaths and uncultivated arid ground. The content of essential oil is between 1 and 2%. The strange name gives a hint at one of its uses in ethnomedicine: it was used as a cure for intestinal worms. Due to its thujone content, pure wormwood oil is a strong neurotoxin and very poisonous. It cannot be used as an internal medicine. That it is on sale is due to its applicability for aromatherapy. The same statement is true for oil of sage, used here for the isolation of α-thujone, which will be discussed later. The assessment and use of the thujonic note from the viewpoint of perfumery are an astonishing manifold. It can be used to add lift, warmth and freshness to fragrances. Thujones have a powerful and penetrating odour, marked by warm−herbaceous and minty−camphoraceous sensations.

Fig. 5.3-1 A thuja tree, the plant from which the name thujone was derived

A less unusual form of intrinsic application is wormwood tea. It is well known to one of the authors from his childhood and was administered by his careful and rigorous mother with emphasis and the obligation to drink it under supervision, and completely. It is easily made from the dried leaves and flowering tops of the plant which are allowed to infuse with hot water for some minutes, only. Immediately send us a note if you know of a beverage which is even more bitter than this tea! Such a brave attack is directed against gastric pain and indigestion. The cure with this stomach medicine is hard but effective. However, it is not a thujone that is responsible for the striking bitter taste, because, as can be seen from the structure, any thujone is too hydrophobic to be well soluble in water. Instead, sesquiterpene lactones such as absinthin and artabsin make the bitter taste.

Definitely, the tea is a traditional means to help pregnant women during pain of labour. This use is the origin of the genus name *Artemisia* due to Greek Goddess Artemis, responsible for birth. The species name *absinthium* is according to Dioskurides related to the word ἄψινθον (apsinthos = unpleasant) or apinthos (undrinkable). It is from this point of view whimsical to read that in the Roman Empire the winner of bull chariot races on the Capitol of Rome received a wormwood drink as a prize. It is likely that this drink was an early form of Vermouth, a fortified wine slightly aromatized with wormwood ingredients.

The plant's ingredients are also effective for the plants themselves. The volatile ones (characteristic smell) serve as a pest repellant. Its roots exude allelochemicals that inhibit the growth of other plants in the close neighbourhood. By this allelopathic effect, wormwood planted just at the fringe of another plantation can serve as a natural weed suppressant. In the past, wormwood leaves were used as "mice death" in pest control in baits for granaries and against moths. Wormwood sap added to ink for handwritten books was known to help against bookworms.

By far the most popular applications of wormwood are as ingredients of alcoholic beverages. Some have always been beyond doubt, with *Vermouth* as the best example. Wines "aromatized" with herbs and spices belong to the Vermouth family with well-known tradenames such as Cinzano, Martini, Distillery Stock, Bartissol (Italy), Noilly Prat and Dubonnet (France). The concept was invented as early as 1786 in Turin driven by the desire to mask insufficient flavours of cheap wine and to imparting at the same time the appearance of a helpful tonic.

On the other hand *Absinthe* underwent an extremely different history with the public. Being a cult drink in the 19th century it ended up as a very dangerous and bad spirit, giving rise to terrible crimes. Therefore, making and selling absinthe was vilified and eventually prohibited in 1915 in most European countries. After a long period of prohibition, it has now started a renaissance, with ethanol as the only real danger therein seen by food chemists. But, in sequence…

Absinthe is an highly alcoholic (from 65 up to 80%) anise-flavoured spirit without added sugar (therefore, it is a liquor and not a liqueur) distilled from several herbs including wormwood [2]. Originally created in Switzerland in 1797 as an elixir, it became very popular in France in the 19th century within of a group artists, who were called "absintheurs". Some of the famous consumers included Vincent van Gogh, Oscar Wilde (who created the phrase *The Green Fairy*), and Pablo Picasso. A number of French painters, such as Edgar Degas, Henri de Tolouse-Lautrec and Edouard Manet, have created well-known pictures all showing absinthe drinkers in a mood of dejection, if not depression. A very instructive recent review [3] discloses, far from cheap sensationalism, the fatal combination of economic and social driving forces that in the France of the 19th century first caused an absinthe boom that finally turned into a complete disaster. An abstract of this tragedy is as follows:

Absinthe, originally a wormwood-based elixir, was administered as a prophylactic to all 100 000 soldiers of the French North African Corps from 1830 on. Soldiers coming home knew the beverage and tried to get it, but that was initially difficult due to harsh restrictions on serving spirits in bars. When these interdictions fell in 1870, the absinthe boom set in and the drink was "in". The consumption exploded in France from 700 000 L in 1873 to 24 000 000 L (!) in 1900. An unexpected side effect additionally pushed the sale of absinthe. Grape louses, introduced from America, caused an epidemic that annihilated France's vines completely between 1860 and 1920. The beverage gap arising was filled with absinthe, the spirit of which was not distilled from wine

Fig. 5.3-2 A wormwood bush

Id quoque enim non ab nulla ratione videtur;
sed vel uti pueris absinthia taetra medentes
cum dare conantur, prius oras pocula circum
contingunt mellis dulci flavoque liquore,
ut puerorum aetas inprovida ludificetur
labrorum tenus, interea perpotet amarum
absinthi laticem deceptaque non capiatur,
sed potius tali facto recreata valescat.

Titus Lucretius Carus (99−55)
De rerum natura, I, 935−950

but from schnapps based on corn or sugar beet, and even industrial ethanol was used. Accordingly, absinthe was cheaper than wine had been and affordable for really everybody.

A ritual for drinking absinthe was developed in France that consisted in adding sugar and water to the highly alhoholic herbal extract. Therefore, a special perforated absinthe spoon was put on the brim of the glass and a cube of sugar placed on the spoon. Then, ice-cold water was carefully dropped on the sugar, allowing dissolution of the sugar and dropwise addition of the sugar solution into the liquor until it was diluted at 1:4 or 1:5. The intention was to change the appearance of the absinthe from a clear solution into a cloudy, opaque liquid. This effect was mysterious for the laity; for a chemist, it it is obvious what happens: diminishing the concentration of ethanol considerably reduces the solubility of the hydrophobic essential oils that only dissolve well in ethanol and eventually leads to the formation of an oil-in-water emulsion which is turbid due to the Tyndall effect. It is noteworthy that this precipitation of essential oils is not a massive thujone deposit but instead is caused by anise ingredients. Additionally, the green colour of absinthe is never caused by addition of a synthetic dyestuff but originates from a coloration step by extraction of crude absinthe distillate with wormwood, hyssop and lemon balm from which hydrophobic leaf dyestuffs such as chlorophylls are extracted.

Cur moriatur homo cui Salvia crescit in horto?

Coat of arms
Medical School of Salerno
(1100−1300)

Fig. 5.3-3 Another subspecies of *Salvia officinalis*

In 1905, a family tragedy occurred in Switzerland which closed the absinthe chapter for a long time. A young, drunken winegrower flipped out in an irrelevant dispute with his pregnant wife and murdered her and two little daughters. At that time, thujone in absinthe was held responsible for the crime. The murderer was punished and absinthe was prohibited in most European countries including Germany at the end in 1923. After a long period of time it became possible to look back, unexcitedly.

Hitherto, the aetiopathology of people depending on absinthe was ascribed to thujone. However, recent research has proved otherwise. First, it has to be considered that the contents of both thujones in the essential oil of wormwood plants may be extremely different (0–70%), depending on the breed and area. Hence absinthe may even contain no thujone at all. It is most astonishing that analyses of both vintage absinthes from around 1900 and of absinthes made recently following the historical procedures pointed out consistently that the concentrations of thujones were low and even below the current prescribed limits. These findings completely change the general opinion on absinthe which had been propagated among the public after its prohibition. In no way is it still reasonable to assume that historical absinthes may have contained thujones in toxic concentrations. The conclusion is most meaningful: absinthism has to be regarded as a type of alcoholism, and so have its effects. [4]

Salvia

Lelifagus prima præfulget fronte locorum,
Dulcis odore, gravis virtute atque utilis haustu.
Pluribus hæc hominum morbis prodesse reperta
Perpetui viridi meruit gaudere iuventa.
Sed tolerat civile malum: nam sæva parentem
Progenies florum, fuerit ni dempta, perurit
et facit antiquos defungier invida ramos.

Walahfrid Strabo (808−849) *Hortulus*

Since 1991, absinthe has again been admitted in the European Union with a maximum level for thujone of 10 mg/kg in beverages containing more than 25 vol.% alcohol and 35 mg/kg in stronger spirits labelled as bitters. There is no doubt that thujone is not the danger in such bottles.

Oil of sage, used here for isolation of α-thujone, is an essential oil obtained by steam distillation of the leaves of common sage, a small evergreen shrub with blue to purple flowers. This plant originates from the Mediterranian region and grows wild and cultivated. The plant was esteemed by both the ancient Greek and Roman cultures. In the Middle Ages, friars brought the plant northwards via the Alps. Other names are kitchen sage, garden sage or Dalmatian sage. The last one, a type of *Salvia officinalis* L., serves as a standard for this species due to its fine and most characteristic sage aroma, with which others are compared. The content of essential oil is between 1.5 and 3.5%. The genus name *Salvia* descends from the Latin *salvare*, which means "to heal". This medical effect is due both to the essential oil and tanning agents as ingredients. Hence common sage is cultivated for use both in medicine and in the kitchen. More than 30 species belong to the genus *Salvia*, e.g. the one which is widespread in Middle Europe, the meadow sage (see photographs in the margin).

The proportion of both thujones in oil of sage is 30–60% with α-thujone prevailing. Dalmatian oil of sage contains a 10:1 ratio of α- to β-thujone. Other main components are camphor and 1,8-cineol. Oil of sage even received the difficultly accessible GRAS status for perfumery ingredients (Generally Recognized As Safe), which means it is acceptable from the toxicological point of view for applications in perfumery, lotions and shampoos and does not show cytotoxic effects or skin irritations. However, pure oil of sage should – similarly to oil of wormwood – not be used in oral applications. The toxicity of *a*-thujone was found to be higher than that of β-thujone. It is also a neurotoxin and can cause an epileptic fit. It is reported that in ancient times oil of sage was taken orally to induce abortion. In all respects this is a high-stakes game which can cost the life of a pregnant woman.

Tea made of common sage can be used as a gargle in the case of a sore throat. Flushing the mouth with sage tea is helpful against stomatitis. Drinking some of this tea is good if one suffers from dyspeptic complaints. The cold tea is also a means against sweating. Obviously, to be able to show all the effects described, sage contains a very complex essential oil.The daily dose of sage herbs to make tea from should not exceed 4−6 g, however. A long-term application as daily tea is also not recommended.

If he smoked too many cigarettes and drank too much absinth it was because he took civilization as he found it, and did the things that he found his civilized brothers doing. The life was a new and alluring one, and in addition he had a sorrow in his breast and a great longing which he knew could never be fulfilled, and so he sought in study and in dissipation − the two extremes − to forget the past and inhibit contemplation of the future.
He was sitting in a music hall one evening, sipping his absinth and admiring the art of a certain famous Russian dancer, when he caught a passing glimpse of a pair of evil black eyes upon him. The man turned and was lost in the crowd at the exit before Tarzan could catch a good look at him, but he was confident that he had seen those eyes before and that they had been fastened on him this evening through no passing accident. He had had the uncanny feeling for some time that he was being watched, and it was in response to this animal instinct that was strong within him that he had turned suddenly and surprised the eyes in the very act of watching him.

Edgar Rice Burroughs (1875–1950)
The Return of Tarzan, Chap. 3

2. Literature

[1] F. W. Semmler, "Ueber Tanaceton und seine Derivate" [On tanacetone and its derivatives] *Ber. Dtsch. Chem. Ges.* **1900**, *33*, 275−277.

[2] D. W. Lachenmeier, S. G. Walch, S. A. Padosch, L. U. Kroener, "Absinthe – A review." *Critical Reviews in Food Science and Nutrition* **2006**, *46*, 365−377.

[3] K. Roth, "Der Zauber der Grünen Fee" [The magic of the green fairy] *Chem. Unserer Zeit* **2005**, *39*, 130−136.

Fig. 5.3-4 Flowering sage

Aber der Mensch lebt nicht vom Brod allein, sondern auch vom Fleische guter Lämmer, deren ich zwei habe: – Die soll man geschwinde schlachten und würzig, mit Salbei, zubereiten: so liebe ich's. Und auch an Wurzeln und Früchten fehlt es nicht, gut genug selbst für Lecker- und Schmeckerlinge; noch an Nüssen und andern Räthseln zum Knacken.

Friedrich Nietzsche (1844–1900)
Thus Spoke Zarathustra, LXXII, The Supper

[4] D. W. Lachenmeier, "Absinthe – History of Dependence to Thujone or to Alcohol?" *Fortschr. Neurol. Psychiatr.* **2007**, *75*, 306–308.

[5] L. Nykänen, "Mass spectra and fragmentation pathways of thujone" *Finn. Chem. Lett.* **1983**, 31–33.

[6] R. J. Abraham, C. M. Holden, P. Loftus, D. Whittaker, "NMR spectra and conformations of cyclic compounds. IX. Conformational studies of bicyclo[3.1.0]hexane derivatives by carbon-13 NMR" *Org. Magn. Reson.* **1974**, *6*, 184–189.

[7] D. A. Lightner, C. S. Pak, B. V. Christ, S. L. Rodgers, J. W. Givens III, "The octant rule. Conformation and circular dichroism of *syn*- and *anti*-2-methylbicyclo[3.1.0]hexan-3-ones, thujone and isothujone" *Tetrahedron* **1985**, *19*, 4321–4330.

3. Isolation

3.1 Principle

The essential plant oils belonging to the class of monoterpenoids, such as thujone, are volatile compounds and can be isolated by simple steam distillation. There are many different steam distillation apparatus available; here we worked with a model of continuous extraction, where the steam is directly extracted with methylene chloride and thus the amount of water is kept to a minimum. The general problem with this isolation is the yield: 100 g of dried plant material give only about 200 mg of essential oil, as we experienced in one run with wormwood leaves from the house garden. Since further purification probably needs much more material, it would be advisable to perform the steam distillation in several large batches. If one wants to avoid the individual steam distillation, one can also buy the essential oils.

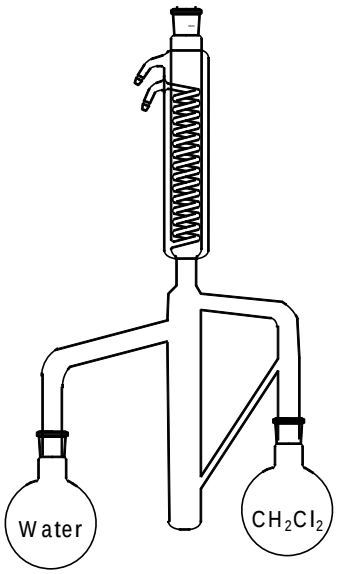


Fig. 5.3-5 Apparatus for continuous steam distillation

We used two commercially available essential oils for our isolation: oil of wormwood, from France, obtained from *Artemisia absinthium* L. by steam distillation, containing 3% α-thujone and 46% β-thujone, distributor: Omikron GmbH Naturwaren; and oil of sage, obtained from *Salvia officinalis* L. by steam distillation, distributor Bioherba Naturprodukte Vertriebsgesellschaft mbh, containing 31% α-thujone and 4% β-thujone.

3.2 Method

Example of an individual steam distillation of wormwood leaves:

A 100 g amount of dried wormwood herbage is placed in a 2 L flask and boiled with water for 5 h. The steam is continously extracted with boiling methylene chloride. This phase is finally dried over $MgSO_4$ and, after the solvent has been evaporated, typically 200 mg of essential oil remain.

3.3 Purification

The essential oils obtained in this manner are a complex mixture of several volatile compounds. For further purification, in the next step a 50 cm spinning band distillation column equipped with a column head allowing regulation of the take-off ratio is used. Typical, 5 mL

of essential oil are distilled under high vacuum, yielding in the case of wormwood oil 0.7 g of a fraction with a bp of 32–34°C (5 Pa), which proves to be mainly β-thujone. This fraction is redistilled. Similarly, spinning band distillation from sage oil gives α-thujone at 31–32°C (5 Pa). Further attempts to separate α- and β-thujone by either preparative GLC, standard column chromatography on silica gel, preparative thin-layer chromatography or HPLC on RP-18 phases or even with chiral columns failed. There seems to be no description in the chromatographic literature where a complete preparative separation of both isomers was successful. The best we could achieve was a separation of the two compounds at about 70% of the chromatographic signals using a 1:1 mixture. The spectra shown below therefore always contain a minor amount of the other isomer.

Fig. 5.3-6 A plant of *Salvia officinalis* (Lamiaceae)

Fig. 5.3-7 Wormwood (Asteraceae)

4. Spectra and Comments

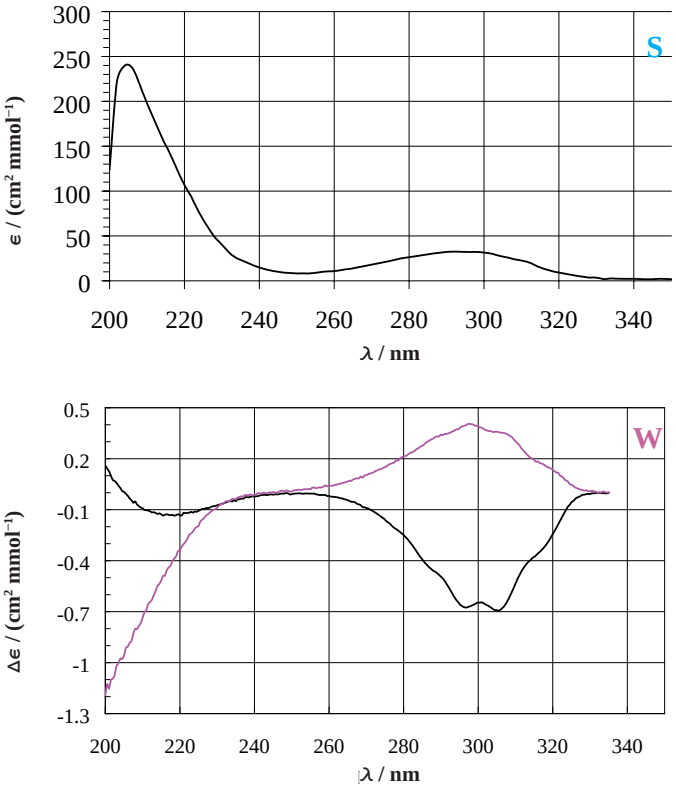
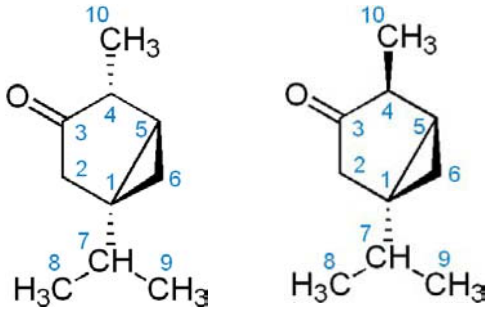


Fig. 5.3-8 UV and CD spectra of the thujones in ethanol

There is no visible difference in the routine UV spectra of α- and β-thujone; therefore, only one spectrum is depicted. The UV spectra are very typical of aliphatic ketones showing the main absorption close to 200 nm and an n→π* transition at about 290 nm. In contrast, the CD spectra of the compounds **S** and **W** show opposite CD effects. They are, however, not mirror images, since the two compounds are diastereomers and not enantiomers. It is also of interest that the n→π* transition apparently reflects the chirality of the molecules stronger than the main band at 205 nm. The sign of the CD bands may be discussed in terms of the octant rules.

De usu eius convenit, herbae facillimae atque inter paucas utilissimae, praeterea sacris populi Romani celebratae peculiariter, siquidem Latinarum feriis quadrigae certant in Capitolio victorque absinthium bibit, credo, sanitatem praemio dari honorifice arbitratis maioribus. stomachum corroborat, et ob hoc sapor eius in vina transfertur, ut diximus.

Pliny the Elder (23–79)
Naturalis Historia Liber XXVII, 28



Scheme 5.3-1

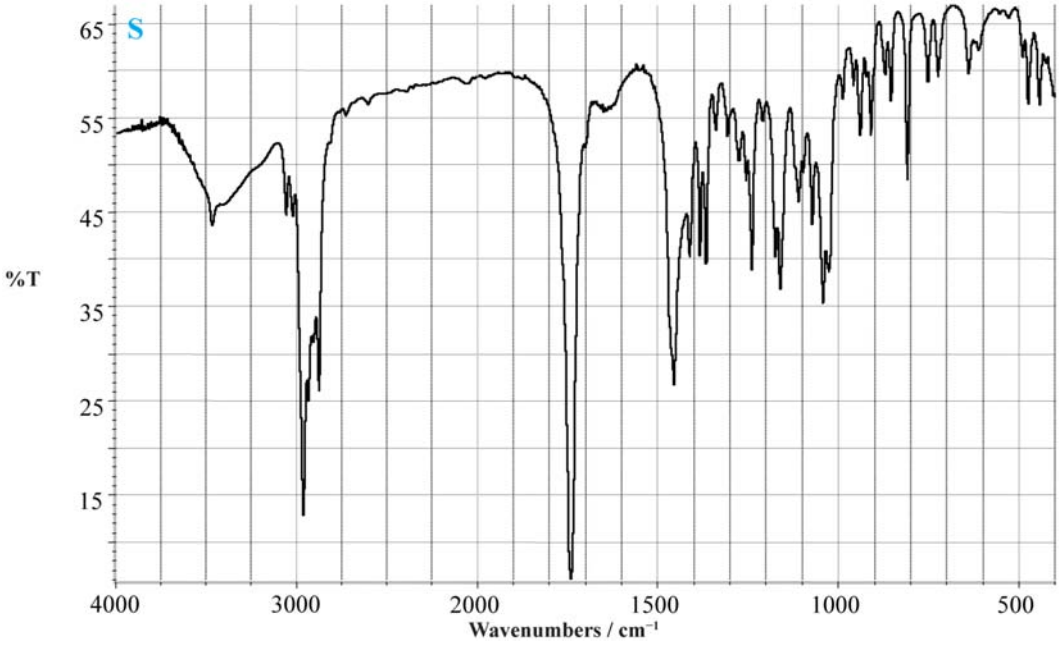


Fig. 5.3-9 IR spectrum as film

As in the UV spectra, there is no visible difference in the routine IR spectra of α- and β-thujone; therefore, only one spectrum is depicted. The C=O band at 1740 cm^{-1} dominates the spectrum. Above 3000 cm^{-1} there are two small but significant CH valence bands, which most likely are due to the three-membered ring system.

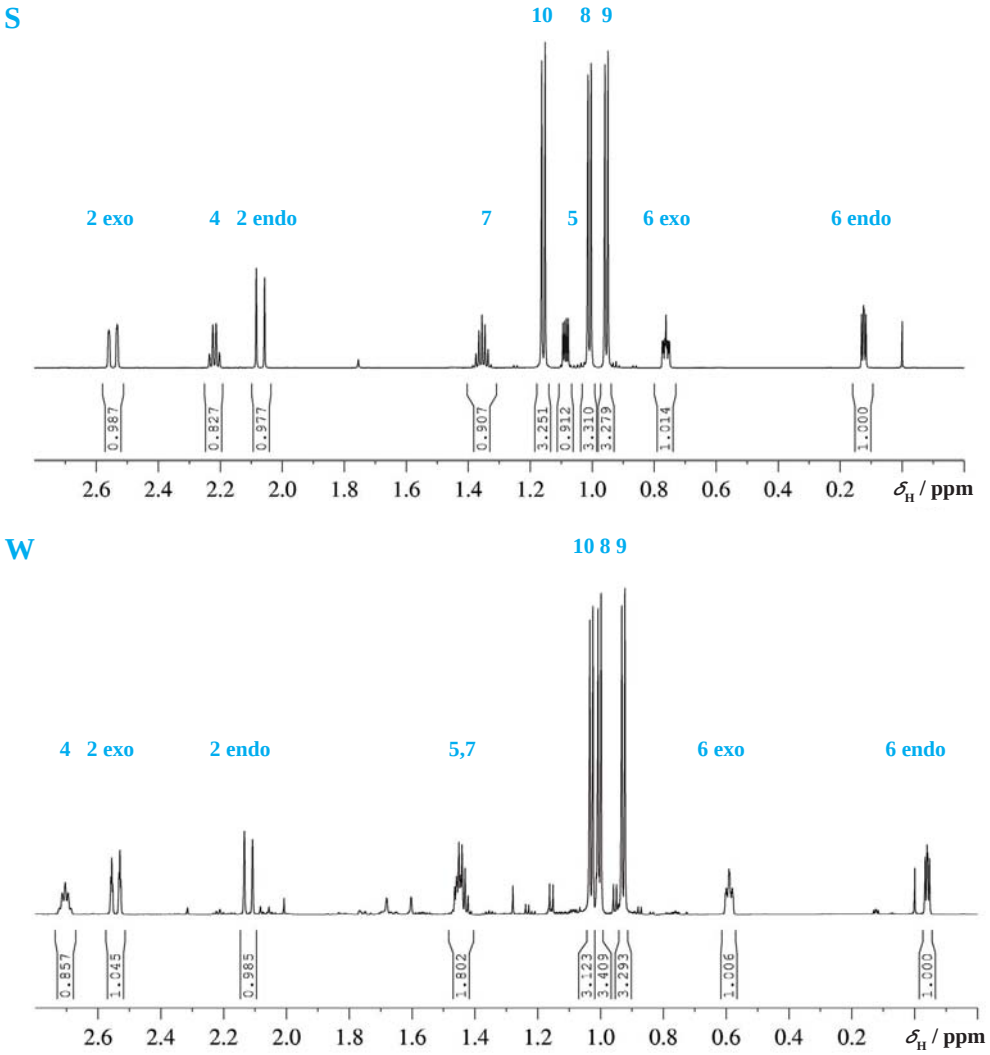


Fig. 5.3-10 ^{1}H NMR spectra at 700 MHz in $CDCl_3$

The ^{1}H NMR spectra of the two compounds isolated from sage (**S**) and from wormwood (**W**) both show minor signals from the other isomer since the complete separation of the isomers proved to be very difficult. Both show the signals of three methyl groups as doublets. The two diastereotopic methylene protons at C-2 form in both compounds an AX spin system centred at about the same chemical shift at 2.3 ppm. From methylene protons adjacent to a carbonyl group in bicyclic systems such as camphor, it is known that an *exo*-proton more in the plane of the carbonyl group is more deshielded and the *endo*-proton more perpendicular to the C=O bond is more shielded. In addition, one has to consider the influence of anisotropy of the three-membered ring. Note that the methine proton H-4 in **W** is the most deshielded one, whereas in **S** it resonates close to the more shielded of the diastereotopic protons at C-2. This provides a hint for its stereochemistry. Furthermore, in **W** it displays an additional spin coupling to H-5, whereas in **S** only a quartet due to H-10 can be seen. Further assignments have to await the discussion of the COSY and HSQC spectra.

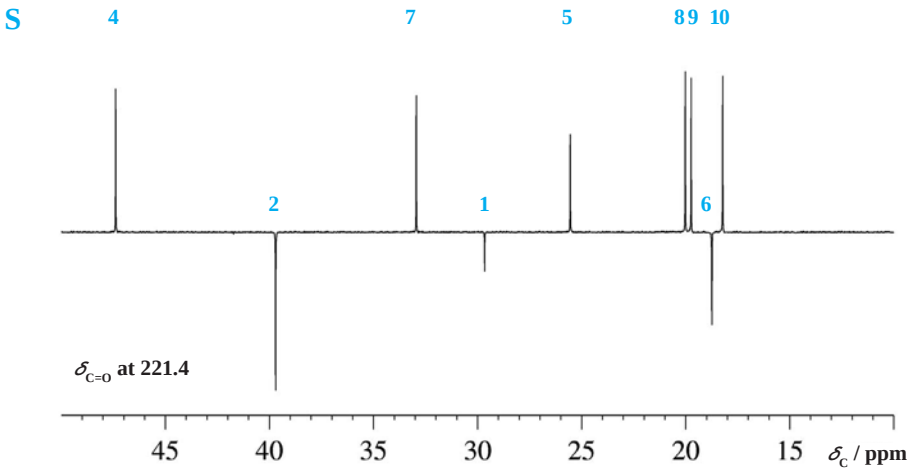
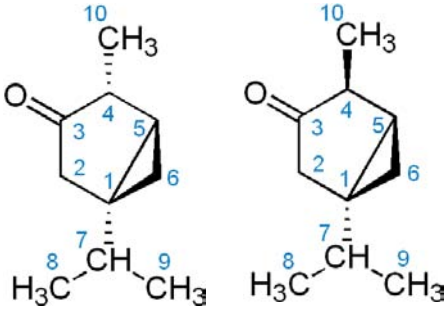


Fig. 5.3-11 APT ^{13}C NMR spectrum at 175 MHz in $CDCl_3$ for compound **S**



Scheme 5.3-2

Comparing the two APT ^{13}C NMR spectra, one first realizes that the sequence of up and down signals is equal, although there are considerable chemical shift differences. For instance, a methyl group signal is about 6 ppm more shielded in **W**. Similarly, the methylene group in the shielded region, which must belong to the cyclopropane ring is considerably more shielded in **W** than in **S**. Both observations can be attributed to a γ-steric effect between the methyl group C-10 and the cyclopropane ring, and this gives a strong hint for assigning the spectra to the stereoisomeric structures.

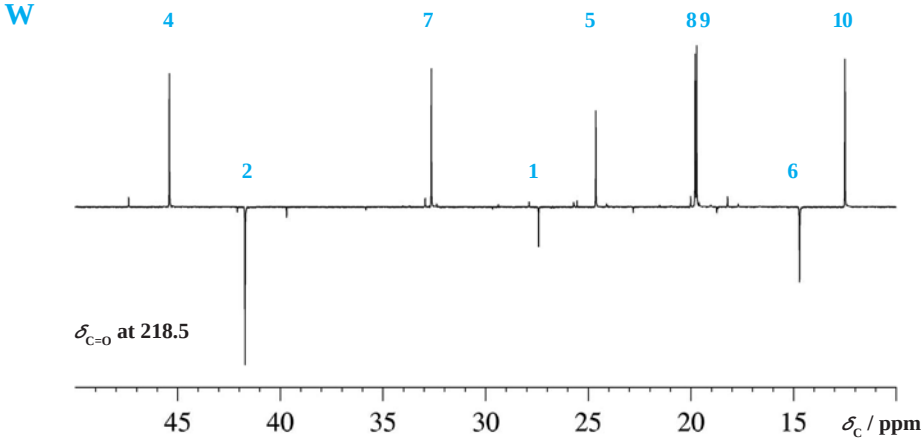


Fig. 5.3-11 APT ^{13}C NMR spectrum at 175 MHz in $CDCl_3$ for compound **W**

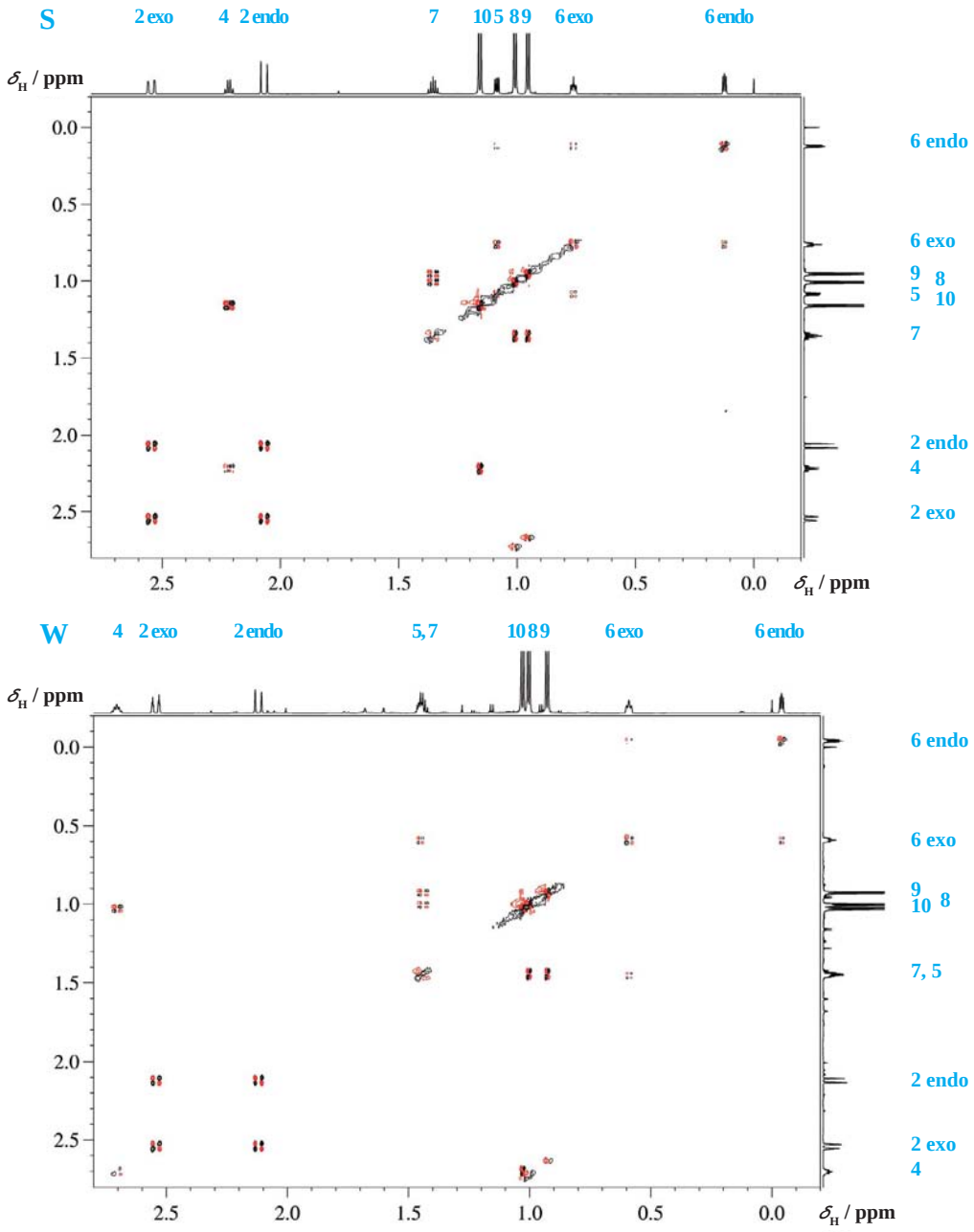


Fig. 5.3-13 Double quantum filtered COSY spectra

From the COSY spectra, it becomes immediately clear which of the methyl group signals belong to the isopropyl group and which to the methyl group C-10 due to the observation of the cross peaks with H-4 and H-7. It is of interest, that in **W** the signal of H-5 collapses with that of H-7, whereas in **S** this signal appears between the methyl group signals. H-5 and the two diastereotopic protons H-6 form an ABX spin system. Remarkably, one of the protons H-6 in **W** is even more shielded than TMS, which clearly demonstrates that it is bound to the cyclopropane unit.

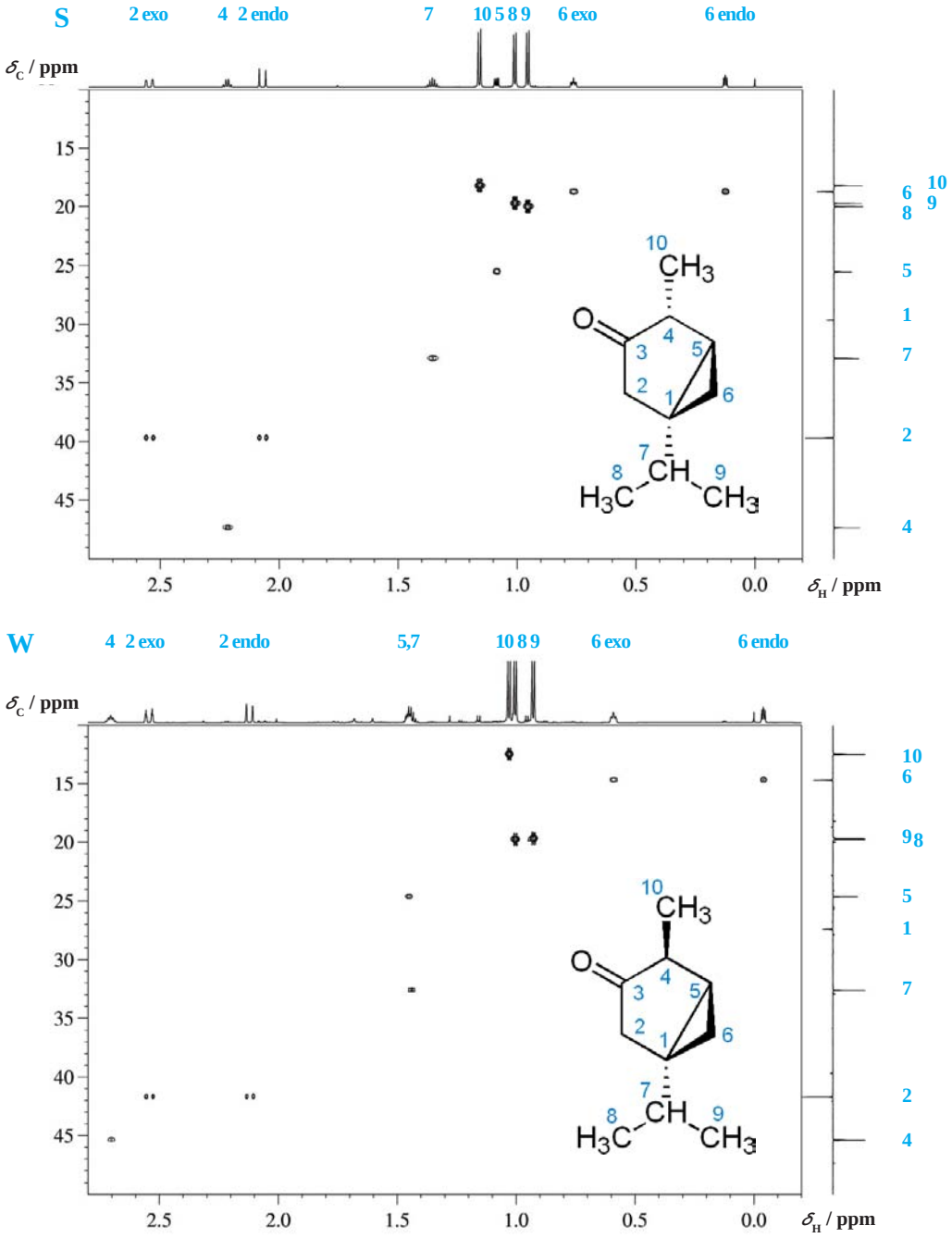


Fig. 5.3-14 HSQC spectra

The HSQC spectra corroborate that the previously assigned protons H-6 are diastereotopic and bound to the same carbon atom. Their individual assignment can again be obtained following the argument of the carbonyl-induced chemical shift anisotropy. In compound **W** the 6-*endo* proton sitting above the C=O plane will be more shielded. In compound **W** the methine proton at C-7 coincides with the proton signal of H-5, whereas in compound **S** these are nicely separated.

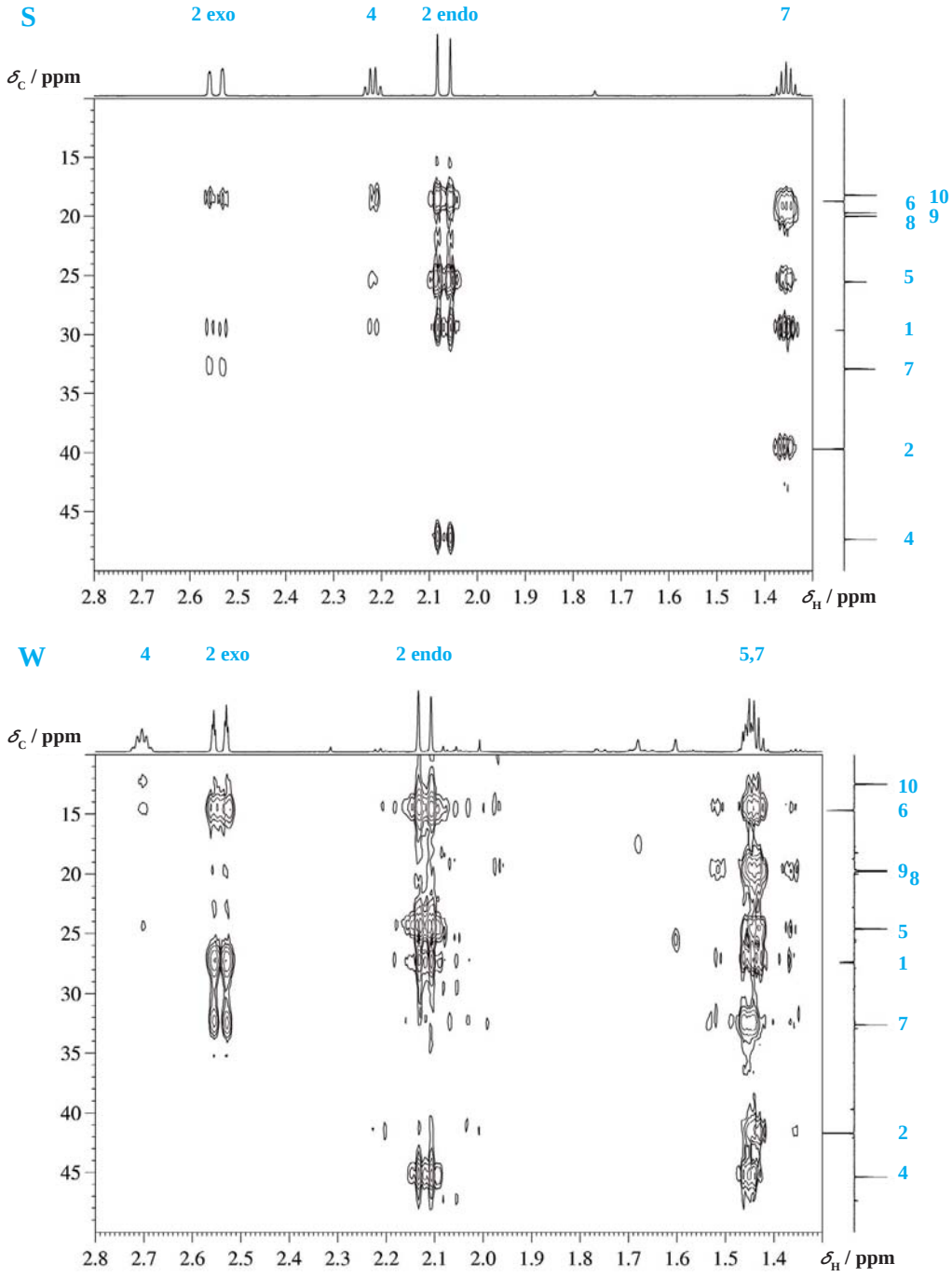


Fig. 5.3-15 Expansions of the HMBC spectra

The above assignments can be corroborated by looking at the expansions of the HMBC spectra. However, the cross peaks displayed here are not very helpful in terms of stereochemistry.

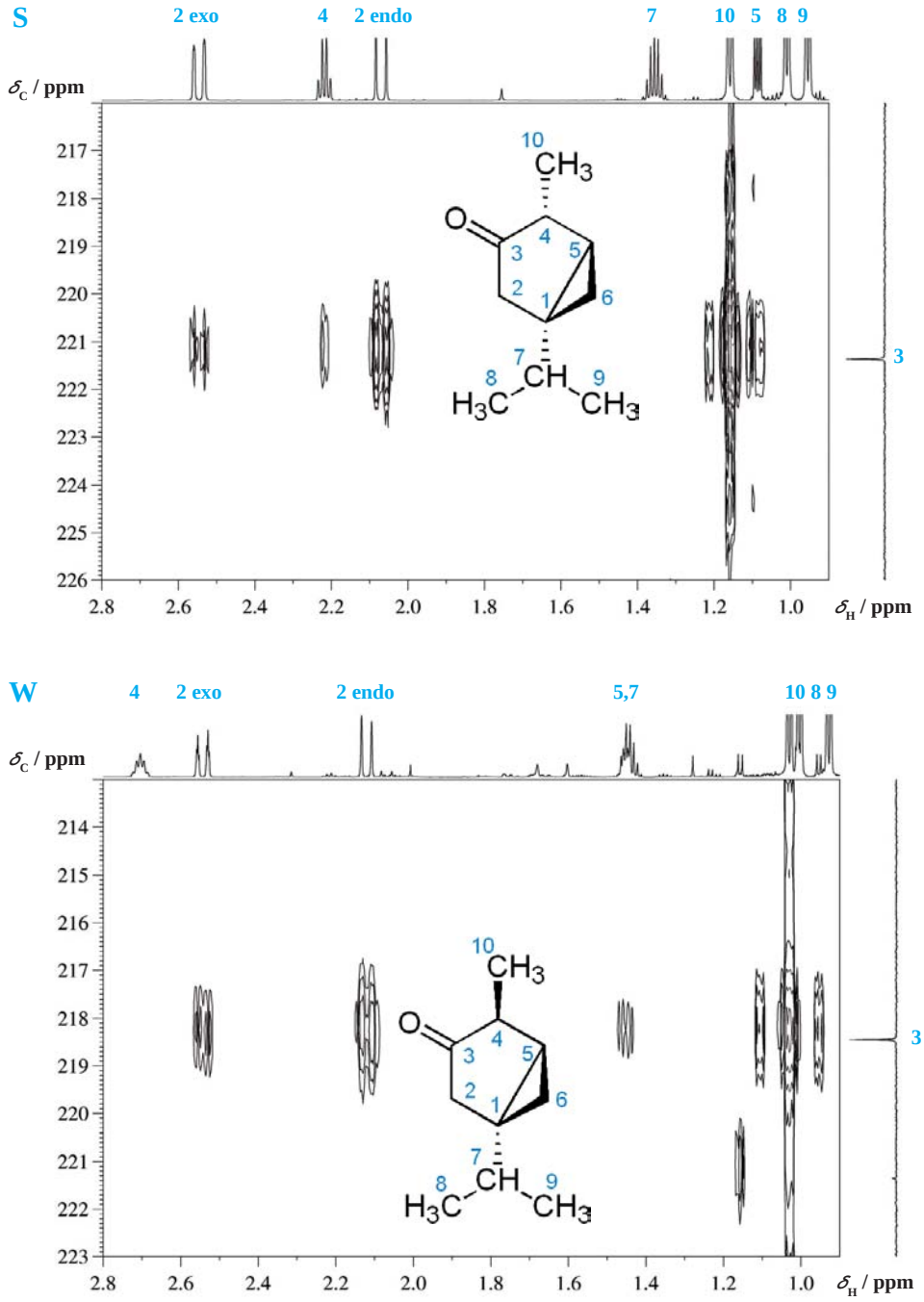


Fig. 5.3-16 Expansions of the HMBC spectra in the CO region

In the second HMBC expansion, it can be seen that the carbonyl group is recognized by both diastereotopic protons H-2, the methyl protons H-10 and the methine protons H-4 and H-5. Again, stereochemical consequences are difficult to judge.

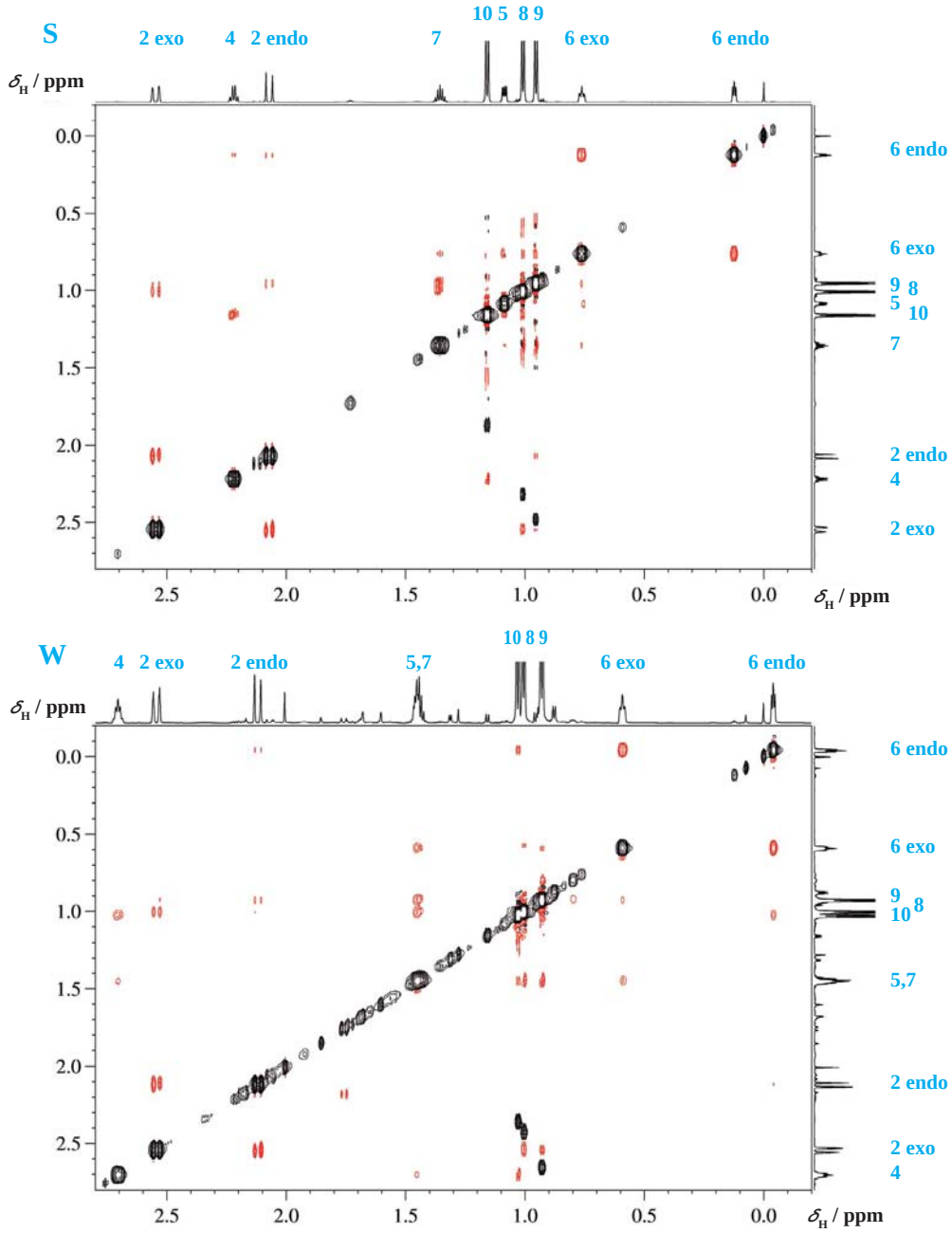


Fig. 5.3-17 NOESY spectra

All this information, however, does not give, at least not in a clear cut way the answer as to which of the spectra belong to α- and which to β-thujone. This can be, however, proven by careful inspection of the NOESY spectra. The most shielded methylene proton *endo*-6 displays an NOE cross peak to methyl group H-10 in compound **W** but not in **S**. In **W** and **S**, proton H-5 gives an NOE cross peak to the less shielded cyclopropane proton of C-6, which secures the relative assignment of these diastereotopic protons. In contrast to **S**, an NOE cross peak between H-5 and H-4 is present in **W**.

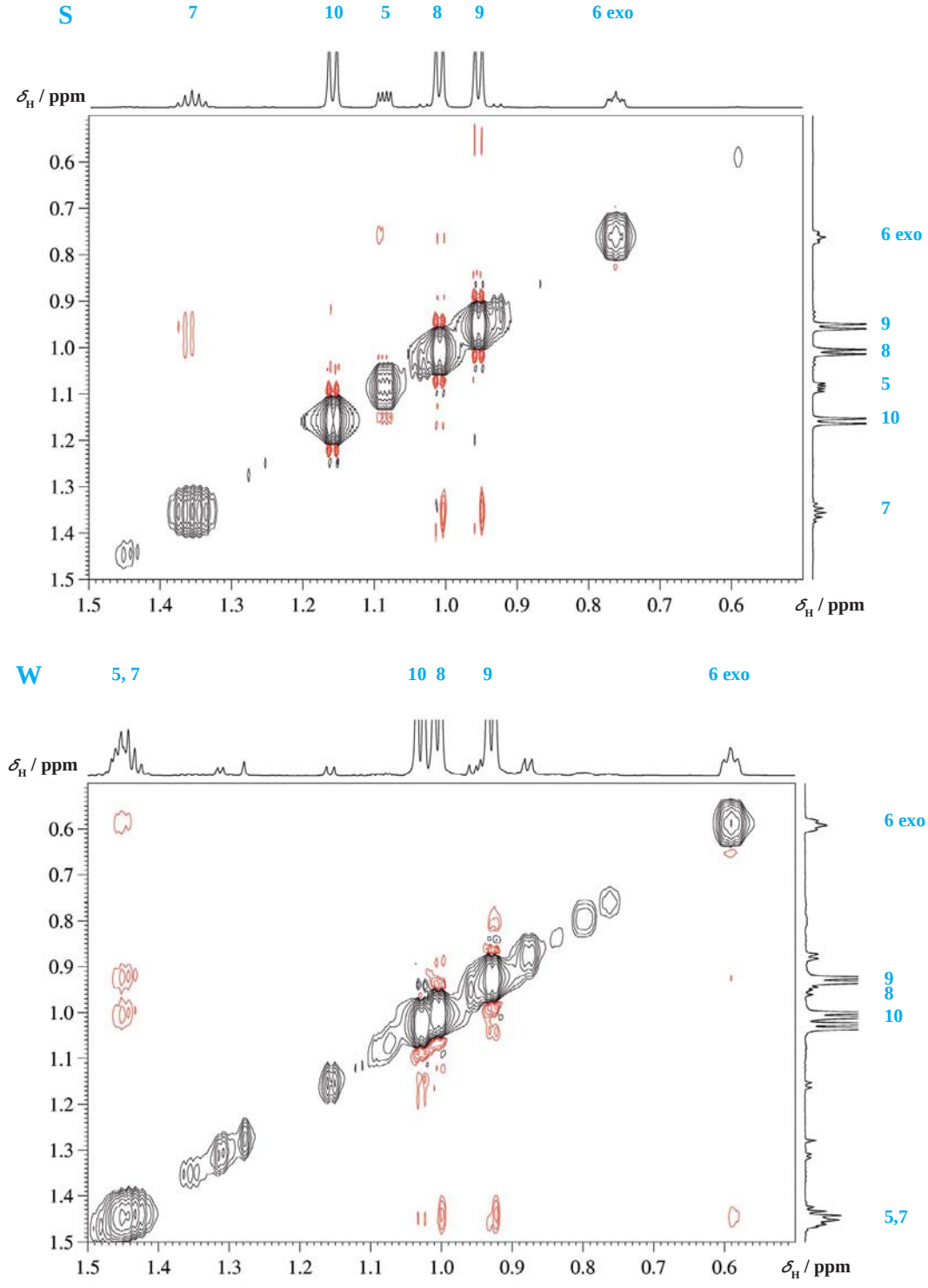


Fig. 5.3-18 Expansions of the NOESY spectra in the aliphatic region

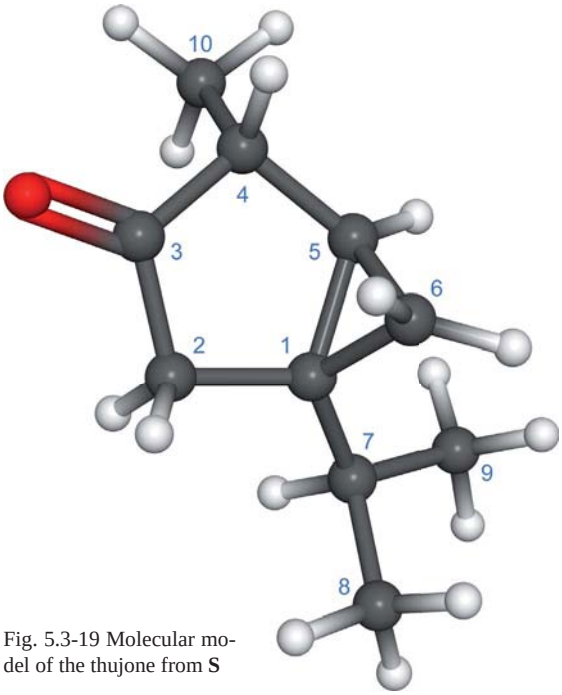


Fig. 5.3-19 Molecular model of the thujone from **S**

Fig. 5.3-20 Meadow sage (*Salvia pratensis* L.)

Fig. 5.3-21 Wormwood bush

Saltimbocca alla Romana

4 veal scaloppini slices
4 prosciutto slices
4 leaves of fresh sage
2 ounces (60 g) flour
salt
2 to 3 tablespoons extra-virgin olive oil
3 tablespoons (40 g) butter
pepper
1/2 cup (120 mL) dry white wine

Instructions
Put the flour on a large plate and add a pinch of salt. Dredge the veal slices in the flour, so that they are all well covered on both sides. Shake away the excess flour. Place a slice of prosciutto and a leaf of sage on each piece of meat. Secure the three together with a toothpick.
In a large frying pan, put the oil and the butter, and turn the heat to medium. When the butter begins foaming, place the meat in the pan, add salt and pepper, and fry gently on both sides until light brown. Add the wine, turn the heat to medium high, and let the wine evaporate.
Place on individual plates, covering the slices with the sauce and serve warm.

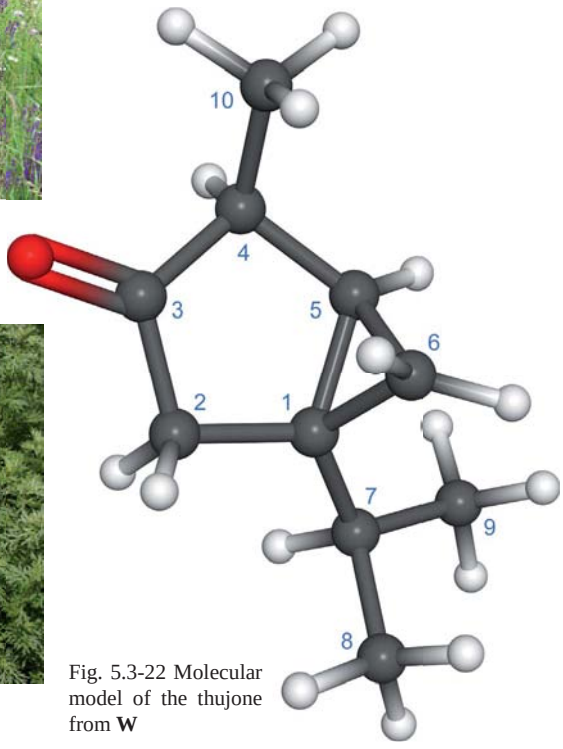


Fig. 5.3-22 Molecular model of the thujone from **W**

Fig. 5.3-23 “Die grüne Stunde” (“The green hour”) − a box of chocolates filled with a green cream flavoured with some absinthe

Fig. 5.3-24 Close-up of a sage blossom

The first stage is like ordinary drinking, the second when you begin to see monstrous and cruel things, but if you can persevere you will enter in upon the third stage where you see things that you want to see.

Oscar Wilde (1854−1900) on absinthe

Fig. 5.3-25 Meadow sage

Thujone from sage			
^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz
221.4	C_q	C-3	
47.4	CH	C-4	2.22, J = 7.6
39.7	CH_2	C-2	2.55/2.07 J = –18.8
32.9	CH	C-7	1.36, J = 6.9
29.7	C_q	C-1	
25.5	CH	C-5	1.09, J = 8.2, 4.0
20.0	CH_3	C-8	0.96, J = 6.9
19.7	CH_3	C-9	1.01, J = 6.9
18.7	CH_2	C-6	0.76/0.12, J = –5.6, 4.1
18.2	CH_3	C-10	1.16, J = 7.6

Thujone from wormwood			
^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz
218.5	C_q	C-3	
45.4	CH	C-4	2.70
41.7	CH_2	C-2	2.54/2.12, J = –18.4
32.6	CH	C-7	1.45
27.4	C_q	C-1	
24.6	CH	C-5	1.44
19.8	CH_3	C-8	1.00, J = 6.85
19.7	CH_3	C-9	0.93, J = 6.85
14.7	CH_2	C-6	0.59/– 0.04, J = – 5.8, 4.2
12.5	CH_3	C-10	1.03, J = 7.05

Table 5.3-1 NMR data for thujones

S

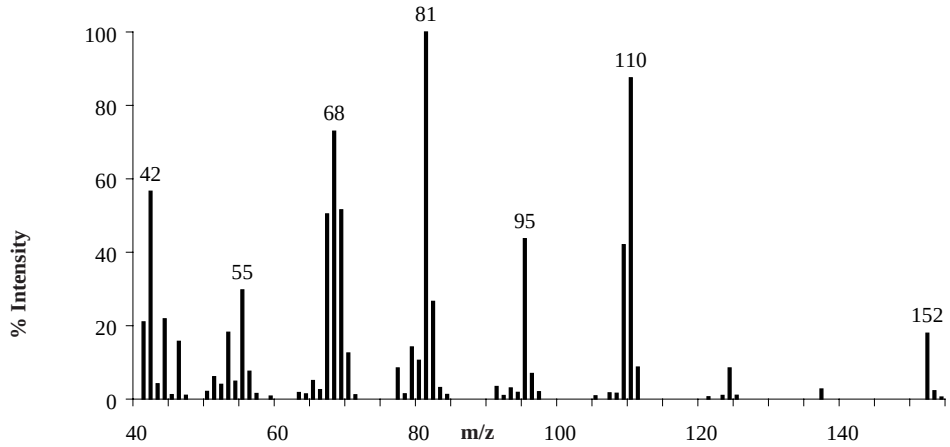
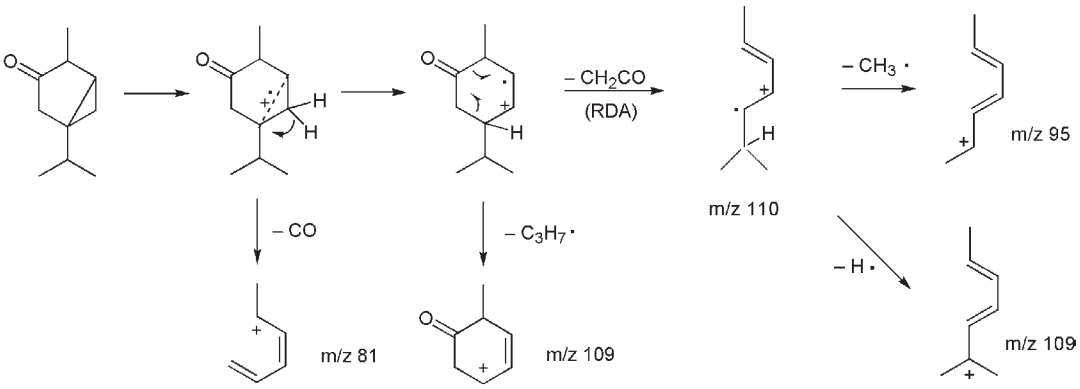


Fig. 5.3-26 Mass spectrum (EI)

The electron impact mass spectra of both isomers are very similar, therefore only one (from sage) is depicted. The peak at m/z = 110 can be explained by loss of ketene from the molecular radical cation after being ionized in the three-membered ring and a hydrogen transfer. This ion can lose a methyl radical to form the ion with m/z = 95. The molecular ion can also split off the isopropyl radical and form the ion with m/z = 109, or eliminate CO and the isopropyl radical to form the base peak at m/z = 81.



Scheme 5.3-3 Fragmentation of thujones

5. Questions

It is essential to build molecular models of both isomers to inspect the stereochemical consequences of the NMR spectra, or to use the webseite given in the preface.

A. Add together all arguments for the decision as to whether the spectra of compounds **S** and **W** belong to either α- or β- thujone. Perform your final assignment.

B. Both thujones have three chiral centres. How many stereoisomers would you on principle expect from this number? Analyse the configurations of all stereoisomers theoretically and decide for which it is possible or impossible to exist as organic compounds. What is the structural reason for the limited number of stereoisomers?

C. In our separation experiments we were unable to achieve a clean separation of α- and β-thujone. It seems that especially higher temperature gives rise to isomerization to a certain degree. Suggest a possible mechanism based on the chemical structure.

D. Camphor is another monoterpene ketone with the molecular formula $C_{10}H_{16}O$. Draw the structure of D-camphor. Which kind of isomerism to the thujones do you find? Analyse the stereochemistry of the camphor constitution similar to that of the thujones in question 1. How many stereoisomers are to be expected, and how many are found in reality?

E. Explain the basic principle of steam distillation.

F. Discuss the sign of the CD effect for both compounds **S** and **W** using the octant rules.

G. How would one be able to assign the diastereotopic methyl groups C-8 and C-9 individually?

H. In the first expansion of the HMBC spectrum shown, are for both compounds there different cross peaks from the two protons H-2 to the different carbon atoms and others missing. Explain.

I. Why has proton H-4 an additional coupling only in one of the compounds?

J. What do the minor peaks in the mass spectrum at m/z = 137 and 124 suggest? Try to find a mechanism for the signal at m/z = 67.

6. Own Observations

5.4 Patchouli alcohol

(1*R*,4*S*,4a*S*,6*R*,8a*S*)-Octahydro-4,8a,9,9-tetramethyl-1,6-methanonaphthalen-1(2*H*)-ol

From patchouli
the essential oil of the patchouli plant
Pogostemon cablin Benth. (Lamiaceae)

$C_{15}H_{26}O$, MW 222.36

CAS RN 5986-55-0, BRN 3537894, 9921816

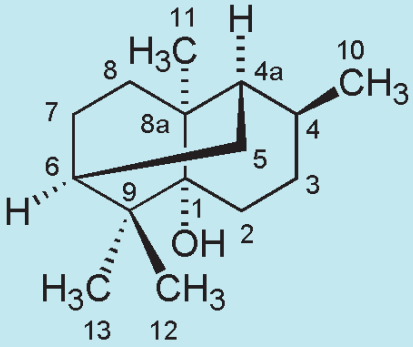
Colourless plates, mp 56 °C, bp 266–271 °C

$[\alpha]_D^{22}$ −119° (*c* 0.1 g/mL, chloroform)
measured on an authentic reference sample.

Patchouli alcohol is commercially available.

Synonymous names:
(–)-Patchouli alcohol, (–)-Patchoulol, Patchoulol

Level: difficult



1. Background: Learn a word of Tamil every day

Often the Greek has been the root for a word of a plant or a natural product discussed in this book. This time the difference could not be greater. Patchouli descends from the words *patchai* (for green) and *ellai* (for leaf) from a classical language of India: Tamil. I remember a refectory in an Indian university that I visited in 1996 on a scientific round trip through India. A large green board was standing at the entrance with a request to any visitor: *Learn a word of Tamil every day!* A large sign was drawn below and my host told me the sense. It was something useful from everyday life (that I forgot), and certainly not patchai ellai. But I found the campaign impressive: *As many languages you know as many lives you live* − the poets say. Tamil is a Dravidian language and official in the Indian state of Tamil Nadu, in Sri Lanka and in Singapore. It is not a specialty today; about 77 million people use it every day. Documents written in Tamil date back for about 2300 years. The writing has a beautiful appearance, if you see it in artistic perfection. Of course, this can be achieved with any language, if the writer has time enough. There is an everyday form that allows mechanical printing of newspapers and books. The characters are rational therein; nevertheless, they look extraordinary (see the Tamil words *patchai ellai* for patchouli in the margin).

ð„¬ê Þ¬ô

Fig. 5.4-1 The words patchai ellai in the Tamil font

The plant *Pogostemon cablin* is a perennial bushy herb. It belongs to the family Lamiaceae and grows in India, for example. It requires warm areas with a temperature higher than 12 °C. The plant is not frost resistant. It is about 1 m in height and has about 7 cm long leaves. The photograph on the first page of this section gives a good impression. Steam distillation of the leaves gives rise to patchouli as the essential oil. This distillation may be done with fresh leaves close to a plantation (preferably) or elsewhere with dried and in this state exported leaves. The yield of essential oil is astonishingly high: only 35–50 kg of fresh leaves yield 1 kg of patchouli. A main component of the viscous oil is patchouli alcohol (patchoulol).

The scent of patchouli is absolutely characteristic, intense, lasting and not to everyone's liking. It reached Europe during the 18th and 19th centuries in colonial times with silk and other fabrics from India and the Middle East. Traders used to pack these goods together with some patchouli leaves to prevent moths from laying their eggs between the fabrics. Thus, patchouli was used as a natural insect repellent. For Europeans, it was just the strange patchouli scent that served as evidence of authenticity for any eastern goods soaked with it. Because only really valuable things were traded over such long distances an association of luxury became firmly connected with this scent. Until today patchouli is an expression of something special. The scent is described as strongly woody, sweet balsamic, herbaceous and earthy. If you got a tiny amount patchouli on your finger you would find it very tenacious, with a lasting smell. It has the unusual property of becoming better in its scent quality on storage. This is likely due to the formation of oxidation products with additional smell properties.

Hier blieb er stehen, sammelte sich und roch. Er hatte ihn. Er hielt ihn fest. Wie ein Band kam der Geruch die Rue de Seine herabgezogen, unverwechselbar deutlich, dennoch weiterhin sehr zart und sehr fein. Grenouille spürte, wie sein Herz pochte, und er wußte, daß es nicht die Anstrengung des Laufens war, die es pochen machte, sondern seine erregte Hilflosigkeit vor der Gegenwart dieses Geruches. Er versuchte sich, an irgendetwas Vergleichbares zu erinnern und musste alle Vergleiche verwerfen. Dieser Geruch hatte Frische; aber nicht die Frische der Limetten oder Pomeranzen, nicht die Frische von Myrrhe oder Zimtblatt oder Krauseminze oder Birken oder Kampfer oder Kiefernnadeln, nicht von Mairegen oder Frostwind oder von Quellwasser…, und er hatte zugleich Wärme.

Patrick Süskind (1949−)
Das Parfüm, Die Geschichte eines Mörders, Teil I

Patchouli's connection to the spirit of the times is interesting. Both incense and patchouli had a surge in popularity with the hippie lifestyle and the devotees of free love in the 1960s. One reason was that the pungent smell of patchouli served as a camouflage for the odour of burning cannabis. Another driving force for the worldwide spread of patchouli was the Hare Krishna movement with its belief that the God Krishna inhabits patchouli. Today, patchouli belongs to the ingredients of perfumery used by the Goth subculture that began in the 1980s and is active, e.g. in the annual German Wave-Gotik meeting. Patchouli is also an ingredient of East Asian incense.

Although patchouli from this end is often associated with an alternative lifestyle, it is widely used also in classic and modern perfumes for high-end products, and also in perfumes with oriental notes for men. A reason is that besides its characteristic smell it has nearly ideal volatility properties.

However, there is more to it than that. Medical science attributes a neural sedative and wound healing effect to patchouli oil. Some also regard it as an aphrodisiac, … some certainly. In Asia, the patchouli plant is used as a medicinal herb with antifungal, antiseptic and antirheumatic effects. The insecticidal use to protect clothing from webbing clothes moths and silverfishes was already mentioned above. Did you expect such diversity when you smelt a breeze of patchouli the last time?

Patchouli alcohol is a tricyclic tertiary sesquiterpene alcohol. The assignment of its complex structure took more than a century. In this case, all attempts to determine the structure by classical chemical means of degradation and comparison of the degradation products with known compounds failed, although leading terpene scientists such as Treibs, Büchi and others were dealt with this challenging question again and again.

In 1869, Gal mentioned that crystals separate from patchouli on standing (mp 54−55 °C, bp 296 °C). They were given the name patchouli camphor and regarded as a homologue of Borneo camphor [1]. The molecular formula was believed to be $C_{30}H_{28}O_2$, which he later corrected to $C_{15}H_{28}O$. The correct molecular formula was found by de Montgolfier [2] to be $C_{15}H_{26}O$. The first chemical feature discovered was the ease of dehydration to form a hydrocarbon, $C_{15}H_{24}$. Wallach was the first to conclude from this behaviour that patchouli alcohol (as he called it) was a tertiary alcohol [3]. The hydrocarbon $C_{15}H_{24}$ formed was named patchoulene. Eventually, this structural problem was resolved by a physical method. In 1963, the groups of Dunitz and Büchi solved the problem by means of an X-ray structure of the chromic acid diester of patchouli alcohol [4]. Pachouli alcohol is not only sold in bulk as a fragrance, however; it also proved to be a valuable member of the chiral pool. In 1984, Holton reported a five-step synthesis of the tricyclic taxane diterpene ring system starting from patchouli alcohol as a source of β-patchouline oxide [5].

The world production per year is about 500 tons of patchouli oil, a freight train filled with an exceptional smell. One can guess that about

Old Jolyon went in for a minute to say good-bye. The little dark hall of the flat was impregnated with a disagreeable odour of patchouli, and on a bench against the wall − its only furniture − he saw a figure sitting. He heard Irene say softly: "Just one minute." In the little drawing-room when the door was shut, he asked gravely: "One of your protegées?" "Yes. Now thanks to you, I can do something for her."

John Galsworthy (1867−1933)
The Forsyte Saga (II)

Fig. 5.4-2 Incense sticks from India

half of it is patchouli alcohol, the compound in this section. If you are interested in classical French techniques of scent extraction apart from steam distillation, such as maceration and enfleurage, and in the emotional effect of fragrances on people, you will be fascinated by the German movie *Perfume: the story of a murderer*, filmed after the international bestseller novel of the same name written by the German writer P. Süskind in 1985. Grenouille, the protagonist of the story is born without having a personal odour, a lack that is balanced by its destiny by endowing him with an incomparable sense of smell. Although a poor devil, he eventually succeeds in becoming an apprentice of an old and unsuccessful perfumer. Soon, his "teacher" becomes rich from the perfumes that Grenouille creates for him in imaginative tempests of creation. Aware of its extraordinary abilities, Grenouille, an anchorite despite his astonishing abilities, had the obsession of creating the perfect smell that would make him fully human and thus equal with his environment. The tragedy consists in murdering a series of young girls to take their personal pheromones. This cannot be hidden and makes him an outlaw. However, the scents thus collected and processed by him prove to be the basis of the most powerful perfume ever made. Some drops sprayed save him even on the scaffold from his execution as a murderer. But nothing makes him a lucky man. The tragic end is inevitable – and triggered by his incomparable last perfume becoming murderous to himself.

2. Literature

[1] Anonymous. "Ueber eine dem Borneocampher homologe Verbindung" [On a compound homologous to Borneo camphor] *J. Prakt. Chem.* **1869**, *107*, 182−183.

[2] A. Henninger. Untitled *Ber. Dtsch. Chem. Ges.* **1877**, *10*, 232−239.

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[4] M. Dobler, J. D. Dunitz, B. Gubler, H. P. Weber, G. Büchi, J. O. Padilla, "The structure of patchouli alcohol" *Proc. Chem. Soc.* **1963**, 383.

[5] R. A. Holton, "Synthesis of the taxane ring system" *J. Am. Chem. Soc.* **1984**, *106*, 5731−5732.

[6] A. Neszmelyi, G. Lukacs, "Natural abundance $^{13}C-^{13}C$ coupling constants for carbon connectivity. Pattern determination and NMR spectral analysis: patchoulol" *J. Chem. Soc. Chem. Commun.* **1981**, 999−1001.

[7] F. Naf, R. Deconant, W. Giersch, G. Ohloff, "A stereocontrolled access to (±)- and (+)-patchouli alcohol" *Helv. Chim. Acta*. **1981**, *64*, 1387−1397.

J'épie Adrienne Septmance. Elle chante, bouscule son travail, court dans la rue, rit haut, sur un ton factice.

Je respire autour d'elle ce parfum commun, qu'on achète ici chez Maumond, le coupeur des cheveux, ce parfum qu'on respire, semble-t-il, avec les amygdales et qui fait penser à l'urine sucrée des chevaux, séchant sur les routes…

– Adrienne, vous sentez le patchouli! décrète ma mère, qui n'a jamais su ce qu'était le patchouli…

Enfin je rencontre, dans la cuisine, un jeune gars noir sous son chapeau de paille blanche, assis contre le mur et silencieux comme un garçon qui est là pour le bon motif. J'exulte, et ma mère s'assombrit.

Sidonie-Gabrielle Colette (1873−1954)
La Maison de Claudine, La Noce

3. Isolation

3.1 Principle

It is really difficult to isolate pure patchouli alcohol from patchouli as a source. The problem is that patchouli consists of ca. 50% patchouli alcohol ($C_{15}H_{26}O$) and a mixture of structurally very similar sesquiterpenoid compounds, e.g. patchoulenol ($C_{15}H_{24}O$), patchoulenone ($C_{15}H_{22}O$), norpatchoulenol ($C_{14}H_{22}O$), nortetrapatchoulol ($C_{14}H_{22}O$), α-patchoulene ($C_{15}H_{24}$), β-patchoulene ($C_{15}H_{24}$), seychellene ($C_{15}H_{24}$), α-guajene ($C_{15}H_{24}$) and α-bulnesene ($C_{15}H_{24}$). The molecular formulae give an idea of the similarities in the vapour pressure of these compounds. Furthermore, such relatives form eutectic mixture, which induces a reduced tendency for the crystallization of a single compound. Although patchouli used for this isolation was stored over a longer period of time in a refrigerator, no crystallization was observed. However, TLC shows that patchouli alcohol is the most polar of these compounds (smallest R_f value).

This corresponds with the finding that on fractional distillation in vacuo it is enriched in the last fraction and shows a comparably high boiling point. Both effects are caused by intermolecular hydrogen bonding between the OH groups. In our hands, a combination of distillation followed by cooling to cause crystallization from the pure distillate or its pentane solution as mentioned in the literature was not successful. However, this may be due to the different qualities of patchouli used as starting material. The starting source always is a critical matter in natural product isolation. The material can differ in its composition for both natural, i.e. seasonal, and regional reasons without a possibility for the user to influence this. The art is to adapt an existing method to the material available by appropriate variations. In the next section we will describe our own efforts at crystallization of patchouli alcohol – maybe they will work well with your material. Eventually, the TLC behaviour suggested a combined procedure of column chromatography on silica gel, followed by a preparative RP-HPLC separation. It gave rise to enough pure patchouli alcohol on the milligram scale for the measurement of all spectra reported.

3.2 Method

Patchouli (80 g) is placed in a 100 mL round-bottomed flask and distilled using a rotary vane vacuum pump over a 20 cm Vigreux column. Nine fractions are made. Table 5.4-1 gives their data.

Qu'est-ce qu'Adam? C'est le royaume d'Ève. Pas de 89 pour Ève. Il y avait le sceptre royal surmonté d'une fleur de lys, il y avait le sceptre impérial surmonté d'un globe, il y avait le sceptre de Charlemagne qui était en fer, il y avait le sceptre de Louis le Grand qui était en or, la révolution les a tordus entre son pouce et son index, comme des fétus de paille de deux liards; c'est fini, c'est cassé, c'est par terre, il n'y a plus de sceptre; mais faites-moi donc des révolutions contre ce petit mouchoir brodé qui sent le patchouli! Je voudrais vous y voir. Essayez. Pourquoi est-ce solide? Parce que c'est un chiffon. Ah! vous êtes le dix-neuvième siècle? Eh bien, après? Nous étions le dix-huitième, nous! Et nous étions aussi bêtes que vous. Ne vous imaginez pas que vous ayez changé grand'chose à l'univers, parce que votre trousse-galant s'appelle le choléra morbus, et parce que votre bourrée s'appelle la cachucha. Au fond, il faudra bien toujours aimer les femmes. Je vous défie de sortir de là.

Victor Hugo (1802−1885)
Les Miserables, Tome V Jean Valjean,
Livre VI, Chapitre II

Fraction No.	Pressure /Pa	Boiling range / °C	Mass / g	Refractive index $n_D^{22\,°C}$
1	0.8–1.3	38–43	0.7569	1.4971
2		40–43	3.7511	1.5003
3		45–54	7.4633	1.5008
4	0.1–1	45–55	6.6033	1.5015
5		48–57	9.2594	1.5022
6		58–63	5.7093	1.5030
7	4.7	63–70	5.3551	1.5040
8		70–80	5.3100	1.5051
9		80–95	4.4233	1.5077

Table 5.4-1 Distillation results for patchouli alcohol

Fractions 2–8 are allowed to stand overnight first in a normal refrigerator (−15 °C) and then in a deep cooling refrigerator (−80 °C). In no case does crystallization occur. *n*-Hexane is added to each fraction in a 1:1 ratio and the samples are again cooled to –80 °C in stoppered flasks. In no case does crystallization occur. The hexane is removed in vacuo, replaced by pentane and the procedure repeated with the same result. The pentane is removed and GC–MS data are taken from fractions 2, 5 and 9. Data for fraction 9 (base peak and fragments) give a hint that patchouli alcohol is present.

Perfumes should be used only in the evening, and then in moderation. Let your perfumes be of the most delicate and recherché kind. Nothing is more vulgar than a coarse ordinary scent; and of all coarse, ordinary scents, the most objectionable are musk and patchouli. Finally, every lady should remember that to dress well is a duty which she owes to society; but that to make it her idol is to commit something worse than a folly. Fashion is made for woman; not woman for fashion.

George Routledge (1812–1888)
Routledge's Manual of Etiquette,
Chap. VII: Dress

The TLC behaviour of fraction 9 is tested with five eluents: *n*-hexane, methanol, *n*-hexane–ethyl acetate (1:1), dichloromethane and chloroform. The last shows the best differentiation of spots on the plate with an R_f value for patchouli alcohol of 0.20 as the slowest spot. For detection, the TLC alumina foils are dipped into vanillin reagent and the spots are visualized by careful heating in the hot air stream from a heat gun. (Recipe for the reagent: dissolve 15 g of vanillin in 200 mL of ethanol and add 2 mL of concentrated sulfuric acid. Handle with care.)

3.3 Purification

Step 1: by preparative column chromatography:

Fraction 9 (3.4 g) is subjected to column chromatography under the following conditions: stationary phase, silica gel 60 (0.040–0.063 mm); eluent, chloroform; column dimensions, 60 × 3 cm. The fractions containing patchouli alcohol are identified by TLC and combined. The solvent is removed on a rotary evaporator and the colourless oily residue is allowed to stand at –15 °C overnight. The residue solidifies to yield 952 mg of colourless crystals. The purity is estimated from an ^{1}H NMR spectrum to be about 90%.

Step 2: by preparative RP-HPLC:

A 250 mg amount of the enriched patchouli alcohol from above is subjected to separation by preparative RP-HPLC under the following conditions: Vertex column Eurospher 100-C18 (5 µm); eluent, methanol; UV detector (210 nm); flow rate, 2 mL/min; sample amounts in the range 13–21 mg in 100 µL of solution. The retention time of patchouli alcohol is 23.5 min under these conditions. The fractions containing the pure patchouli alcohol are collected, combined and the solvent is removed. All spectra shown here were measured with this material.

4. Spectra and Comments

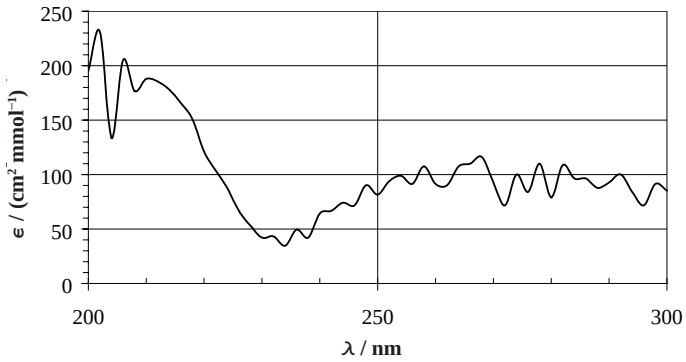


Fig. 5.4-3 UV spectrum in ethanol

Patchouli alcohol is an aliphatic alcohol and, just like ethanol, lacks any chromophore. Therefore, no UV absorption is expected, as is found in the depicted spectrum. For the same reason, one does not obtain a CD spectrum, although the compound is chiral.

"I am afraid I must be going," exclaimed Lady Henry, after an awkward silence, with her silly sudden laugh. "I have promised to drive with the duchess. − Good-by, Mr. Gray. − Good-by, Harry. You are dining out, I suppose? So am I. Perhaps I shall see you at Lady Thornbury's."
"I dare say, my dear," said Lord Henry, shutting the door behind her, as she flitted out of the room, looking like a bird-of-paradise that had been out in the rain, and leaving a faint odor of patchouli behind her. Then he shook hands with Dorian Gray, lit a cigarette, and flung himself down on the sofa.
"Never marry a woman with straw-colored hair, Dorian," he said, after a few puffs.

Oscar Wilde (1854−1900)
The Picture of Dorian Gray
[13-Chapter Version, 1890,
Chapter III]

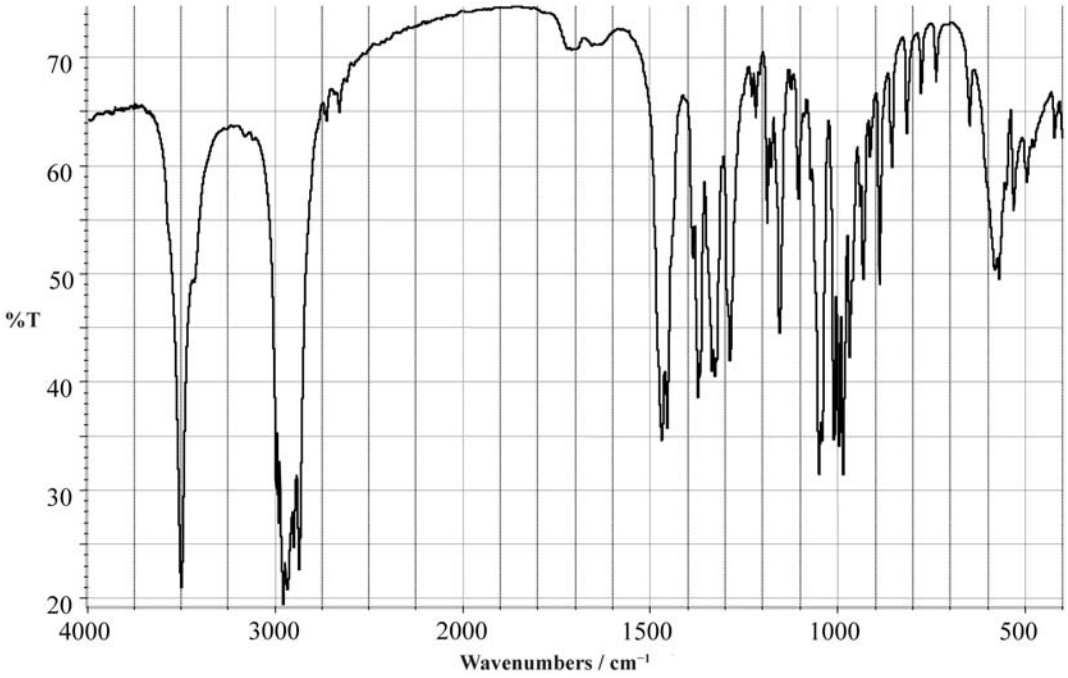
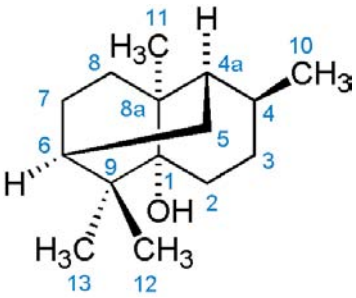


Fig. 5.4-4 IR spectrum in KBr

The compound reveals a remarkable sharp OH valence vibration band at 3500 cm^{-1}. This is probably due to the fact that patchouli alcohol is a tertiary alcohol with a rather isolated OH group. The CH valence vibrations are all well below 3000 cm^{-1} as required for a pure aliphatic compound and the CH bending vibrations start below 1500 cm^{-1}.

Fig. 5.4-5 Leaves of the patchouli plant *Pogostemon cablin* Benth



Scheme 5.4-1

Sur la toilette, les bouquets, des roses, des lilas, des jacinthes, mettaient comme un écroulement de fleurs, d'un parfum pénétrant et fort; tandis que, dans l'air moite, dans la fadeur exhalée des cuvettes, traînait par instant une odeur plus aiguë, quelques brins de patchouli sec, brisés menu au fond d'une coupe. Et, se pelotonnant, ramenant son peignoir mal attaché, Nana semblait avoir été surprise à sa toilette, la peau humide encore, souriante, effarouchée au milieu de ses dentelles.

Emile Zola (1840−1902)
Nana, Chap. II

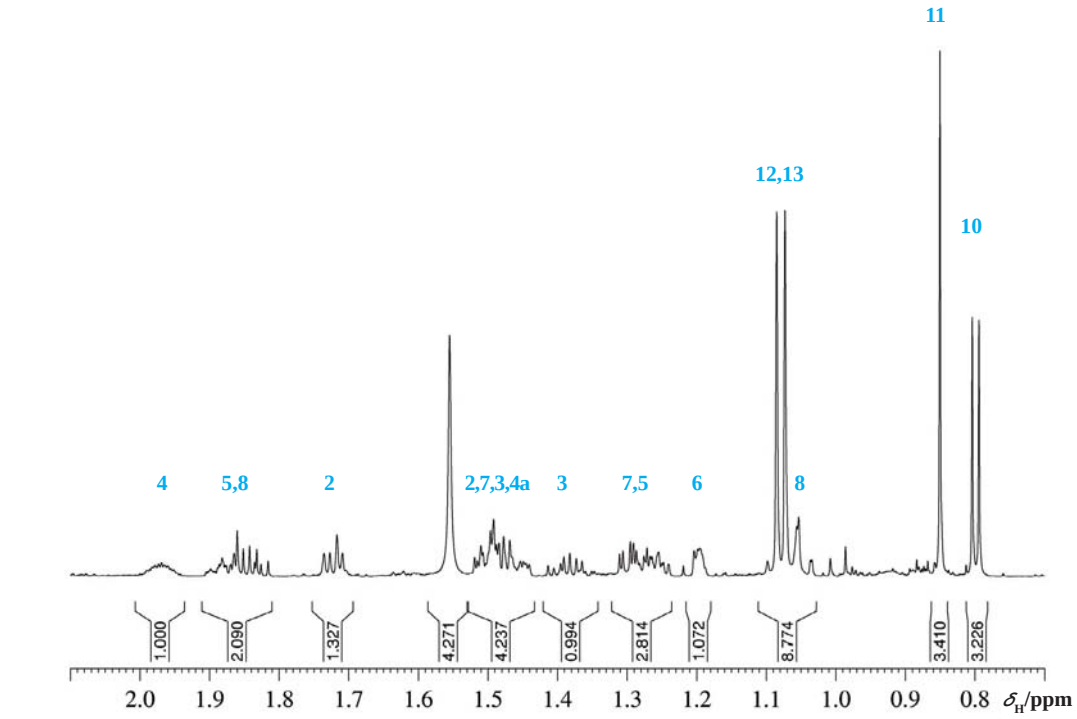


Fig. 5.4-6 ^{1}H NMR spectrum at 600 MHz in $CDCl_3$

Typically for a pure alicyclic compound, we only find resonances between 0.8 and 2 ppm; these are, however, well dispersed. Looking at the four methyl group signals, their assignment is straightforward since only the methyl group H-10 will form a doublet at 0.8 ppm with H-4. The angular methyl group H-11 forms a sharp singlet at 0.85 ppm and the two diastereotopic methyl groups H-12 and H-13 located on the same carbon atom C-9 resonate closely together at 1.08 ppm, in comparison with H-10 somewhat deshielded due to the vicinity of the OH group. Their individual assignment is not yet possible at this stage.

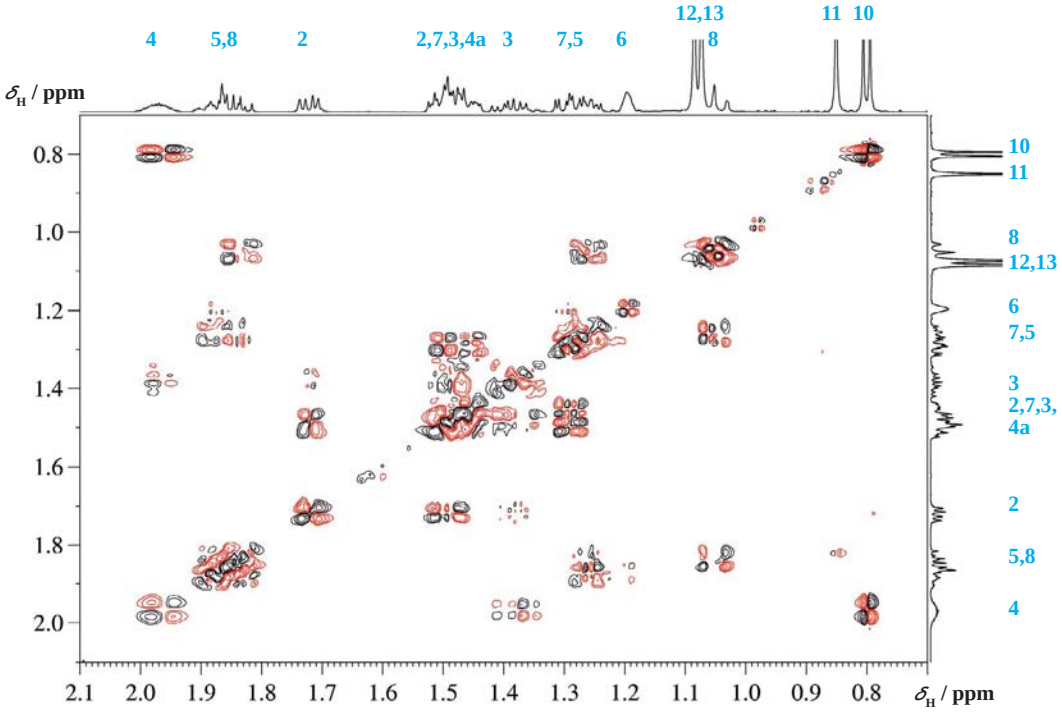
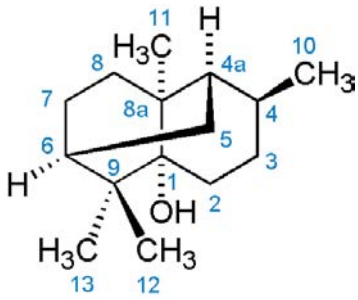


Fig. 5.4-7 Double quantum filtered COSY spectrum

Since we have already safely assigned H-10, the COSY spectrum identifies H-4 at 1.97 ppm by the corresponding cross peaks. A further cross peak starting from H-4 leads to the multiplet at 1.38 ppm integrating for one proton. Inspecting the edited HSQC, we find that this belongs to a methylene group with the other proton at 1.48 ppm, and we therefore assign these two multiplets to the methylene group H-3. From the proton H-3 at 1.38 ppm a faint cross peak leads to the diagonal signal at 1.72 ppm, which is therefore tentatively assigned to H-2. The corresponding H-2 proton on the same carbon atom can then be found at 1.49 ppm. Further assignments from the COSY spectrum alone are insecure at this point of the discussion.

Calyste lui disposa dans cette direction un grand fauteuil gothique et ouvrit la croisée à vitraux. Camille Maupin, qui partageait le goût oriental de l' illustre écrivain de son sexe, alla prendre un magnifique narghilé persan que lui avait donné un ambassadeur; elle chargea le cheminée de patchouli, nettoya le bochettino, parfuma le tuyau de plume qu'elle y adaptait, et dont elle ne se servait jamais qu'une fois, mit le feu aux feuilles jaunes plaça le vase à long col émaillé bleu et or de ce bel instrument de plaisir à quelques pas d'elle, et sonna pour demander du thé .

Honore de Balzac (1799−1850)
Beatrice, Chap. VII



Scheme 5.4-2

Fig. 5.4-8 Offers of a perfumery specializing on the Goth subculture

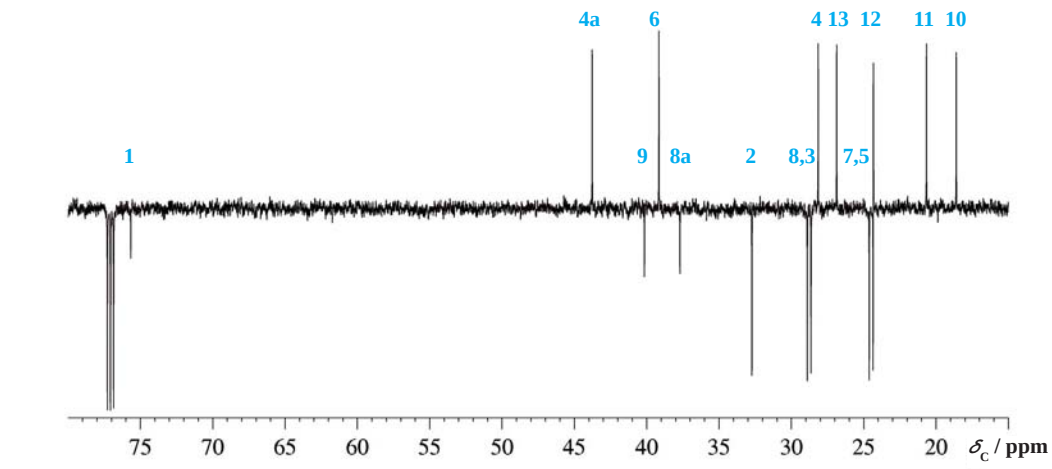


Fig. 5.4-9 APT ^{13}C NMR spectrum

The spectrum reveals 15 signals as required by the 15 different carbon atoms of patchouli alcohol. Three signals are small and negative and therefore belong to the three tertiary carbon atoms. Immediately assigned can be C-1 at 75.7 ppm due to its typical chemical shift for a quaternary alcoholic carbon atom. The five methylene groups give the corresponding five and strong negative signals; however, a strict differentiation between the positive CH_3 group signals and the CH signals is not yet possible at this stage.

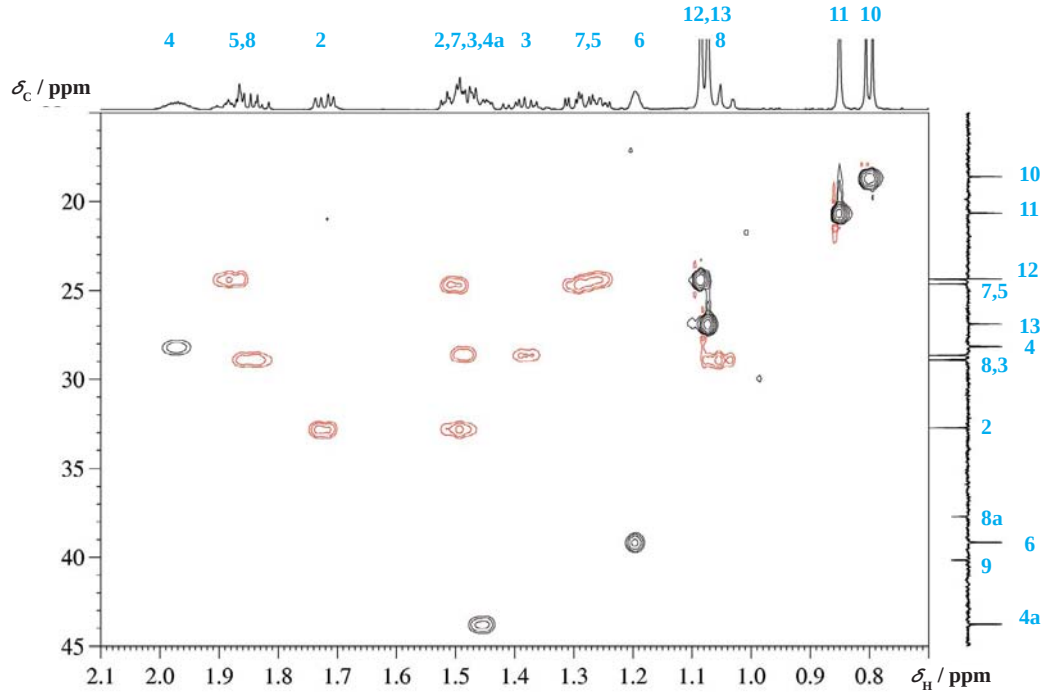


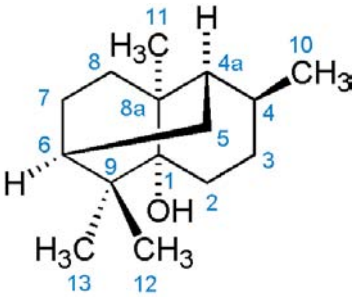
Fig. 5.4-10 CH-edited HSQC spectrum

From this spectrum, we can directly take the assignments for C-10 at 18.6 ppm and C-11 at 20.7 ppm, since the proton spectrum has already been assigned for these methyl groups. The ^{13}C NMR signals, for the methyl groups C-12 and C-13, however, are far more apart from each other than the corresponding proton signals and we have to await the discussion of the NOESY spectrum for a safe differentiation.

C-4 at 28.2 ppm can be assigned with the help of the proton spectrum, whereas the assignment of the two remaining CH signals at 43.8 and 39.2 ppm is insecure at this stage. All CH_2 groups display rather pronounced diastereotopicity, as is revealed by the CH-edited HSQC spectrum. A secure assignment cannot be given yet, although it is safe to assume that the most deshielded methylene group at 32.7 ppm belongs to C-2 due to its vicinity to the hydroxyl group, and this is in accordance with the arguments made earlier for the COSY spectrum. Since one of the H-3 protons has already been assigned in the ^{1}H NMR spectrum, C-3 can be secured at 28.6 ppm.

Первое, что поразило его при входе в переднюю, был запах пачули, весьмаему противный; тут же стояли какие-то высокие сундуки и баулы. Лицовыскочившего к нему навстречу камердинера показалось ему странным. Неотдавая себе отчета в своих впечатлениях, переступил он порог гостиной...Ему навстречу с дивана поднялась дама в черном шелковом платье с воланами и,поднеся батистовый платок к бледному лицу, переступила несколько шагов,склонила тщательно расчесанную душистую голову — и упала к его ногам... Туттолько он узнал ее: эта дама была его жена.

Ivan Turgenev (1818—1883)
Дворянское гнездо, Chap. XXXVI



Scheme 5.4-3

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz
75.7	C_q	C-1	
43.8	CH	C-4a	1.45
40.2	C_q	C-9	
39.2	CH	C-6	1.20
37.7	C_q	C-8a	
32.7	CH_2	C-2	1.72, 1.49, dd, J = 5.75, 12.75
28.9	CH_2	C-8	1.85, 1.06
28.6	CH_2	C-3	1.48, 1.38
28.2	CH	C-4	1.97
26.9	CH_3	C-13	1.07
24.6	CH_2	C-7	1.50, 1.29
24.4	CH_2	C-5	1.87, 1.26
24.3	CH_3	C-12	1.09
20.7	CH_3	C-11	0.85
18.6	CH_3	C-10	0.80

Table 5.4-2 NMR data for patchouli alcohol

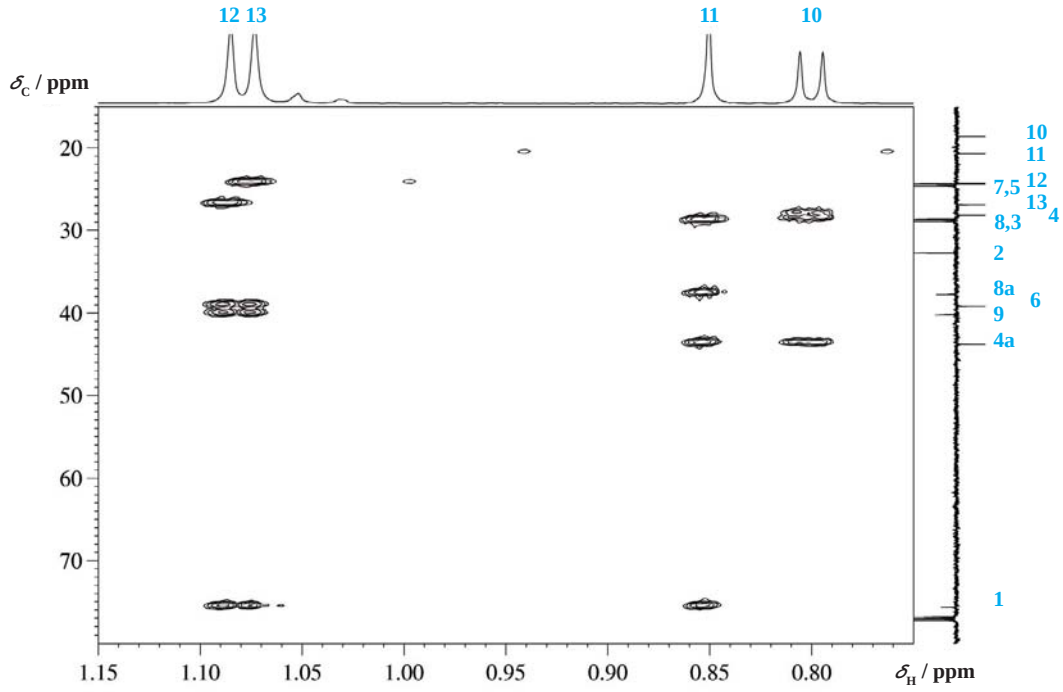


Fig. 5.4-11 Expansion of the HMBC spectrum in the methyl group region

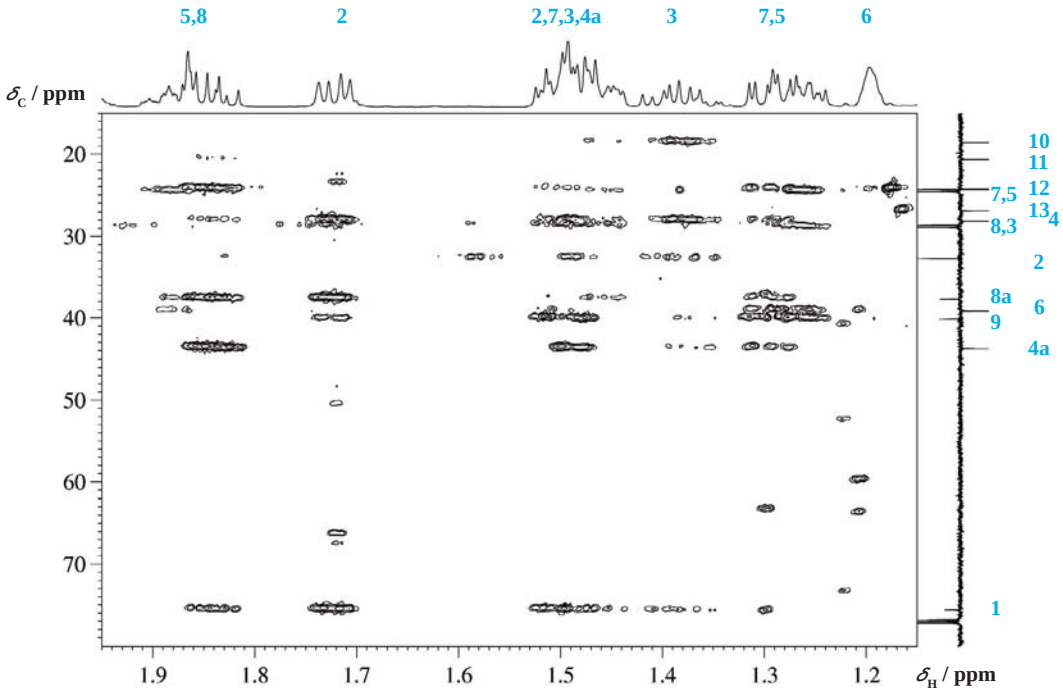
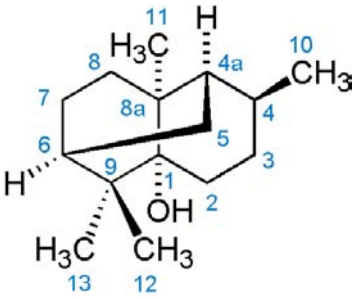


Fig. 5.4-12 Expansion of the HMBC spectrum between 2 and 1 ppm



Scheme 5.4-4

The discussion of the HMBC spectrum will give, as in many other examples in this book, the final arguments for the assignments of the as yet unassigned signals.

Starting from the proton signal of the methyl group H-10, we find two correlation signals, one to C-4 at 28.2 which was already assigned and one to a CH signal at 43.8 ppm which must therefore be C-4a. This signal is, as expected, also seen from H-11 over three bonds. The protons of H-11 also are connected to C-1 at 75.7 and to C-8a at 37.7 ppm. A further correlation signal is present, which leads to a methylene group, which is therefore assigned to C-8 at 28.9 ppm in close vicinity to C-3. If one now goes back to the HSQC spectrum, one can identify the protons H-8 at 1.85 and 1.06 ppm showing the largest diastereotopicity in this molecule. Going further back to the COSY spectrum, one easily identifies by the cross peaks H-8/H-7 the protons H-7 at 1.29 and 1.5 ppm with the corresponding carbon value of 24.6 ppm from the HSQC spectrum. Typical for methyl groups situated on the same carbon atom, the HMBC spectrum reveals cross peaks from the protons of one methyl group to the carbon atom of the other via three bonds. Both methyl groups see, of course, C-1, C-6 and C-9.

In the second expansion of the HMBC spectrum, H-2 at 1.7 ppm shows a correlation to C-1, C-8a and C-4, which confirms its assignment. Similarly, the other overlapping proton multiplets show HMBC cross peaks which confirm their assignments.

Fig. 5.4-13 An ignited incense stick

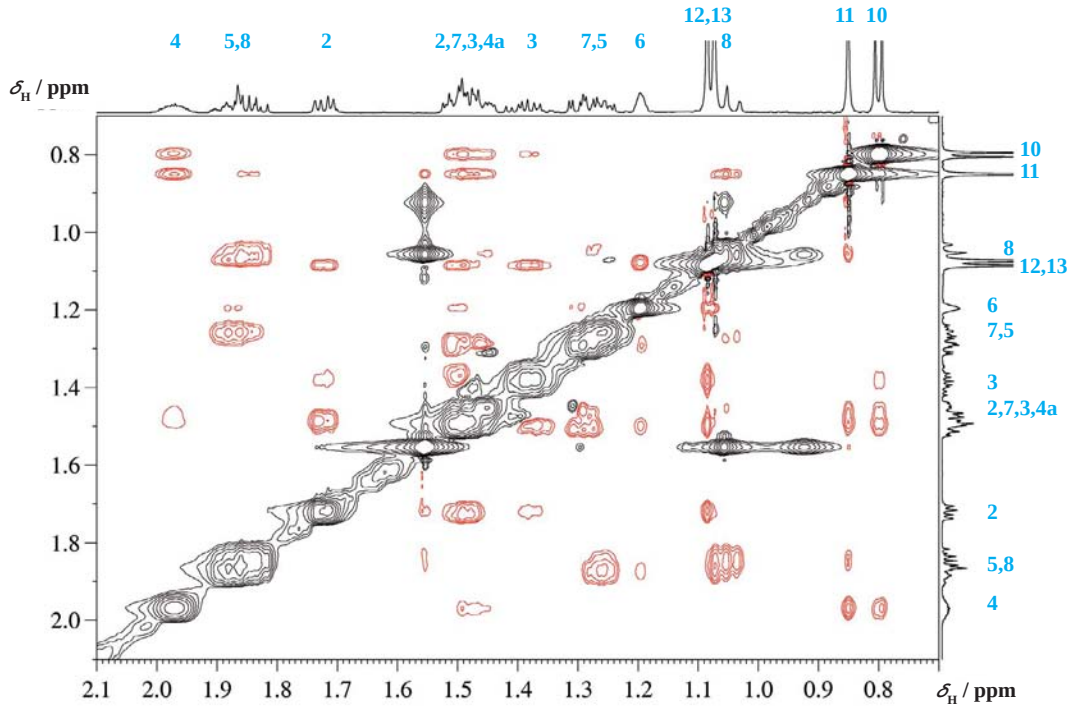


Fig. 5.4-14 NOESY spectrum

For the discussion of the NOESY spectrum, it is essential to build a molecular model and inspect the stereochemically important cross peaks in relation to the three-dimensional structure. The only open assignment question at this stage is the relative assignment of the methyl group signals H-12 and H-13.

The NOESY spectrum reveals that the more deshielded methyl group shows cross peaks to the single H-3 proton at 1.38 ppm, to both H-2 protons and to H-6. The more shielded methyl group, in contrast, shows no NOE cross peaks at all. As our structure is drawn, the more deshielded methyl group at 1.09 ppm is assigned to C-12. Note that the corresponding carbon chemical shifts are in reversed order, as seen from the HSQC spectrum.

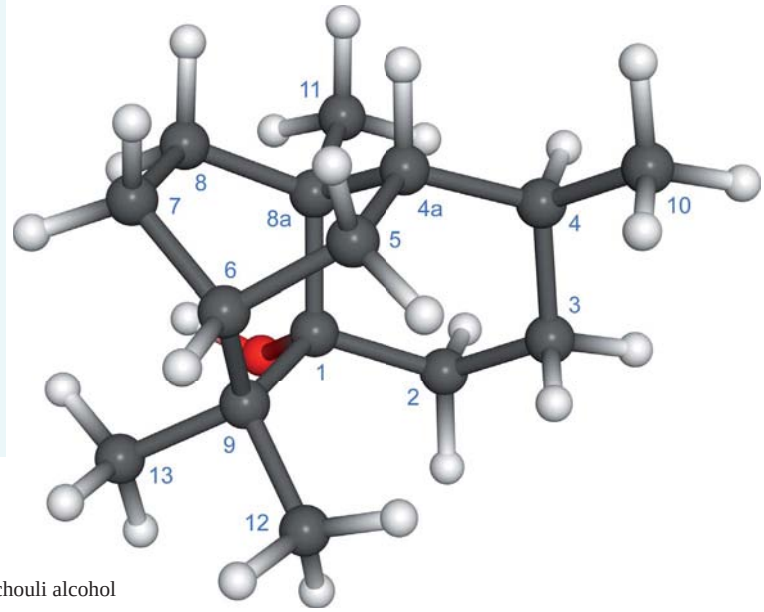


Fig. 5.4-17 Molecular model of patchouli alcohol

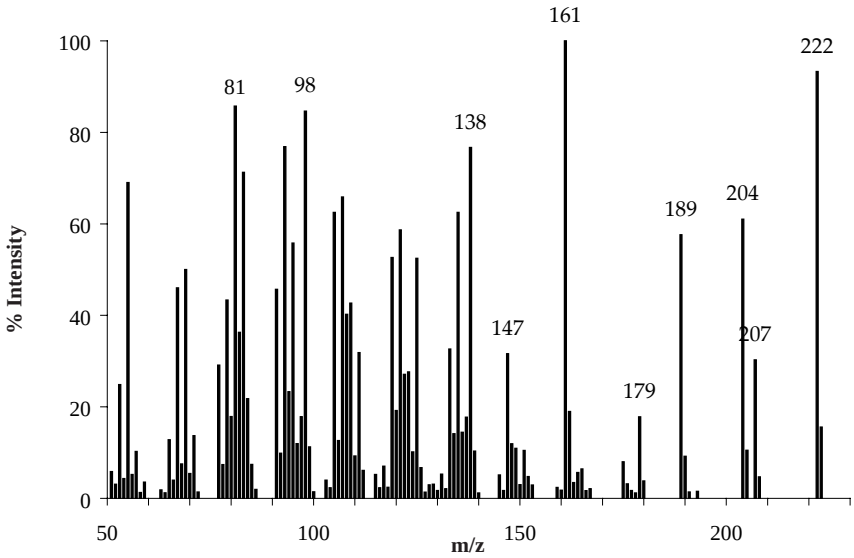
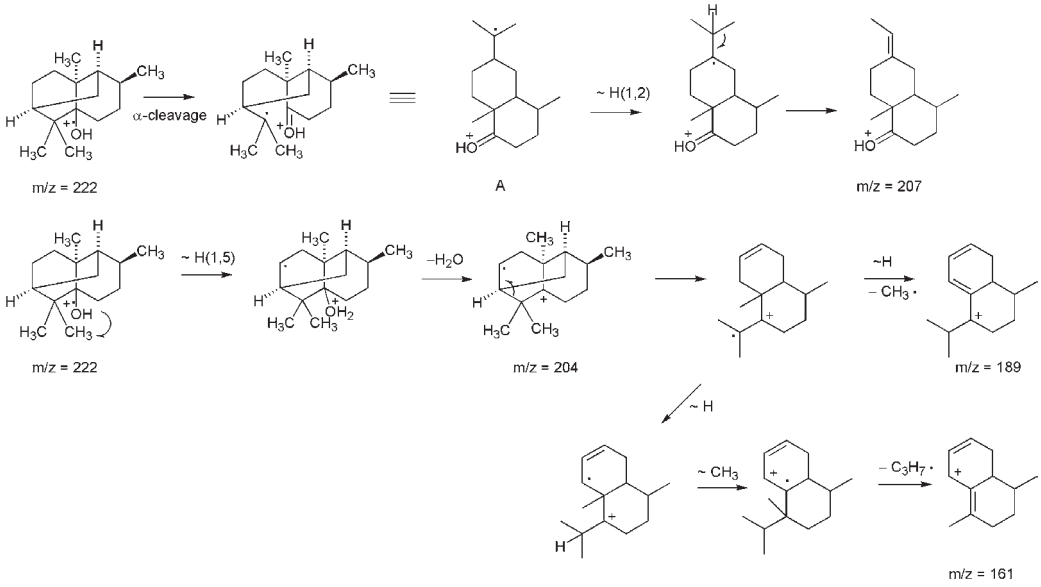


Fig. 5.4-16 Mass spectrum (EI)

The signal at m/z = 207 indicates the loss of a methyl group and a suggestion of the probable mechanism is given in the first line of the fragmentation scheme. The ion labelled A can also undergo a 1,3-hydrogen shift and subsequently lose C_3H_7 to form an ion with m/z = 179. There is also a peak at m/z = 204 which indicates loss of H_2O, and this is interpreted in the second line of the scheme. Subsequent loss of a methyl group would form the signal at m/z = 189. In the third line, the signal at m/z = 161 is interpreted as a loss of an isopropyl radical.



Scheme 5.4-5 Fragmentation of patchouli alcohol

5. Questions

A. Draw the formula of patchouli alcohol and indicate the three isoprene units present.

B. The ingredients of a fragrance are divided into three groups according to their volatility: top-note (quickly evaporating), middle-note (evaporating over up to 10 hours) and base-note (very long perceptible, does not consist of more than 10% of the ingredients). Patchouli belongs to the base-notes, similar to sandalwood. The volatility in turn depends on the structure of a compound. What in the structure of patchouli alcohol is within the ideal frame for a base-note?

C. On dehydration, dehydrogenation and rearrangement, patchouli alcohol is transformed into a blue, stable, pure hydrocarbon: guaiazulene (1,4-dimethyl-7-isopropylazulene; $C_{15}H_{18}$). Azulenes are in a valley of stability, obviously. A related transformation takes place on steam distillation of chamomile. Here, matricin as precursor is transformed into chamazulene (7-ethyl-1,4-dimethylazulene; $C_{14}H_{16}$). Draw the structure of guaiazulene and describe the general driving force for the formation of azulenes.

D. Why do azulenes show a blue colour whereas regioisomeric naphthalenes (having the same number of π-electrons) and the same substituents are colourless?

E. Whether azulene (bicyclo[5.3.0]decapentaene; $C_{10}H_8$) or its regioisomer naphthalene is thermodynamically more stable can today be investigated by calculations, but was proven by experiment long ago. The experiment is simple but impressive: on long-term standing of a flask with azulene and a flask with naphthalene, a change of colour takes places in one bottle. The question is: in which bottle, and why?

F. Suggest a reason why the methylene group of C-8 shows the highest diastereotopicity in this molecule.

G. Discuss, on the basis of the NOESY spectrum, which of the two protons H-2 is located towards the geminal methyl groups H-12 and H-13.

H. The proton multiplet at 1.86 ppm, assigned to H-5 and H-8, displays four significant HMBC cross peaks. Assign them to the corresponding carbon atoms.

I. Suggest a mechanism leading to the fragment ion of m/z = 179 in the mass spectrum.

6. Own Observations

5.5 Onocerin

(2*S*,2'*S*,4a*R*,4'a*R*,5*S*,5'*S*,8a*R*,8'a*R*)-5,5'-(1,2-Ethanediyl)bis(decahydro-1,1,4a-trimethyl-6-methylene-2-naphthalenol)

From the roots of the spiny restharrow
Ononis spinosa L.
(Fabaceae or Leguminosae)

$C_{30}H_{50}O_2$, MW 442.72

CAS RN 511-01-3, BRN 2065241, 2308843, 2631686, 3223825

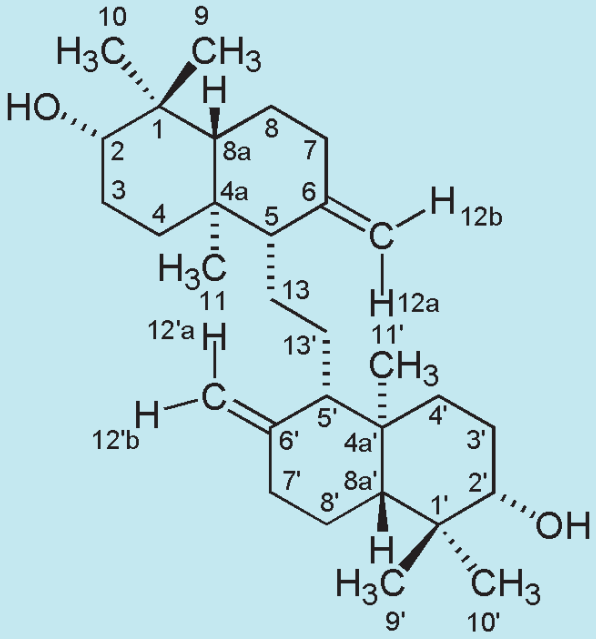
Colourless crystals, mp 208−210 °C

$[\alpha]_D^{21}$ +17.1° (*c* 0.0007 g/mL, chloroform)

α-Onocerin is commercially available.

Synonymous names:
(+)-Onocerin,
Onocera-8(2),14(27)-diene-3β,21α-diol

Level: easy



1. Background: Thorns, spines and *Weiberkrieg*

ἀνωνίς. οἱ δὲ ὀνωνίδα καλοῦσι. κλῶνες σπιθαμιαῖοι καὶ μείζονες, θαμνοειδεῖς, πολυγόνατοι, μασχάλας ἔχοντες πολλάς, κεφάλια περιφερῆ, φυλλάρια λεπτά, μικρά, πρὸς τὰ τοῦ πηγάνου ἤλωτοῦ τοῦ ἐν χορτοκοπίοις, ὑποδασέα, εὐώδη. ἁλμεύεταιδὲ πρὸ τοῦ ἀκανθοφορῆσαι καὶ ἔστιν ἡδίστη. ἔχουσι δὲ οἱ κλάδοι ἀκάντας ὀξείας, σκολοποειδῆς, στερειάς, ῥίζαν δὲ θερμαντικήν, λευκήν, ἧς ὁ φλοιὸς πινόμενος σὺν οἴνῳ οὖρα ἄγει καὶ λίτους τρύπτει, ἐσχάρας περιρρήσει. ἀφεψηθεῖσα δὲ ἐν ὀξυκράτῳ καὶ διακλυζομένη πόνον ὀδόντων πραΰνει.

Pedanius Dioscorides (ca. 40–90)
Περὶ ὕλης ἰατρικῆς,
Book III, Chap 18

Usually we are not fond of them: thorns and spines. If you do not really grow cacti and enjoy their bizarre network of thorns, you will likely have no other opinion. Maybe we love the fruits of such plants such as blackberries or the flowers of roses (both having spines from the botanical point of view) or the liqueur made of blackthorn fruits (*Prunus spinosa* L.) that carries thorns, to be exact − and no spines. In any other respect, we try to avoid physical contact and injury. For the botanist, the difference exists in the construction: whereas a thorn has a deep anchorage in the plant tissue of the stem or branch due to its metamorphosis from this tissue, a spine does not have this origin; it is a formation of the epidermis. The difference is obvious: you can easily break away the spine of a rose, to remove a thorn is impossible, a pocket knife is necessary to resect a thorn of a cactus. And, although its name contains *spinosa*, i.e. spiny, a restharrow has thorns. The genus name *Ononis* is borrowed from the Greek word ὄνος for donkey, an animal that obviously likes to eat the restharrow leaves.

The use of spiny restharrow plant parts is known from the Middle Ages. It was part of herb gardens. Fresh salted slips of the plant were eaten. The diuretic activity of root extracts was also known. A decoction of the root was also regarded as a remedy against toothaches. In the common parlance in Germany, the plant acquired the drastic name *Weiberkrieg*. This word means that wives are at war with the thorns of the restharrows because they get caught on them with their skirts.

The spiny restharrow, the root of which is used here for the isolation of α-onocerin, is a rather inconspicuous plant. A farmer would likely rank it with weeds. The meaning of this plant for him is that it is an obstacle. Due to its tough and spiny structure, it hinders his work on the field, e.g. ploughing and harrowing. Therefrom, the English name restharrow was formed, *Ononis spinosa* plants rested the harrow by felting it. The plant is about 50 cm in height (see photographs) and belongs to the so-called chamaephytes – plants whose buds can survive adverse seasons with different stress factors (cold, dryness) near the ground, but not directly on the ground. A well-known tough plant of this kind is the huckleberry. The spiny restharrow is found throughout much of Europe, is modest to its environment and also accepts nutrient-poor soils. We will in a moment see how this is possible. Before this riddle is resolved, let us look at restharrows in a meadow with the eyes of a stock farmer breeding cattle. Also for him, a restharrow colony is a nuisance, because the spines of the plants hurt the feet of his range animals and may cause ulcers.

What is so special about this imprecated plant? Belonging to the legume family, restharrows host specialized bacteria in nodules in their roots. These rhizobia are able to assimilate gaseous nitrogen from the air, to split reductively the triple bond in N_2 and deliver ammonium ions. Hence this nitrogen fixation is a source of species with single N atoms that can be utilized in the biosynthesis of N-containing compounds such as proteins. Everybody knows the eatable protein-rich fruits of related

Leguminosae plants such as peas, lentils and beans. For restharrows, the symbiosis just means a rich ammonium offer for the plant despite a poor soil.

The first to deal intensively with the chemical constituents of the spiny restharrow root was the Austrian chemist Hlasiwetz, who had already isolated a crystalline compound [1]. He named it onocerin and found a molecular formula of $C_{12}H_{20}O$. In 1896, Thoms revised the composition to $C_{26}H_{44}O_2$ [2]. This work already took note of the dimeric nature of onocerin. Additionally, by oxidation to a diketone he found that he was dealing with a diol, that he brought in close relationship to the phytosterins. The correct structure was not reported earlier than in 1955, by Barton and Overton [3]. They confirmed the correct molecular formula $C_{30}H_{50}O_2$, established that there are two exocyclic methylene groups and two secondary alcohols, that it is tetracyclic and described the unique symmetry of the molecule. Apart from IR and UV spectra, no other spectroscopic techniques were avalaible, used or necessary. At that time, masters of natural products chemistry were able to elucidate all structural relations to compounds of known configuration by purely chemical means in reactions on the bench.

The title compound is a triterpenoid, with squalene being the lead compound for triterpenes in the acyclic series (compare answer B in the Answers section 5.8 on betulinic acid). α-Onocerin was the first tetracyclic triterpene isolated from the vegetable kingdom, a rather rare case. Furthermore, α-onocerin has been a target of interest for synthetic studies for a long time [4]. The most recent work published describes an elegant four-step, four-component coupling leading in the final step from an open chain bis-allylic silane precursor directly to the tetracyclic dimeric final product α-onocerin [5].

In phytomedicine, α-onocerin is a phytochemical lead compound and serves as a reference for quality control in *Ononis spinosa* roots, which are used as a diuretic drug to scour out the kidneys and for prophylaxis against nephrolits. Therefore, its structure was eventually also subjected to detailed spectroscopic investigations by NMR and MS [6]. Recently, α-onocerin has also been found in a poisonous plant named wolfpaw clubmoss (*Lycopodium clavatum* L.). It is now under investigation due to its activity as an acetylcholinesterase inhibitor, a property that could e.g. be of interest in the treatment of Alzheimer's disease, characterized by memory deficits [7].

One of the most charming of the "old-fashioned" border flowers, having been grown in this country since 1570. It came from the Pyrenees, is hardy, evergreen, and shrubby. The common name of the genus, Restharrow, is in reference to the long, tough, and woody roots and branches. According to Gerarde, these properties "maketh the oxen, whilst they be in plowing, to rest or stand still." Although this species has tough roots and branches, it seems more likely that the name would be from the trouble caused by the weedy species of the genus of his time.

John Wood
Hardy Perennials and Old Fashioned Flowers

2. Literature

[1] H. Hlasiwetz, "Ueber die Wurzel der Ononis spinosa" [About the root of Ononis spinosa] *J. Prakt. Chem.* **1855**, *65*, 419−450.

[2] H. Thoms, "Über das Onocerin" [About onocerin] *Ber. Dtsch. Chem. Ges.* **1896**, *29*, 2985−2991.

[3] D. H. R. Barton, K. H. Overton, "Triterpenoids. Part XX. The constitution and stereochemistry of a novel tetracyclic triterpenoid" *J. Chem. Soc.* **1955**, 2639−2652.

Maid of the wilderness,
Sweet in thy rural dress,
Fond thy rich lips I press
Under this tree.

Morning her health bestows,
Sprinkles dews on the rose,
That by the bramble grows:
Maid happy be.
Womanhood round thee glows,
Wander with me.

The restharrow blooming,
The sun just a-coming,
Grass and bushes illuming,
And the spreading oak tree;

Come hither, sweet Nelly,
* * *
The morning is loosing
Its incense for thee.
The pea-leaf has dews on;
Love wander with me.

We'll walk by the river,
And love more than ever;
There's nought shall dissever
My fondness from thee.

Soft ripples the water,
Flags rustle like laughter,
And fish follow after;
Leaves drop from the tree.
Nelly, Beauty's own daughter,
Love, wander with me.

John Clare (1793–1864)
The Northamptonshire Peasant Poet

[4] G. Stork, A. Meisels, J. E. Davies, "Total synthesis of polycyclic triterpenes. Total synthesis of (+)-α-onocerin" *J. Am. Chem. Soc.* **1963**, *85*, 3419–3425.

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[6] G. F. Pauli, "Comprehensive spectroscopic investigation of α-onocerin" *Planta Med.* **2000**, *66*, 299–302.

[7] I. Orhan, S. Terzioglu, B. Sener, "α-Onocerin: an acetylcholinesterase inhibitor for *lycopodium clavatum*" *Planta Med.* **2003**, *69*, 265–267.

[8] E. Stahl, W. Schild, "Isolierung von Onocol (α-Onocerin) aus Hauhechelwurzel" [Isolation of α-onocerin from roots of spiny restharrow], In: *Isolierung und Charakterisierung von Naturstoffen*, Gustav Fischer Verlag, Stuttgart, **1986**, pp. 49–51.

3. Isolation

3.1 Principle

Shreds of spiny restharrow roots can be bought and are dry. These woody shreds are milled to a powder with a kitchen grinder. In a Soxhlet extraction all lipophilic constituents of the root are dissolved in dichloromethane, in total ca. only 1%. α-Onocerin is very lipophilic as a look at the structure shows. Two hydroxy groups do not alter its hydrophobicity, appreciably. The next step consists in the crystallization of α-onocerin from the extract. This is brought about by removing the dichloromethane and replacing it by a 1:1 solution of dichloromethane–ethyl acetate. The crystalline α-onocerin thus obtained is pure according to TLC and NMR and no further purification is required for these crystals. The purity of this material is an example of the excellent selectivity that can be achieved during the formation of a crystal lattice. If one has the chance to include such a separation step during the isolation of a solid natural product, it is always worthwhile at least to try it. In industrial processes, many efforts are made to establish preparative methods that include crystallization steps for purification. Also, stirring of crude solids with a suitable solvent is used, i.e. on stirring a slurry of impure crystals in such a solvent, eventually the crystal lattice is refined, molecules which do not fit are dropped off into the solvent whereas molecules that fit are incorporated into the crystal. The procedure leads to purification without the necessity to heat and dissolve all the material. In principle, it would of course also be possible for a natural product's purification – if enough of it were be available.

In our case, another crop of material can be obtained by column chromatography of the remaining extract material.

3.2 Method

The method is based on the procedure described in [8]. Shreds of spiny restharrow roots (100 g) are milled in a kitchen grinder. The powder obtained is placed in the thimble of a Soxhlet apparatus and extracted with 700 mL of dichloromethane for 4 h. The brown solution obtained has a pepper-like odour and is dried over $MgSO_4$ (5 g), filtered by suction and the solvent removed in vacuo to yield 1 g of a brown solid. This is dissolved in a 1:1 (v/v) mixture of ethyl acetate–dichloromethane (20 mL). On cooling and standing, crystals separate. They are filtered by suction using a small funnel and carefully washed by means of a pipette and 1 mL of the solvent of crystallization.

After drying in vacuo, the mass of the crystals was 73 mg; mp 208–210 °C; $[\alpha]_D^{21}$ +17.1° (*c* 0.0007 g/mL, $CHCl_3$) at 21 °C. Analyses proved this to be pure α-onocerin. No further purification was necessary for this material.

To improve the yield, column chromatography with the remaining extract was performed as described below.

3.3 Purification

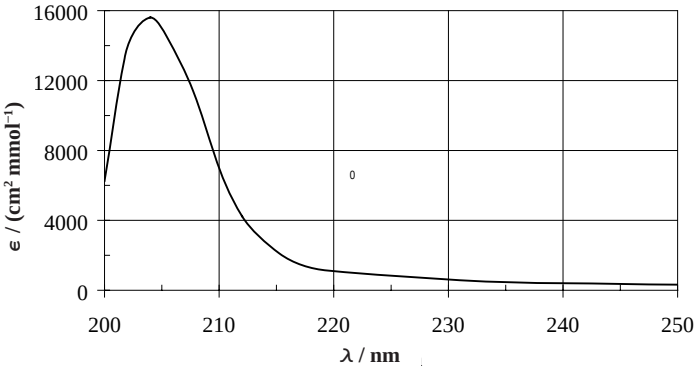
The mother liquor from above was reduced to dryness in vacuo. The remainder was dissolved in a small amount of a 1:1 (v/v) mixture of ethyl acetate–dichloromethane (ca. 10 mL).

Conditions: column, 60 × 3 cm; stationary phase, silica gel 60 (90 g, 0.063–0.200 mm); eluent, as mentioned above for the dissolution of the crude extract, 1 L; fractions containing α-onocerin were identified by TLC, collected and the solvent removed to obtain another crop of 36 mg of α-onocerin.

Fig. 5.5-1 A restharrow bush

4. Spectra and Comments

Fig. 5.5-2 Shreds of spiny restharrow roots as used for α-onocerin isolation



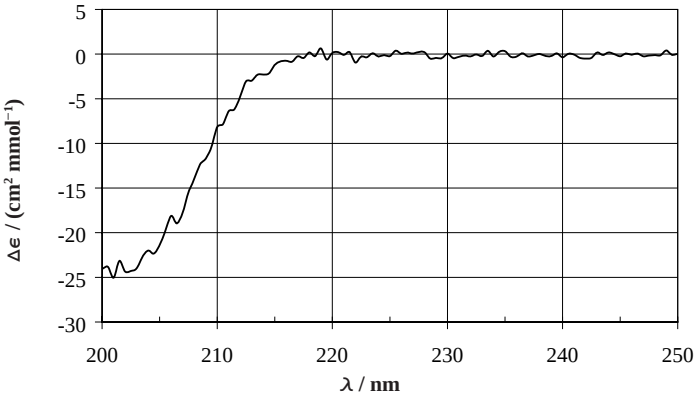
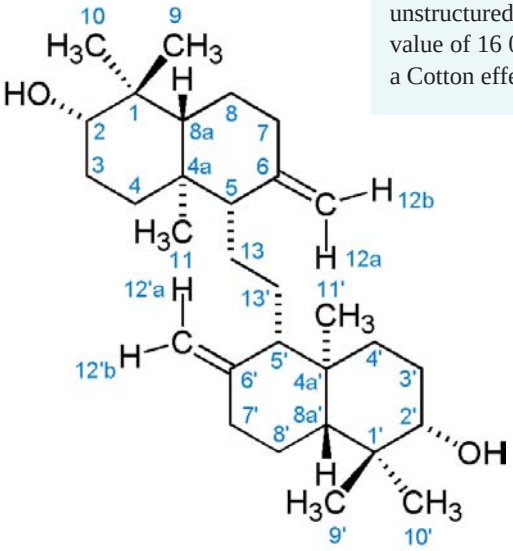


Fig. 5.5-3 UV and CD spectra

Onocerin has, due to its isolated *exo*-methylene group a strong but unstructured UV band with a maximum at 204 nm showing an ε value of 16 000 cm^2 $mmol^{-1}$. Since the compound is chiral, there is a Cotton effect at the position of the chromophore band.



Scheme 5.5-1

Here the barley has taken a different tint now the beard is out; here the oats are straggling forth from their sheath; here a pungent odour of mustard in flower comes on the air; there a poppy faints with broad petals flung back and drooping, unable to uphold its gorgeous robes.
The flower of the field pea, here again, would make a model for a lady's hat; so would a butterfly with closed wings on the verge of a leaf; so would the broom blossom, or the pink flower of the restharrow. This hairy caterpillar, creeping along the hawthorn, which if touched, immediately coils itself in a ring, very recently was thought a charm in distant country places for some diseases of childhood, if hung about the neck. Hedge mustard, yellow and ragged and dusty, stands by the gateway.

Richard Jefferies (1848–1887)
Nature Near London

Fig. 5.5-4 *Ononis spinosa*

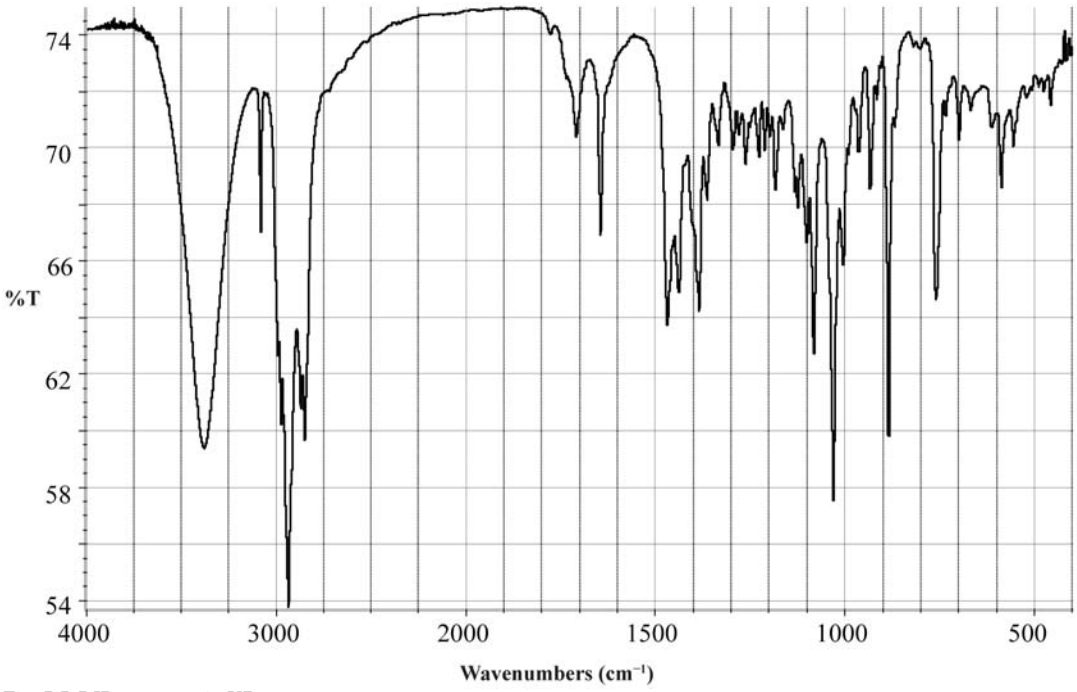


Fig. 5.5-5 IR spectrum in KBr

Onocerin is a perfect example of the special behaviour of end-located olefinic hydrogens. Their CH valence vibrations appear rather separate, displaying a sharp resonance at about 3100 cm^{-1}. Also the corresponding C=C vibration seems to be split and at higher wavenumbers, namely 1650 cm^{-1}.

Fig. 5.5-6 Spiny restharrow plants with typical pink flowers

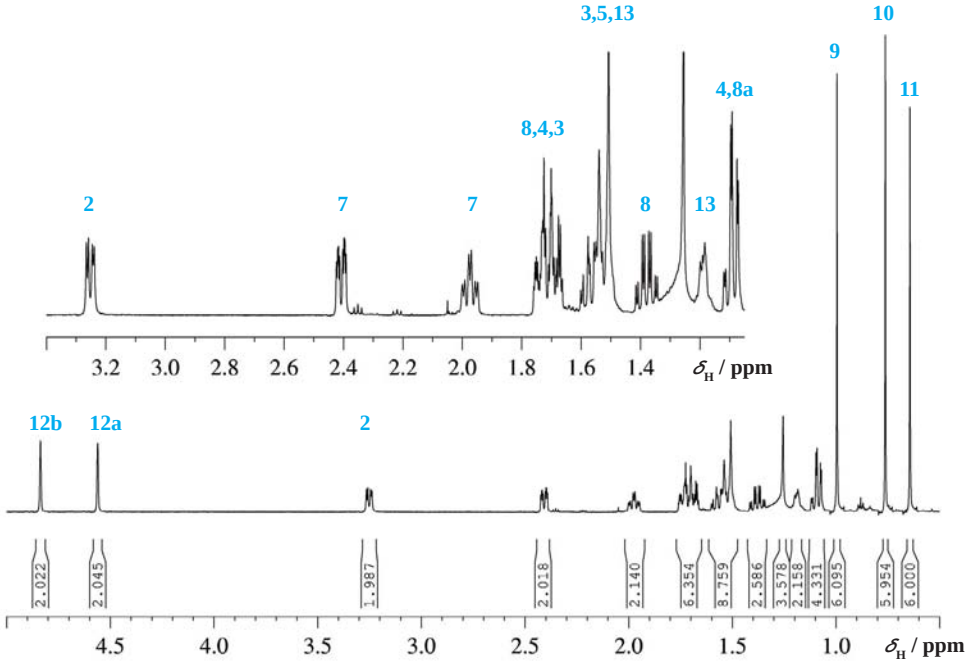


Fig. 5.5-7 ^{1}H NMR spectrum at 600 MHz in $CDCl_3$

Onocerin contains a symmetry axis, hence from the 30 carbon atoms and 50 protons we expect only half of the signals. A first view at the proton spectrum reveals three singlets for the methyl groups, since all three methyl groups do not couple to other protons. Their individual assignment has to await the discussion of the HMBC spectrum. Similarly, we see in the olefinic region two singlets for the *exo*-methylene group, without being able yet to assign these peaks individually. The signal at 3.2 ppm can safely be assigned to H-2 due to its chemical shift in the vicinity of an OH group. Further assignments in the 1D proton spectrum will be insecure at this stage of the discussion. Note that there are considerable chemical shift differences compared with ref. [6], which are due to the different solvents used. The data here are in $CDCl_3$ only and referenced to internal TMS.

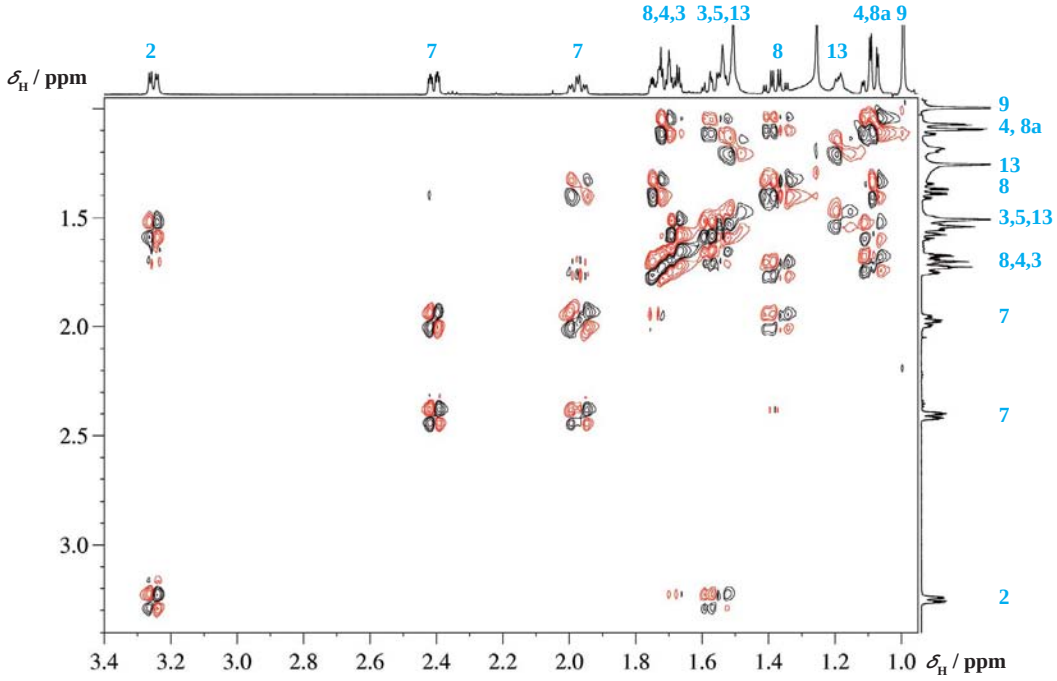


Fig. 5.5-8 Double quantum filtered COSY spectrum

Having already assigned H-2, we find in the COSY spectrum a cross peak leading to the signal of one H-3 at 1.58 ppm. H-3 is part of a diastereotopic methylene group and we can assume the other H-3 to resonate at 1.7 ppm, as indicated by the two cross peaks close to the diagonal peak of H-3. Another cross peak from H-3 leads to the signal of one H-4 at 1.05 ppm. The diastereotopic partner proton of H-4 can be seen at 1.7 ppm and this concludes the assignment of the ABB'CC' spin system within the first ring of onocerin. There is another spin system of the same type, comprised of H-8a, H-8 and H-7. The two allylic protons H-7 can be assumed to resonate at 2.4 and 2.0 ppm; the latter displays a cross peak to one H-8 at 1.4 ppm, which in turn leads to its diasterotopic partner proton H-8 at 1.7 ppm. As the cross peak of H-8 at 1.4 ppm indicates, the final proton of this spin system, H-8a, must be underneath the signal of H-4 at 1.05 ppm. The remaining protons will be assigned with the help of the HSQC spectrum to the bridge H-5−H-13−H-13' and H-5'.

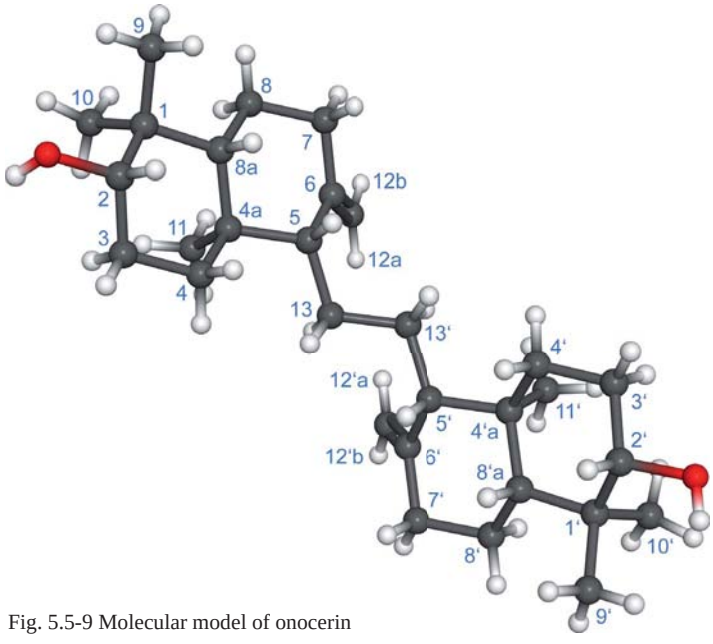


Fig. 5.5-9 Molecular model of onocerin

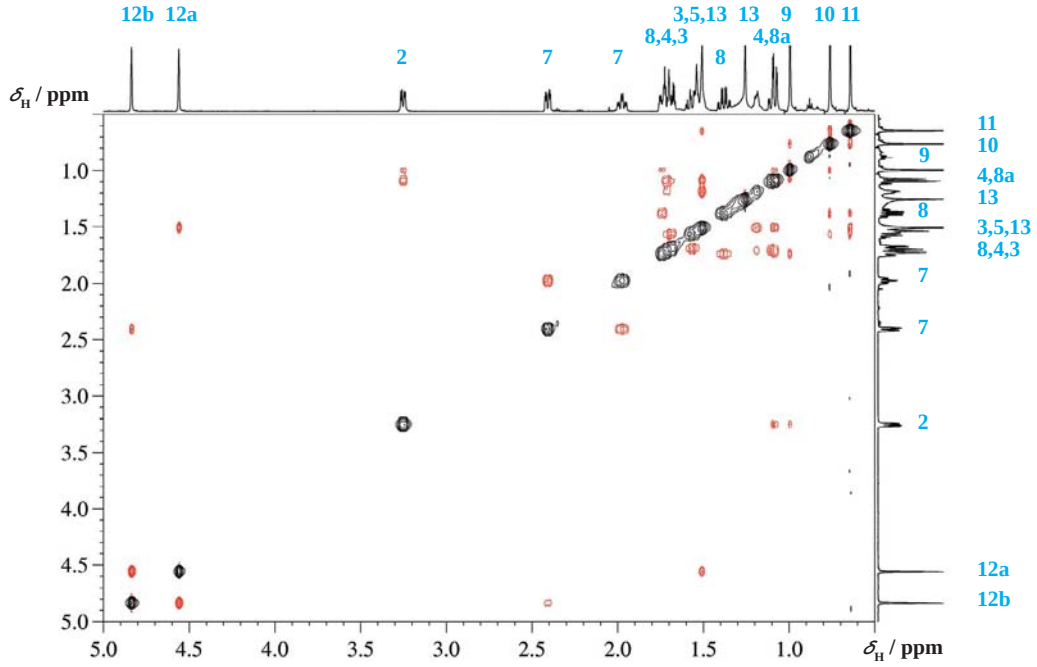


Fig. 5.5-10 NOESY spectrum

The NOESY spectrum is very helpful for assigning H-12a and b individually. The signal of H-12 at 4.8 ppm displays a cross peak to H-7 at 2.5 ppm and therefore we assign this signal as H-12b, being in close vicinity to the methylene group C-7. Correspondingly, the signal of H-12 at 4.5 ppm displays a cross peak to the signal group at 1.5 ppm which is comprised of H-5 and H-13. In comparison with the crowded COSY spectrum, the NOESY spectrum is once again easier to read and corroborates the assignments for the diastereotopic methylene groups.

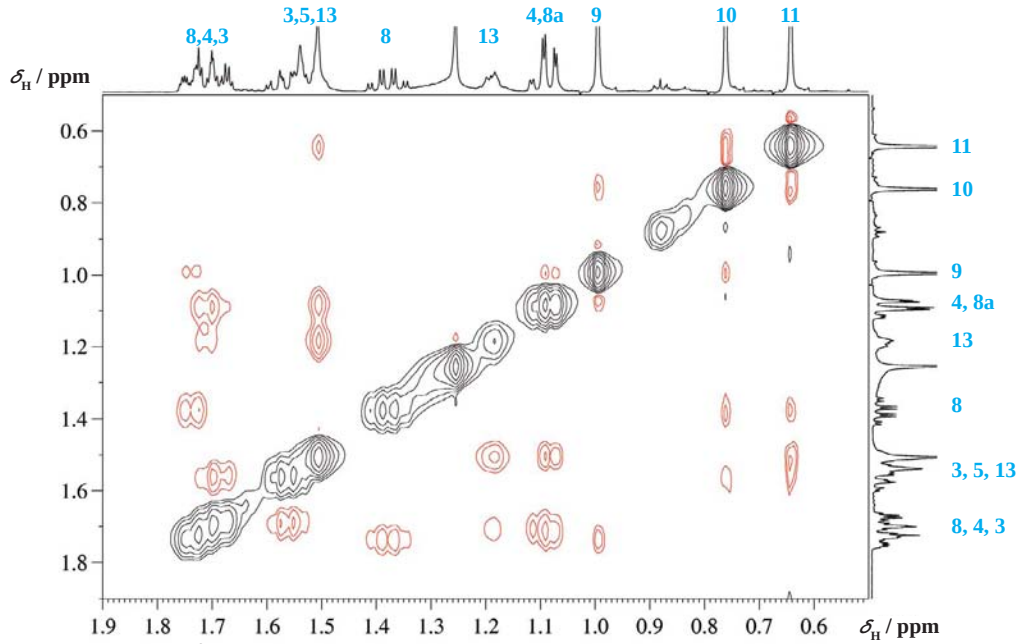


Fig. 5.5-11 Expansion of the NOESY spectrum in the aliphatic region

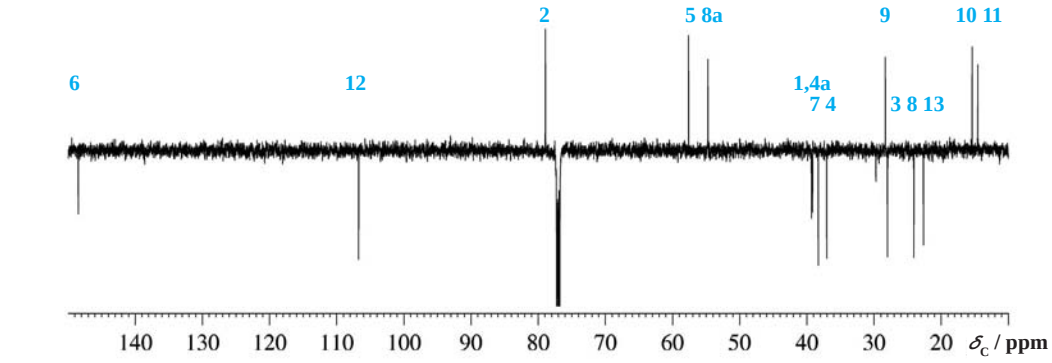


Fig. 5.5-12 APT ^{13}C NMR spectrum

The edited ^{13}C NMR spectrum reveals 15 signals in accordance with the symmetrical structure of onocerin. The signals of C-6 at 150, C-12 at 110 and C-2 at 78 ppm can be directly assigned. The individual assignment of the two methine carbon atoms C-5 and C-8a must await the inspection of the HSQC spectrum. Arguments for the correct assignment of the five methylene groups and the three methyl groups will also be derived from the HMBC spectrum.

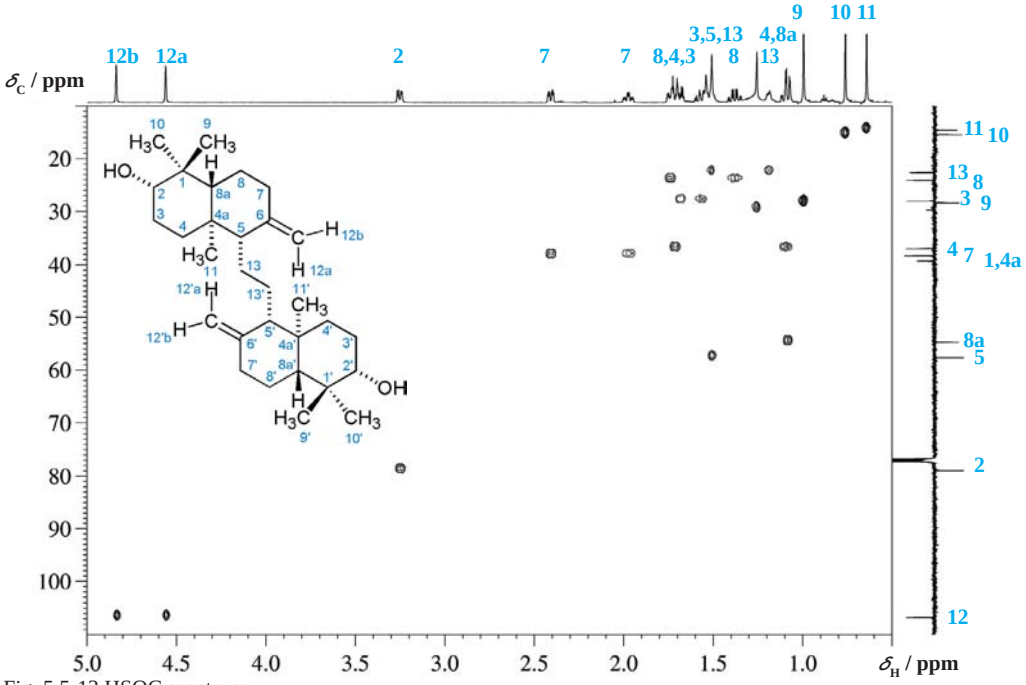


Fig. 5.5-13 HSQC spectrum

In addition to the obvious connectivities for C-12 and C-3, the HSQC spectrum indicates five further diastereotopic methylene groups. From our assignments in the proton spectrum, we know already C-3, C-4, C-7 and C-8, hence we find easily C-13 at 22 ppm. It is interesting that the methyl groups in this compound have the same order of their chemical shifts for both ^{1}H and ^{13}C. The HSQC spectrum reveals a significant impurity at 1.3/30 ppm, likely from chromatography.

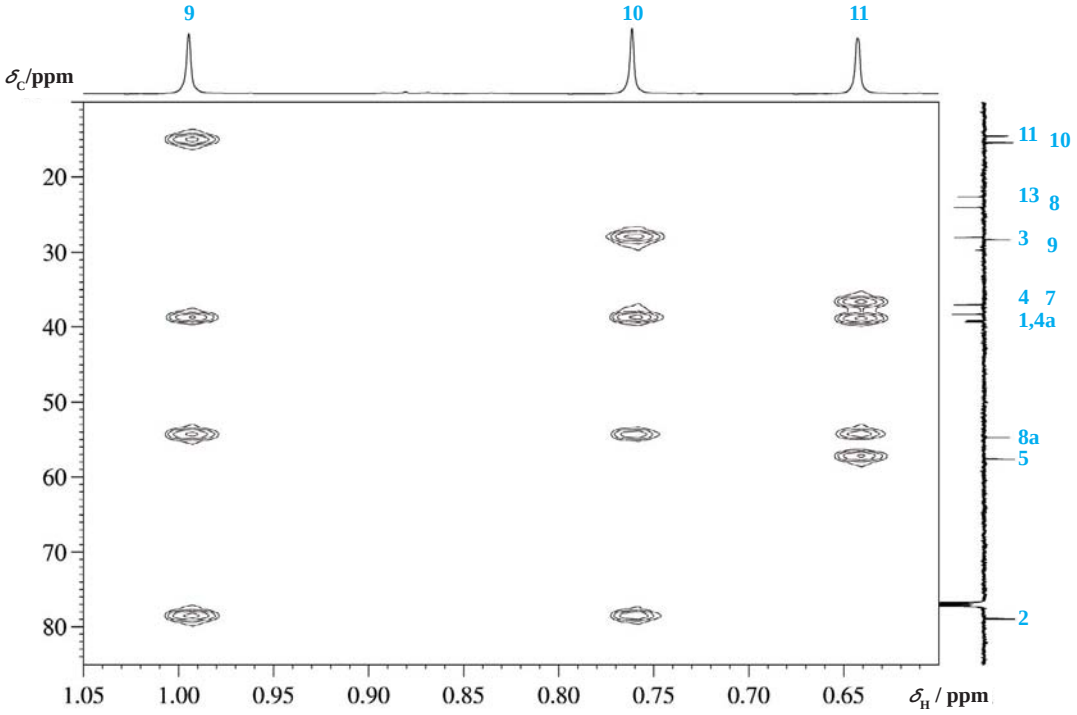


Fig. 5.5-14 Expansion of the HMBC spectrum for the methyl groups

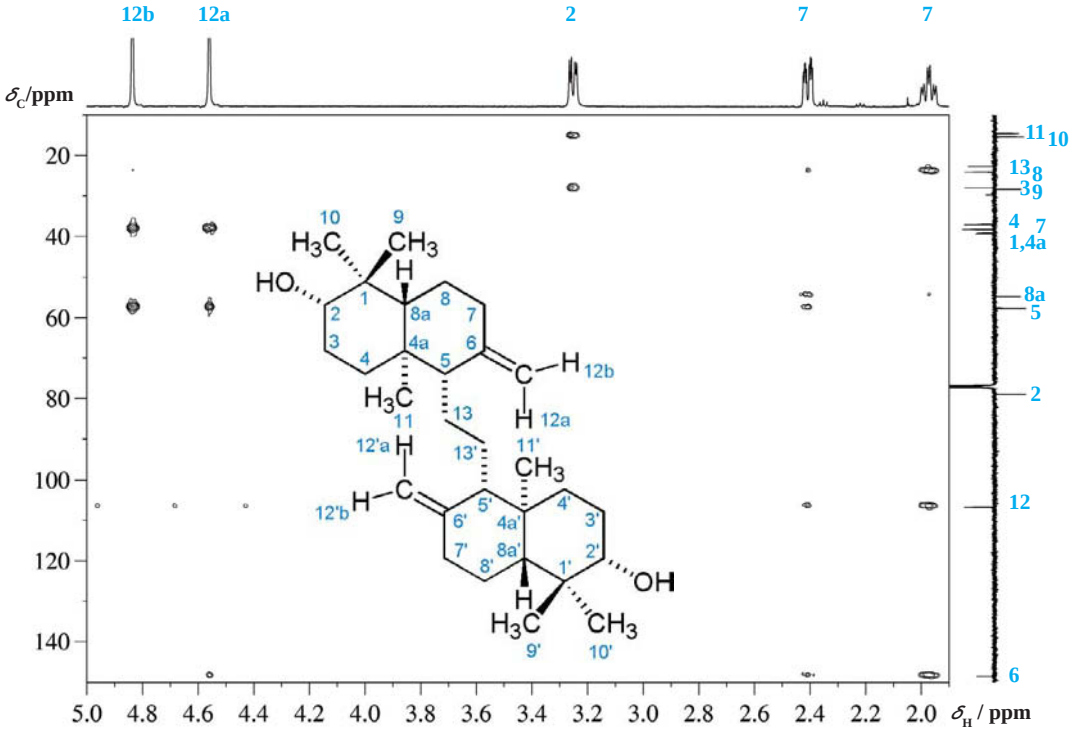


Fig. 5.5-15 Expansion of the HMBC spectrum for the olefinic and allylic protons

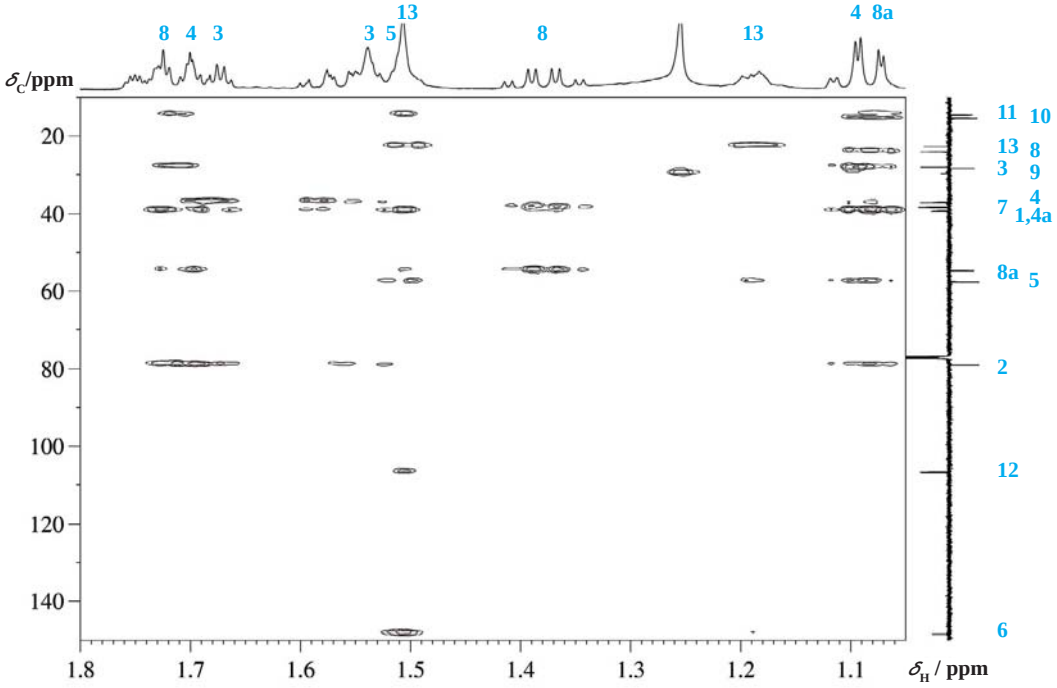
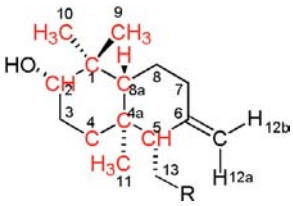


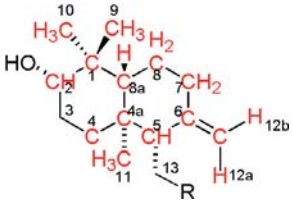
Fig. 5.5-16 Expansion of the HMBC spectrum between 2 and 1 ppm

We start with the discussion of the expansion given for the methyl group region. As required for geminal methyl groups, two methyl group signals must show cross peaks to each other, and this is the case for the signals at 1.0 and 0.75 ppm. Their individual assignment is not possible from the HMBC spectrum, since both show three more but cross peaks to identical carbon signals. These include a connection to a quaternary carbon atom at 39.0 ppm, which is therefore assigned to C-1, to a methine carbon atom at 52 ppm, which must be therefore C-8a, and of course to C-2. The most shielded methyl group signal at 0.6 ppm, now assigned to H-11, couples with a methylene group at 38 ppm, confirmed as C-4, a quaternary carbon C-4a, at 39.0 ppm which is nearly superimposed on the signal of C-1, and to the two methine carbons at 52 and 53 ppm, which confirms the assignment for C-8a and C-5. In the partial structure given in the margin, the confirmed assignments from this HMBC expansion are shown in red.

The second HMBC expansion is given for the protons H-12, H-2 and H-7. The correlation signals from H-12 define the assignment of C-6, C-5 and C-7, and those of H-7 confirm the assignment of C-8, and this again is shown in the partial structure in red. This leaves only C-3 and C-13 for an additional corroboration. In the third expansion, we inspect the signals of H-4 and H-8a, which are overlaid. Both cannot have a connection to C-13, but they display a correlation signal to a methylene group at 30 ppm, which therefore must be C-3, seen from H-4. The signal of H-5 has a strong correlation signal to C-13 and C-13', via ^{2}J and ^{3}J(C,H), as have the signals of H-13 to C-5.



Scheme 5.5-2



Scheme 5.5-3

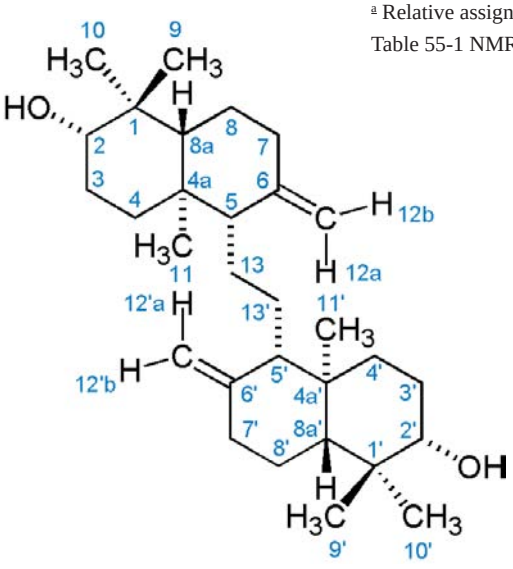
ОРЕ моме Трандафиле!
Що се вршиш, не се мъжиш,
три години шес пръстени!
— А друженки невернци! —
Що ме мене не вервите?
Змех ме любит три години,
я не можам да г' оделям.
— Море моме Трандафиле, —
я пойди си во градина,
да набериш гърмотърнье ;
та пойди си нови пазар,
та купи си ново гърне,
да 'и вариш спроти стреда,
да 'и пиеш спроти петок;
така да се змех оделит.

Dimitar Miladinov (1810−1862) and Konstantin Miladinov (1830−1862)

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz
148.4	C_q	C-6	
106.7	CH_2	C-12	H-12a: 4.56, H-12b: 4.84
78.9	CH	C-2	ax: 3.25, $^{3}J(H_2,H_3)$ = 11.92, 4.30
57.6	CH	C-5	1.51
54.7	CH	C-8a	ax: 1.08, $^{3}J(H_{8a},H_8)$ = 12.65, 2.93
39.3	C_q	C-1[a]	
39.1	C_q	C-4a[a]	
38.3	CH_2	C-7	eq: 2.41, $^{2}J(H_7,H_7)$ = 12.9, $^{3}J(H_7,H_8)$ = 4.2, 2.6 , ax: 1.97, $^{2}J(H_7,H_7)$ = 12.9, $^{3}J(H_7,H_8)$ = 12.9, 5.30
37.0	CH_2	C-4	eq: 1.71, ax: 1.09
28.3	CH_3	C-9	0.995
28.0	CH_2	C-3	eq: 1.68, ax: 1.57
24.1	CH_2	C-8	eq: 1.74, ax: 1.38
22.6	CH_2	C-13	1.51, 1.19
15.3	CH_3	C-10	0.76
14.5	CH_3	C-11	0.64

[a] Relative assignment insecure.

Table 55-1 NMR data for onocerin



Scheme 5.5-4

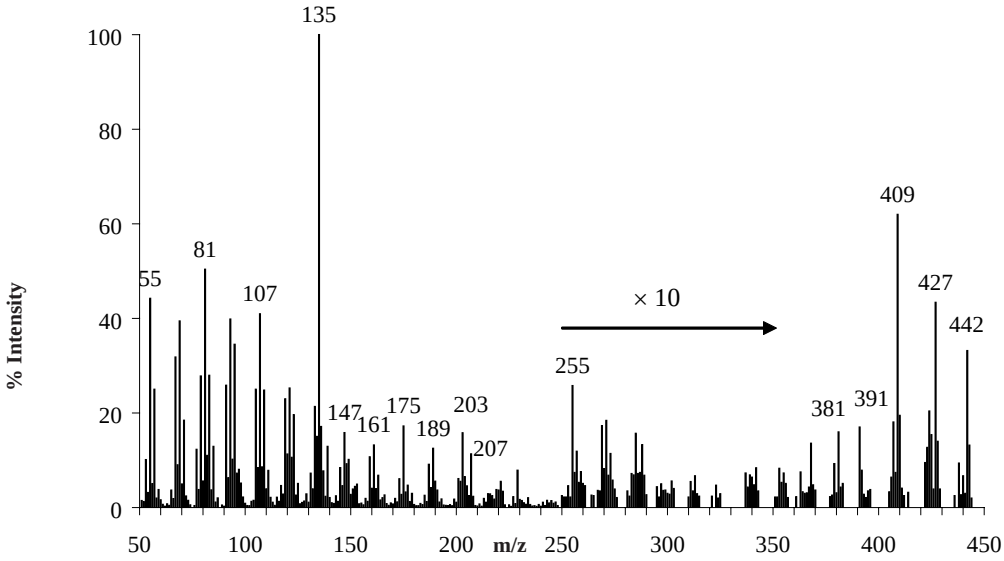
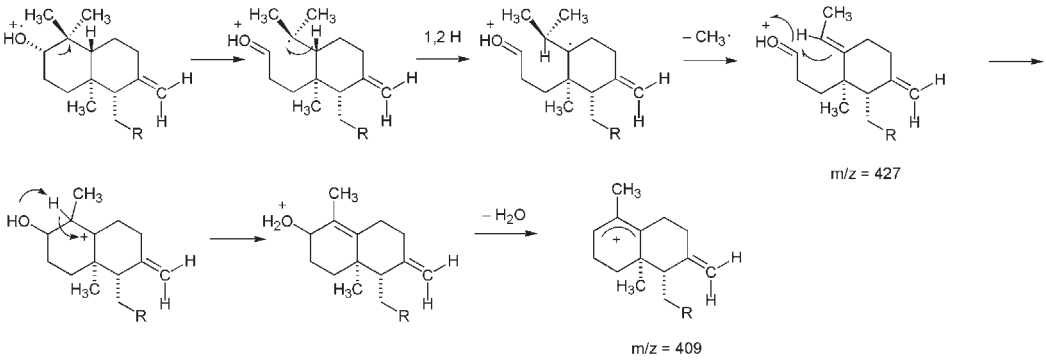


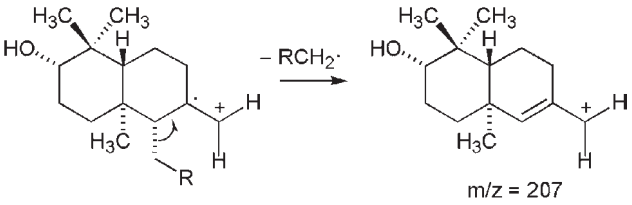
Fig. 5.5-17 Mass spectrum (EI)

The mass spectrum displays the signal of the molecular ion, although only readily observable after amplification by a factor of 10 for m/z values higher than 250. One observes the loss of a methyl group (m/z = 427) and an additional loss of water leading to the signal at m/z = 409. This fragmentation can be rationalized as given in the scheme below:



Scheme 5.5-5 Fragmentation of onocerin

Ionization at the *exo*-methylene group explains the formation of the ion at m/z = 207, as can be easily formulated. The atomic composition of this particle with $C_{14}H_{23}O^+$ = 207.17434 was confirmed by high-resolution MS. From this ion, the base peak at m/z = 135 may be generated.



Scheme 5.5-6 Further fragmentation

5. Questions

A. Indicate the six isoprene units present in α-onocerin.

B. Search for the structure of β-onocerin (CAS RN 6049-24-7). Explain why α-onocerin easily rearranges in the thermodynamically more stable β-onocerin.

C. Suggest a laboratory experiment by which one could easily prove that both α- and β-onocerin are unsaturated.

D. What determines that the methyl groups H-11 and H-10 are on the same side of the molecule?

E. Give an argument for the relative assignment of the methyl group H-9 being transoid to the OH group at C-2.

F. If you did not know that onocerin is a symmetrical dimer, which feature in the spectra points to this fact?

G. Give a mechanism for the fragmentation which leads to the base peak in the mass spectrum at m/z = 135.

6. Own Observations

5.6 Cnicin

(3*R*)-(3a*R*,4*S*,6*E*,10*Z*,11a*R*)-2,3,3a,4,5,8,9,11a-Octahydro-10-(hydroxymethyl)-6-methyl-3-methylene-2-oxocyclodeca[*b*]furan-4-yl 3,4-dihydroxy-2-methylenebutanoic acid ester

From the leaves of blessed thistle

Cnicus benedictus L. (Asteraceae)

$C_{20}H_{26}O_7$, MW 378.42

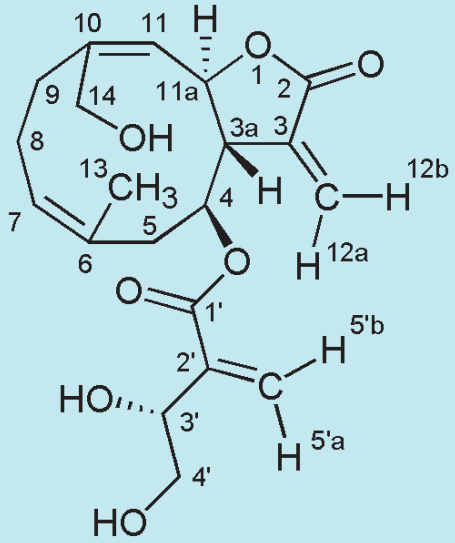
CAS RN 24394-09-0, BRN 7661758

Off-white fine needles, mp 330−335 °C (dec.)
$[a]_D^{22}$ +142° (*c* = 0.01 g/mL, ethanol)

Cnicin is commercially available.

Synonymous names:
(+)-Cnicin, 3,4-Dihydroxy-2-methylenebutyric acid 8-ester with 6α,8α,15-trihydroxygermacra-1(10),4,11(13)-trien-12-oic acid γ-lactone

Level: medium



1. Background:
Ora et labora – the Benedictines and their herbs

Otiositas inimica est animae, et ideo certis temporibus occupari debent fratres in labore manuum, certis iterum horis in lectione divina.
Ideoque hac dispositione credimus utraque tempore ordinari:
id est ut a Pascha usque kalendas Octobres a mane exeuntes a prima usque hora paene quarta laborent quod necessarium fuerit;
ab hora autem quarta usque hora qua sextam agent lectioni vacent;
post sextam autem surgentes a mensa pausent in lecta sua cum omni silentio, aut forte qui voluerit legere sibi sic legat ut alium non inquietet;
et agatur nona temperius mediante octava hora, et iterum quod faciendum est operentur usque ad vesperam.
Si autem necessitas loci aut paupertas exegerit ut ad fruges recolligendas per se occupentur, non contristentur,
quia tunc vere monachi sunt si labore manuum suarum vivunt, sicut et patres nostri et apostoli.

Benedictus de Nursia (480−547)
Regula Benedicti Caput XLVIII:
De opera manuum cotidiano

Do you have vision? Imagine a col in the high Alps during the Middle Ages. Do you see the small group of Black Monks climbing up from the South? Yes, indeed their black cowls identify them as Benedictines. Maybe they even come from the monastery of Monte Cassino, the place where Benedict of Nursia founded their Order in 529. The Benedictine motto is *ora et labora* (pray and work). And so they did. It is well known that monks, being among the few early travellers, not only distributed their beliefs but also useful practical things to enrich everday life, such as seeds of new plants from Southern Europe unknown north of the Alps.

The plant in this section belongs to these. *Cnicus benedictus* [*cnicus* arises from the Greek word κνίζειν which is best translated by "to scratch", *benedictus* (Latin) stands for the word blessed] is a thistle-like plant from the Mediterranean region, distributed from Turkey to Portugal. The plant is annual, grows to ca. 60 cm tall and has long, hairy leaves with spines on their margins which are responsible for the botanical name. The colour of their flowers is yellow (see photographs). Dried leaves and flowers are both used for medical purposes. A feature is the bitter taste of all plant parts, caused by cnicin, which is present at a level of around 0.5% of the dry mass. Early on, cnicin attracted the attention of chemists and some properties were reported in detail [1]. However, the correct structure was not published earlier than in 1960 [2].

Astonishingly, the plant was not mentioned by writers of ancient times at all. Its time came later. Blessed thistle – the name became a programme, it tells us all about the attitude of the mediaeval doctors to the properties of this plant. It was regarded as a real mercy due to its healing power. Its use was really widespread: it reached from bitter elixirs against lack of appetite, dyspeptic troubles, liver diseases, and biliousness to external local remedies against ulcers and chilblains. None of this was wrong.

Still today, liver and bile tea compositions contain blessed thistle herb. It is known that the herb stimulates salivary secretion and gastric juice formation. Another proven fact is the strong anti-inflammatory effect of cnicin, which explains the traditional use as a remedy against external wounds. Cnicin and other sesquiterpene lactones have antibacterial and antifungal effects and are also antibiotically active against trichomonades. Cnicin is also known to be cytotoxic [3].

The sesquiterpene skeleton of cnicin belongs to the germacranolide group. The exocyclic α-methylene group on the γ-butyrolactone ring is the structural feature and it is this reactive group that undergoes Michael additions with nucleophilic groups from biomolecules. This feature is held responsible for the physiological effects, which may even cause allergic reactions in the worst cases. In cnicin, a similar α-methylene unit is present in the ester side chain. It has been proved to be the molecular basis for the irreversible inhibition of the bacterial enzyme MurA and hence for the antibacterial activity by suppression

of the assembly of the bacterial cell wall [4]. Finally, sesquiterpenes of the germacrane type such as cnicin can be regarded as 1,5-dienes and are thus suitable precursors for [3,3] sigmatropic Cope rearrangements leading to compounds of the elemanolide group of sesquiterpenes [5].

2. Literature

[1] F. Scribe, "Cnicin" *Ann. Chem. Pharm*. **1842**, *44*, 298−299.

[2] M. Suchy, V. Benesova, V. Herout, F. Sorm, "Über Terpene, CXIX. Die Struktur des Cnicins, eines Sesquiterpen-Lactons aus Cnicus benedictus L." [Terpenes. CXIX. The structure of cnicin, a sesquiterpene-lactone of cnicus benedictus L.] *Chem. Ber.* **1960**, *93*, 2449−2456.

[3] M. Bruno, S. Rosselli, A. Maggio, R. A. Raccuglia, K. F. Bastow, C.-C. Wu, K-H. Lee, "Cytotoxic activity of some natural and synthetic sesquiterpene lactones" *Planta Medica* **2005**, *71*, 1176−1178.

[4] A. Bachelier, R. Mayer, C. D. Klein, "Sesquiterpene lactones are potent and irreversible inhibitors of the antibacterial target enzyme MurA" *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5605−5609.

[5] S. Rosselli, A. Maggio, R.A. Raccuglia, M. Bruno, "Rearrangement of germacranolides. Synthesis and absolute configuration of elemane and heliangolane derivatives from cnicin" *Eur. J. Org. Chem.* **2003**, 2690−2694.

[6] E. Stahl, W. Schild, "Isolierung von Cnicin aus Kardobenediktenkraut" [Isolation of cnicin from the leaves of blesses thistle] In: *Isolierung und Charakterisierung von Naturstoffen*, Gustav Fischer Verlag, Stuttgart, **1986**, pp. 73−75.

[7] M. Suchy, V. Herout, F. Sorm, "Cnicin: a new guaianolide-type lactone" *Chem. Ind.* (*London*) **1959**, 517−518.

[8] V. A. Raldugin, M. M. Shakirov, S. M. Adekenov, "Hydrogen and carbon-13 NMR spectra of sesquiterpene lactone cnicin" *Sibirskii Khimicheskii Zhurnal*, **1993**, 73−75.

3. Isolation

3.1 Principle

Cnicin is a lipophilic compound with a certain slight polarity, the isolation of which is not simple. On the one hand it has the full hydrophobic sesquiterpenoid skeleton and five carbons in the acid side chain, on the other hand also three OH groups. The problem is that the plant leaves contain both merely lipophilic compounds (chlorophyll, carotenoids) and hydrophilic compounds (saccharides) and that cnicin is in between them in terms of polarity. To separate cnicin and dyestuffs from glycosides and pure saccharides is easy. They are excluded when working with highly lipophilic solvents such as dichloromethane−methanol mixtures. Cnicin is not polar enough to dissolve in water in amounts useful for isolation methods.

Beatrice. By my troth, I am sick.
Margaret. Get you some of this distilled Carduus Benedictus, and lay it to your heart: it is the only thing for a qualm.
Hero. There thou prickest her with a thistle.
Beatrice. Benedictus! why Benedictus? you have some moral in this Benedictus.
Margaret. Moral! no, by my troth, I have no moral meaning; I meant, plain holy-thistle.

William Shakespeare (1564−1616)
Much Ado About Nothing III, 4

Fig. 5.6-1 A blessed thistle flower

The trick consists in finding a pathway of isolation that allows the complete separation between cnicin and the carotenoids of the leaves. In this respect, none of the published procedures that we repeated proved really effective in discriminating cnicin from the carotenoids–chlorophylls. Also, we were not satisfied on repeating the original method in [6] because the column chromatographic separation reported therein uses a dichloromethane–methanol (95:5) mixture that does not give rise to a cnicin fraction free of carotenoids, as can be shown by NMR spectra. What is a good approach in such a difficult case?

Dr. Montfords Cordial Water.

Take Angelica leaves twelve handfuls, six leaves of Carduus Benedictus, Balm & Sage, of each five handfuls, the seeds of Angelica and sweet Fennil, of each five ounces bruised, scraped and bruised Liquorish twelve ounces, Aromaticum Rosatum, Diamoscus dulcis, of each six drams; the Herbs being cut small, the seeds and Liquorish bruised, infuse them into two gallons of Canary Sack for twenty four hours, then distill it with a gentle fire, and draw off onely five pints of the spirits, which mix with one pound of the best Sugar dissolved into a Syrup in half a pint of pure red Rose-water.

Anonymous
A Queens Delight; or The Art of Preserving, Conserving and Candying. As also A Right Knowledge of Making Perfumes, and Distilling the Most Excellent Waters.
London, 1671

We recommend testing some pure solvents rather than solvent mixtures for their eluting abilities on the mixtures that have to be separated on the column just using TLC plates. Often, the results are amazing, surprising and convincing at the same time. In this case, the idea was to find a highly lipophilic solvent that may transport only chlorophylls and xanthophylls without influencing cnicin. We tested the interaction of the initial blessed thistle leave extract with chloroform, *n*-hexane, and diethyl ether. The last one was able to fulfil our aim. Therefore, the crude plant extract was first flushed free of all highly lipophilic plant dyes on the column, leaving the cnicin unaffected. In a second step, changing to a solvent mixture with higher elution power, the cnicin was slowly transported to the outlet, nearly free of colorations. Those contained in minute amounts in the eluate did not contaminate the cnicin crystals because the colour remained in solution (compare the isolation of patchouli alcohol to find another variation of this principle).

In summary: in the case of difficult separations, it is worthwhile to deal thoroughly with the separation problem first on TLC plates, before wasting time and money with “macroscopic” experiments on a column.

3.2 Method

This procedure is a variation of the method described in [6].

Dried leaves of blessed thistle (100 g) are stirred at ambient temperature in a 2 L round-bottomed flask with a dichloromethane–methanol mixture (9:1, v/v; 1250 mL) for 4 h. The herbs are filtered by suction and the dark green extract is reduced to dryness in vacuo to yield 4.7 g of a dark green solid. The leaves remaining in a swollen state are discarded. The dark green extract is placed in a mortar with Kieselgel 60 (25 g) and diethyl ether (30 mL) and finely mixed with the pistils to yield a dark green slurry that was placed by means of a spatula on the top of a column prepared for column chromatographic separation.

Conditions: column: 25 × 5 cm; stationary phase, silica gel 60 (45 g, 0.040–0.063 mm); first eluent: diethyl ether (3000 mL). The ether is passed through the column and removes all the highly lipophilic plant dyestuffs, a procedure which lets all of the cnicin remain on the column. This was ensured before the chromatography by a TLC check. The chronological order of coloured dyestuff fractions eluted from the column is: black–green, green, pale green, yellowish green, greenish yellow, yellow and pale yellow. Finally, the eluent is nearly colourless.

All these ethereal fractions are discarded. They do not contain any cnicin. For the final purification, the eluent has to be changed as mentioned in Section 3.3.

A suitable TLC system for supervision of the run and detection of cnicin is the following: Kieselgel 60_{F254} plates; eluent, dichloromethane–methanol (9:1, v/v). The spot of cnicin is colourless and has an R_f value of 0.4–0.5. A track of pure hydroquinone placed on a separate lane on the same plate gives a suitable orientation. It will be slightly faster than cnicin if a chromatogram of the whole original blessed thistle leaves extract is taken. Under 254 nm UV irradiation the cnicin spot quenches the emission of the fluorescence indicator integrated on the TLC plate and can thus be detected with absolute certainty as a violet spot.

Fig. 5.6-2 A column after cnicin has been eluted

3.3 Purification

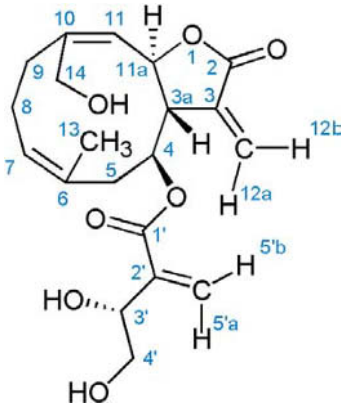
The eluent is now changed to a dichloromethane–methanol mixture (95:5, v/v) and the elution is continued until 3000 mL of the eluent have been passed through the column. Fractions of 20 mL are collected in test-tubes. Some fractions are colourless; others show a very pale greenish or yellowish colour that does not disturb the further isolation in any way. The fractions containing cnicin are subjected to TLC. Finally, in our case fractions 60–120 are combined (the first of them contain massive amounts of cnicin) to yield a pale green eluate rich in cnicin. The solvent is removed in vacuo to a remaining volume of 25 mL. During this operation, crystallization of cnicin begins, which is completed by immersing the flask for 1 h in an ice–water bath. The cnicin crystals are filtered off by suction through a small funnel and washed with chloroform to remove coloured impurities from the surface of the crystals, which turn out to be tiny needles under the microscope; 636 mg of off-white pure cnicin remain after drying in vacuo.

The behaviour of cnicin under the microscope with a Boetius micro-hot-stage is different from the literature description: cnicin does not melt at 143 °C [6]; instead, it only sinters between 140 and 143 °C. The crystals also do not melt at 330 °C as reported [7], but decompose to a brown material in the range 330–335 °C.

The optical rotation $[a]_D^{22}$ +142° (c = 0.01 g/mL, ethanol) is in accordance with database values.

Fig. 5.6-3 Flowering blessed thistles

Fig. 5.6-4 Material for extraction



Scheme 5.6-1

This representative flower will always be a wild one, and of the simplest form which completely expresses the character of the plant; existing divinely and unchangeably from age to age, ungrieved by man's neglect, and inflexible by his power.

And this divine character will be expressed by the epithet 'Sacred,' taking the sense in which we attach it to a dominant and christened majesty, when it belongs to the central type of any forceful order; − 'Quercus sacra,' 'Laurus sacra,' etc., − the word 'Benedicta,' or 'Benedictus,'being used instead, if the plant be too humble to bear, without some discrepancy and unbecomingness, the higher title; as 'Carduus Benedictus,' Holy Thistle.

John Ruskin (1819−1900)
Proserpina, Vol. 1 Studies of Wayside Flowers

4. Spectra and Comments

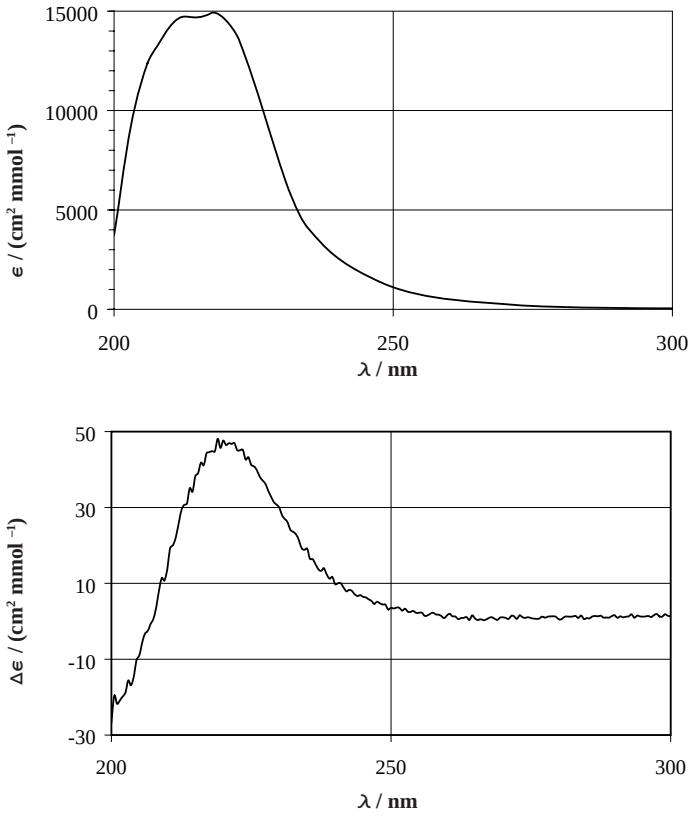
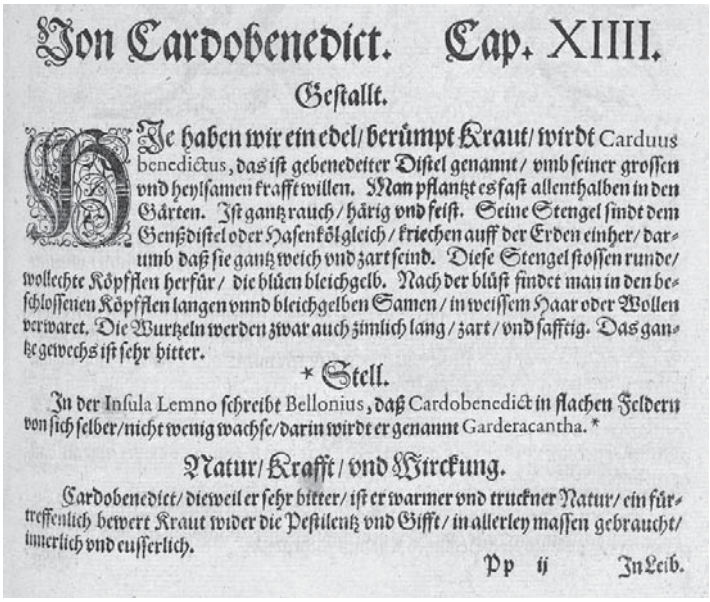


Fig. 5.6-5 UV and CD spectra in ethanol

The compound contains as chromophoric groups two double bonds, each of which is conjugated with a carbonyl group and in addition two isolated double bonds. Therefore, we find in the UV spectrum one very strong transition at 220 nm with an ε value of 15 000 cm^2 $mmol^{-1}$. The transition is, however, unstructured due to the flexibility of the side chains. The compound is chiral, having four stereogenic centres rather close to the chromophoric groups. Therefore, we expect a CD spectrum with a rather strong Cotton effect, which is indeed the case, with a positive Δε value of 50 cm^2 $mmol^{-1}$.



Von Cardobenedict. Cap. XIIII.

Gestallt.

DJe haben wir ein edel/ berümpt Kraut/ wirdt Carduus benedictus, das ist gebenedeiter Distel genannt/ vmb seiner grossen vnd heylsamen krafft willen. Man pflantzt es fast allenthalben in den Gärten. Ist gantz rauch/ härig vnd feist. Seine Stengel sindt dem Genßdistel oder Hasenkölgleich/ kriechen auff der Erden einher/ darumb daß sie gantz weich vnd zart seind. Diese Stengel stossen runde/ wollechte Köpfflen herfür/ die blüen bleichgelb. Nach der blüst findet man in den beschlossenen Köpfflen langen vnnd bleichgelben Samen/ in weissem Haar oder Wollen verwaret. Die Wurtzeln werden zwar auch zimlich lang/ zart/ vnd safftig. Das gantze gewechs ist sehr bitter.

* Stell.

In der Insula Lemno schreibt Bellonius, daß Cardobenedict in flachen Feldern von sich selber/ nicht wenig wachse/ darin wirdt er genannt Garderacantha. *

Natur/ Krafft/ vnd Wirckung.

Cardobenedict/ dieweil er sehr bitter/ ist er warmer vnd truckner Natur/ ein fürtreffenlich bewert Kraut wider die Pestilentz vnd Gifft/ in allerley massen gebraucht/ innerlich vnd eusserlich.

Pp ij Jn Leib.

Fig. 5.6-6 Facsimile taken from Pietro Andrea Mattioli (1501−1577), *Kreutterbuch Deß Hochgelehrten vnnd weitberühmten Herrn D. Petri Andreae Matthioli*: 1586
University Library, Leipzig

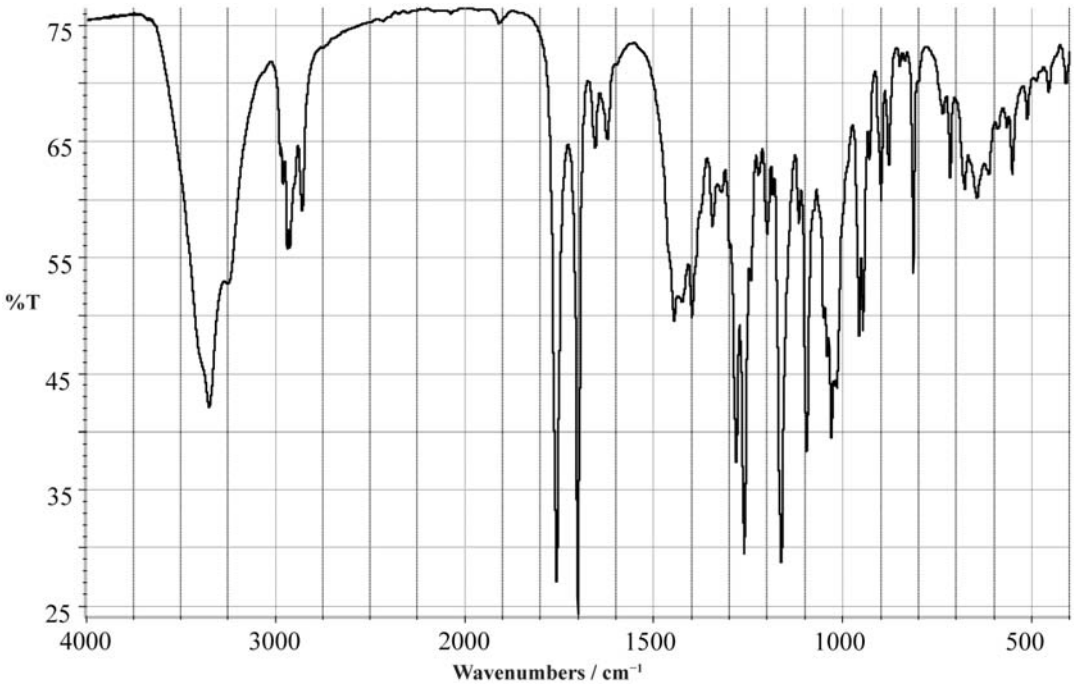


Fig. 5.6-7 IR spectrum in KBr

The broad OH valence vibration at 3300 cm^{-1} dominates the IR spectrum and this probably is the reason why the sp^2 CH vibrations of the terminal methylene groups expected at 3100 cm^{-1} cannot be seen. Two carbonyl valence bond vibrations can be detected at 1760 and 1700 cm^{-1}, and also two C=C bond vibrations at 1660 and 1620 cm^{-1}.

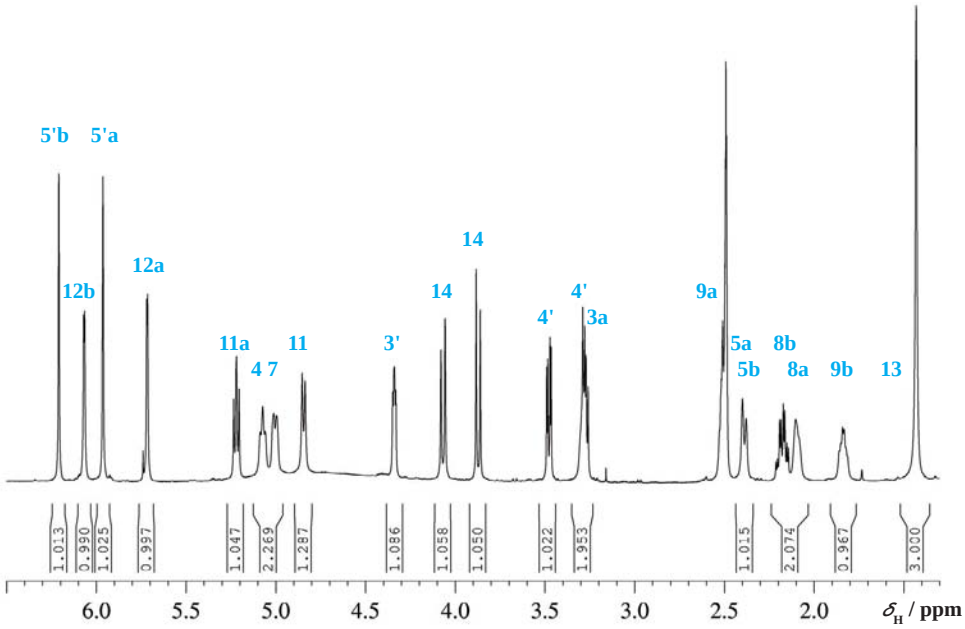
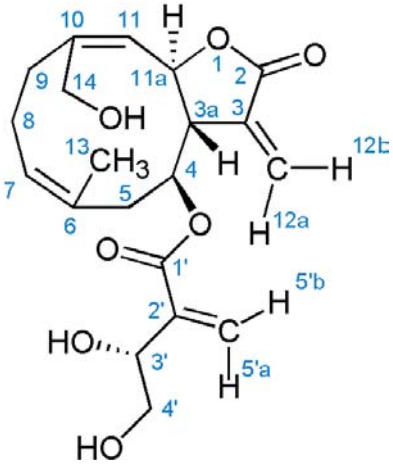


Fig. 5.6-8 ^{1}H NMR spectrum at 600 MHz in DMSO-d_6

In the rather complicated structure, 26 protons should be observable as individual signals. Counting the integral values in the displayed ^{1}H NMR spectrum, we find 21 protons and a rather broad hump from the exchanging three OH groups between 5.3 and 4.5 ppm. It is fair to assume at present that the two missing signals might be hidden near or under the solvent signal.

The structure requires four absorptions of the terminal methylene groups as apparent singlets, since the olefinic $^{2}J_{HH}$ is usually rather small and we find these four signals between 6.3 and 5.7 ppm without assigning them in detail at present.

The next five signals between 5.3 and 4.3 ppm obviously stem from CH moieties and they are either olefinic =CH or CHO groups. Their relative assignment will be easily possible with the help of the HSQC spectrum due to the significant ^{13}C chemical shift difference between =CH and CHO groups. The molecular structure further requires two diastereotopic methylene groups H-14 and H-4' also bound to oxygen. We find these two AB spin systems centred at 4.0 and 3.4 ppm. The integral value of the shielded part of the latter reveals that there is another CH proton underneath and this can therefore be safely assigned to H-3a, in agreement with its chemical shift of 3.28 ppm. With the assumption that two proton signals are hidden by the DMSO signal, we assign these and the four signals between 2.5 and 1.8 ppm to the three diastereotopic methylene groups 5, 8 and 9. Finally, the methyl group H-13 at 1.43 ppm can of course be directly assigned, displaying a chemical shift value very typical for a methyl group attached to a double bond.



Scheme 5.6-2

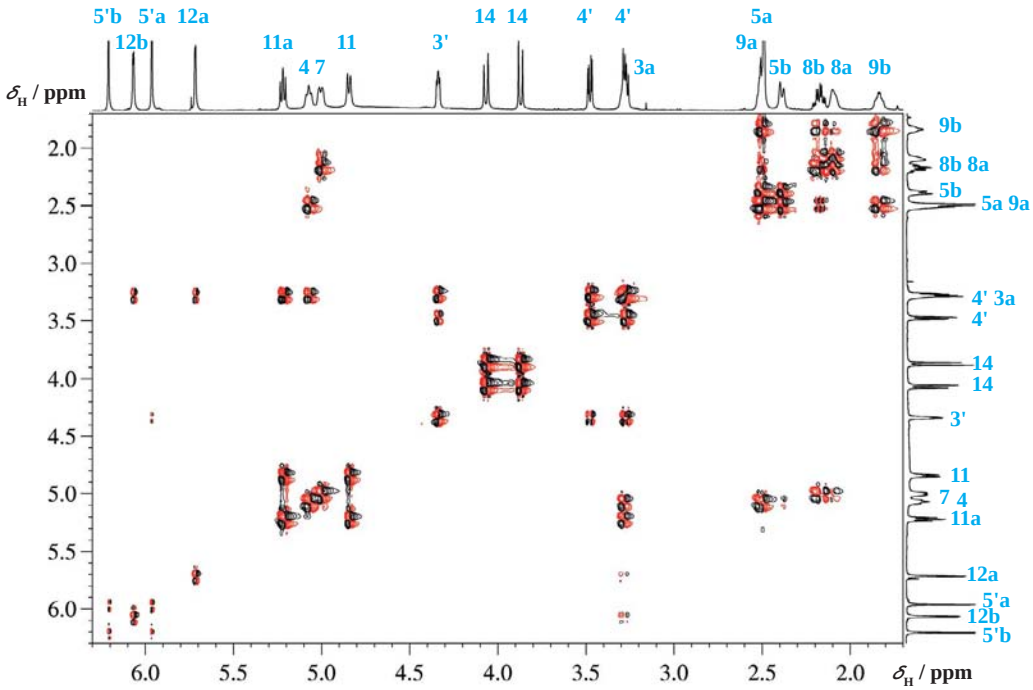


Fig. 5.6-9 Double quantum filtered COSY spectrum

Although the olefinic $^{2}J_{HH}$ of terminal methylene protons is very small, the COSY spectrum reveals in its lower left corner that the first and third lines in the proton spectrum form one olefinic methylene group and the second and fourth signals another. From the last two signals at 6.07 and 5.72 ppm, a COSY cross peak leads to the signal of H-3a at 3.28 ppm, and this therefore assigns the signals of this olefinic methylene group to H-12a and H-12b. The other methylene group H-5' does not show cross peaks to the side chain. Having already assigned proton 3a at 3.28 ppm, we find from this proton two cross peaks to CHO moieties at 5.22 and 5.07 ppm, which must therefore be H-11a and H-4. Since H-11a is coupled to an olefinic proton, but H-4 should reveal a cross peak into the aliphatic region to proton H-5, these two signals can be distinguished. One of the H-5 protons is found underneath the DMSO signal at 2.49 ppm, whereas the other is close by at 2.39 ppm, as revealed by the strong COSY cross peaks between them. The protons 4' should form an ABC spin system by couplings to each other and spin coupling to H-3'. Such a spin system is easily found at 3.27 and 3.48 ppm for the protons H-4' coupled to H-3' at 4.34 ppm. Thus, the signals of the remaining diastereotopic methylene group bound to oxygen, H-14 can be located at 3.87 and 4.07 ppm. In the aliphatic region, the protons H-8 should show a connection between each other and to the olefinic proton H-7. This is the case for the signals at 2.18 and 2.1 ppm, giving a cross peak to H-7 at 5.0 ppm. The left over olefinic signal at 4.84 ppm must therefore stem from H-11, which is of course connected by a cross peak to H-11a as described above. Finally, it can be seen that the protons H-8 are further coupled to the most aliphatic signal at 1.83 ppm, which reveals a cross peak to a signal very close to the DMSO signal, and these two signals are therefore assigned to H-9. In summary, the COSY spectrum, due to the rather well-dispersed chemical shift situation in this compound, is already sufficient to assign all proton signals with safety.

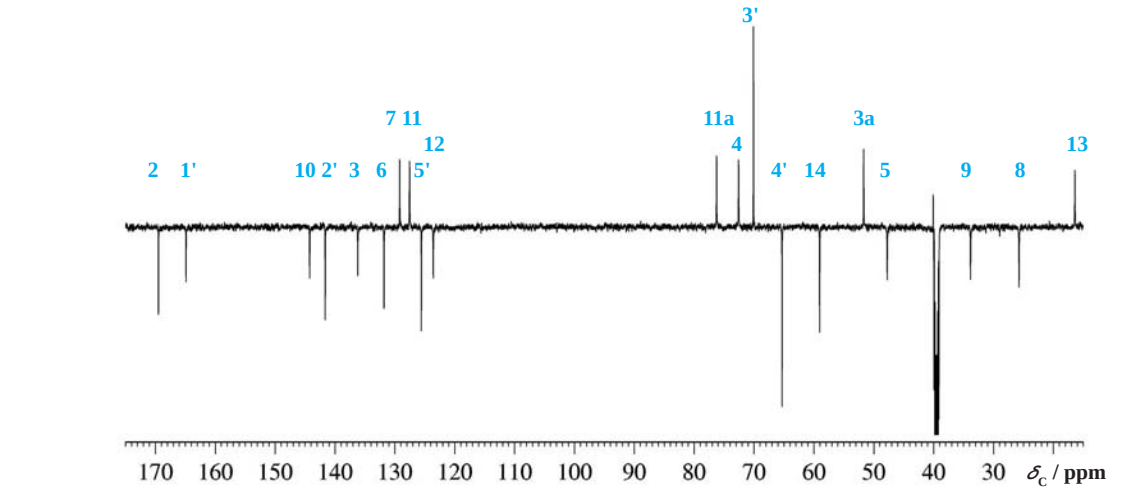
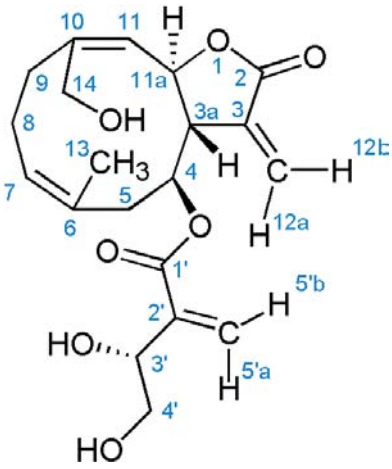


Fig. 5.6-10 APT ^{13}C NMR spectrum

The edited ^{13}C NMR spectrum reveals the expected 20 signals of this compound having, due to its chirality, no symmetry. Going from left to right we find first, of course, the two carbonyl groups at 169.5 and 164.8 ppm, which cannot yet be individually assigned. They are followed by four quaternary olefinic signals, which are negative, and two positive peaks. These must therefore belong to H-7 and H-11, the only olefinic CH moieties in this molecule. As the HSQC spectrum reveals later, the next two negative signals at 125.6 and 123.6 ppm belong to the terminal methylene groups C-5' and C-12. In the CHO region, we find, as required by the structure, four positive and two negative signals between 80 and 50 ppm, followed by the three aliphatic methylene groups C-5, C-9 and C-8 and the final signal of the methyl group C-13. Individual assignments are only possible with the help of the HSQC and HMBC spectra.

Fig. 5.6-11 Historical drawing of the blessed thistle plant



Scheme 5.6-3

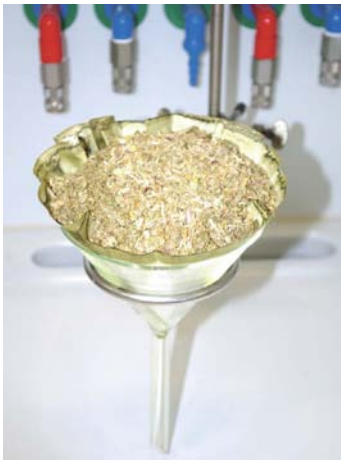


Fig. 5.6-12 A funnel with blessed thistle leaves after extraction with CH_2Cl_2–CH_3OH (9:1)

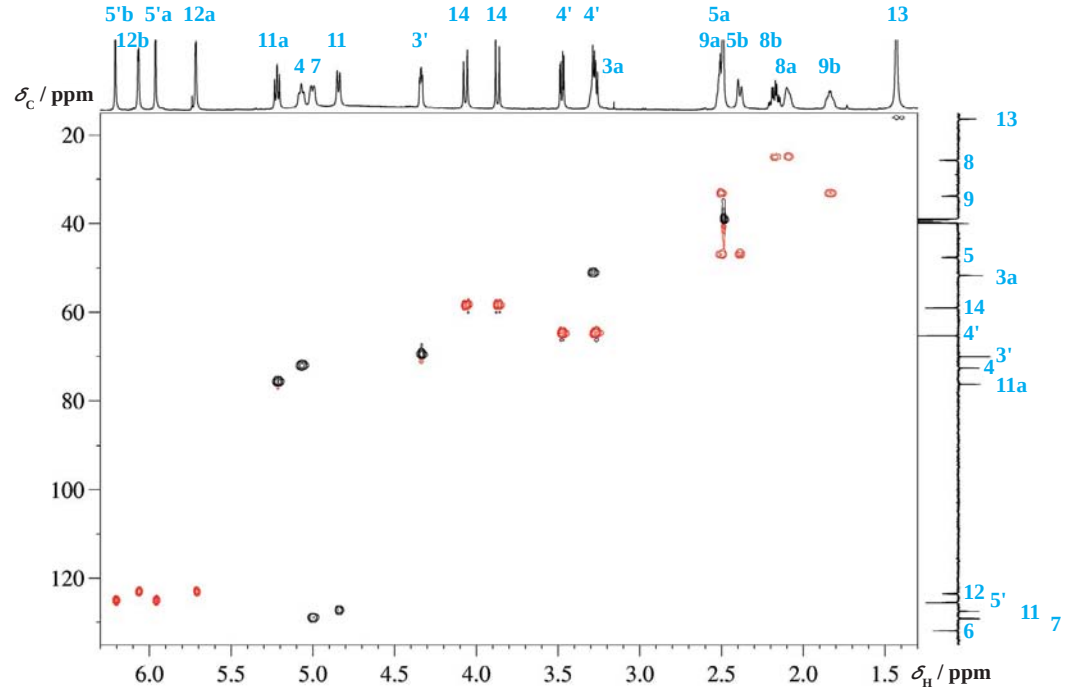


Fig. 5.6-13 HSQC spectrum

Since we have already assigned all proton signals with the help of the COSY spectrum, the HSQC spectrum will transfer this assignment information to carbon. At the same time, we may inspect the HSQC spectrum to check for any inconsistencies. In the lower left corner, we find four red correlation signals for the two terminal olefinic methylene groups H-5' and H-12. The four proton signals at about 5 ppm are split into two groups by the ^{13}C chemical shifts, the two olefinic carbon atoms 7 and 11 and the two CHO carbon atoms 11a and 4. Very nicely revealed by the red colour of the edited HSQC spectrum are the signals of the five diastereotopic methylene groups. Their inspection confirms the assignments made from the COSY spectrum.

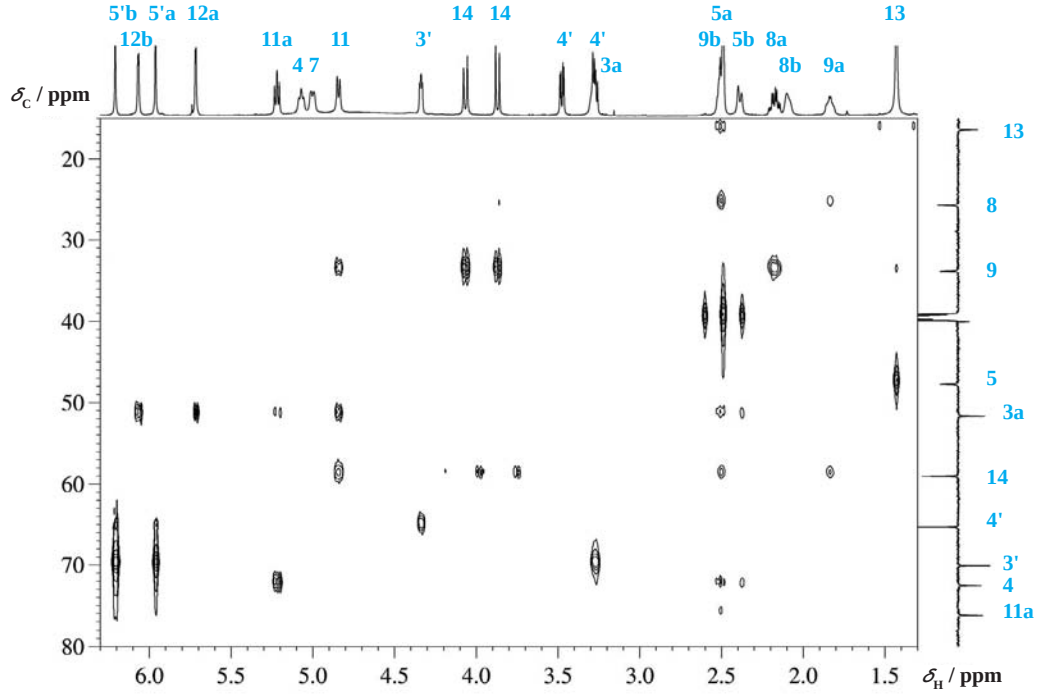


Fig. 5.6-14 Expansion of the HMBC spectrum for the aliphatic carbon atoms

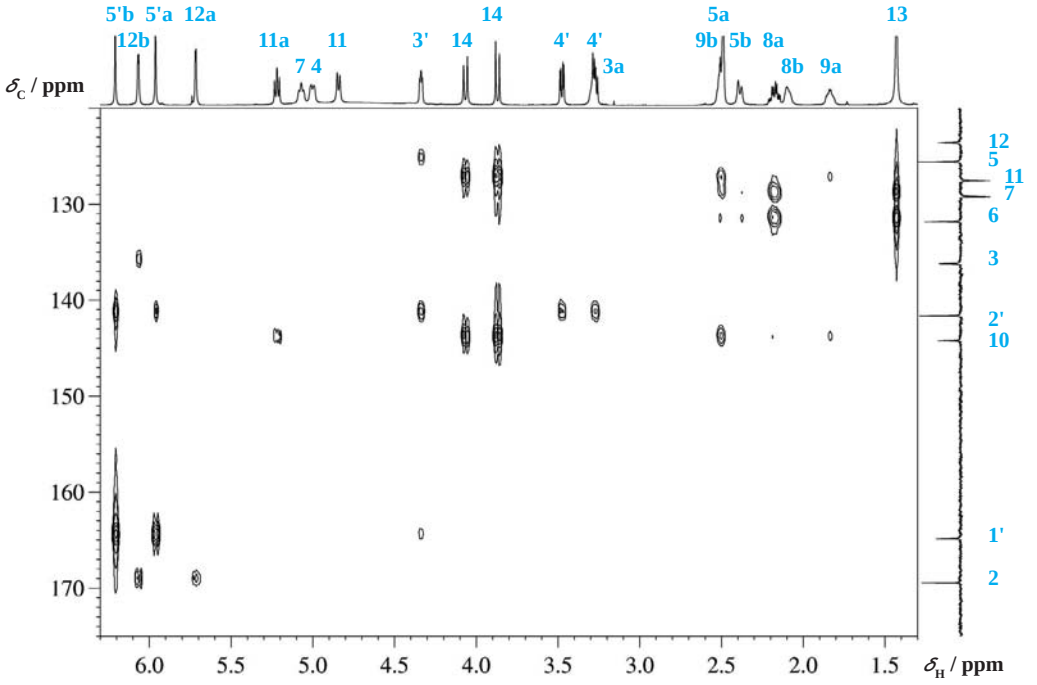
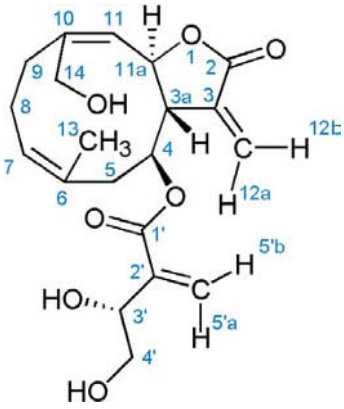


Fig. 5.6-15 Expansion of the HMBC spectrum for the sp^2-hybridized carbon atoms

Cnicin contains six quaternary carbon atoms and their individual assignment is possible only with the help of the HMBC spectrum. We show two HMBC expansions, one for the aliphatic carbon chemical shifts and one for the olefinic and carbonyl region. In the lower left corner of the latter the assignment of the two carbonyl groups becomes evident, C-2 is more deshielded and appears at 170 ppm. Compared with this, the chemical shift ratio is reversed for the quaternary olefinic carbon atoms C-2' and C-3. The signal at 140 ppm is seen from H-5, H-4' and H-3' and is therefore assigned to C-2', whereas the signal at 135 ppm is only seen by H-12 and therefore assigned to C-3. The distinction between C-6 and C-10 is very easy since C-6 is connected to the methyl group and C-10 is seen from the protons H-14. The HMBC expansion in the aliphatic chemical shift region of the carbon atoms confirms in detail the assignments made above.

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz
169.5	C_q	C-2	
164.8	C_q	C-1'	
144.2	C_q	C-10	
141.6	C_q	C-2'	
136.2	C_q	C-3	
131.8	C_q	C-6	
129.2	CH	C-7	5.00, J = 11.1
127.5	CH	C-11	4.84 $J_{11,11a}$ = 9.6
125.6	CH_2	C-5'	a: 5.96, b: 6.21
123.6	CH_2	C-12	a: 5.72, b: 6.07, $J_{12a,12b}$ = 3.2
76.2	CH	C-11a	5.22, $J_{11a,3a}$ = 8.3, $J_{11a,11}$ = 9.6
72.6	CH	C-4	5.07, J = 8.3
70.1	CH	C-3'	4.34, $J_{3'4'}$ = 4.3, 6.0
65.3	CH_2	C-4'	3.48, $J_{3'4'}$ = 4.3, $J_{4'4'}$ = –11.1, 3.27, $J_{3'4'}$ = 6.0, $J_{4'4'}$ = –11.1
59.0	CH_2	C-14	4.07, 3.87, J = –13.6
51.7	CH	C-3a	3.28
47.7	CH_2	C-5	a: 2.50, b: 2.39, J = –12.2
33.8	CH_2	C-9	a: 1.83, b: 2.51
25.7	CH_2	C-8	a: 2.10, b: 2.18, J = 4.9, 5.2, –12.2
16.4	CH_3	C-13	1.43

Tabel 5.6-1 NMR data for cnicin



Scheme 5.6-4

Am Brunnen stand ein großer Hund,
Trank Wasser dort mit seinem Mund.
Da mit der Peitsch' herzu sich schlich
Der bitterböse Friederich;
Und schlug den Hund, der heulte sehr,
Und trat und schlug ihn immer mehr.
Da biß der Hund ihn in das Bein,
Recht tief bis in das Blut hinein.
Der bitterböse Friederich,
Der schrie und weinte bitterlich.
Jedoch nach Hause lief der Hund
Und trug die Peitsche in dem Mund.

Ins Bett muß Friedrich nun hinein,
Litt vielen Schmerz an seinem Bein;
Und der Doktor sitzt dabei
Und gibt ihm bitt're Arzenei.

Heinrich Hoffmann (1809–1894)
Struwwelpeter,
Die Geschichte vom bösen Friederich

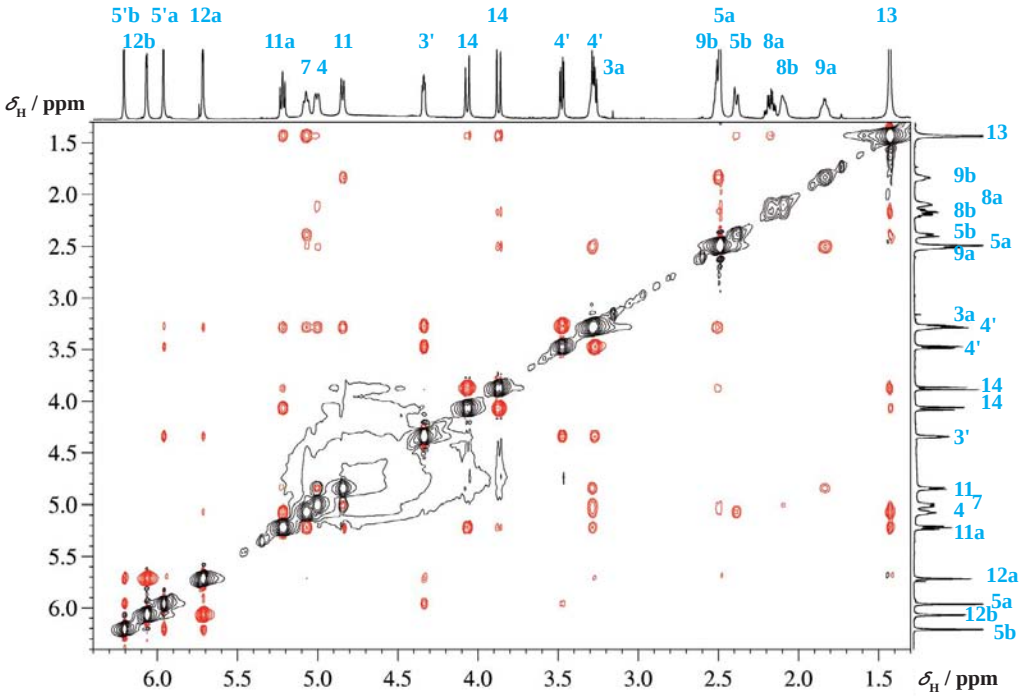


Fig. 5.6-16 NOESY spectrum

So far we have not decided which of the signals for protons H-5' and H-12 belong to H-5'a or H-12a. There are NOE cross peaks for the signal of H-5' at 5.96 ppm leading to H-3' and both H-4', and this will only be possible for H-5'a. A distinctive NOE cross peak connects the signals at 6.21 ppm with that at 5.72 ppm and this is easily explained if we assign the former to H-5'b and the latter to H-12a. The olefinic protons H-11 and H-7 are connected by NOE cross peaks, as are the signals of H-11a and H-4 within the 10-membered ring. One of the protons signals, H-9 at 1.83 ppm, displays an NOE cross peak to the olefinic proton H-11 and this should therefore be H-9a due to the geometry of the double bond.

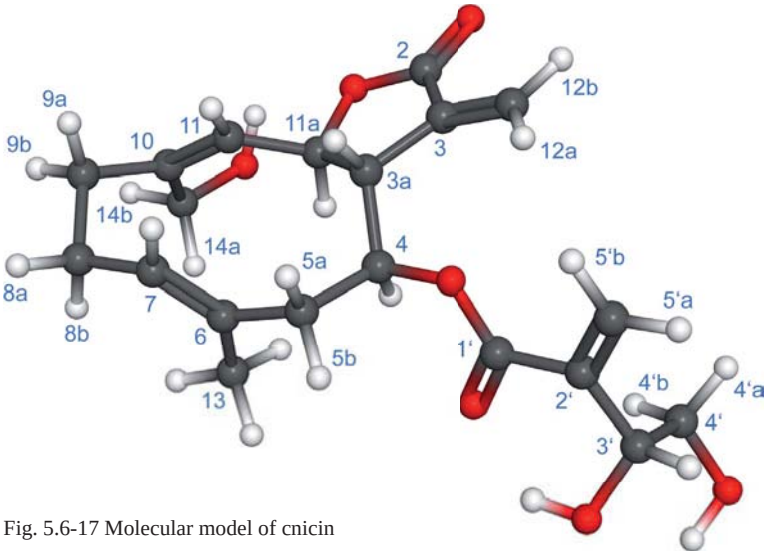


Fig. 5.6-17 Molecular model of cnicin

The methyl group H-13 displays cross peaks to one of the H-8 signals, which is therefore assigned to H-8b at 2.10 ppm. H-5a at 2.50 ppm is distinguished from H-5b due to the NOE cross peak to H-3a. The cross peaks between the methyl group H-13 and the protons H-11a and H-14 indicate that these are on the same side of the molecule. H-5'a shows cross peaks to both H-4' as required from the molecular model.

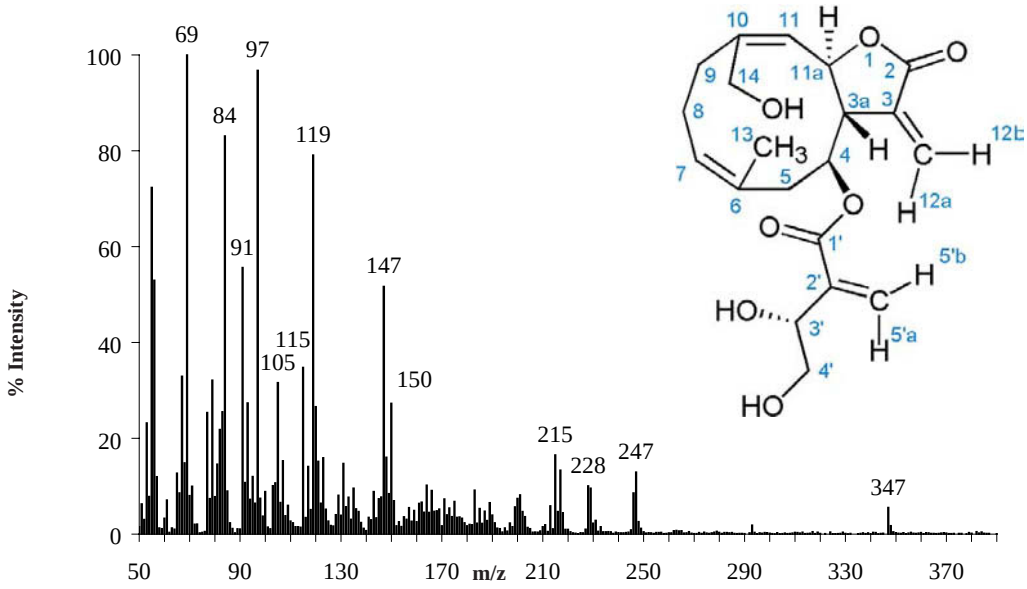
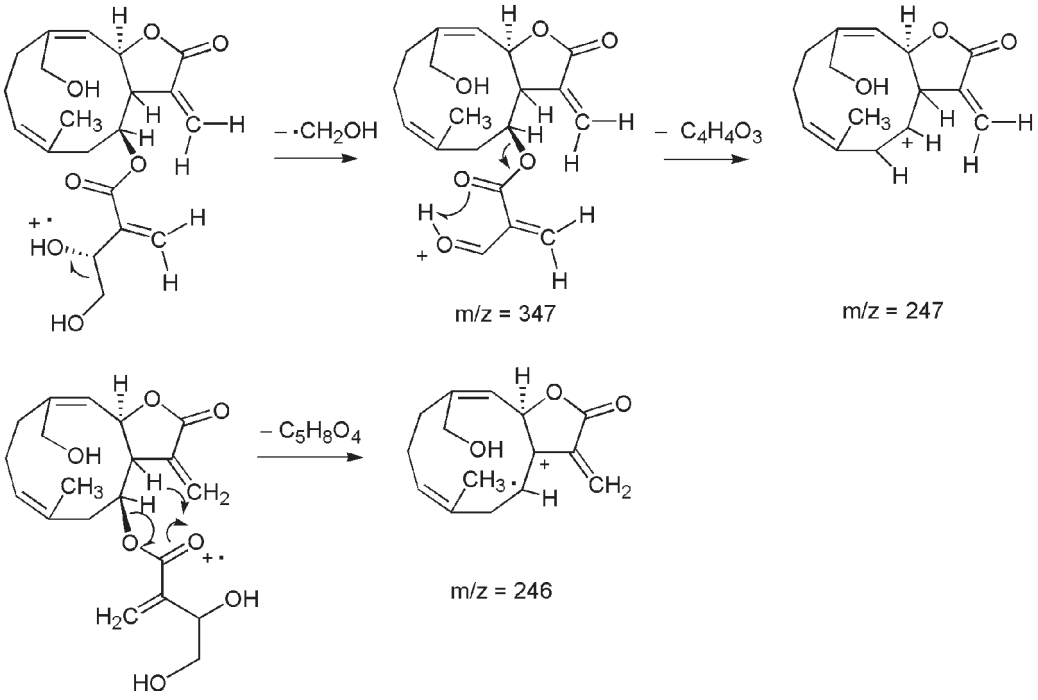


Fig. 5.6-18 Mass spectrum (EI)

The EI mass spectrum does not show the molecular ion signal, even after amplification. The reason is probably that directly after ionization a hydroxymethylene radical is split off, as indicated in the scheme below. Removal of the side chain leads to the ion with m/z = 247. Cnicin can also be ionized at the carbonyl atom of the side chain. A McLafferty-type rearrangement can then lead to an ion with m/z = 246.



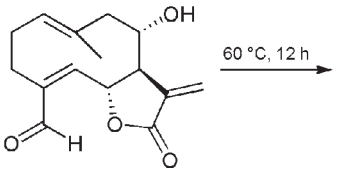
Scheme 5.6-6 Fragmentation of cnicin

5. Questions

A. To which class of natural products do the following four bitter compounds belong: limonin, humulon, absinthin and quinine, and in which plants do they occur? Make a note on their use or problems.

B. Which are the five basic taste sensations and by which kind of compounds are they caused?

C. Draw the formula of the product of the following thermal Cope rearrangement described in ref. [5]:



Scheme 5.6-7

D. Indicate the four isoprene units present in cnicin using the formula given at the first page of this section.

E. Which of the IR carbonyl vibrations belong to the lactone ring?

F. Why is C-2 more deshielded than C-1'?

G. The protons H-9 show the largest diastereotopicity in this molecule. Give a reason and suggest why H-9a is considerably deshielded.

H. Can you differentiate between the two protons H-14?

6. Own Observations

5.7 Abietic Acid

(1*R*,4a*R*,4b*R*,10a*R*)-1,2,3,4,4a,4b,5,6,10,10a-Decahydro-1,4a-dimethyl-7-(1-methylethyl)-1-phenanthrenecarboxylic acid

From rosin (colophony) of pine trees
Pinus sylvestris (Pinaceae)

$C_{20}H_{30}O_2$, MW 302.45

CAS RN 514-10-3, BRN 1915575, 2059296, 2221449, 2221451

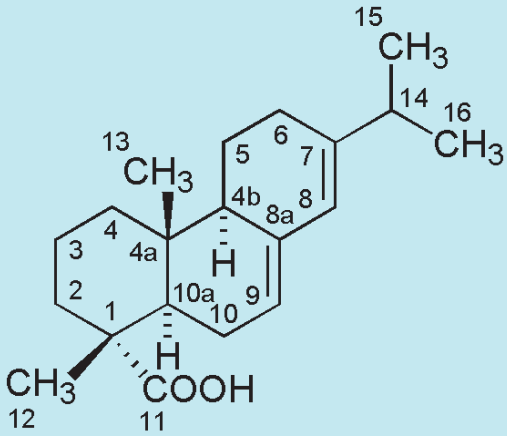
$[\alpha]_D^{15}$ –102° (ethanol) (database value)

Colourless plates, mp 172–175 °C,

Abietic acid is commercially available in enriched form.

Synonymous names:
(–)-Abietic acid, 7,13-Abietadien-18-oic acid, 13-Isopropylpodocarpa-1,13-dien-15-oic acid, Sylvic acid

Level: difficult



1. Background: Greek fire and mystic smoke

Fig. 5.7-1 Mountain pine

Abietic acid in direct translation means “acid from the firs”. This name is due to the fact that firs (Genus *Abies*) and also pines (Genus *Pinus*) as coniferous trees produce an oleoresin as a hydrocarbon secretion from which rosin (synonym colophony or Greek ῥητίνη) is made. It is the source for the isolation of abietic acid, one of several resin acids that are structurally shared in two groups, the abietic-type acids and the pimaric-type acids.

When walking through a forest of coniferous trees, one can often see on their trunks where drops of resin have been oozing out of the tree. This gives a hint at the natural purpose of resin production by the plant, namely the sealing of wounds by a viscous secretion that can finally become a solid and is at the same time a pest repellent against infections of the wood. The solid parts, which remain on the trees, consist mainly of abietic acid. When this happened in the prehistory of the Earth it was − under special circumstances − the source of the formation of a valuable “mineral” called amber, which may be up to 250 million years old. It is interesting that the English word refers to the colour of this fossil resin. In contrast, the German word refers to the properties of amber, its combustibility and its (relative) hardness. Amber in German is *Bernstein*; originally it was *börnsteen*, which means *burning stone*. The Greeks had yet another look at the material; for their name, see the Questions section.

The natural oleoresin is a viscous liquid and consists of a mixture of volatile mono- and sesquiterpenes (α- and β-pinene, limonene, terpinolene, caryophyllene, to name just a few) and non-volatile resin acids as protectants and wood preservatives. Resin arises from the parenchymatous epithelial cells surrounding the resin ducts in the trunks. Abietic acid is a tricyclic diterpene carboxylic acid with the molecular formula $C_{20}H_{30}O_2$ that fits correctly to four isoprene units. Biosynthetically, the uncyclized precursor of abietic acid is geranylgeranyl diphosphate that cyclizes to abieta-7,13-diene, wherein one of the geminal methyl groups undergoes oxidation to form abietic acid.

Fig. 5.7-2 Amber necklace

As with many higher terpenoid compounds, the definitive elucidation of the structure was not easy and took decades in a stepwise process. However, the problem could be solved with classical chemical means before the entry of NMR spectroscopic methods into structural assignment. Reductive and oxidative treatment of natural products belonged to the standard methods. It was the father of one of us who investigated abietic acid in his dissertation work [1]. One of the classical steps reported there is heating of abietic acid with sulfur to achieve aromatization by dehydrogenation of all rings. Hence the hydrocarbon retene (1-methyl-7-isopropylphenanthrene, $C_{18}H_{18}$) was obtained (with loss of two C as CO_2 and CH_4), and gave the first information on the C skeleton. The well-known group of Ružička at the ETH Zürich was dealing with the structural assignment of many terpenes and also determined the structure of abietic acid [2]. Later, the same group

used abietic acid in a successful attempt to show experimentally the connection of the steroids with the di- and triterpenes [3]. The principal method was that by sophisticated stepwise oxidative degradations links to compounds of known stereochemistry were established that chemically gave access to an unknown structure. However, in this book the abietic acid structure is assigned spectroscopically. As a member of the chiral pool, abietic acid is a valuable starting material for synthesis [4].

Rosin is mainly produced in Indonesia, China, Vietnam and Mexico. In the USA it is made in the South Atlantic and Eastern Gulf states. In Europe, France is a main supplier. The world production is about 1 000 000 tons per year, i. e. rosin is a real bulk product. Different species of pine trees yield the resin from which rosin is separated. Sodium abietate ("Kraft soap"), a so-called resin soap, is released together with tall oil ("liquid rosin", "pine oil") as a chemical pulping byproduct of the Kraft process, which is directed at the isolation of cellulose fibres in the form of wood pulp from wood chips with removal of lignin under strongly basic conditions.

The author remembers that in the 1960s in German forests, resin was obtained from coniferous trees by the following method: Two series of three or four sloping grooves were carved by hand from above the left and from above the right sides at a height of about 1 m in the healthy tree trunk half way around the tree. The grooves joined together at the lowest point, where a small empty terracotta flowerpot was hung, tied by means of a wire around the tree. The tree, thus wounded, began to ooze stringy resin, which dropped into the flowerpot. Occasionally, lumbermen came, collected the pots and replaced them with new, empty ones. There was a rule for the forester that a tree needed to have a certain age before exudation of resin was possible. Also, it was not permitted to allow the tree to recover for too long a time because another material was expected from it: wood.

Early in history humans recognized the value of the oleoresin. It was handled and traded in Colophon, an ancient Ionic city. The oleoresin was separated by distillation in copper stills into two parts, a liquid one, called oil of turpentine, and a solid one, called colophony or rosin. The essential oil is carried off between 100 to 160 °C, leaving the hot fluid resin as the remainder, which is purified by pouring it through straining wadding. Rosin can be obtained in different colours depending on the natural source and the manner of distillation, which can also be done as a steam distillation to obtain oil of terpentine. The colours can vary from black to water clear: the value is higher the paler the colour is. We used a resin with a honey-like colour, i.e. of fairly good quality.

At ambient temperature, colophony is brittle and has a very faint piny odour. It becomes soft at the temperature of boiling water. Understandably for a resin-derived material, it is still slightly sticky. It is used in many ways, some of them being well known and others almost unknown. Maybe the best known, which is due to its stickiness, is its use for rosining the bows of string instruments. For example, a bow to

οἱ δ' ἐνιαυτὸν ἅπαντα παρ' ἡμῖν αὖθι μένοντες
ἐν νηῒ γλαφυρῇ βίοτον πολὺν ἐμπολόωντο.
ἀλλ' ὅτε δὴ κοίλη νηῦς ἤχθετο τοῖσι νέεσθαι,
καὶ τότ' ἄρ' ἄγγελον ἦκαν, ὃς ἀγγείλειε γυναικί.
ἦλυθ' ἀνὴρ πολύϊδρις ἐμοῦ πρὸς δώματα πατρὸς

χρύσεον ὅρμον ἔχων, μετὰ δ' ἠλέκτροισιν ἔερτο.
τὸν μὲν ἄρ' ἐν μεγάρῳ δμῳαὶ καὶ πότνια μήτηρ
χερσίν τ' ἀμφαφόωντο καὶ ὀφθαλμοῖσιν ὁρῶντο,
ὠνον ὑπισχόμεναι: ὁ δὲ τῇ κατένευσε σιωπῇ.
ἦ τοι ὁ καννεύσας κοίλην ἐπὶ νῆα βεβήκει,

ἡ δ' ἐμὲ χειρὸς ἑλοῦσα δόμων ἐξῆγε θύραζε.

Homer (8th century BC)
οδθσσειασ, 15, 455–465

play a violin is strung with horsehair. To obtain an acceptable vibration of the string, a piece of rosin is regularly applied to this horsehair to increase the friction between the bow and string as the vibrating element, from which the tone arises. Another use in the area of the fine arts is that ballet dancers may rub their shoes in rosin to reduce slipping on the stage. For the same reason, baseball pitchers increase the grip of their throwing hand with rosin powder. Similarly, rock climbers use rosin for better friction between their hands and the rock.

Of course, these special applications do not consume a major part of the colophony. Other applications are far more common, and to mention just a few: rosin is used as a flux in soldering and it is a component of certain soaps (as resin acid salts), varnishes, glazing agents, adhesives, printing inks and sealing wax. For the author, its most amazing implementation is as part of the so-called "mystic smoke". This is a gummy mixture of rosin, oil and wax. When it is rubbed and then suddenly stretched between the fingertips, it gives off small puffs of "smoke", a real miracle when you see it for the first time. However, all who have to work constantly with rosin need to do so with care. Rosin and abietic acid are known to have adverse effects and to act as irritants. Therefore, in the EU abietic acid is classified as a harmful and irritant compound (Xi). Soldering workers, who are exposed long-term to the fumes of the colophony added to the soldering wire to obtain flux, are at risk. Such fumes may cause bronchial asthma. All who have repeated direct skin contact (musicians, dancers) may be in danger of contact dermatitis [5]. Searching for safe substitutes is therefore necessary.

Sed et mare scrutantur, ac soli omnium sucinum, quod ipsi glesum vocant, inter vada atque in ipso litore legunt. Nec quae natura quaeve ratio gignat, ut barbaris, quaesitum compertumve; Diu quin etiam inter cetera eiectamenta maris iacebat, donec luxuria nostra dedit nomen. Ipsis in nullo usu: rude legitur, informe perfertur, pretiumque mirantes accipiunt. Sucum tamen arborum esse intellegas, quia terrena quaedam atque etiam volucria animalia plerumque interlucent, quae implicata humore mox durescente materia cluduntur. Fecundiora igitur nemora lucosque, sicut Orientis secretis, ubi tura balsamaque sudantur, ita Occidentis insulis terrisque inesse crediderim, Quae vicini solis radiis expressa atque liquentia in proximum mare labuntur ac vi tempestatum in adversa litora exundant. Si naturam sucini admoto igni temptes, in modum taedae accenditur alitque flammam pinguem et olentem; mox ut in picem resinamve lentescit.

Publius Cornelius Tacitus (55−115)
Germania, 45

A historical application of resin was as part of Greek fire (liquid fire), a feared incendiary device invented in the Byzantine Empire as a weapon for defence and attack. The capital of this so-called Eastern Roman Empire was Constantinople (Byzantium); its population spoke Greek, hence the name Greek fire. Its exact original formula was a military secret and is unknown today. However, the general composition is understood and it surely must have contained highly combustible hydrophobic liquids such as petroleum, naphtha and resin. The Byzantines used it in naval battles with strong effect as it could continue burning on water. For the first time it was used during the first siege of Constantinople (674–678) to defend the walls of the city and repel the besieging forces of the Arabs; it was later used against the Russians and the Venetians. It could be thrown in the form of burning flasks by light catapults as far as 400 m and later it was discharged by a pump mechanism at close distances in the manner of a flamethrower. Hence Greek fire is to a large extent responsible for the many Byzantine military victories and it is even regarded as the reason why the whole Empire survived as long as it did. After more than a millennium, Constantinople was captured by the Ottoman Empire in 1453.

2. Literature

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Fig. 5.7-3 Pine needles

3. Isolation

3.1 Principle

It is difficult to isolate pure abietic acid from rosin as a source. Although it is the main component, it is accompanied by some isomers (see Question C) and other very related compounds. Therefore, it was a breakthrough when Harris and Sanderson discovered that the amount of abietic acid could be enhanced to nearly 50% by an acid-catalysed isomerization process of rosin [6]. The isolation described here is based on their optimized procedure reported later [7].

Fig. 5.7-4 Mountain pine

The crucial steps are:

(1) Precipitation of the abietate as diamylammonium salt. This ensures that non-acidic terpenoids are excluded by this salt formation. However, it is not certain if isomeric resin acids will not be precipitated to some extent also.

(2) Liberation of abietic acid by acidification of the dissolved salt with acetic acid. This operation requires rapid filtration once the abietic acid is precipitated to avoid isomerization of abietic acid as far as possible. The practical result shows the abietic acid not to be completely pure.

(3) A series of recrystallizations improves the purity only to a certain degree.

(4) Chromatographic methods of separation cannot be avoided; if possible, a preparative HPLC is recommended; although this will not deliver much material it will be of the highest purity for the purpose of measuring NMR spectra.

(5) The hints about storage given in ref. [7] should be kept in mind, especially storage with exclusion of air to avoid oxidation.

3.2 Method

Rosin (53 g) is placed in a 1 L round-bottomed flask, ethanol (150 mL) and concentrated HCl (8.5 mL) are added and the solution is refluxed for 2.5 hours under an N_2 atmosphere to avoid air oxidation. Ethanol and water are then removed in vacuo. The remaining dark orange residue is dissolved in diethyl ether (200 mL), washed with water to remove any HCl and dried over Na_2SO_4 (30 g). The Na_2SO_4 is removed by filtration and the ether by distillation in vacuo. The remaining residue is warmed in a hot water bath and poured on to a clean ceramic plate on a laboratory bench to cool to a brittle mass.

This mass is broken, transferred into a 500 mL Erlenmeyer flask and dissolved in acetone (75 mL). At incipient boiling of the solution, dipentylamine (25.4 g) is added slowly with vigorous stirring.

The flask is flushed with nitrogen, stoppered and allowed to stand overnight for cooling to ambient temperature. Crystals separate. The flask is then immersed in an ice bath, the mixture is stirred to increase crystallization and finally the crystals of the crude diamylammonium abietate are filtered by suction; 44.3 g of orange coloured material are obtained. This solid is recrystallized four times. In each case, ca. 20 mL of acetone are used per gram of solid. The acetone volumes of the single recrystallizations are, 900, 710, 650 and 440 mL. A final crop of 17.0 g of pure diamylammonium abietate is obtained. The last mother liquor is slightly reduced in vacuo to yield another crop of 5.0 g.

All 22.0 g of this salt were placed in a 1 L Erlenmeyer flask and dissolved in hot ethanol (200 mL). After cooling, glacial acetic acid (7.8 g) is added in one portion with vigorous stirring. Water (180 mL) is added slowly with vigorous agitation until the first crystals of abietic acid appear. The remaining water is then added more rapidly. Immediately after this

operation, the abietic acid is filtered by suction (to avoid isomerization) and washed with distilled water until indicator paper does not show an acidic reaction of the washing water. The abietic acid is recrystallized by dissolution in boiling ethanol (160 mL), followed by addition of distilled water (190 mL) and cooling. The pale yellow crystals are filtered by suction and dried in vacuo. The yield is 17.1 g.

This material is recrystallized from methanol (100 mL) with addition of charcoal powder (5 g) for decoloration. Another recrystallization from ethanol follows. Finally, 10.7 g of abietic acid are obtained, which, however, is still off-white and not colourless. The mp is 160 °C. Therefore, yet another recrystallization is necessary, this time from a methanol–cyclohexane mixture (25 mL; 4:1, v/v).

3.3 Purification

(a) By preparative thin-layer chromatography: Eluent: ligroin (bp 50–80 °C)–ethyl acetate (5:1, v/v). A 35 mg amount of the above abietic acid is dissolved and separated on a preparative TLC plate (silica gel 60). Detection is by UV irradiation.

(b) By preparative column chromatography: Eluent: ligroin (bp 50–80 °C)–ethyl acetate (5:1, v/v). A 0.2 g amount of the above acid is dissolved in a small amount of glacial acetic acid. The fractions obtained are analysed by TLC as above and fractions containing abietic acid are combined. The solvent is removed in vacuo and 100 mg abietic acid remain.

c) By preparative HPLC: Eurospher preparative 100-C-18 column, 250 mm × 16 mm, 5 μm material. Eluent: isocratic methanol, aliquots of 5 mg of raw abietic acid, flow rate 2 mL/min, retention time 30 min.

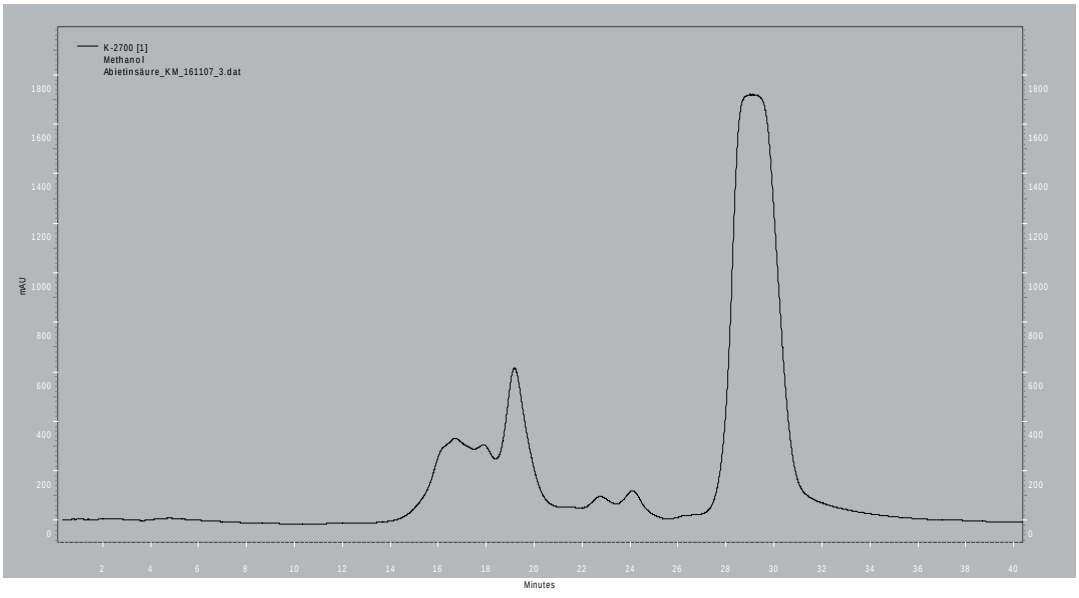


Fig. 5.7-5 Preparative HPLC trace of commercial abietic acid (Aldrich) sold in 75% purity

4. Spectra and Comments

Fig. 5.7-6 Colophony

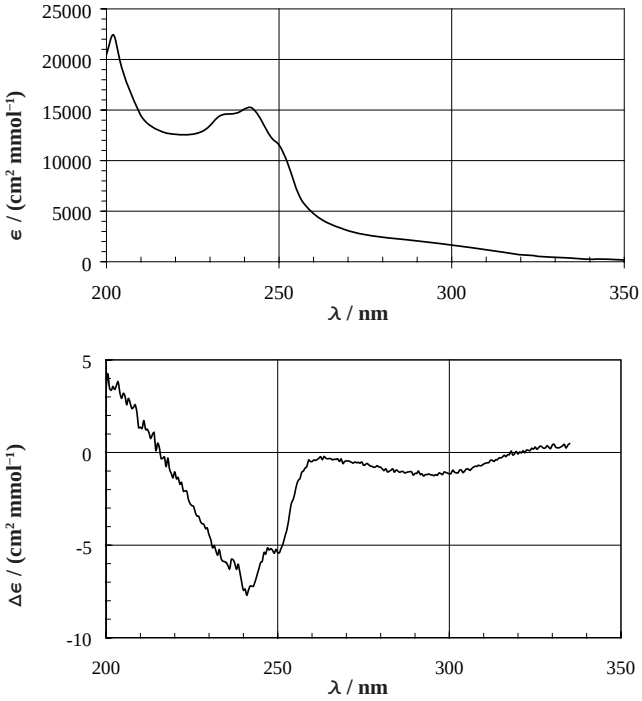


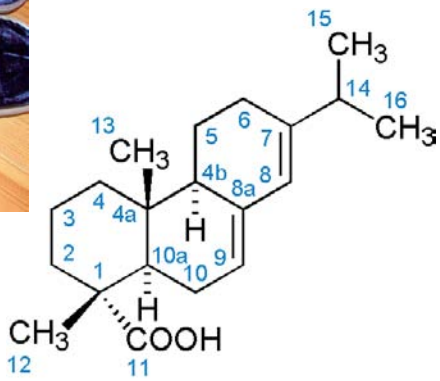
Fig. 5.7-7 UV and CD spectra in ethanol

Abietic acid has two isolated chromophores, the carboxyl group and the transoid butadiene unit. The former gives rise to the UV absorption at 202 nm and the latter is likely responsible for the broad band at 240 nm. Both have rather strong absorption coefficients. Even the long tail at 290 nm still has an absorption coefficient of 2000 cm^2 $mmol^{-1}$. As abietic acid is chiral with four stereogenic centres it is not surprising that it shows a rather strong and negative Cotton effect at the main band at 240 nm. The shoulder at 290 nm also shows a weak negative Cotton effect.

Fig. 5.7-8 Colophony used for violin strings

Fig. 5.7-9 A violin and its case

Scheme 5.7-1



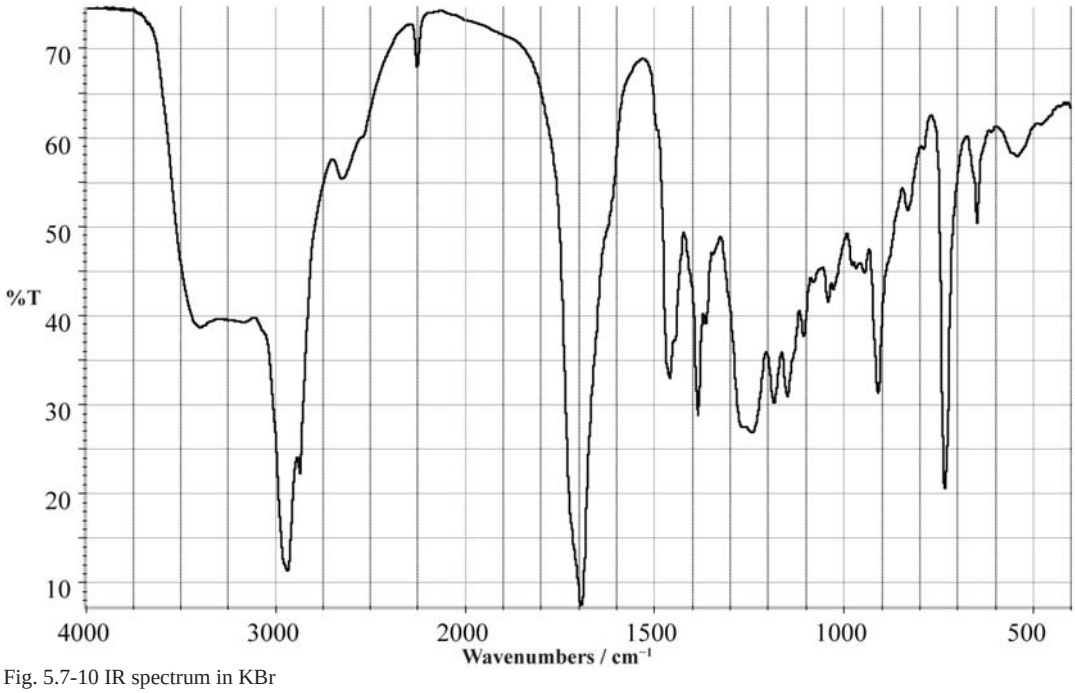


Fig. 5.7-10 IR spectrum in KBr

The IR spectrum is rather unstructured and shows a very broad OH absorption typical of carboxylic acids. The many sp^3 hybridized CH valence vibrations give a broad signal at 2950 cm^{-1}. The carbonyl vibration is also very broad and overlaps with the double bond vibration band, which is hardly seen as a shoulder at 1600 cm^{-1}. Note the sharp absorption at 2100 cm^{-1}, which is an overtone vibration also often seen in amino acids.

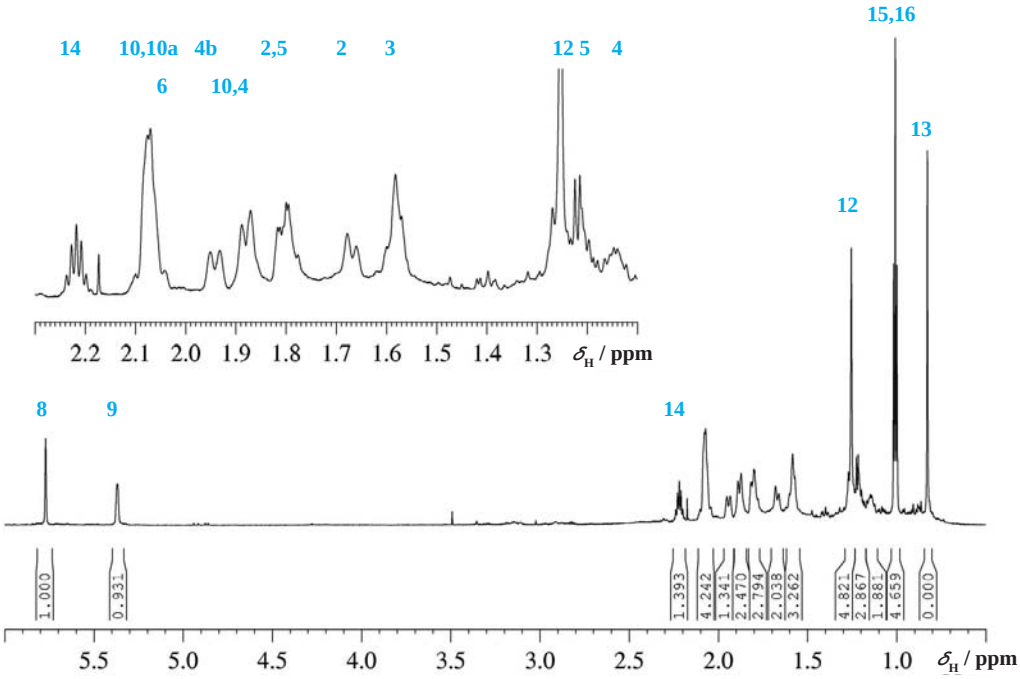
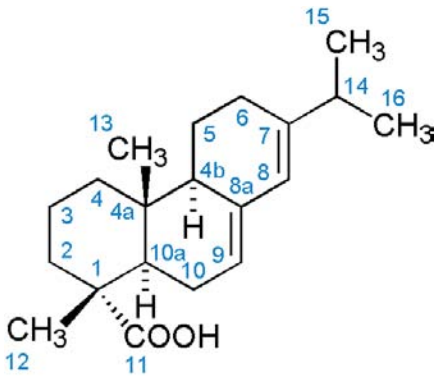


Fig. 5.7-11 ^{1}H NMR spectrum at 700 MHz in $CDCl_3$

Looking at the molecular structure, one expects to see in the proton spectrum two olefinic signals: the isolated and hence sharp signal should stem from H-8, whereas H-9 is coupled to the methylene group of C-10 and therefore gives a broadened signal. We expect to see an isopropyl group with two different methyl group signals, since abietic acid is chiral and therefore the methyl groups will be diastereotopic. The methine proton H-14 of the isopropyl group can be readily identified at 2.22 ppm by its multiplicity. The remaining two methyl group signals of H-13 and H-12 embrace the isopropyl group signals and it is safe to assume that the angular methyl group H-13 at 0.83 ppm is more shielded whereas H-12 will be deshielded by the electron demand of the carboxyl group.



Scheme 5.7-2

Parce, precor: nostrum laceratur in arbore corpus. iamque vale" − "Parce cortex in verba novissima venit. Inde fluunt lacrimae, stillataque sole rigescunt de ramis electra novis, quae lucidus amnis excipit et nuribus mittit gestanda Latinis.

Publius Ovidius Naso (43−18)
Metamorphoses II, 361−366.

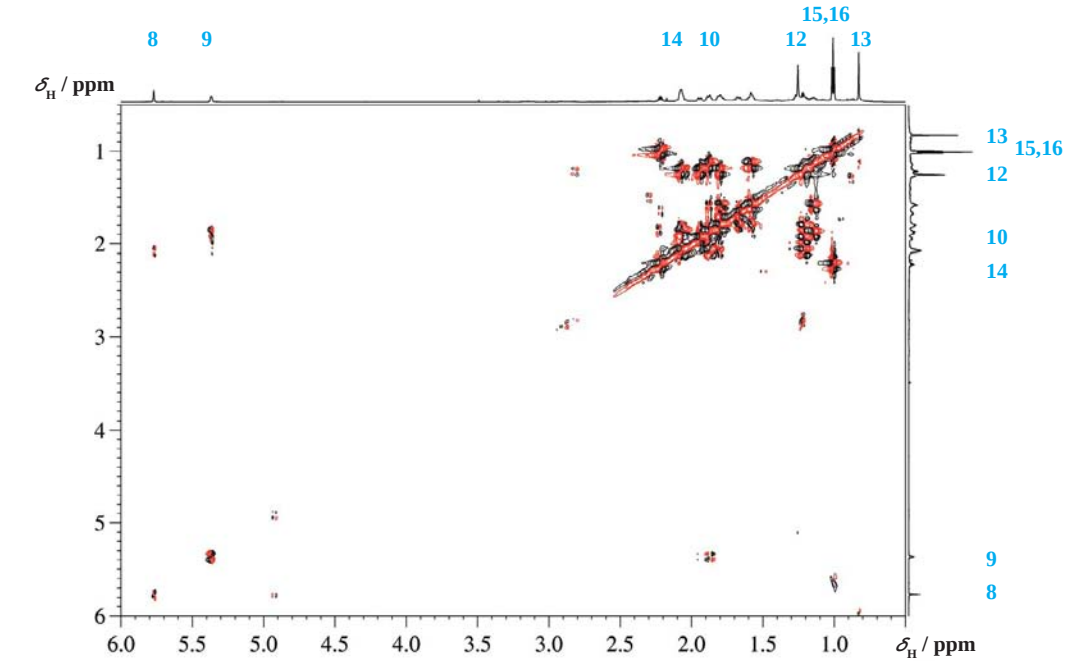


Fig. 5.7-12 Double quantum filtered COSY spectrum

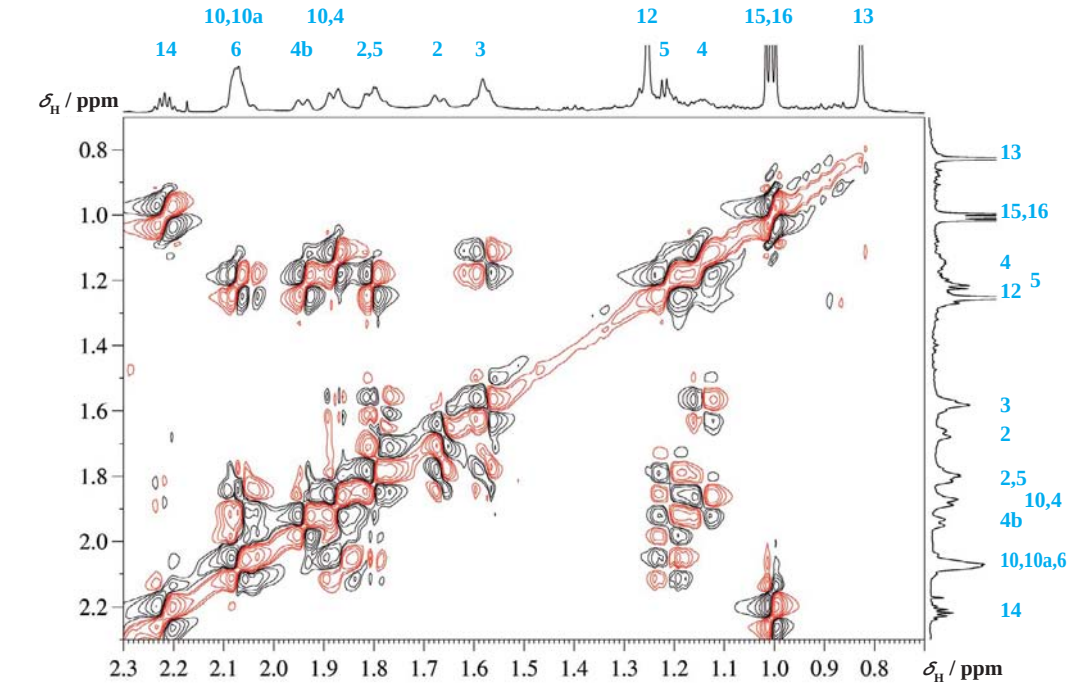


Fig. 5.7-13 Expansion of the COSY spectrum

The double quantum filtered COSY spectrum shows the signal of one of the H-10 protons at 1.88 ppm due to its coupling with the olefinic proton H-9. In the expansion of the COSY spectrum, the other H-10 proton can be located at 2.07 ppm. For further evaluation at this point, the COSY spectrum is too complicated due to the six diastereotopic methylene groups and overlapping signals.

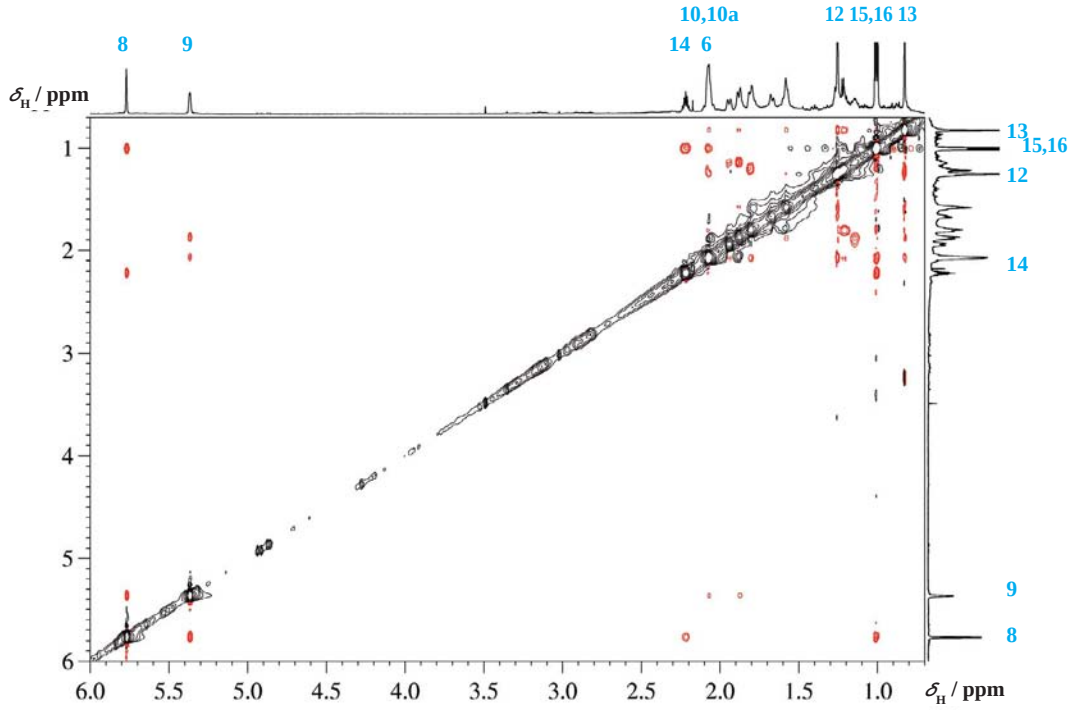


Fig. 5.7-14 NOESY spectrum

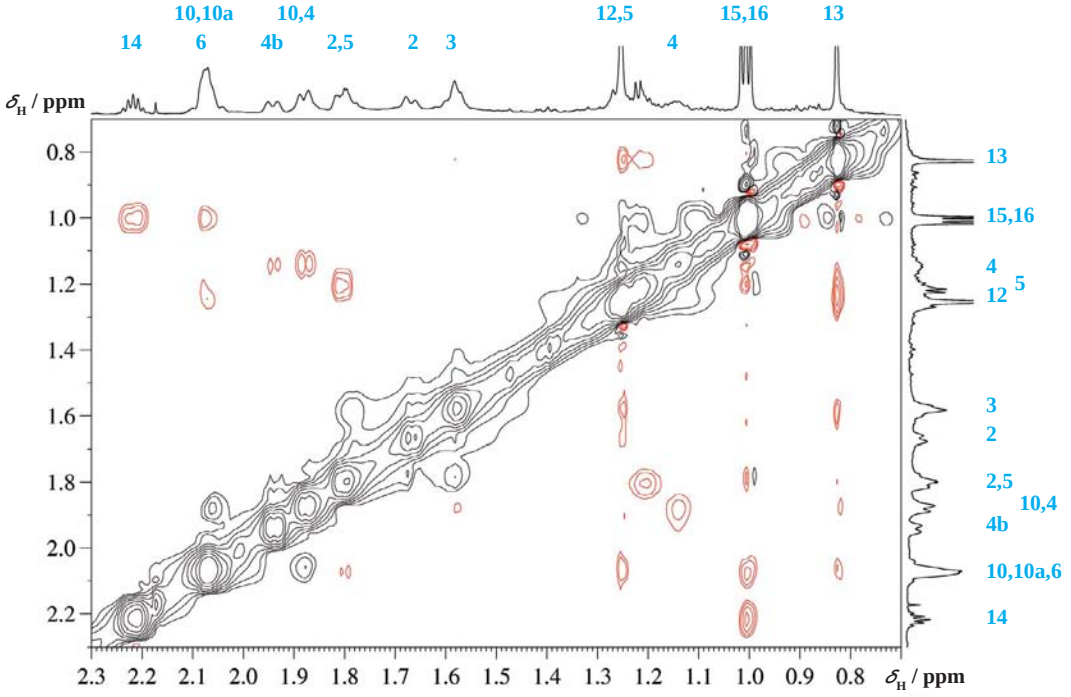


Fig. 5.7-15 Expansion of the NOESY spectrum

Forma e materia, congiunte e purette,
usciro ad esser che non avia fallo,
come d'arco tricordo tre saette.

E come in vetro, in ambra o in cristallo
raggio resplende sì, che dal venire
a l'esser tutto non è intervallo,

così 'l triforme effetto del suo sire
ne l'esser suo raggiò insieme tutto
sanza distinzïone in essordire.

Dante Alighieri (1265−1321)
Divina Commedia, *Paradiso*, XXIX, 9

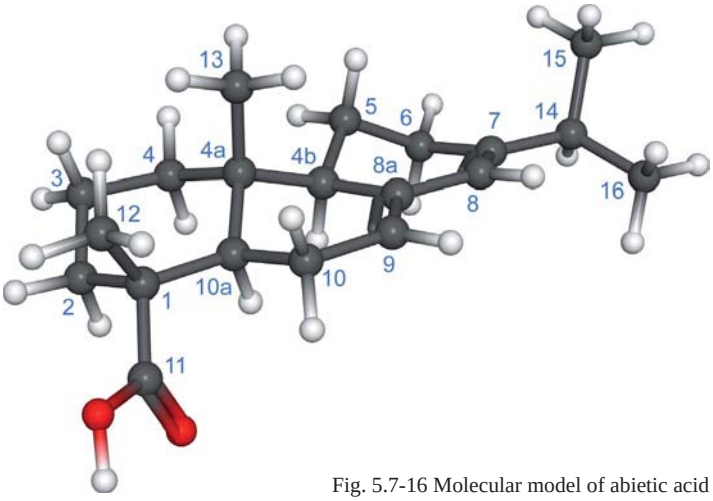


Fig. 5.7-16 Molecular model of abietic acid

In contrast to the COSY spectrum, the NOESY spectrum is less crowded and therefore much more helpful in this case. Starting in the olefinic region, we recognize that H-8 and H-9 “see” each other, as expected from the structure. H-8 shows in addition two cross peaks with the aliphatic region, both to H-14 at 2.22 ppm and to H-15/16, thus H-8 is indeed close to the isopropyl group. H-9 displays connectivities to both signals of the methylene group C-10 at 2.07 and 1.88 ppm, which assures their correct assignment. In the expansion of the NOESY spectrum, one observes besides the trivial cross peaks significant and stereochemically important information. For example, the methyl group signal H-13 at 0.83 ppm displays a strong cross peak to one of the H-5 protons at 1.22 ppm and to the methyl group H-12 at 1.25 ppm, indicating that both methyl groups are in a cisoid position at the six-membered ring. The methyl group signals of the isopropyl group also see the methylene group H-6 at 2.07 ppm, which assures this assignment. The diastereotopic protons of the two methylene groups H-4 and H-5 have, of course, large NOE effects between each other.

Fig. 5.7-17 Pine trees in the Pyrenees

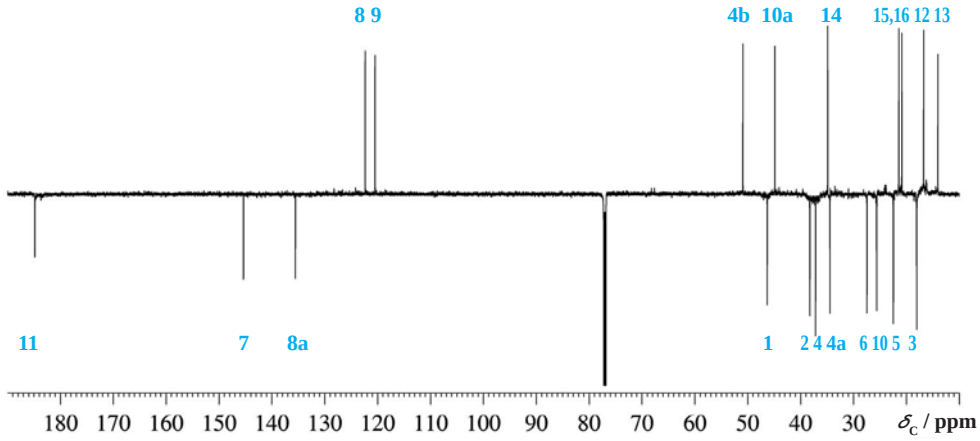
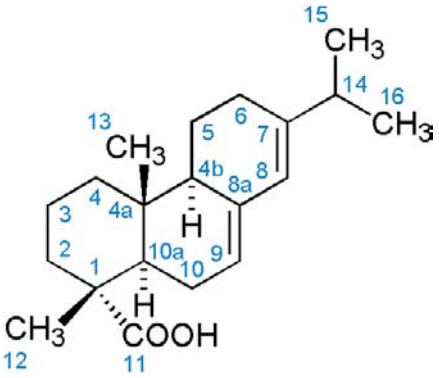


Fig. 5.7-18 APT $^{1\text{-}3}$C NMR spectrum at 175 MHz in $CDCl_3$

The ^{13}C APT NMR spectrum nicely displays all signals of the 20 different carbon atoms. As expected, we find 11 negative signals from the six methylene groups and the five quaternary carbon atoms and nine positive signals from the four methyl groups and the five methine carbon atoms of the molecule. However, apart from the assignment of the carboxyl group at 184.8 ppm, at this point we cannot individually assign the other signals by simple inspection.

Fig. 5.7-19 Pine trees at a beach

Fig. 5.7-20 Pine needles



Scheme 5.7-3

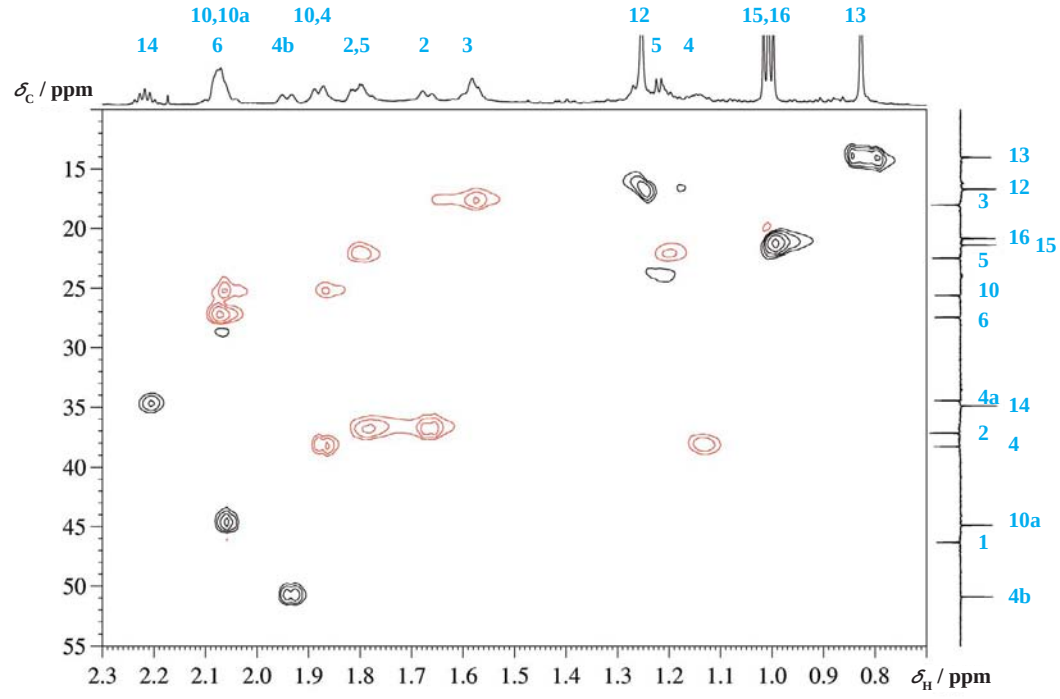


Fig. 5.7-21 CH-edited HSQC spectrum

The CH-edited HSQC spectrum is most helpful for further assignment. Since the olefinic region is trivial, it is not shown here. As we have already assigned the proton signals of the methyl groups, their ^{13}C assignment follows accordingly. Displayed in red are the signals of the six diastereotopic methylene groups. The largest diastereotopicity is found for methylene group C-4 at 37.2 ppm followed by C-5 at 22.5 ppm and then by C-10 at 25.6 ppm and C-2 at 38.3 ppm, whereas C-3 at 18.0 ppm and C-6 at 27.5 ppm hardly show any diastereotopicity. This can be easily understood from their relative proximity to the next chiral centre. Three methine carbon atoms remain in the aliphatic region. Whereas the assignment of C-14 at 34.9 ppm is obvious, the individual assignment between C-10a and C-4b can be made by assuming that C-4b should be more deshielded due to its allylic position. The proof of all these assumptions, however, will come from the discussion of the HMBC spectrum.

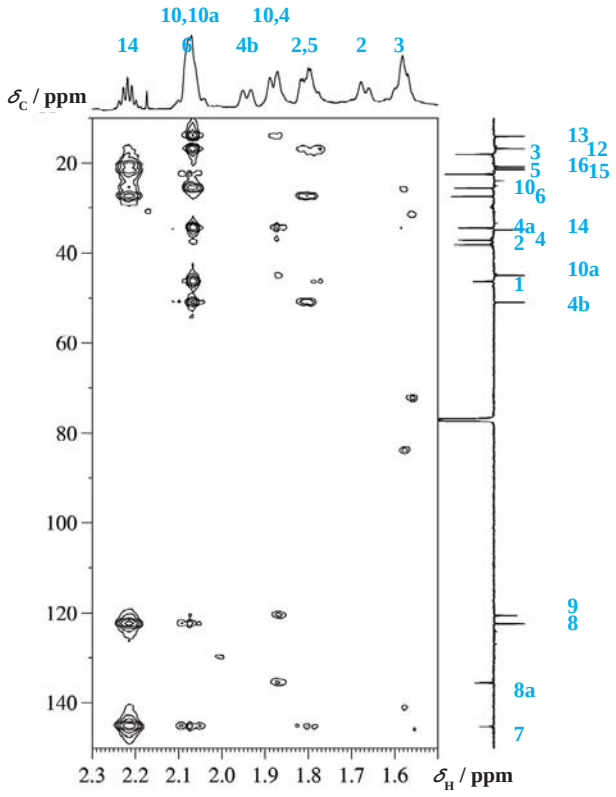


Fig. 5.7-22 Expansion of the HMBC spectrum in the aliphatic region

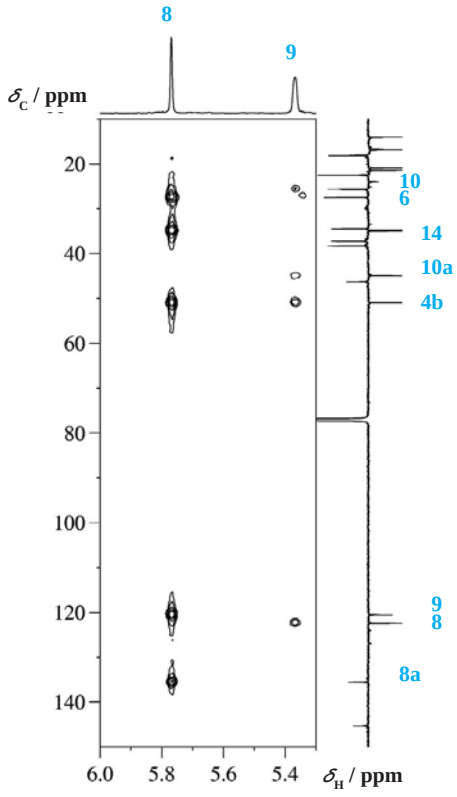


Fig. 5.7-23 Expansion of the HMBC spectrum in the olefinic region

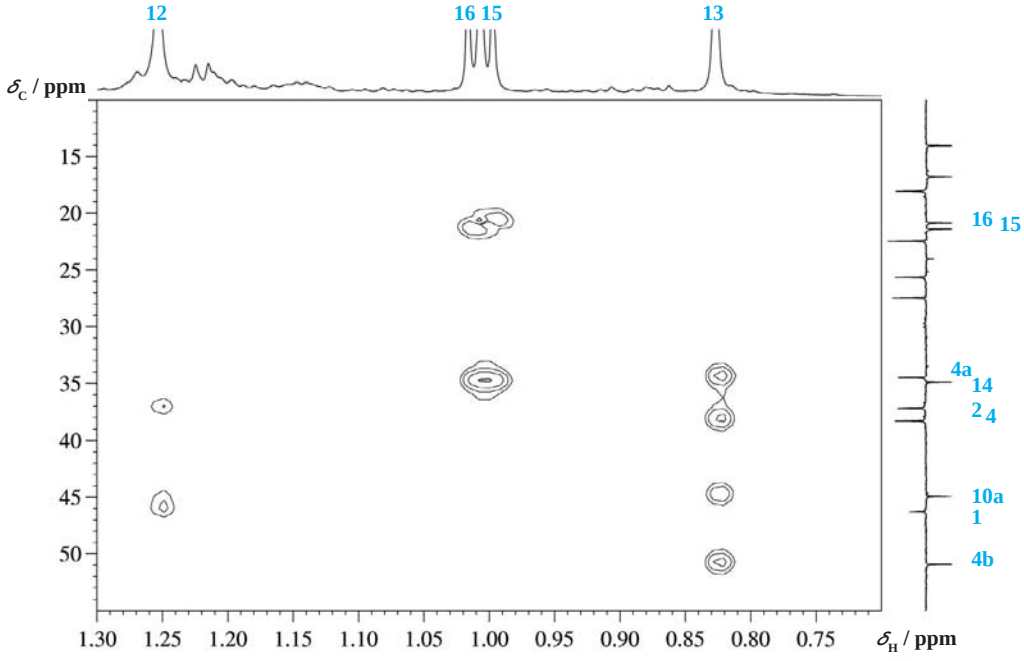


Fig. 5.7-24 Expansion of the HMBC spectrum for the methyl groups

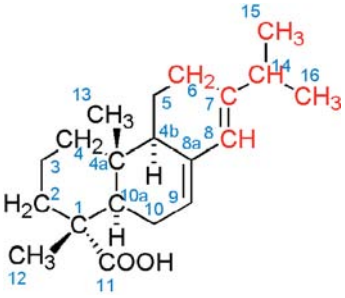
The analysis of the HMBC spectrum of abietic acid will again demonstrate the power of this method for organic structure elucidation. We start with the methyl group region. The two proton signals of the isopropyl methyl groups show cross peaks to the carbon atom of the neighbouring methyl group, thus H-15 "sees" C-16 and H-16 "sees" C-15 over three bonds. In addition, of course, C-14 is found at 34.9 ppm. H-14 at 2.22 ppm shows cross peaks to one quaternary and one methine carbon atom in the olefinic region, thus these signals at 145.3 and 122.4 ppm can be identified as C-7 and C-8. At this time, it helps to draw in red in the structure of abietic acid those carbon atoms whose assignment has been fixed by the HMBC cross peaks starting from the isopropyl group.

The angular methyl group H-13 at 0.83 ppm displays four HMBC cross peaks; one of these is to a quaternary carbon atom and hence C-4a at 34.4 ppm. The next cross peak leads to a methylene group at 38.3 ppm and this is therefore assigned to C-4. The corresponding two protons are located in the HSQC spectrum at 1.15 and 1.88 ppm. The remaining two cross peaks lead to the methine carbon atoms 10a at 44.9 and 4b at 50.9 ppm and their relative assignment was already discussed above. Again, the HSQC spectrum reveals the corresponding proton positions at 2.07 and 1.94 ppm. In the molecular formula we can therefore mark four additional carbon atoms in red.

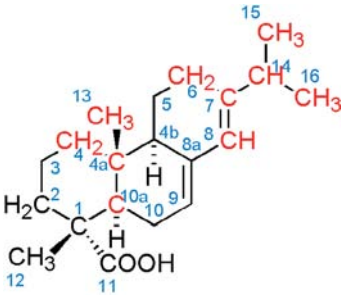
The remaining methyl group H-12 displays three HMBC cross peaks; one of these (not shown) is to the carboxyl atom C-11, which, of course, assures the correct assignment for these methyl group protons. The other two cross peaks go to a quaternary carbon atom, which must be C-1 at 46.3 ppm, and to a methylene group, which is therefore C-2 at 37.2 ppm. The corresponding protons are found in the HSQC spectrum at 1.67 and 1.8 ppm. Now only six carbon atoms have still to be assigned.

We now look into the olefinic region, where from the proton spectrum H-9 at 5.38 ppm and hence C-9 at 120.5 ppm via the HSQC spectrum are already known. In the HMBC expansion given we find therefore, starting from H-9, C-8 at 122.4 ppm and the remaining quaternary olefinic carbon atom at 135.6 ppm must be C-8a. H-9 shows three cross peaks in the aliphatic region. Two lead to methine carbon atoms and they have already been discussed, C-4b at 50.9 ppm and C-10a at 44.9 ppm. The remaining cross peak leads to a methylene group at 25.6 ppm, which is C-10. Once again, the HSQC spectrum reveals the proton positions for C-10 at 1.88 and 2.07 ppm.

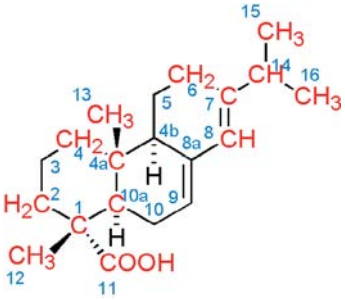
Now only two carbon atoms, C-3 and C-5, are not yet assigned. These can be distinguished by the large diastereotopicity of the H-5 protons at 1.22 and 1.8 ppm and by the chemical shift of C-3, which has to be the most aliphatic, i.e. the most shielded methylene group of the molecule.



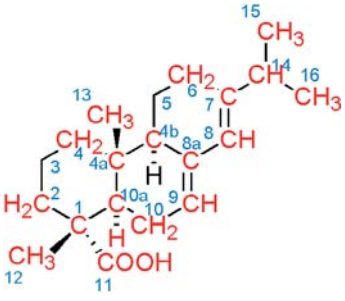
Scheme 5.7-4



Scheme 5.7-5



Scheme 5.7-6



Scheme 5.7-7

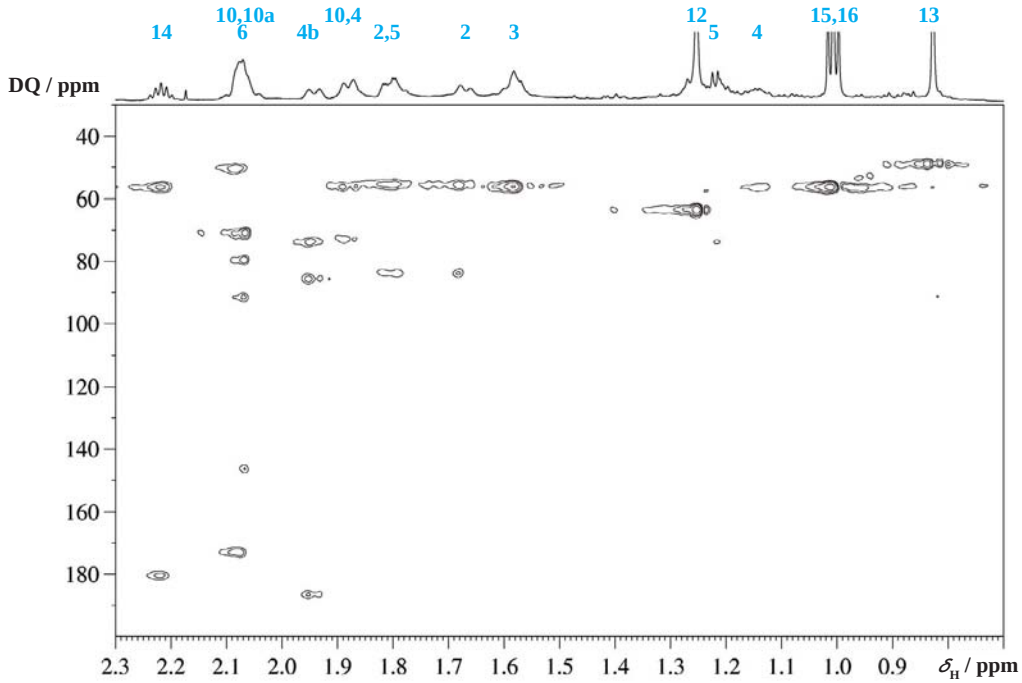
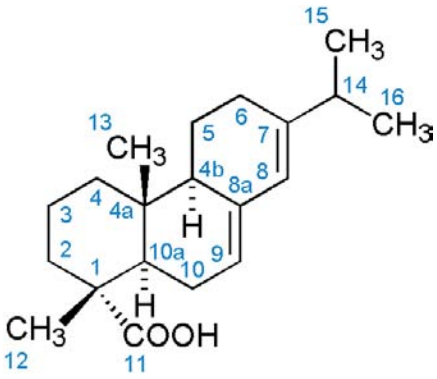


Fig. 5.7-25 ADEQUATE spectrum

Fortunately, for more complicated cases such as abietic acid, there is now a fairly recent technique available, which independently leads to a safe assignment of the carbon NMR spectrum and then of course to a corroboration for the proton assignment via HSQC. This is the proton-detected INADEQUATE technique, called ADEQUATE. In the spectrum displayed, a methine proton will give three cross peaks, a methylene proton two and a methyl proton only one. These cross peaks appear at the double quantum frequency of carbon; this is the sum of the chemical shifts of the carbon atoms which are connected to the proton in question by one and two bonds.

For example, the methine proton H-4b displays three cross peaks at the double quantum frequencies δ_{DQ} = 73.4, 85.3 and 186.5 ppm. The signal at 186.5 ppm is due to the connectivity of H-4b with C-4b and C-8a and therefore the signal appears at the sum of the chemical shifts of C-4b and C-8a = 186.5 ppm. Similarly, the carbon C-4b is also connected to C-4a and to C-5, therefore two additional cross peaks appear at the double quantum frequencies of C-4b + C-4a = 85.3 ppm and C-4b + C-5 = 73.5 ppm. By analysing all the peaks in the spectrum, an unequivocal assignment even of large and complicated molecules such as abietic acic can be obtained.



Scheme 5.7-8

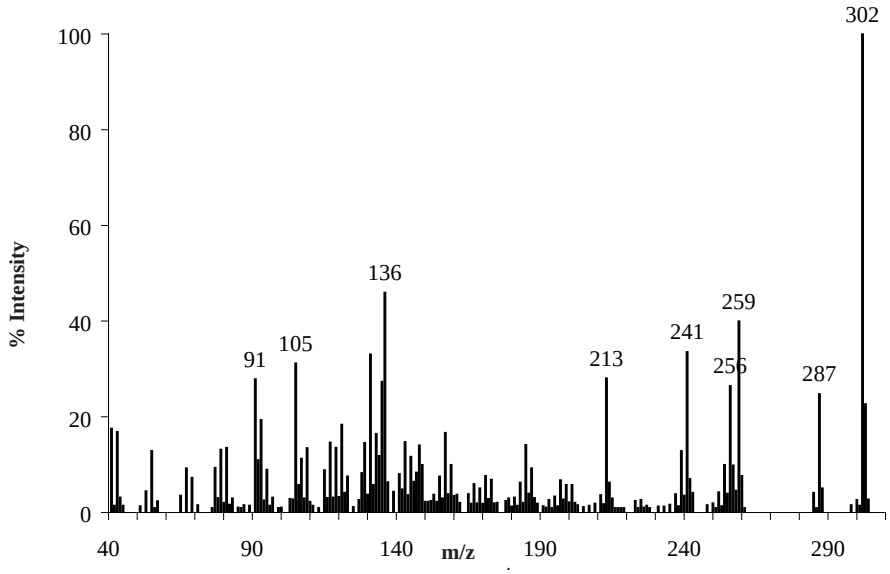
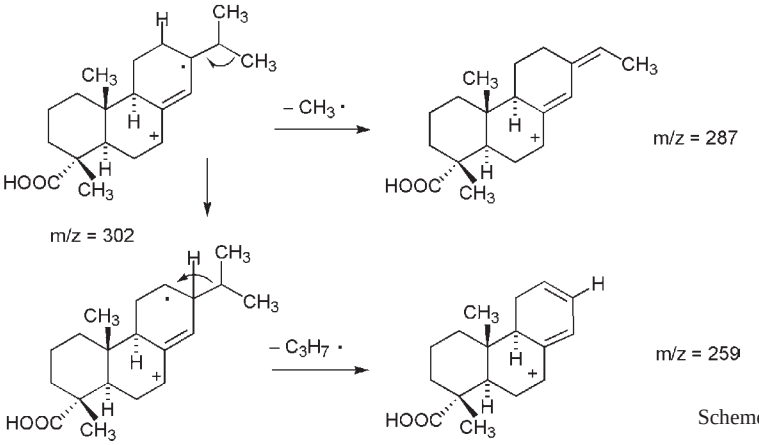


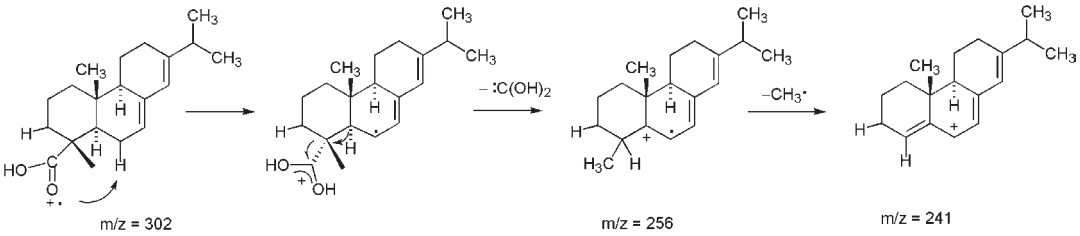
Fig. 5.7-26 Mass spectrum (EI)

The EI mass spectrum of abietic acid shows the $M^{+\cdot}$ peak at m/z = 302 as the base peak, indicating the relatively high stability of this resin acid. Losses of a methyl group or of the isopropyl group are shown by the peaks at m/z = 287 and 259.



Scheme 5.7-9 Fragmentation of abietic acid

The signal at m/z = 256 may be explained by a McLafferty-type rearrangement and loss of a stable dihydroxycarbene, as indicated.



Scheme 5.7-10 Further fragmentation

Один из говоривших был штатский, с морщинистым, желчным и бритым худым лицом, человек, уже приближавшийся к старости, хотя и одетый, как самый модный молодой человек; он сидел с ногами на отоманке с видом домашнего человека и, сбоку запустив себе далеко в рот янтарь, порывисто втягивал дым и жмурился.
Это был старый холостяк Шиншин, двоюродный брат графини, злой язык, как про него говорили в московских гостиных.

Lev Nikolayevich Tolstoy
(1828–1910)
Война и мир, Book I, Chap. 18

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz
184.8	C_q	C-11	
145.3	C_q	C-7	
135.6	C_q	C-8a	
122.4	CH	C-8	5.77
120.5	CH	C-9	5.38
50.9	CH	C-4b	1.94
46.3	C_q	C-1	
44.9	CH	C-10a	2.07
38.3	CH_2	C-4	1.15, 1.88
37.2	CH_2	C-2	1.80, 1.67
34.9	CH	C-14	2.22
34.4	C_q	C-4a	
27.5	CH_2	C-6	2.07
25.6	CH_2	C-10	2.07, 1.88
22.5	CH_2	C-5	1.80, 1.22
21.4	CH_3	C-15	1.00
20.9	CH_3	C-16	1.01
18.0	CH_2	C-3	1.58
16.7	CH_3	C-12	1.25
14.0	CH_3	C-13	0.83

Table 5.7-1 NMR data for abietic acid

Fig. 5.7-27 Pine trees in the Mediterranean mountains

Fig. 5.7-28 Amber bracelet

Sie schloß uns darauf selbst den Glasschrank auf, worin die Arbeiten in Bernstein aufbewahrt standen. Der sizilianische unterscheidet sich von dem nordischen darin, daß er von der durchsichtigen und undurchsichtigen Wachs- und Honigfarbe durch alle Abschattungen eines gesättigten Gelbs bis zum schönsten Hyazinthrot hinansteigt. Urnen, Becher und andere Dinge waren daraus geschnitten, wozu man große, bewundernswürdige Stücke des Materials mitunter voraussetzen mußte. An diesen Gegenständen sowie an geschnittenen Muscheln, wie sie in Trapani gefertigt werden, ferner an ausgesuchten Elfenbeinarbeiten hatte die Dame ihre besondere Freude und wußte dabei manche heitere Geschichte zu erzählen. Der Fürst machte uns auf die ernsteren Gegenstände aufmerksam, und so flossen einige Stunden vergnügt und belehrend vorüber.

Johann Wolfgang Goethe (1749−1832)
Italienische Reise, Catania, 3.5. 1787

5. Questions

A. Draw the formula of abietic acid and indicate the four isoprene units present.

B. Search for the structural formulae of the following resin acids (CAS RN given in parentheses) isomeric to abietic acid ($C_{20}H_{30}O_2$): neoabietic acid (471-77-2), palustric acid (1945-53-5), pimaric acid (127-27-5), isopimaric acid (5835-26-7) and levopimaric acid (79-54-9). What tendency is expressed by the formation of dehydroabietic acid (1740-19-8)?

   Compare these formulae with that of abietic acid and comment on the graduation of the thermodynamic stability in some obvious cases and on the message that can be derived for the unclear cases from the equilibration step done prior to isolation.

C. Compare the structural patterns of regioisomeric abietic-type resin acids and pimaric-type resin acids of molecular formula $C_{20}H_{30}O_2$. Which type can be regarded as being thermodynamically more stable and for what reason?

D. Alkali metal salts of resin acids such as sodium abietate and sodium pimarate are called resin soaps, and indeed they are surfactants and have surface-active properties. Explain why. What are the general structural requirements that make a compound surface-active? Which group of isoprenoid acids with surface-active properties occurs in the human body, from which organ is it secreted, where is it used and for what purpose?

E. A piece of amber that we admire arose from a chunk of prehistoric colophony over millions of years – what is a likely explanation for the kind of chemical transformation that took place inside the material? What property can be regarded today as a macroscopic proof? Why did it take so long? What proves that amber has formed from prehistoric resin? What is copal?

F. Amber is a fascinating material, and from the names that it was given by different peoples it is obvious that they were fascinated by different properties. The Greek name for amber is evidence for this statement. What is this name? What is its connection to physics and chemistry?

G. Where is Europe's most famous place of recovery for amber? Why was it valuable already in ancient times from the Iron Age on?

H. Study the expansion of the COSY spectrum and locate the cross peaks between the methylene groups 4, 3 and 2, and also the cross peaks starting from then more shielded H-5 at 1.2 ppm to H-6, H-4b and the other H-5.

I. Build a molecular model and decide which of the H-5 protons is axial and equatorial by looking at the NOESY spectrum.

J. Comment on the cross peaks between the H-10 protons and those between H-2 and H-3 which have the same sign as the diagonal.

K. Why does H-9 show a spin coupling only to H-10 at 1.88 ppm and not to the other H-10 at 2.07 ppm?

L. There is an NOE cross peak between H-4b and H-4 at 1.15 ppm. Is this proton axial or equatorial?

M. Can you make an assumption about the conformational preference of the isopropyl group?

N. In the ADEQUATE spectrum, there are six cross peaks identified for the proton absorption of H-10, H-10a and H-6 at 2.07 ppm. Calculate the corresponding double quantum frequencies for the carbon atoms and compare with the experiment.

O. Suggest a mechanism for the formation of the peak in the mass spectrum at m/z = 213.

6. Own Observations

5.8 Betulinic Acid

3-Hydroxy-3β-lup-20(29)-en-28-oic acid

From the bark of the plane-tree

Platanus hybrida L. (Platanaceae)

$C_{30}H_{48}O_3$, MW 456.71

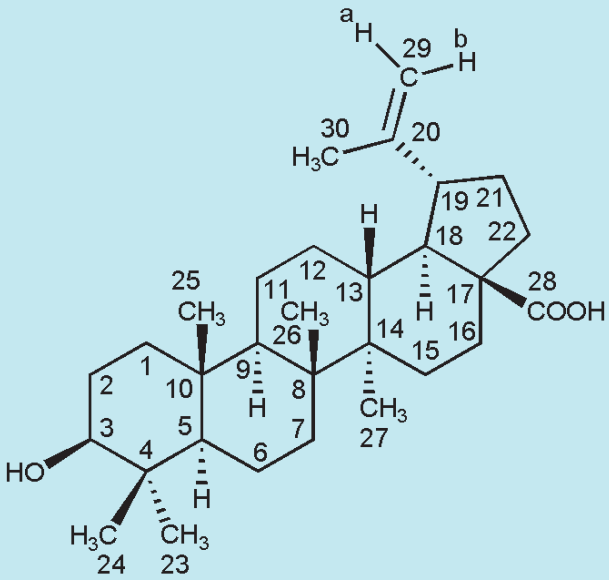
CAS RN 472-15-1,
BRN 2228573, 2228575, 2711110

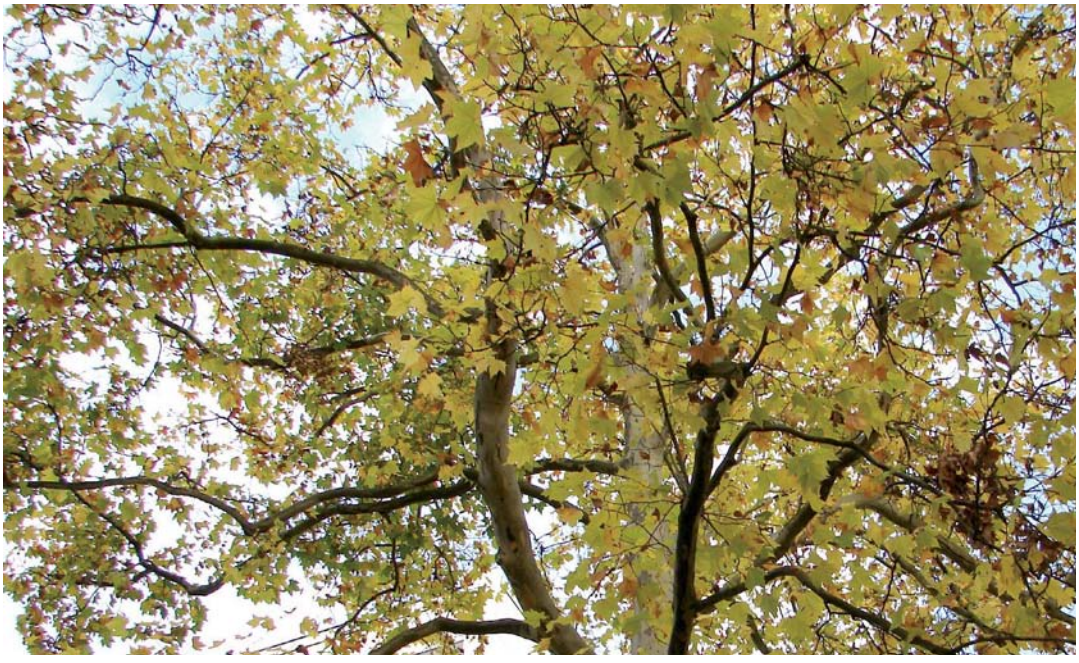
Colourless crystals, mp 293–295 °C
$[\alpha]_D^{25}$ +11.1° (*c* 0.00036 g/mL, chloroform)
(with sublimation at normal pressure between two glass plates); 316 °C (without sublimation, sealed in a Fischer cuvette)

Betulinic acid is commercially available.

Synonymous names:
(+)-Betulinic Acid, β-Betulinic acid, Betulic acid, Lupatic acid, Lup-20(29)en-28-oic acid

Level: easy





1. Background: A surface story: how barks cure skin

Then Jacob took fresh rods of poplar and almond and plane trees, and peeled white stripes in them, exposing the white which was in the rods.

Old Testament, Genesis, 30, 37

Betulinic acid (source: plane tree bark) and its close relative betulin (with a CH_2OH group instead of COOH) from birch bark were both named after the birch trees of the genus *Betula* L. Our story starts with birch trees. The birch bark consists of two layers, an outer white one, called birch cork, and an inner darker one, the real rind. White birch cork contains betulin to about 25% of its dry mass. For a certain reason, betulin is a particular compound in organic chemistry. It is well known that the famous chemist C. W. Scheele was the first to discover a variety of organic compounds from natural sources in the late 18th century, e.g. uric acid, oxalic acid (1776), glycerol (1779), lactic acid (1780) and gallic acid (1786). However, it is not as well known that soon afterwards, betulin was described from birch bark [1]. Betulin was discovered by chance as a sublimate from birch cork and described as a "modified resin in form of a salt". This opinion on betulin was obviously due to the crystalline shape of sublimed betulin which reminded the finder of a crystalline salt. From our contemporary point of view, this betulin can be regarded as the first bioactive principle ever isolated from a plant source. The first detailed investigations of this strange material were reported only 88 years later [2]. The correct stereochemistry was elucidated in 1953 by chemical means only, i.e. before spectroscopic methods were used for structural assignments [3].

Betulin is an abundant triterpene diol occurring extremely widely in hundreds of different plant species, e.g. in the European hornbeam (*Carpinus betulus* L.) and the common hazel (*Corylus avellana* L.), as outlined in a review [4]. The natural purpose for betulin seems to be that it prevents the tree from drying out. Both betulin and betulinic acid belong to the pentacyclic triterpenes with a lupane skeleton. For lupane, a procedure of numbering exists which differs from the numeration necessary for the related stem hydrocarbon chrysene. From this point of view, the CA index name 3-hydroxy-3β-lup-20(29)-en-28-oic acid is a typical example of how a complicated compound can be named shortly in the manner of a semi-trivial nomenclature by using the abbreviated term lupane.

Fig. 5.8-1 Huge plane tree

Birch tree products have been used in ethnomedicine for a very long time by different cultures, both in the Indogerman habitat and in the Northern America Indian region. The biological activity of birch bark constituents is manifold. Pliny the Elder mentioned the birch as "gallica arbor" in his 37-volume encyclopaedia *Naturalis Historia* written around 77. Interestingly, birch bark was known as a cure for skin diseases at that time. Birch bark powder was also used by the healer Hildegard of Bingen in the 12th century to treat open wounds. Birch trees were worshiped as symbols of life and fertility over the centuries. Even the traditional maypole has its origin in such ancient rituals. However, it is a rare and therefore rather special problem for making dermatologically useful preparations that the hydrophobic betulin is also a lipophobic compound which cannot be integrated easily in creams or unguents. This special behaviour was recently overcome by the development of

an oleogel in which a betulin- and betulinic acid- containing birch bark extract is incorporated by means of a vegetable oil and water [5].

Betulinic acid, our target compound, has been found to be biologically more active than betulin. Betulinic acid was shown to be a selective inhibitor of human melanoma by inducing apoptosis. Furthermore, it possesses anti-malarial, anti-HIV, antibacterial and anti-inflammatory properties and is active against several other tumour cell lines at a level comparable to drugs for clinical use [6–8]. Obviously, a principle reason for this is the structural relationship of these natural products to compounds produced naturally in the body, in other words the ability of cell structures to interact with this kind of triterpene skeleton.

In principle, it can be made chemically by oxidation of betulin as a primary alcohol. However, despite the fact that birch bark is very rich in betulin, it is not an easy task to isolate pure betulin. The problem is that betulin is always accompanied by a number of structurally related triterpenoids such as lupeol, erythrodiol and oleanolic acid. It is reported and consistent with our own experience from attempts to isolate betulin in a pure state that crystallization is an insufficient means, and instead elaborate chromatography is required. Based on this finding, methods have been developed for the isolation of pure betulinic acid from the bark of plane trees as the best source. It can be done on a technical scale in procedures without chromatographic steps [9]. In 2006, a Betulin Institute was founded in Darmstadt whose work is dedicated to research on betulin, betulinic acid and their use for pharmaceutical purposes [10].

Fig. 5.8-2 Plane tree avenue at San Miguel, Azores, planted a long time ago to protect carts against rainy weather

2. Literature

[1] J. T. Lowitz, “Über eine neue, fast benzoeartige Substanz der Birken” [On a novel, nearly benzoin resin like substance of the silver birches] *Chemische Annalen für die Freunde der Naturlehre, Arzneygelahrtheit, Haushaltungskunst und Manufakturen* **1788**, *2*, 312–316.

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[3] J. M. Guider, T. G. Halsall, E. R. H. Jones, “Triterpenes and related compounds. XX. Stereochemistry of ring E of betulin and related compounds” *J. Chem. Soc*. **1953**, 3024–3028.

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[5] M. Laszczyk, S. Jaeger, B. Simon-Haarhaus, A. Scheffler, C. M. Schempp, “Physical, chemical and pharmacological characterization of new oleogel-forming triterpene extract from the outer bark of birch (Betulae Cortex)” *Planta Medica*, **2006**, *72*, 1389–1395.

[6] P. A. Krasutsky, “Birch bark research and development” *Nat. Prod. Reports*, **2006**, *23*, 919–942.

Fig. 5.8-3 Bark of a plane tree

Qui properant, nova musta bibant:
mihi fundat avitum
Consulibus priscis condita testa merum.
Nec platanus, nisi sera, potest obsistere Phoebo,
Et laedunt nudos prata novella pedes.

P. Ovidius Naso (43 BC–17 AD)
Ars Armatoria Lib. II, 695

Ich stellte also mehrere Scheite von jungen Birken mit weißer Rinde, aufgerichtet, so nahe an ein sehr ruhiges Feuer, bis das Holz stark zu dampfen und die Rinde braun zu werden anfing; da denn nach etwa 10 Minuten die Flocken ziemlich zu erscheinen anfingen, die ich mit einem Papier öfters abnahm. Wenn keine Flocken mehr erschienen nahm ich ander Holz u. f. f. Auf diese Weise sammlete ich in einem Tage ein offenes Glas voll, welches 1 Pfd. Wasser hielt; die Flocken aber wogen nur etwan 8 bis 10 Gran.

J. T. Lowitz (1757−1804)
Chemische Annalen, see [1]

[7] S. Alakurtti, T. Makela, S. Koskimies, J. Yli-Kauhaluoma, "Pharmacological properties of the ubiquitous natural product betulin" *Eur. J. Pharm. Sci*. **2006**, *29*, 1−13.

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[9] B. Draeger, T. Galgon, R. Neubert, W. Wohlrab, "Method of producing betulinic acid by the fractional extraction of ground plane tree bark using dichloromethane" US Patent (**2001**), US 6175035 B1 200110116, Application: US 99-310163 19990510 based on the German Patent Ger. Offen (**1998**) DE 19713768 A1, Application: DE 97-19713768 19970403.

[10] http://www.betulininstitut.de/.

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3. Isolation

The method follows the procedure described in ref. [9].

3.1 Principle

Betulinic acid, due to its steroid-like skeleton a highly hydrophobic carboxylic acid, is isolated from the dried and milled bark of plane trees by Soxhlet extraction with dichloromethane. Purification is possible just by recrystallization. Chromatographic separations are not necessary.

It is advantageous that the bark of a plane tree comes off unassisted each year in autumn at the end of the growth period. A tree of 15 m height drops several kilograms of bark just following its biological rhythm without having a problem. In contrast, greater efforts are necessary to obtain the white bark of birch trees. Moreover, betulinic acid cannot be isolated from it directly, but only the corresponding alcoholic precursor betulin. Furthermore, it is our experience that betulin obtained from birch tree bark by extraction is not nearly as pure as betulinic acid obtained by the method described below. Purification of crude betulin is impossible without chromatography. This clear drawback and the circumstance that plane tree bark is a renewable raw material make it a preferred source for betulinic acid.

3.2 Method

Bark of plane trees is air dried and ground by means of a grinder. The milled bark (70 g) is placed in the thimble of a Soxhlet apparatus and extracted with 700 mL of dichloromethane for 6 h. The yellow extract obtained is filtered to remove a few mechanical impurities coming from the bark. The filtrate is concentrated to half of its volume and allowed to stand in a stoppered flask in a deep freezer (−18 °C) overnight for crystallization. Filtration yields 1.0 g of yellow crystals of crude betulinic acid (mp 290−293 °C, see text below). On work-up of the mother liquor, another crop of 100 mg of crude product can be obtained.

Fig. 5.8-4 Air-dried plane tree bork

3.3 Purification

The crude betulinic acid obtained is dissolved in boiling methanol (100 mL) and a small amount of undissolved material removed by filtration. The filtrate is poured into an Erlenmeyer flask, stoppered and allowed to stand in a deep freezer (−18 °C) overnight. Colourless crystals separate and are filtered off, rinsed with 5 mL of ice-cold methanol and dried to yield 600 mg of pure betulinic acid, $[\alpha]_D^{25}$ +11.1° (*c* 0.00036 g/mL, chloroform).

Melting point: 293−295 °C (with sublimation at normal pressure between two glass plates); 316 °C (without sublimation, sealed in a Fischer cuvette).

See the detailed description given in Section 1.2 on caffeine for the handling of a Fischer cuvette.

Fig. 5.8-5 In the shadow of plane tree

4. Spectra and Comments

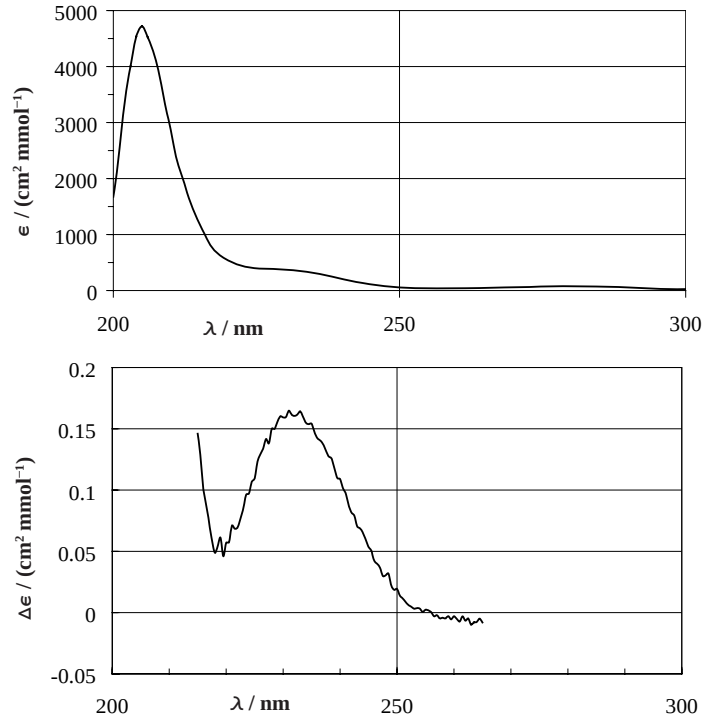


Fig. 5.8-6 UV and CD spectra in ethanol

Fig. 5.8-7 Pure betulinic acid

The two isolated chromophores, the double bond and the carboxyl group, give rise to a UV spectrum with a maximum at 205 nm ($\varepsilon \approx 5000$). A weak and a very weak shoulder can be seen at 240 and 280 nm.

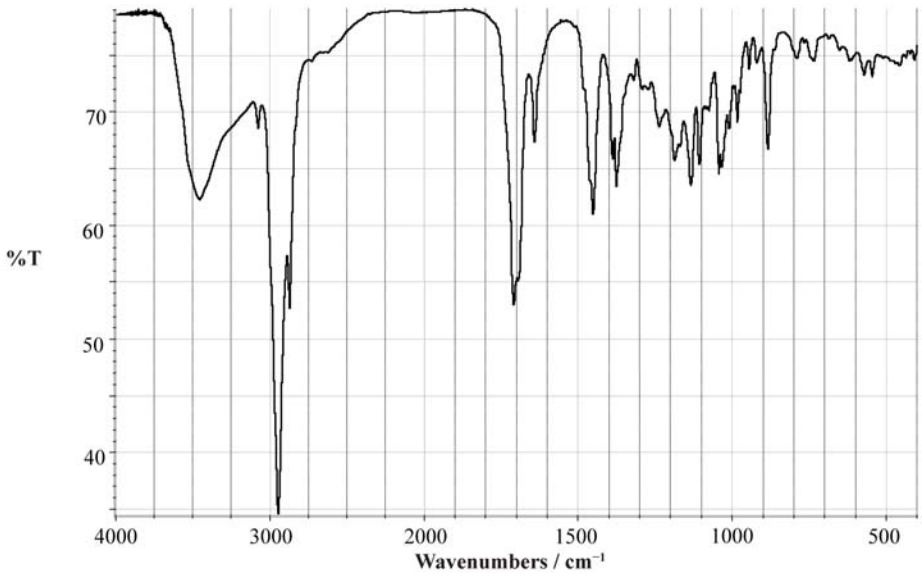


Fig. 5.8-8 IR spectrum in KBr

In the IR spectrum, the OH valence vibration of the carboxylic acid, the sp^2 and sp^3 CH valence vibrations, the C=O double bond and the C=C double bond can be clearly identified. Note the typical wavenumber of about 3100 cm^{-1} for the terminal $=CH_2$ group of C-29.

Fig. 5.8-9 Autumn

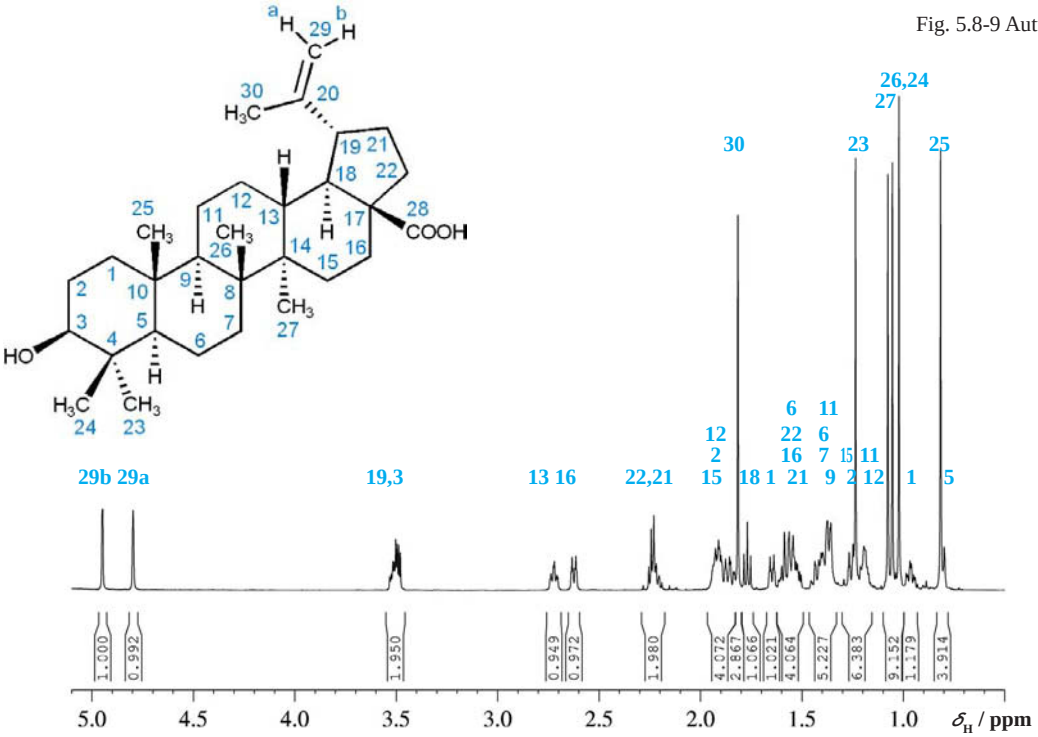


Fig. 5.8-10 ^{1}H NMR spectrum at 700 MHz in pyridine-d_5

Betulinic acid, as a triterpene, consists of 30 carbon atoms and is only sparingly soluble in the usual NMR solvents. The spectra shown here were therefore recorded in pyridine-d_5 and it is advisable to use an NMR spectrometer with the highest available field strength due to the complexity of the spectra; here a 700 MHz spectrometer was applied.

For the assignment strategy, it is best to start with the vinyl group C-20/C-29, since here the chemical shifts are obvious, and then try to get into the inner part of the molecule as far as possible. In a second step, one starts from C-3, because also here the assignments are easy. Hopefully, one arrives from these two ends of the molecule at a correct assignment also for the inner part.

The two olefinic protons H-29a and H-29b resonate at δ_H = 4.80 and 4.95 ppm and their relative assignment can be made from the NOESY spectrum due to the vicinity of H-29a to the methyl group C-30. The next signal in the proton spectrum at 3.5 ppm integrates for two protons, which from their chemical shifts are assigned to H-19 and H-3. The remaining aliphatic protons will not be assigned at this stage; only the signal of the methyl group C-30 attached to a sp^2 centre can be safely identified from its chemical shift of 1.82 ppm.

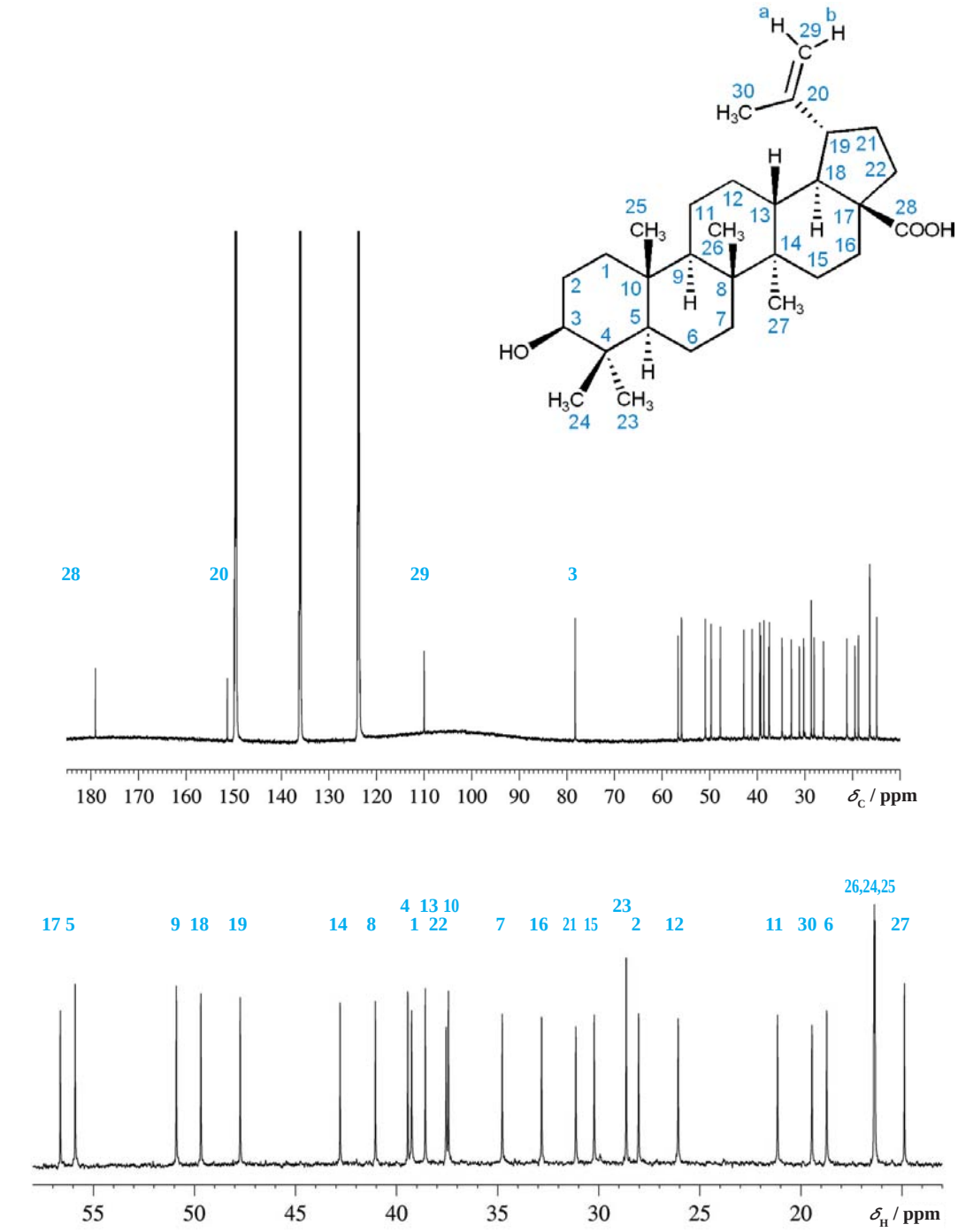


Fig. 5.8-11 ^{13}C NMR spectrum at 175 MHz in pyridine-d_5

Similarly to the proton spectrum, only four signals in the ^{13}C NMR spectrum can be easily assigned from their typical chemical shifts, C-28 at 179.1, C-20 at 151.3, C-29 at 111.0 and C-3 at 78.2 ppm. The forest of signals between 60 and 10 ppm has to await the discussion of the HSQC and HMBC spectra.

Fig. 5.8-12 Plane tree bark

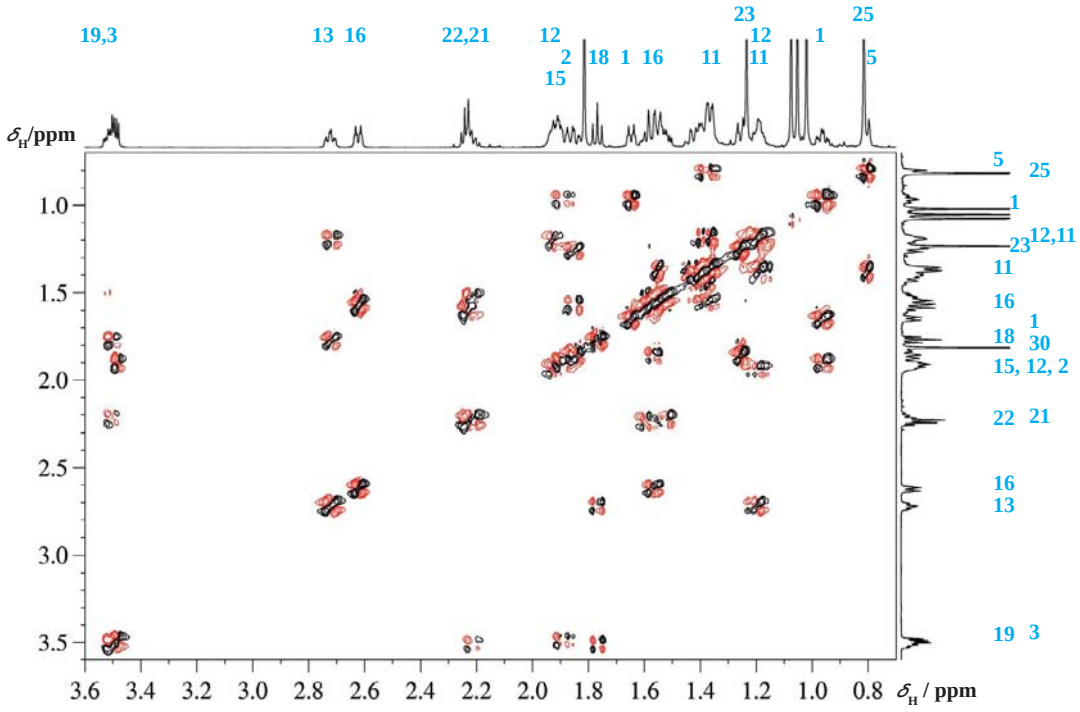


Fig. 5.8-13 Double quantum filtered COSY spectrum

The closely resonating protons H-19 and H-3 at δ_H = 3.5 ppm show two cross peaks for the slightly deshielded signal and only one cross peak for the slightly more shielded signal. The former is assigned to H-19 and first displays a COSY connection to a proton triplett at δ_H = 1.77 ppm which can be safely assigned as H-18. This must have a large trans-axial spin coupling to H-13, which is found at δ_H = 2.72 ppm. H-13 displays another COSY cross peak leading to the diastereotopic methylene group H-12 at 1.93 and 1.20 ppm which in turn leads to the methylene group H-11. The other cross peak of the allylic proton H-19 leads to the multiplet of H-21 at δ_H= 2.22 ppm.

The more shielded signal at 3.5 ppm is assigned to H-3 and leads to a multiplet at δ_H = 1.89 ppm, which is therefore attributed to H-2, giving a further connection to the multiplet of H-1 at δ_H = 0.96 ppm. Both H-13 and H-16 may be influenced by the anisotropy of a double bond and therefore the signal at 2.62 ppm which is close to the signal of H-13 is assigned to H-16.The diastereotopic partner proton H-16 at δ_H = 1.56 ppm displays a COSY cross peak leading to the assignment of H-15 at δ_H = 1.85 ppm.

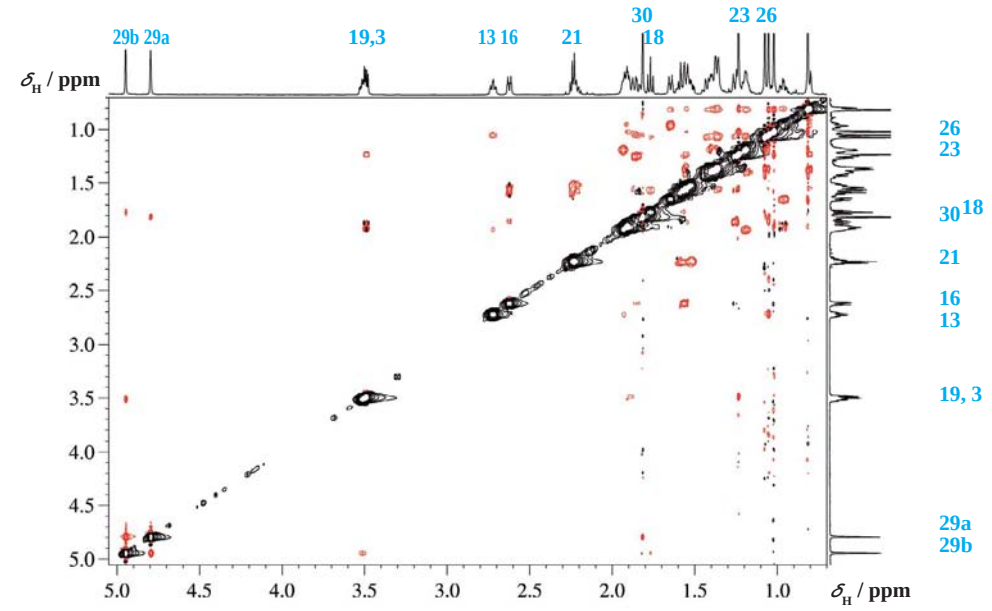


Fig. 5.8-14 NOESY spectrum

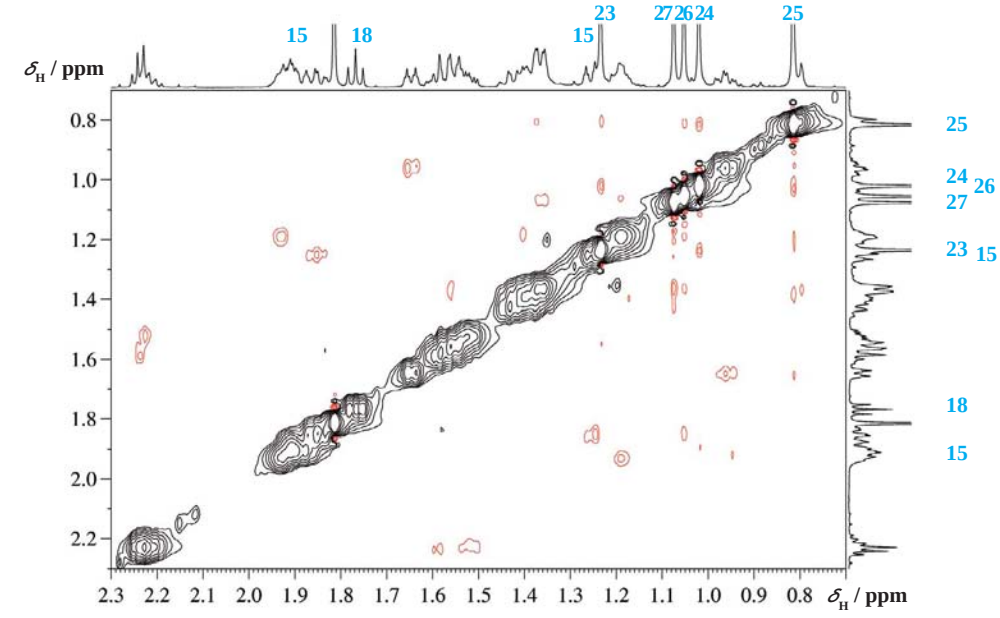


Fig. 5.8-15 Expansion of the NOESY-spectrum

In the NOESY spectrum, we find a confirmation of the assignments described so far. The proton at δ_H = 4.80 ppm shows a cross peak to the olefinic methyl group C-30 at 1.82 ppm, whereas the other proton at δ_H = 4.95 ppm displays a cross peak to the allylic proton H-19 at δ_H = 3.52 ppm, but also to H-18 at δ_H = 1.77 ppm, and this tells us something about the conformation of the side chain. H-13 is connected, as seen in the NOESY spectrum, with the methyl group H-26 at δ_H = 1.05 ppm, being in a *syn*-1,3 position. H-3 shows in the NOESY spectrum a cross peak to the methyl group protons H-23 at δ_H = 1.23 ppm; these in turn show a cross peak to the methyl group signal of H-24 at δ_H = 1.02 ppm, and this is expected due to the vicinity of these two methyl groups.

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz
179.1	C_q	C-28	
151.3	C_q	C-20	
110.0	CH_2	C-29	4.95/4.80
78.2	CH	C-3	3.49
56.6	C_q	C-17	
55.9	CH	C-5	0.80
50.9	CH	C-9	1.36
49.7	CH	C-18	1.77
47.7	CH	C-19	3.52
42.8	C_q	C-14	
41.1	C_q	C-8	
39.4	C_q	C-4	
39.3	CH_2	C-1	1.65/0.96
38.6	CH	C-13	2.72
37.5	CH_2	C-22	2.24/1.59
37.4	C_q	C-10	
34.8	CH_2	C-7	1.42/1.37
32.8	CH_2	C-16	2.62/1.56
31.1	CH_2	C-21	2.22/1.52
30.2	CH_2	C-15	1.85/1.26
28.6	CH_3	C-23	1.235
28.0	CH_2	C-2	1.89
26.1	CH_2	C-12	1.93/1.20
21.0	CH_2	C-11	1.40/1.19
19.4	CH_3	C-30	1.82
18.7	CH_2	C-6	1.54/1.37
16.38	CH_3	C-26	1.05
16.36	CH_3	C-24	1.02
16.32	CH_3	C-25	0.80
14.9	CH_3	C-27	1.08

Table 58-1 NMR data for betulinic acid

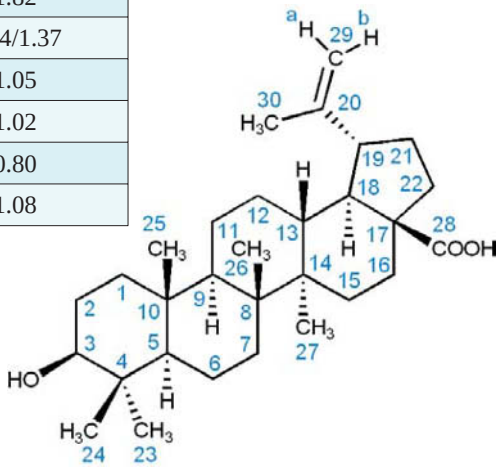
Tu penches, grand Platane, et te proposes nu,
Blanc comme un jeune Scythe,
Mais ta candeur est prise, et ton pied retenu
Par la force du site.

Ombre retentissante en qui le même azur
Qui t'emporte, s'apaise,
La noire mère astreint ce pied natal et pur
A qui la fange pèse.

De ton front voyageur les vents ne veulent pas;
La terre tendre et sombre,
O Platane, jamais ne laissera d'un pas
S'émerveiller ton ombre!

Paul Valery (1871–1945)
Au Platane

Fig. 5.8-16 Plane tree in front of the Chemistry Institute at Leipzig



Scheme 5.8-1

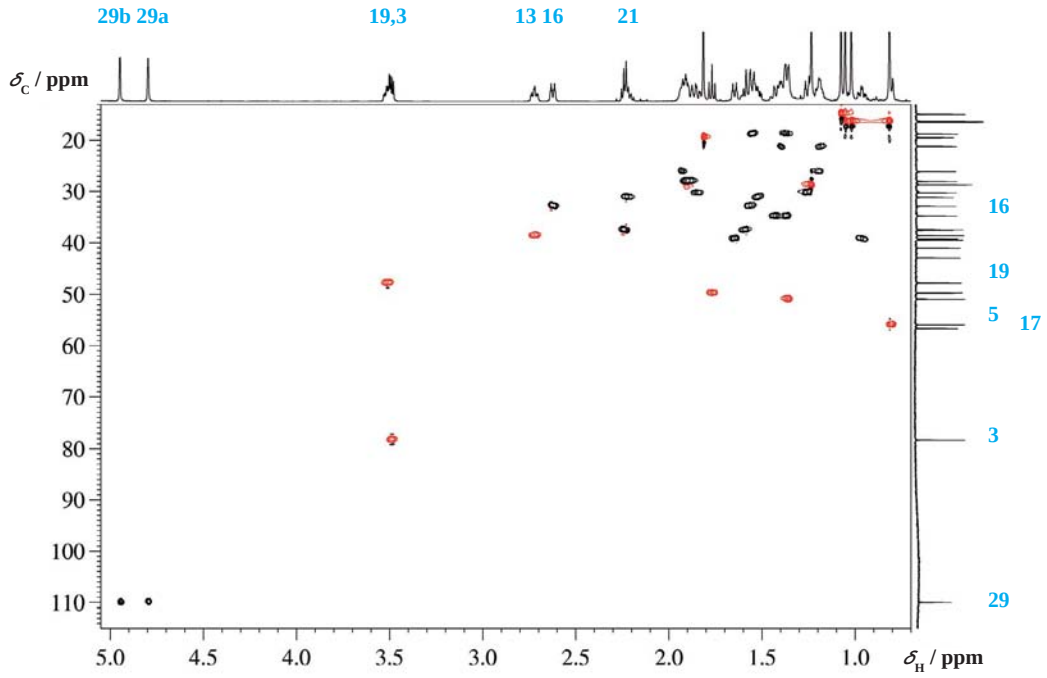
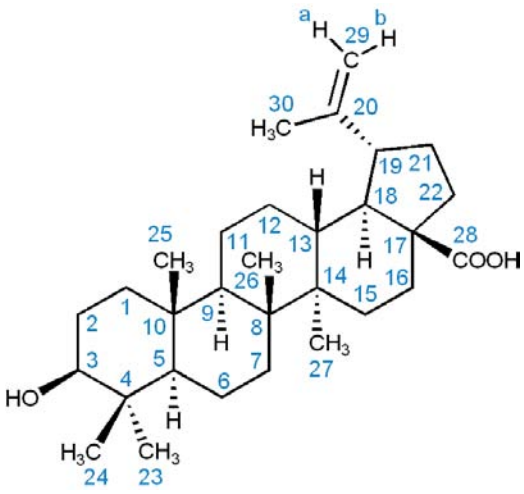


Fig. 5.8-17 CH-edited HSQC spectrum

At this point we take a look to the HSQC spectrum, which was recorded with proton editing; the red signals stem from CH or CH_3 groups whereas the black signals indicate CH_2 groups, thus clarifying the large diastereotopicities of methylene groups present in this compound. The proton doublet at δ_H = 2.62 ppm belongs, according to the HSQC spectrum, to a diastereotopic methylene group connected with a proton signal at δ_H = 1.56 ppm. We assign these signals to H-16, where one of these diasterotopic protons is rather deshielded due to the anisotropic effect of the carboxy group. The expansion of the HSQC spectrum also shows that the multiplet at δ_H = 2.25–2.22 ppm belongs to two different methylene carbon atoms both with diastereotopic protons. The other corresponding protons resonate at δ_H = 1.52 and 1.59 ppm. These two pairs are assigned to H-22 and H-21, both situated in the five-membered ring. As the COSY spectrum, in this region, is very crowded, the edited HSQC spectrum helps to identify C-9 and the resonance position of its methine proton at 1.36 ppm.

Now we start at the other end of the molecule. According to the HSQC spectrum the other geminal proton of C-1 can be found at δ_H = 1.65 ppm. Very astonishing at first sight is the chemical shift of the last unassigned methine proton, which can be seen in the HSQC spectrum at 0.80 ppm connected to C-5 at 56.6 ppm, and this leads further to the methylene groups C-6 and C-7.



Scheme 5.8-2

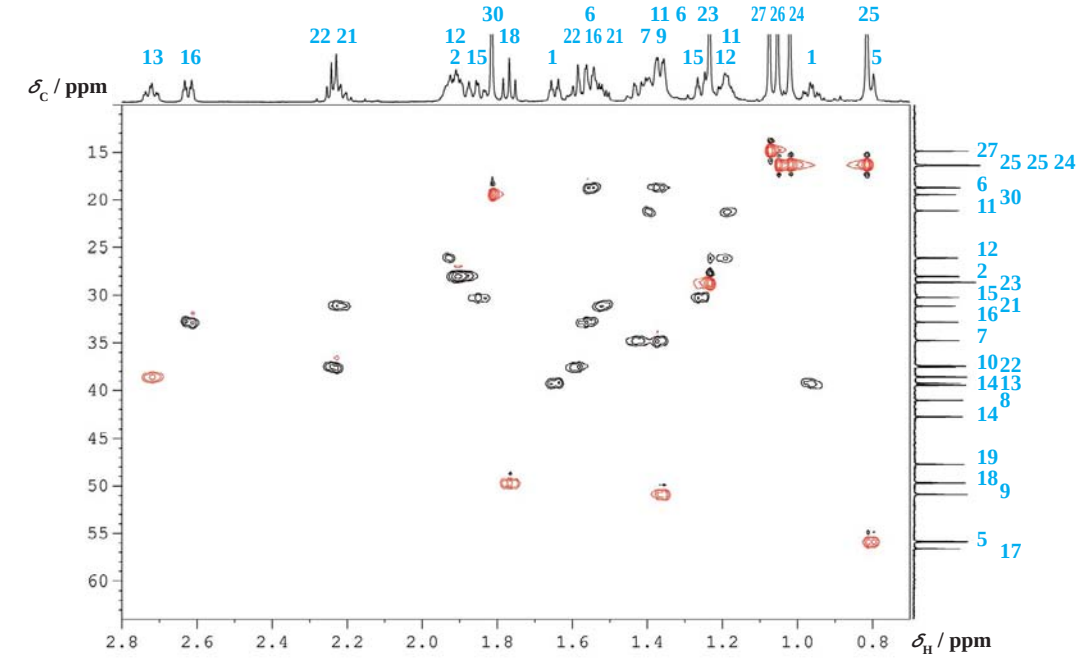


Fig. 5.8-18 Expansion of the HSQC spectrum

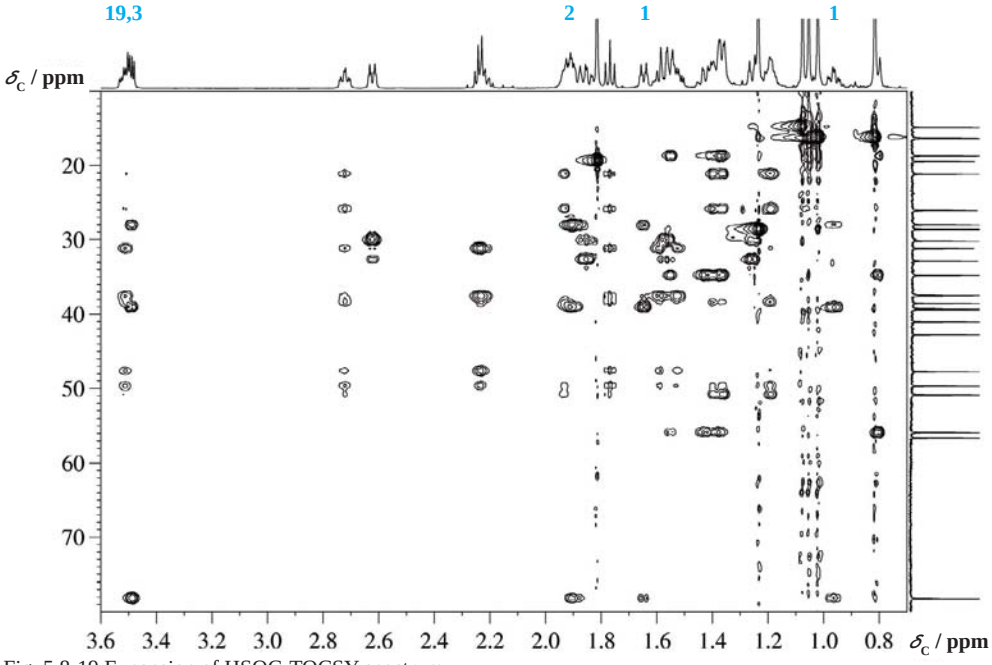


Fig. 5.8-19 Expansion of HSQC-TOCSY spectrum

Several of the assignments discussed above can also be inspected in the HSQC-TOCSY spectrum, a pseudo-3D method yielding combined TOCSY and HSQC information useful for complicated molecules. Consider, e.g., the four signals at the bottom of the spectrum for C-3 at 78.2 ppm. Starting from the diagonal peak for H-3 (78.2/3.49 ppm) one finds in the horizontal three cross peaks, stemming from H-2 (δ_H = 1.89 ppm), and the diastereotopic methylene group H-1 (δ_H = 1.65, 0.96 ppm).

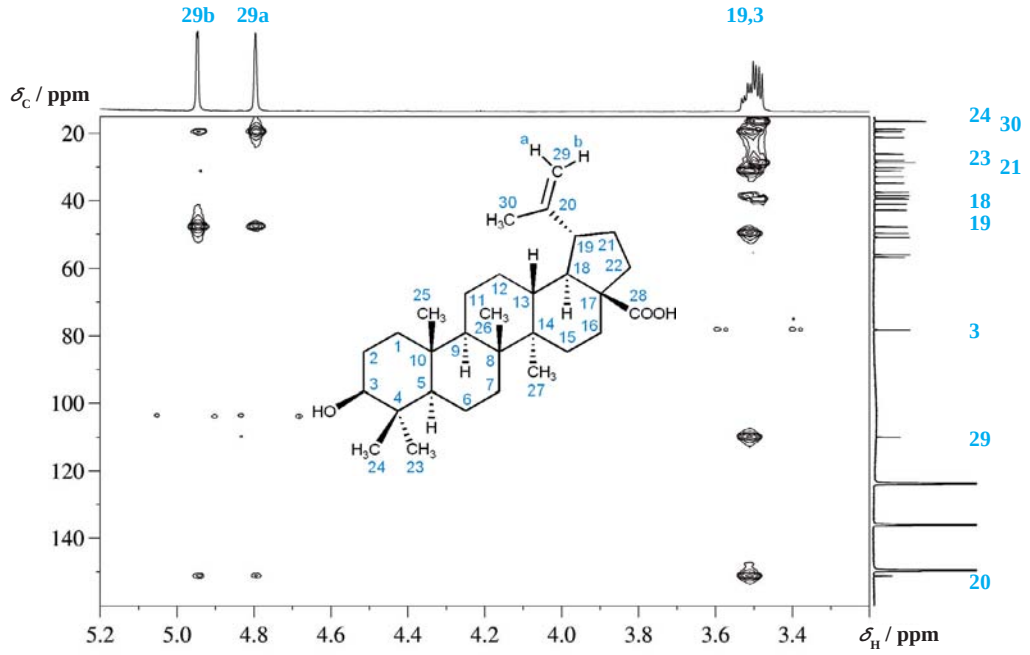


Fig. 5.8-20 Expansions of the HMBC spectrum

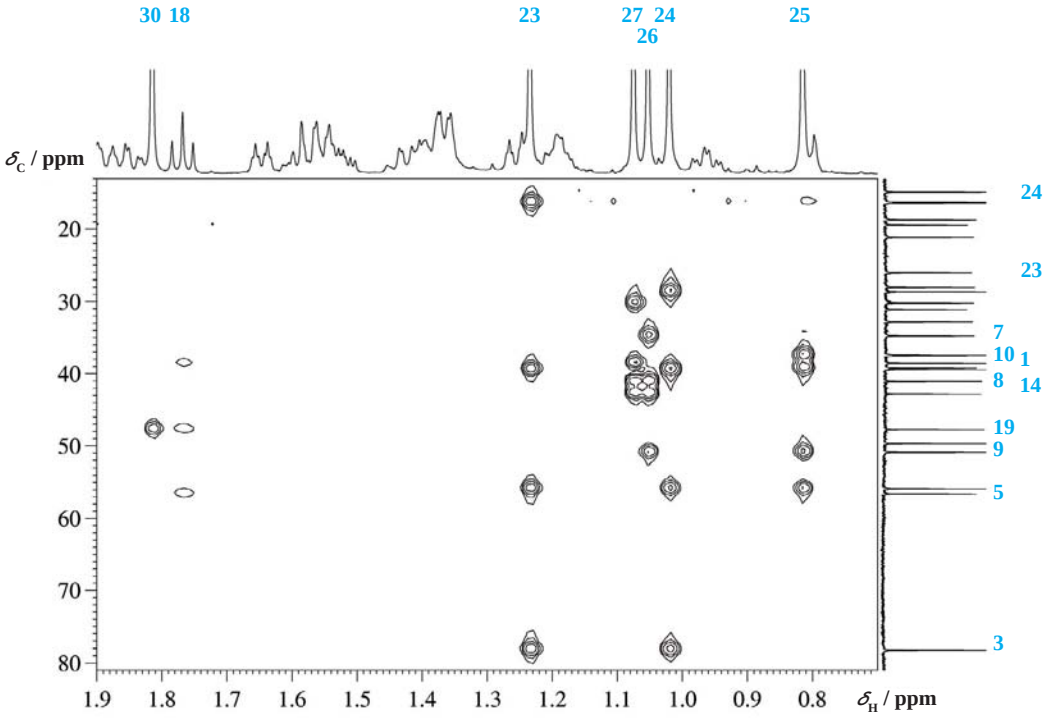


Fig. 5.8-21 Expansion of the HMBC spectrum for the methyl groups

In the first HMBC expansion shown, the olefinic protons H-29b and H-29a display connectivities to the methyl group C-30, the allylic carbon C-19 and the olefinic carbon C-20. The allylic proton H-19 shows five HMBC connections to C-30, C21, C-18 C-29 and C-20. H-3 is connected to C-23 and C-24.
The next HMBC expansion is adjusted in its intensity for the inspection of the methyl groups. H-30 is connected to C-19. The methyl group at 1.235 ppm displays four HMBC cross peaks. Three of them are identical with the cross peaks of the methyl group at 1.02 ppm, hence these two methyl groups must be the geminal methyl groups C-23 and C-24. The remaining two different cross peaks lead to the respective carbon positions of C-23 and C-24 due to their geminal situation. The next two methyl group signals at 1.08 and 1.05 ppm each display four HMBC cross peaks and again two of them are identical for both methyl groups. This identifies these methyl groups as H-27 and H-26, both seeing C-8 and C-14 over two and three bonds. The quaternary carbon atom at 42.8 ppm is seen in the HMBC spectrum also from the signals of H-18, H-13 and H-16 (not shown in this expansion), hence it can safely be assigned as C-14. The methyl group signals H-27 and H-26 can be differentiated by their connection to C-15 and C-13, or C-7 and C-9, respectively. The final methyl group at 0.8 ppm displays four connectivities to C-1, C-5, C-9 and C-10, and this identifies this signal for methyl group H-25. C-10 can be confirmed at 37.4 ppm via HMBC cross peaks from H-1 and H-2.

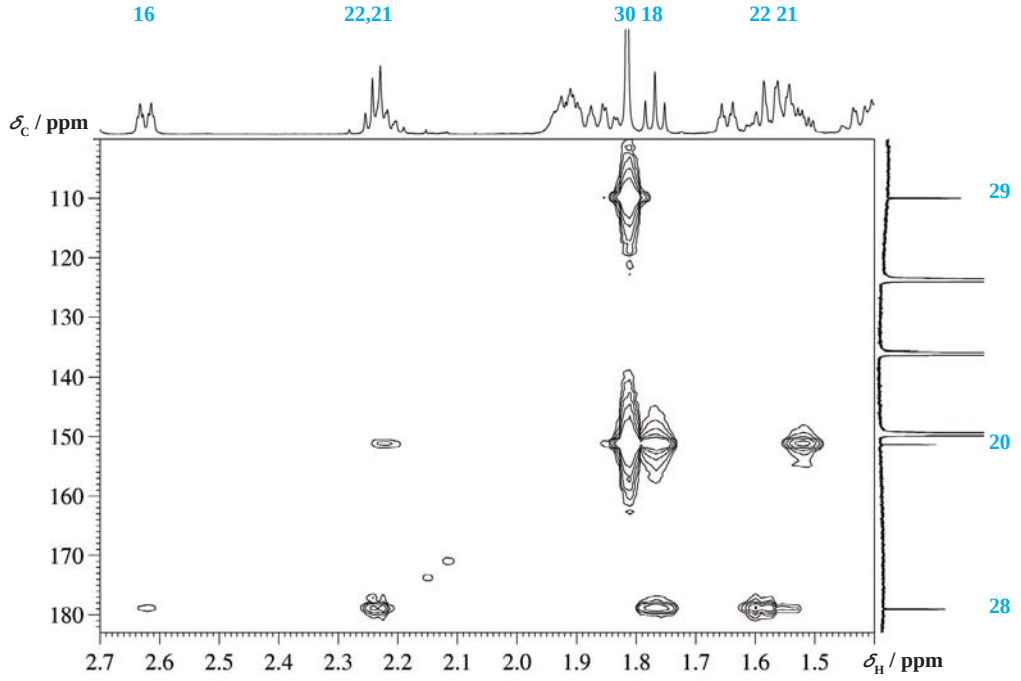


Fig. 5.8-22 Expansion of the HMBC spectrum between 3 and 1.5 ppm

Inspection of the final HMBC expansion reveals that the more shielded part of the multiplet at δ_H = 2.25–2.22 ppm belongs to H-21 due to its connectivity with C-20. The expansion of the HMBC spectrum, however, demonstrates also that the multiplet at δ_H = 2.25–2.22 ppm has a connectivity to the carboxyl atom C-28. It is therefore reasonable to assume that the other proton of this multiplet belongs to H-22. H-18 is confirmed by its connectivity to C-20 and C-28. The quaternary carbon atom at 56.6 ppm is seen in the HMBC spectrum from the signals of H-18, H-21 and H-22, hence it can safely be assigned as C-17 and thus we have concluded the assignment of the side chain and of the five-membered ring, and also the assignment of the six-membered ring attached to the five-membered ring. For a molecule of this size, many more detailed expansions of the HMBC spectra are required which are not given here for space reasons.

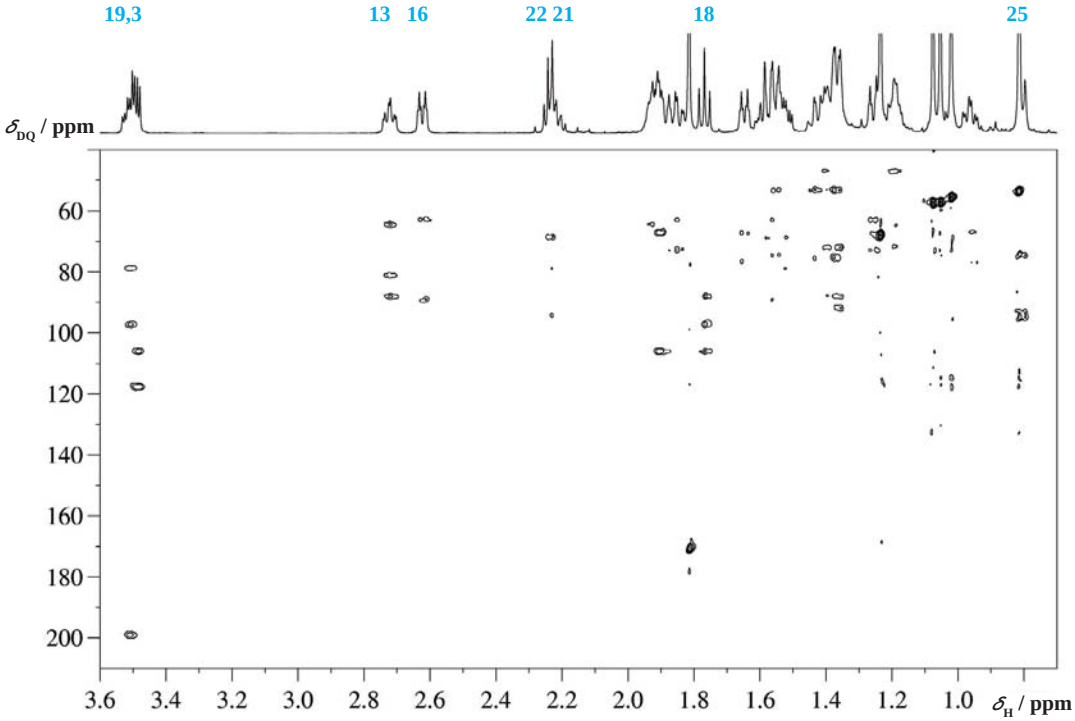


Fig. 5.8-23 ADEQUATE spectrum

As one moves away from the functional groups, the assignment of the inner protons and carbons of betulinic acid becomes more and more difficult. Fortunately, there is now a fairly recent technique available, which independently leads to a safe assignment of the carbon NMR spectrum and then, of course, to a corroboration for the proton assignment via HSQC. This is the proton-detected INADEQUATE technique, called ADEQUATE. In the spectrum displayed, a methine proton will give three cross peaks, a methylene proton two and a methyl proton only one. These cross peaks appear at the double quantum frequency of carbon, which is the sum of the chemical shifts of the carbon atoms which are connected to the proton in question by one and two bonds.

For example, the allylic proton H-19 displays three cross peaks at the double quantum frequencies δ_{DQ} = 78.8, 97.4 and 199.0 ppm. The signal at 199 ppm is due to the connectivity of H-19 with C-19 and that of C-19 with C-20, therefore the signal appears at the sum of the chemical shifts of C-19 and C-20 = 199 ppm. Similarly, the proton signal of H-3 displays two cross peaks at δ_{DQ} = 106.2 and 117.6 ppm due to the sum of C-3 and C-2 and the sum of C-3 and C-4. Note the cross peak for the methyl proton H-29 at δ_{DQ} = 170.7 ppm. This is due to the sum of the chemical shifts of C-29 and C-20. By analysing all the peaks in the spectrum, an unequivocal assignment even of such a large and complicated molecule can be obtained, and this can be found in the table.

ΣΩΚΡΑΤΗΣ. Ἀτάρ, ὦ ἑταῖρε, μεταξὺ τῶν λόγων, ἆρ᾽ οὐ τόδε ἦν τὸ δένδρον ἐφ᾽ ὅπερ ἦγες ἡμας;
ΦΑΙΔΡΟΣ. Τοῦτο μὲν οὖν αὐτό.
ΣΩΚΡΑΤΗΣ. Νὴ τὴν Ἥραν, καλή γε ἡ καταγωγή. Ἥ τε γὰρ πλάτανος αὕτη μάλ᾽ ἀμφιλαφής τε καὶ ὑψηλή τοῦ τε ἄγνου τὸ ὕψος καὶ τὸ σύσκυον πάγκαλον, καὶ ὡς ἄκμὴν ἔχει τῆς ἄνθης, ὡς ἄν εὐωδέστατον παρέχοι τὸν τόπον. Ἥ τε αὖπηγὴ χαριεστάτη ὑπὸ τῆς πλάτανου ῥεῖ μάλα ψυχροῦ ὕδατος, ὥς γε τῷ ποδὶ τεκμήρασθαι. Νυμφῶν τέ τινων καὶ Ἀχελῴου ἱερὸν ἀπὸ τῶν κορῶν τε καὶ ἀγαλμάτων ἔοικεν εἶναι. Εἰ δ᾽ αὖ βούλει, τὸ εὔπνουν τοῦ τόπου ὡς ἀγαπητὸν καὶ σφόδρα ἡδυ θερινόν τε καὶ λιγυρὸν ὑπηχεῖ τῷ τῶν τεττίγων χορῷ. Πάντων δὲ κομψότατον τὸ τῆς πόας, ὅτι ἐν ἠρέμα προσάνται ἱκανὴ πέφυκε κατακλινέντι τὴν κεφαλὴν παγκάλως ἔχειν. Ὥστε ἀριστά σοι ἐξενάγηται, ὦ φίλε Φαῖδρε.

Plato (428–348 BC)
Phaedrus

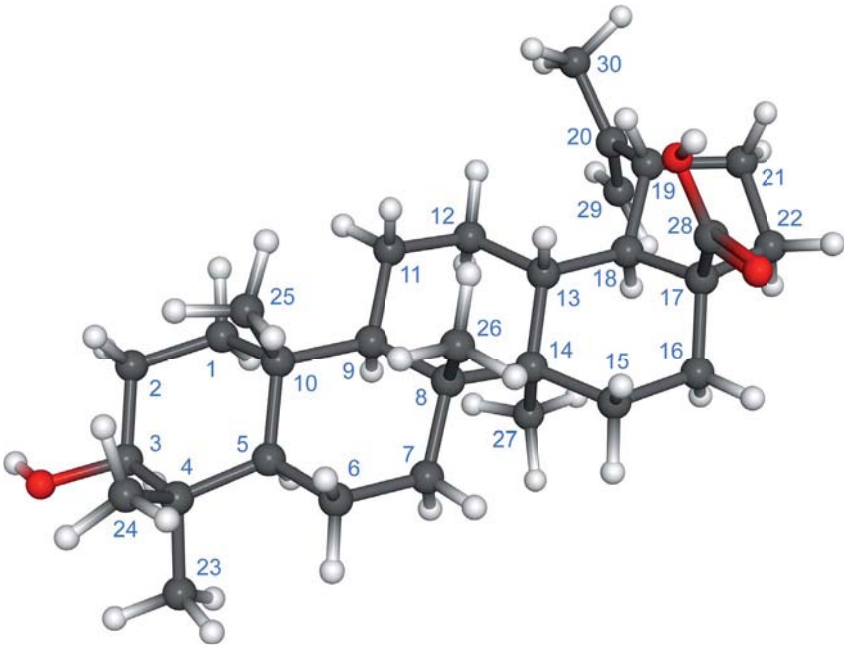


Fig. 5.8-24 Molecular model of betulinic acid

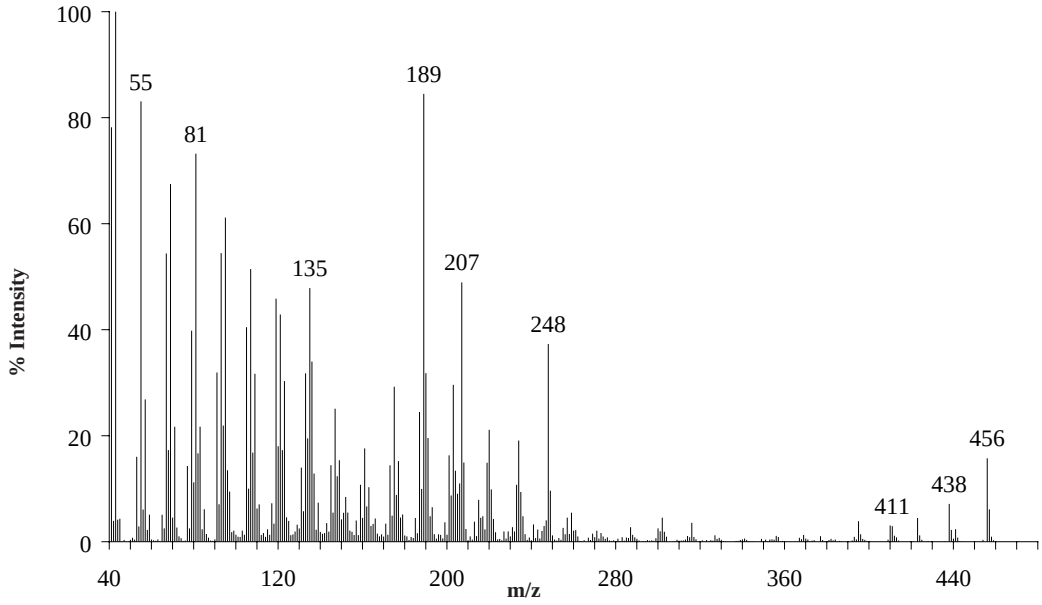
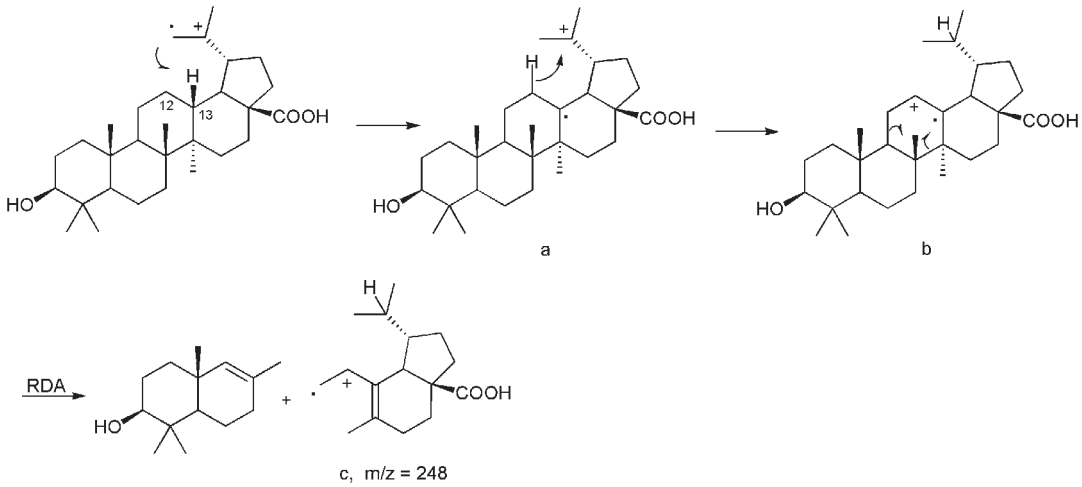


Fig. 5.8-25 Mass spectrum (EI)

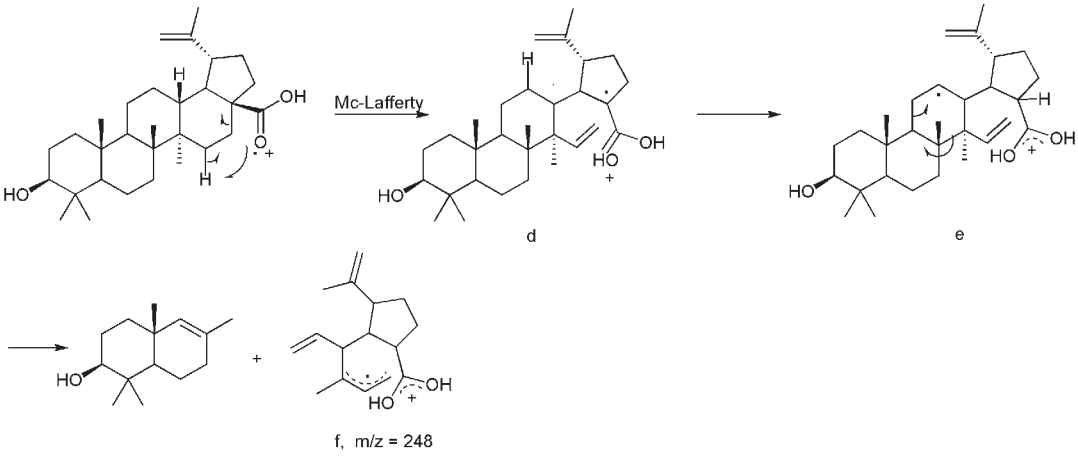
The mass spectrum displays the typical pattern of a polycyclic compound with many CH_2 and CH groups leading to an array of signals separated by 13 and 14 mass units. The M−18 peaks indicates the elimination of water and the M−45 peak the elimination of the carboxyl group. Characteristic is the m/z value of 248, which indicates that after ionization probably at C-28 or C-20, the molecule is cleaved by breaking the two bonds C-8−C-14 and C-9−C-11.

In detail this can be formulated (we are indebted to Prof. K. P. Zeller, University of Tübingen, for this suggestion) either by ionization at the isopropenyl group and a hydrogen transfer leading to ion a which undergoes a second hydrogen shift leading to b. From b, a retro-Diels−Alder reaction forms the radical ion c with m/z = 248.



Scheme 5.8-3 Fragmentation of betulinic acid

A second possibility starts by ionization at the carboxyl group, two McLafferty rearrangements lead to ions d and e, which is finally cleaved to the radical ion f, again with m/z = 248.



Scheme 5.8-4 Further fragmentation

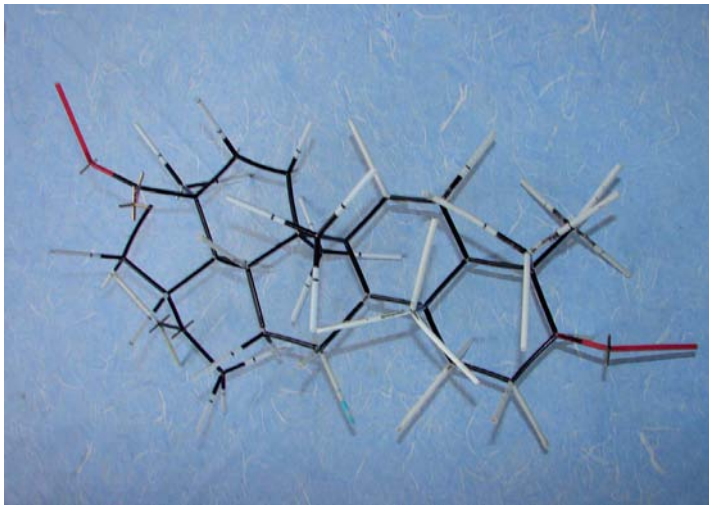


Fig. 5.8-26 Molecular model build to inspect the stereochemical relationships

5. Questions

A. What is the name of the monomer from which all terpenes are made up – if you consider it as a pure hydrocarbon? In which form is it used in terpene synthesis in the cell?

B. A triterpene containing 30 C atoms is based on a non-cyclized open-chain C_{30} precursor. What is the name of this precursor? Astonishingly, although reactive, this compound has also been isolated from a living organism. Search from what source.

C. A certain animal triterpene is a C_{30} steroid occurring among other compounds in lanolin, a hydrophobic secretion of the sebaceous glands of the sheep excreted into wool. The compound of interest is regarded as the biosynthetic precursor of, for example, cholesterol, the sex hormones and bile acids. Give the trivial name and the structure of this compound.

D. It is mandatory in such a complicated case to build a molecular model. Inspect the model and give a reason why proton H-5 is the most shielded proton.

E. How could one ascertain the stereochemical situation of H-5?

F. Despite the fact that H-5 is the most shielded proton, C-5 is the most deshielded methine carbon atom not bearing a hetero substituent. Explain.

G. Which of the diastereotopic protons H-16 and H-22 is the more deshielded?

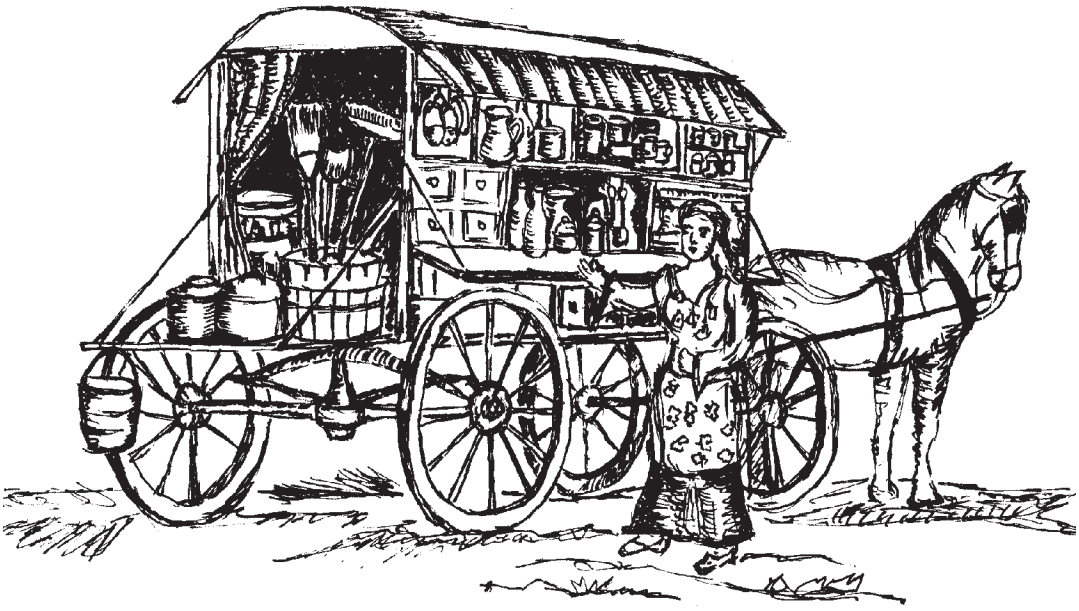
H. Give a reason why one of the diastereotopic protons H-16 is somewhat more deshielded than one of the pair H-22.

I. How could one ascertain that the conformation of the vinyl side chain is as drawn in the molecular formula?

J. How do you interpret the m/z values 207 and 189?

6. Own Observations

Chapter 6 Miscellaneous



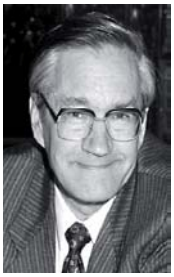
Miscellaneous items on sale

The two major analytical tools demonstrated in this book are Nuclear Magnetic Resonance and Mass Spectrometry. Researchers using these techniques have several times been awarded the Nobel Prize:

Nuclear Magnetic Resonance:

Felix Bloch
1952

Edward M. Purcell
1952



Richard R. Ernst
1991

Kurt Wüthrich
2002

Paul C. Lauterbur
2002

Peter Mansfield
2002

Mass Spectrometry:

Joseph John Thomson
1906

Francis William Aston
1922

Wolfgang Paul
1989

John Bennet Fenn
2002

Koichi Tanaka
2002

6.1 Shikimic Acid

(3*R*,4*S*,5*R*)-3,4,5-Trihydroxy-1-cyclohexene-1-carboxylic acid

From star aniseed
Illicium verum Hook. F. (Schisandraceae)

$C_7H_{10}O_5$, MW 174.15

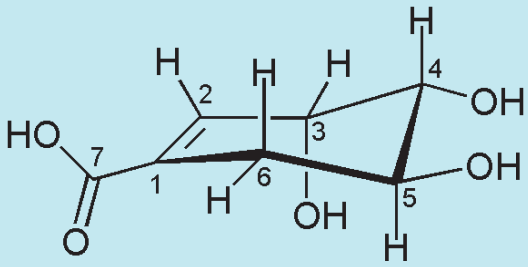
CAS RN 138-59-0, BRN 2210055, 2692131, 3118358, 4782717

Colourless crystals, mp 185−187 °C,
$[\alpha]_D^{21}$ = −184.2° (*c* = 0.0104 g/mL, water)

Shikimic acid is commercially available.

Synonymous names:
(−)-Shikimic acid, L-Shikimic acid

Level: medium



1. Background: Against the big fear: pandemic

Schlaflied

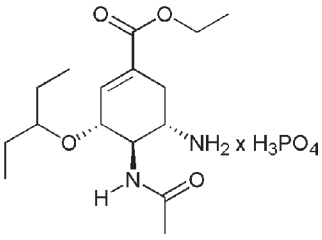
EINMAL wenn ich dich verlier,
wirst du schlafen können, ohne
daß ich wie eine Lindenkrone
mich verflüstre über dir?

Ohne daß ich hier wache und
Worte, beinah wie Augenlider,
auf deine Brüste, auf deine Glieder
niederlege, auf deinen Mund.

Ohne daß ich dich verschließ
und dich allein mit Deinem lasse
wie einen Garten mit einer Masse
von Melissen und Stern-Anis.

Rainer Maria Rilke (1875−1926)

Shikimic acid – for many readers this name will have an exotic, strange and mysterious touch. Maybe it seems Asiatic to you. No wonder – the name has its origin in the Japanese flower *shikimi* (Japanese star anise, *Illicium anisatum* L.) from which the acid was first isolated in 1885 [1]. At first glance the molecule seems tiny and simple in its structure. One might expect that it would be possible to be chemically synthesized and, if it should be needed, even on the bulk scale. However, the reality is different: the demand for shikimic acid is extraordinarily high, but there is no efficient synthetic access. Shikimic acid serves as a precursor for the synthesis of the drug oseltamivir (INN), developed by Gilead Sciences, which is sold under the trade name Tamiflu® by Hoffmann-La Roche (Roche). It is used in the treatment of both influenza viruses A and B and is expected to be effective also in the treatment of the dangerous H5N1 avian influenza (called avian flu or bird flu). Tamiflu received its official approval in the USA and Japan in 2000, and in the EU in 2002. It is allowed to use this medicinal drug, which has to be prescribed, also for prevention, and also for children from the age of one year on. According to the present knowledge, oseltamivir (see formula below) acts as a virostatic, but is not virucidal, which means that it suppresses the reproduction of the virus but does not eliminate it. Many experts consider that a pandemic of avian flu has to be expected or at least seems likely in the coming years. In the globalized world this may cause dramatic effects. This possibility caused increasing fear and induced governments, corporations and private individuals to stockpile the drug. Correspondingly, the current production and sales are not small [2]. In 2006, Roche sold Tamiflu for 2.6 billion Swiss Francs and the drug climbed to fourth position among the Roche blockbusters. The production is currently sufficient to treat seasonal influenza and to meet the demand for the official stockpiling of governments. Shortages seem possible in the event of an actual avian influenza pandemic.

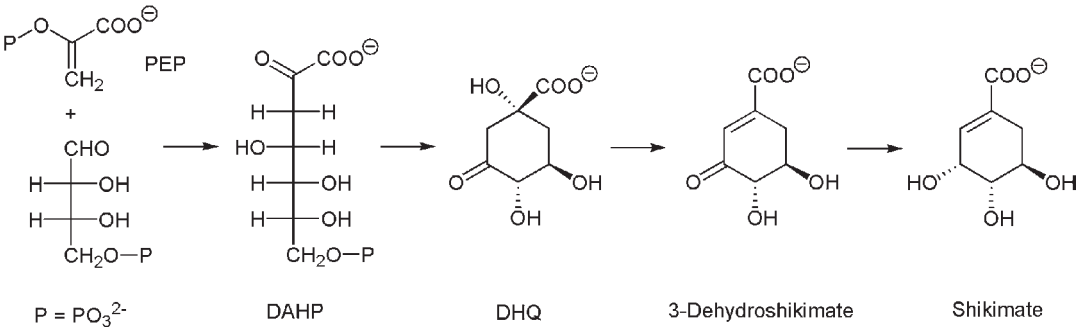


Scheme 6.1-1 Oseltamivir

However, there is still a bottleneck in this business, and it is a chemical one: shikimic acid. It is more efficient to isolate the compound from natural sources than to synthesize it. If isolation is the approach, this shifts the bottleneck into the Nature: How is it made? Where does it occur? Are there enough natural sources of shikimic acid? For the last question, a rapid answer is: No! This makes natural sources of shikimic acid more or less a strategic raw material. There are other examples in the past of natural products becoming of strategic importance. Think of the combat for access to cinchona trees (*Cinchona officinalis* L.) during World War II. The bark of this trees contains quinine as an anti-

malaria agent, necessary to support troops in tropical regions. This led to safeguarding operations by the USA. But step by step.

Shikimic acid is made by autotrophic organisms, i.e. plants and some bacteria, but not by animals. That means it is in principle made by many organisms. Its biosynthesis combines a C_3- and a C_4-unit and starts from phosphoenolpyruvate (PEP) and D-erythrose-4-phosphate, which form 3-deoxy-D-*arabino*-heptulosonate-7-phosphate (DAHP). DAHP is cyclized to form 3-dehydroquinate (DHQ), which in turn is dehydrated to 3-dehydroshikimate. Reduction of the enone unit leads to shikimic acid.



Scheme 6.1-2 Biosynthesis of shikimate (no enzymes or cosubstrates shown)

The importance of shikimic acid cannot be overestimated. It is the precursor to form aromatic rings, and thus it supplies the aromatic amino acids tyrosine and phenylalanine even for all animal proteins. Indole, another central biochemical precursor, is derived from it, and hence the amino acid tryptophan. Finally, a lot of other aromatics and many alkaloids as well as tannins, lignin and flavonoids are shikimic acid-based compounds.

However, the point is that shikimic acid serves mostly as a biosynthetic intermediate only, on the way to the compounds mentioned. Usually, it is not stored as an intermediate and hence it cannot be easily isolated due to a too low concentration in almost any plant. Of course, there are exceptions. The Japanese star anise, a plant that is treated with care because it contains toxins such as anisatin, shikimitoxin and shikimin, has already been mentioned above. Star aniseed (also called Chinese star anise) used in this preparation is used as a spice and serves as the best natural source for shikimic acid isolation. However, its occurrence as an evergreen tree that is grown for commercial use in China, Vietnam, India and other Asian countries is limited. Again, we are at a bottleneck: the abundance of the occurrence of the Chinese star anise trees (*Illicium verum* Hook. F.).

Shikimic acid is industrially extracted from star aniseed in an optimized 10-stage process. It is reported that from 3 to 10% of a given mass of star aniseed could be obtained as shikimic acid. However, this amount will not be obtained by the method described here. About 1 g of shikimic acid is necessary for the chemical synthesis of as much oseltamivir as present in seven of its 75 mg capsules. The drawback is the shortage of star aniseed in comparison with the demand of the

pharmaceutical industry. A search for alternatives has led to classical and modern alternatives.

Home-made medicines was all de go. Oil and turpentine, camphor, assfiddy, cherry bark, sweetgum bark; all dem things was used to make teas for grown folks to take for deir ailments. Red oak bark git sho' 'nough 'ligion. De wooden bowls what slave chillun et out of was made out of sweetgum trees. Us et wid mussel shells 'stid of spoons. Dem mussel shells was all right. Us could use 'em.

Slave Narratives: a Folk History of Slavery in the United States
From Interviews with Former Slaves
Georgia Narratives, Part 1

Alternatively, shikimic acid can be isolated in 1.5% yield from the seeds in sweetgum balls of the sweetgum tree (*Liquidambar styraciflua* L.), that grows widespread around the world and is abundant in warm, temperate areas of eastern North America. The prickly sweetgum balls are regarded as the urban forest's counterpart to the spiny sea urchin and are correspondingly unpopular. They are a bane for all who like barefoot living. Therefore, the trees have been cursed and their seeds esteemed as useless. Whether this waste could really be turned into a profitable raw material for shikimic acid is still questionable. For isolation in bulk, not the whole ripe ball but only the granular, infertile seeds inside are required, which would mean harvesting the husks while they are still green and picking out the seeds before they disperse [3]. Whether this can become an effective business is much in doubt.

The fermentative production of shikimic acid by metabolic engineered microorganisms is an excellent alternative. The principles of such methods were summarized in 2003 [4], and some more recent patents are pending. The demand for shikimic acid has led to intensive work to establish synthetic approaches to oseltamivir based upon cheap commercially available sources. Various methods have been developed with a recent concise procedure in eight steps with 30% overall yield [5].

Fig. 6.1-1 Flower of the Japanese star anise (*Illicium anisatum* L.)

The country in which the neuraminidase inhibitor Tamiflu has been prescribed and used on the largest scale is Japan. It is therefore no great surprise that if any problems with Tamiflu were to occur, reports about such incidents would have to be expected from Japan. Of course, this does not mean that the approval procedure for Tamiflu would not have been carried out with the utmost care. It is just the very large number of patients treated in real life, much greater than could ever be possible in a clinical study, that produces a vast amount of new data. There are many analogous examples in pharmacy with similar findings. Indeed, Japanese paediatricians are now concerned with whether Tamiflu prescribed to children or teenagers might cause mental instability or even suicidal tendencies [6]. Clearly, the number of suspicious deaths is tiny when set in comparison with the number of patients who received the drug. Therefore, the whole context is under detailed consideration at present. Some chemists have also looked at the matter again and there is a line of argumentation that the active principle of the drug shows structural and hence possibly functional relationships to the inhibitory neurotransmitter GABA (γ-aminobutyric acid), a compound active in inhibitory synapses in the brain of vertebrates. Another hypothesis is that a recently found Asian single polynucleotide polymorphism, i.e. a genetic reason leading to a different enzymatic equipment in part of the Japanese population, but not among Europeans or African Americans, may be of importance for some side-effects. In summary, this debate has only just started. Hopefully, the epidemic situation with H5N1 avian influenza will stay as it is, so that drugs can remain in the stockpile and do not have to prove their effectiveness in a pandemic.

2. Literature

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Tom was swimming in bliss. He said:
"Do you love rats?"
"No! I hate them!"
"Well, I do, too − LIVE ones. But I mean dead ones, to swing round your head with a string."
"No, I don't care for rats much, anyway. What I like is chewing-gum."
"Oh, I should say so! I wish I had some now."
"Do you? I've got some. I'll let you chew it awhile, but you must give it back to me."
That was agreeable, so they chewed it turn about, and dangled their legs against the bench in excess of contentment.

Mark Twain (1835−1910)
The Adventures of Tom Sawyer,
Chap. 7
(The resin of the American sweetgum tree was earlier used for chewing gum)

3. Isolation

3.1 Principle

This follows the method described in [7] with two alterations mentioned in the text.

Shikimic acid is a natural product that can be isolated from entire star aniseeds. It is a highly hydrophilic, polar and clearly acidic compound. These properties can be used for stepwise separation from any other side components contained in the star aniseeds.

The first step consists in a Soxhlet extraction of any polar components with ethanol from the star aniseeds, except for pure carbohydrates. However, this step also includes the extraction of ethereal oils because they are also soluble in ethanol.

In the next step, these ethereal oils are removed from more hydrophilic compounds. To achieve this, the ethanolic extract is dissolved in warm water, causing complete solution of shikimic acid (solubility in water 180 g/L at 20 °C!). Cooling to ambient temperature gives rise to a cloudy emulsion with oily droplets on the surface, all due to the water-insoluble ethereal oil. It is then removed by extraction with diethyl ether (alteration 1). Then, heating of the aqueous phase with a small amount of formalin reductively protects the compounds dissolved in water. The aqueous phase is then filtered through a Celite pad for a first decoloration, which, however, is not yet complete.

The clear solution obtained is then passed through a column filled with an anion-exchange resin in its acetate form (Amberlite IRA-400). By this means, shikimic acid is selectively transferred from the solution onto the resin and acetate ions are released into the solution. After washing with water, shikimic acid is set free by elution with 25% aqueous acetic acid. Water and acetic acid are completely distilled off from the eluate in vacuo, finally very carefully in the fine vacuum of an oil pump. The brown amorphous mass is not allowed to have any smell of acetic acid!

This mass is then heated under reflux with ethyl acetate in the presence of charcoal to achieve complete decoloration (alteration 2). Contrary to the literature cited, methanol was not used either for this step or for the crystallization procedure to avoid formation of methyl shikimate, which had occurred to some extent in another run. After filtration, pure shikimic acid crystallized on slow evaporation of part of the ethyl acetate in vacuo as tiny colourless crystals which were recrystallized from ethyl acetate. Any difficulties in solidification that occasionally may occur by crystallization attempts from a mixture of methanol and toluene are avoided by this method.

3.2 Method

An 80 g amount of star aniseed is crushed by means of a kitchen grin-der (Moulinette) to a coarse semolina-like meal. This material is placed in the thimble of a Soxhlet apparatus and extracted with ethanol (500 mL) for 4 hours (number of circulations about 50). The brown extract obtained is clear and becomes cloudy on cooling to ambient temperature. It is filtered and the ethanol removed in a 1 L round-bottomed flask on a rotary evaporator, and finally for 5 min with an oil pump. A viscous brown residue remains and smells intensively of anised. Its mass is 20.1 g.

Distilled water (400 mL) is added to the residue in the 1 L round-bottomed flask and the mixture is warmed to 80 °C in a water bath with pivoting. A cloudy, pale brown emulsion is obtained with a few millilitres of an oily liquid at the surface. The mixture is cooled to ambient temperature and transferred into a separation funnel. The lipophilic components are extracted with diethyl ether (2 × 150 mL). Both ethereal phases are discarded. To the orange-coloured aqueous layer (at pH 3) 37% formalin solution is added (0.5 mL), the mixture refluxed for 5 min and then allowed to cool to 25 °C, whereupon it turns

cloudy. The suspension is filtrated by suction through a sintered glass filter funnel containing a 3 cm pad of Celite. A clear, orange-coloured filtrate is obtained.

A glass column (size 30 cm × 3 cm) with a glass filter funnel in front of the outlet is prepared for ion exchange as follows: 80 g of Amberlite IRA-400 anion-exchange resin (chloride form) are mixed with distilled water (250 mL) in a beaker and poured wet into the column via a funnel. A 2 molar solution of sodium acetate (500 mL) is passed 20 times through the resin charge. Finally, the column is flushed once with distilled water (150 mL). Then, the 400 mL of filtrate obtained above are passed through the column five times. An orange−brown liquid remains which is initially stored. Later, the water is removed in vacuo to give a remainder of 4.3 g of a brown viscous residue. TLC shows that it still contains some shikimic acid together with four less polar components in considerable amounts. Therefore, this residue is finally discarded. In the next step, the shikimic acid elution is done with 25% acetic acid (400 mL). The rate of elution is adjusted at the outlet bleeder to allow only 100 mL to pass within 5 min. The column is then flushed with distilled water (100 mL). The eluate and wash water are combined to give a clear, pale orange solution. Water and acetic acid are first distilled off using a rotary evaporator (bath temperature 40 °C, pressure down to 14 mbar). A brown oil remains (mass 6.4 g), which smells intensely of acetic acid. This residual acetic acid is carefully removed using an oil pump equipped with a nitrogen cooling trap by evaporation in a water bath (50 °C) at a pressure of 0.2 mbar for 4 h. A brown extract (mass 4.9 g) of crude shikimic acid remains, which forms an amorphous solid.

3.3 Purification

In a 500 mL round-bottomed flask, the crude shikimic acid from above (4.9 g) is placed together with charcoal powder (3.0 g) and ethyl acetate (250 mL). The suspension is heated under reflux for 15 min for decoloration. Then it is filtered through a very close-grained glass filter funnel to yield a completely colourless solution of shikimic acid. The filtrate is reduced to dryness in vacuo to give rise to shikimic acid as a colourless solid (461 mg) with a mp of 175−180 °C. The residue at the filter funnel has a dry mass of 7.2 g and obviously still contains shikimic acid, whose solubility in hot ethyl acetate is rather low. Hence a second extraction with ethyl acetate (200 mL, reflux for 10 min) gives rise to another 251 mg of shikimic acid. A final third extraction with ethyl acetate (800 mL, reflux for 10 min) yields a final crop of 1.1 g of shikimic acid. The remaining solid of charcoal etc. is then discarded.

A TLC analysis of the three crops of product obtained is made on silica gel 60_{F254} alumina foils [eluent: $CHCl_3$−CH_3OH (5:1, (v/v)]. Shikimic acid shows a trail-like spot with R_f 0.1. Almost none of other less polar compounds with higher R_f which are visible on TLC plates during the extraction procedure are present here. Shikimic acid shows a slight fluorescence quenching and a blue spot when dipped into an oxidizing reagent and heated with a fan heater. The reagent is obtained

Star Anise Tea

4 green tea bags
one 3-inch cinnamon stick, broken into pieces
6 whole star anise or 1 tablespoon broken star anise pieces
4 cups boiling water

Let the cinnamon and the star anise infuse for two minutes in freshly boiled water.
Add this infusion through a sieve into a pre-warmed teapot, containing the green tea bags.
Let it infuse for an additional 2 minutes and serve directly.

From the authors

by dissolving 25 g of molybdophosphoric acid monohydrate, 10 g of cerium(IV) sulfate tetrahydrate and 60 mL of conc. sulfuric acid in 940 mL of water.

Finally, the three crops of shikimic acid (1.812 g) are recrystallized by dissolving them in boiling ethyl acetate (1 L) for 10 min. Then, the clear solution is allowed to cool to 40 °C (no precipitation occurs with this procedure). The solution is subjected to slow rotary evaporation and concentrated to a volume of 200 mL. A fine, colourless precipitate of microcrystalline shikimic acid is formed, which is filtered off and carefully dried in vacuo using an oil pump.

Yield: 1.16 g, mp 185−187 °C, pure according to TLC and NMR; $[\alpha]_D^{21} = -184.2°$ (c = 0.0104 g/mL, water).

4. Spectra and Comments

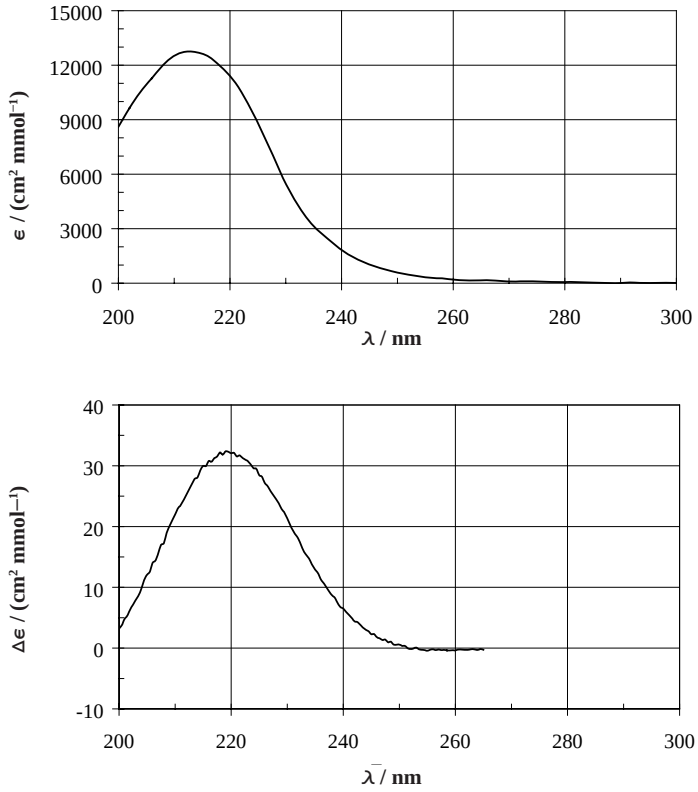


Fig. 6.1-2 UV and CD spectra in ethanol

Due to the conjugation of the C=O bond with the C=C bond, shikimic acid has a rather strong chromophore giving rise to an absorption band at 215 nm showing an ε-value of about 13 000 $cm^2\ mmol^{-1}$. This absorption band has also a rather strong and negative Cotton effect, as revealed in the CD spectrum.

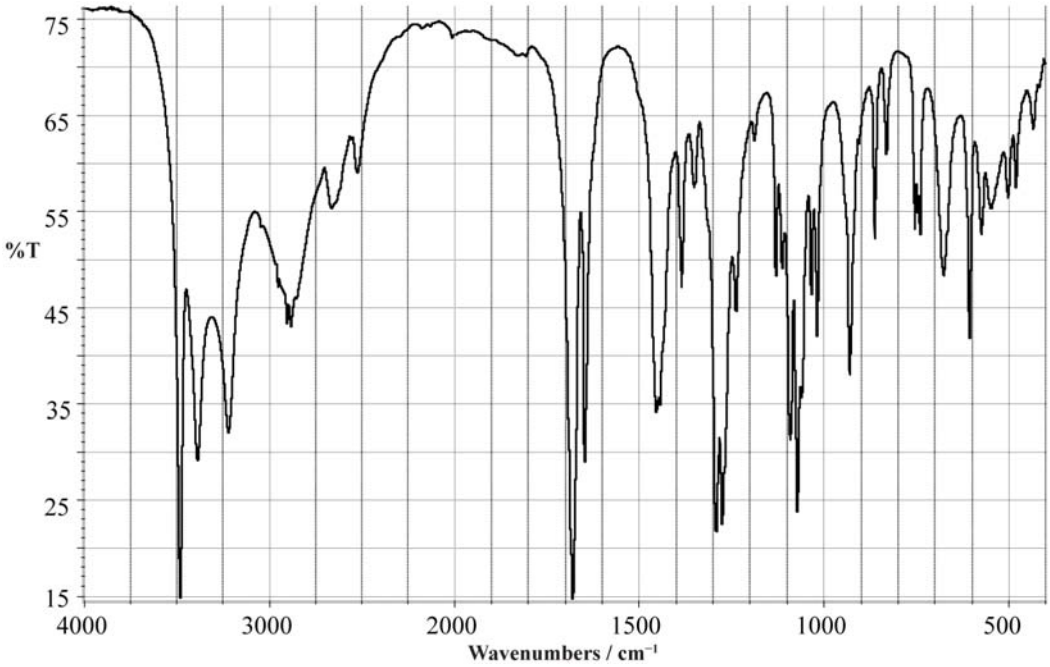
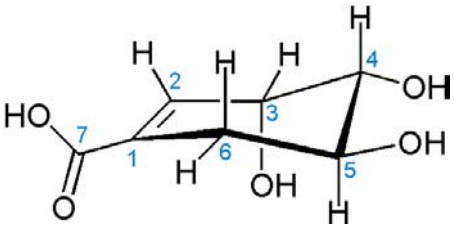


Fig. 6.1-3 IR spectrum in KBr

Inspecting the IR spectrum, one observes a rather structured OH region with unusual sharp signals. In contrast, the area of the sp^3-CH valence vibrations is rather broad. Probably due to the conjugation of the C=O and C=C bonds, their frequencies become rather close and somewhat low for a carboxylic acid at 1690 cm^{-1} and slightly high for a C=C double bond at 1650 cm^{-1}.



Scheme 6.1-3

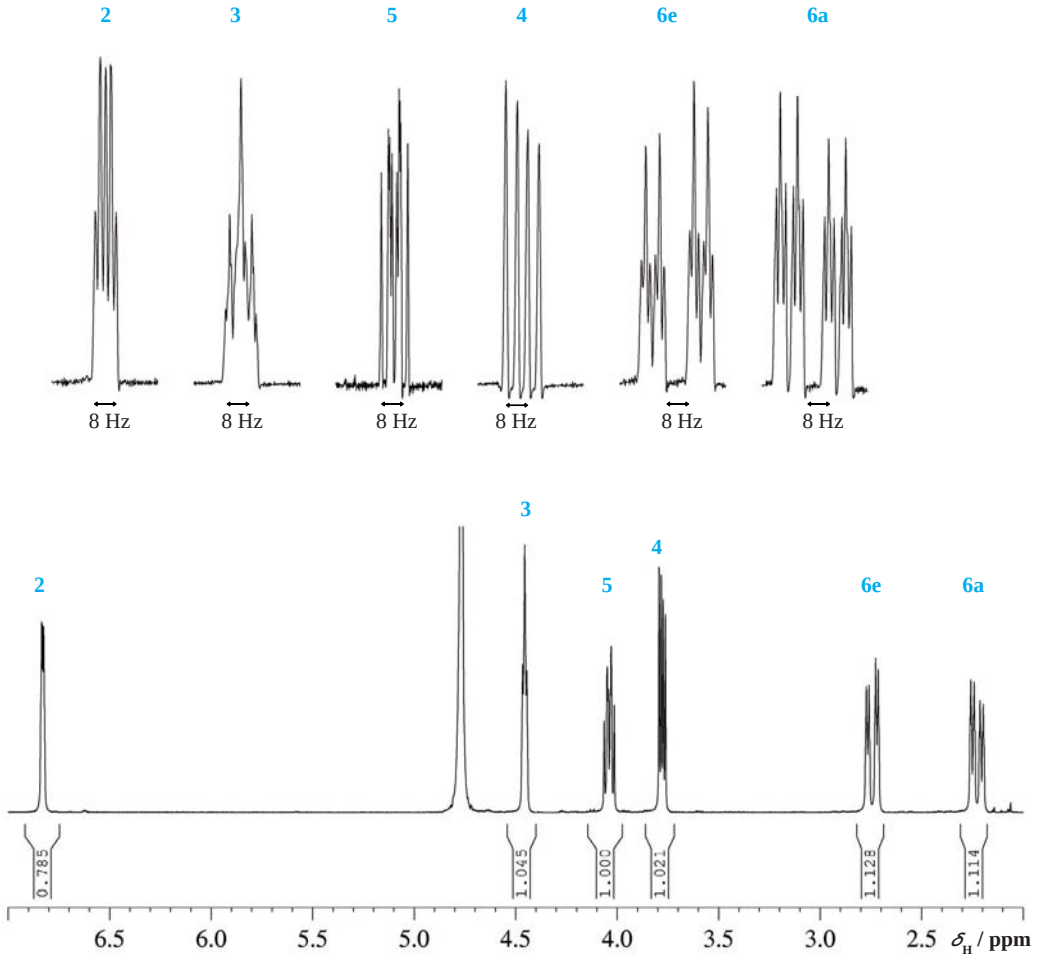
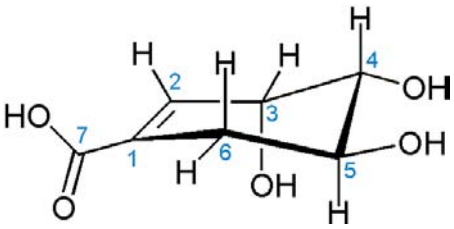


Fig. 6.1-4 ^{1}H NMR spectrum at 400 MHz in D_2O

Shikimic acid has six non-exchangeable CH protons and therefore the ^{1}H NMR spectrum shows six distinct absorptions. The diastereotopic methylene group at C-6 can be directly recognized and is centred at 2.5 ppm. Also, the olefinic proton H-2 at 6.83 ppm can be directly assigned. The individual assignment of the three CH(OH) protons between 4.5 and 3.7 ppm has to await the analysis of the COSY spectrum. The conformation of the six-membered ring can be deduced from the spin coupling constants and therefore the expansions for each multiplet are given.



Scheme 6.1-4

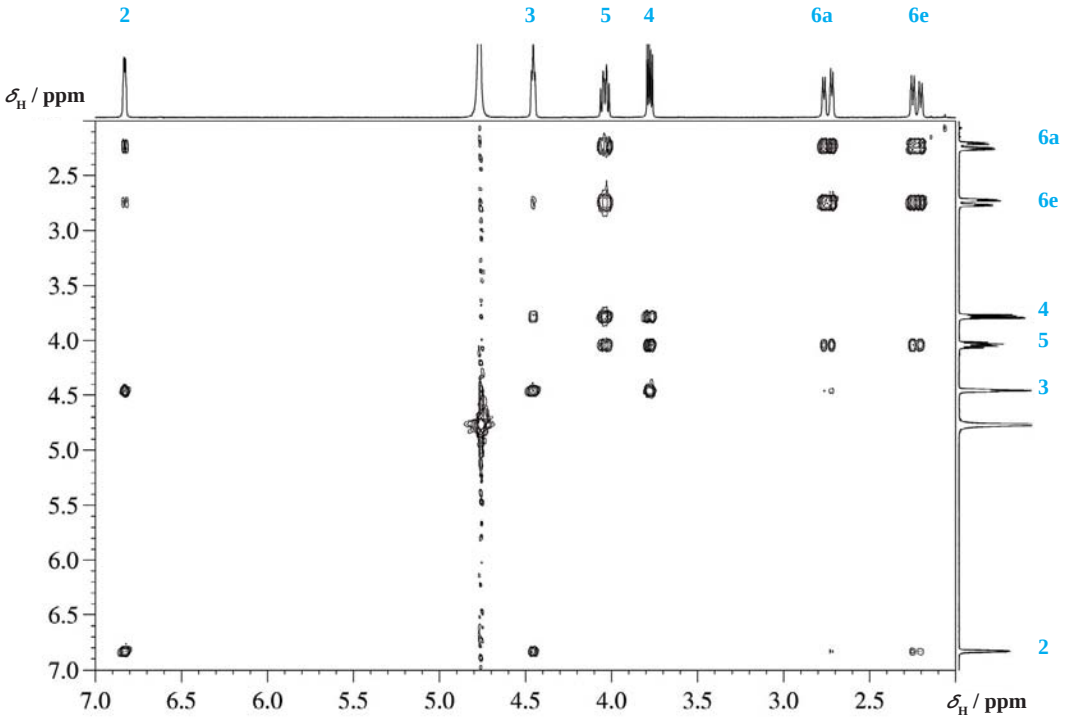


Fig 6.1-5 COSY spectrum

The olefinic proton H-2 displays a cross peak leading to the assignment for H-3 at 4.46 ppm, but also shows two further cross peaks caused by an allylic spin coupling to both H-6e and H-6a. The signal of H-3 leads the way to H-4 at 3.78 ppm, and this in turn is coupled to H-5 at 4.04 ppm, which shows again cross peaks to both H-6a and H-6e. In addition, we find very weak cross peaks between H-3 and H-6e/H-6a working over five bonds. The corresponding splitting can also be seen in the expanded patterns for the protons H-6. Thus, the COSY spectrum gives a very clear picture of the connectivities of the six different proton signals.

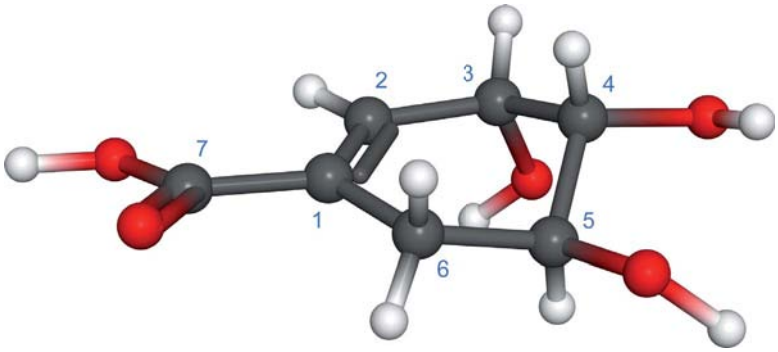


Fig. 6.1-6 Molecular model of shikimic acid

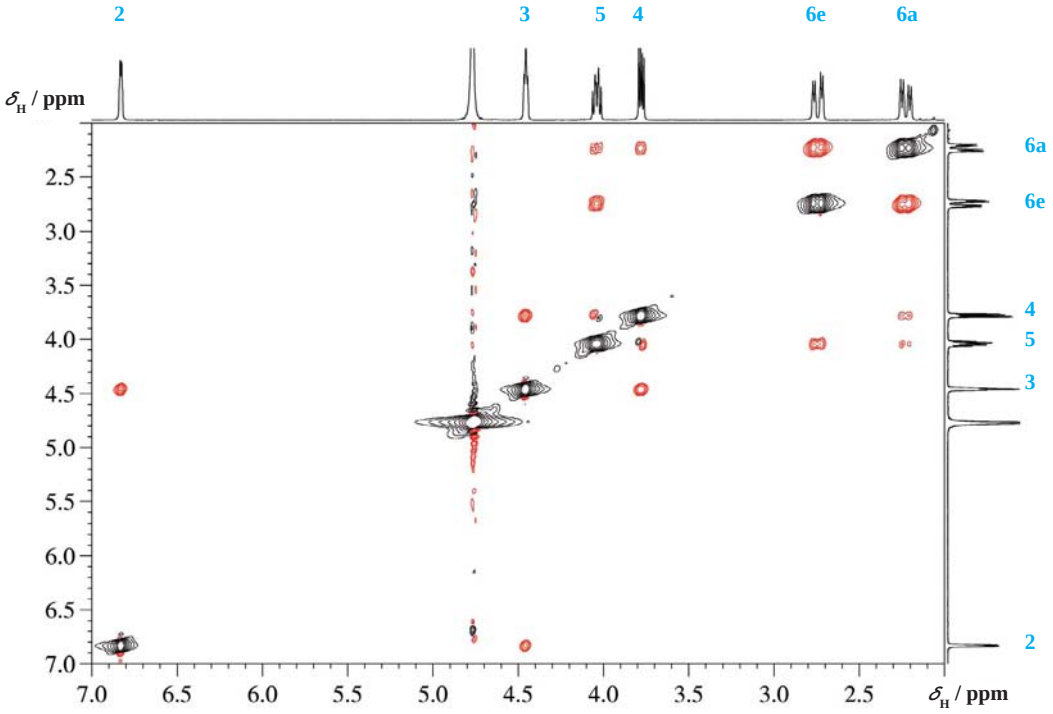


Fig. 6.1-7 NOESY spectrum

This is also true for the NOESY spectrum, which gives the same information as the COSY spectrum, showing all the trivial connectivities. The only stereochemically relevant information is the cross peak between H-4 and one of the H-6 protons, which is caused by a 1,3 diaxial interaction and thus this cross peak demonstrates that both of these protons are in axial positions. Looking at the intensities of the NOE cross peaks between H-5 and both H-6 one observes that the cross peak to H-6e has a considerably higher intensity. Also, the intensities of the cross peaks between H-3 and H-4 are larger than those between H-4 and H-5 and this confirms the drawing of the molecule as given.

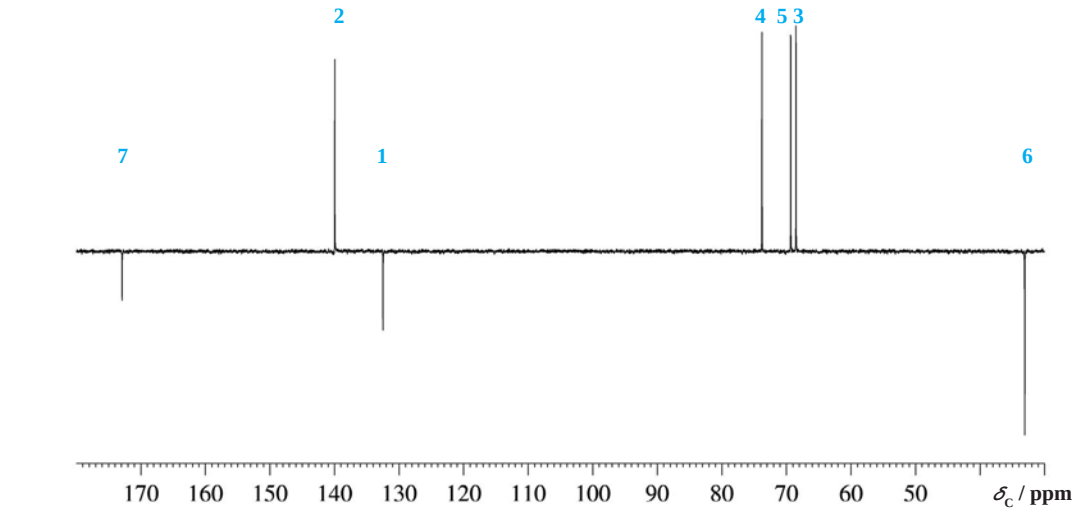


Fig. 6.1-8 APT ^{13}C NMR spectrum

By simple inspection one can directly assign four of the seven signals in the APT ^{13}C NMR spectrum. The carboxyl atom at 172.9 ppm, the two olefinic signals C-2 at 140 and C-1 at 132.5 ppm and the methylene group at 33.1 ppm pose no assignment problem. The three CH(O) signals are best assigned by evaluating the HSQC spectrum.

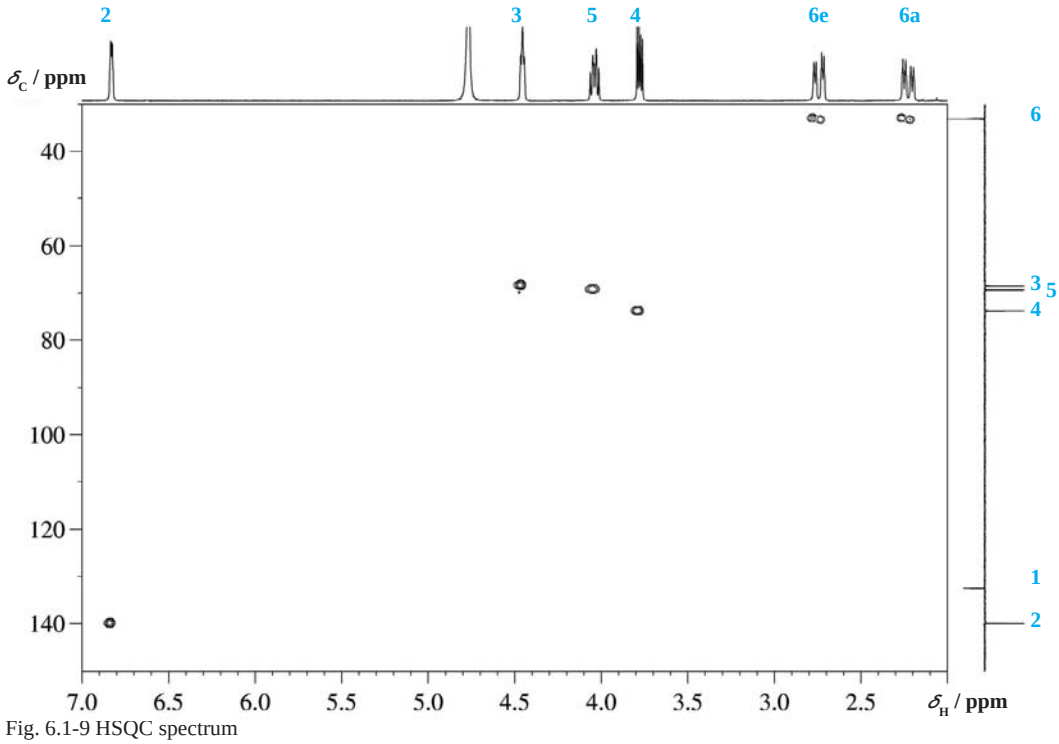


Fig. 6.1-9 HSQC spectrum

The HSQC spectrum is only used to assign the three very closely resonating CH(O) carbon signals at about 75 ppm, since we have identified their corresponding proton signals.

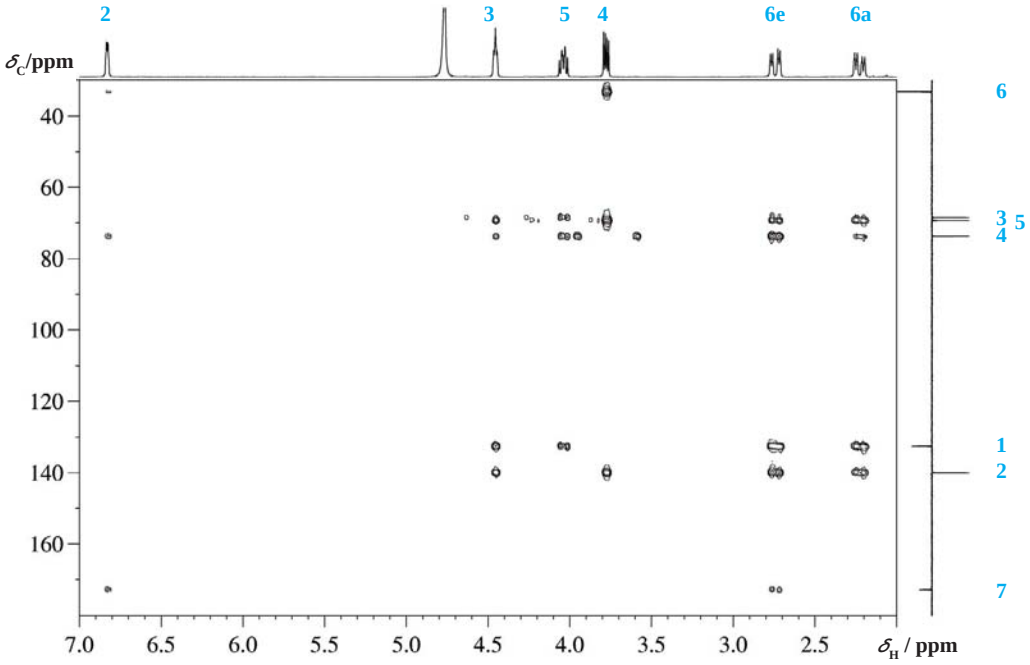
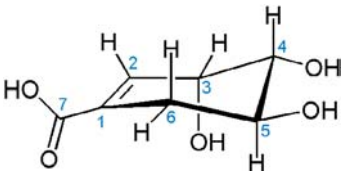


Fig. 6.1-10 HMBC spectrum

The HMBC spectrum corroborates all the assignments discussed above. It is a very good exercise to go through all cross peaks and verify the connectivities indicated. There are two stereochemical indicators: note that the signal of H-6e shows a cross peak to C-7, but not H-6a. Similarly, H-6a shows a much weaker cross peak to C-4 than H-6e. At this stage we can also discuss the H, H spin coupling constants given in the table. These have been confirmed by complete iterative spin simulation of the experimental spectrum. $^3J_{2,3}$ = 4.2 Hz. $^3J_{3,4}$ of 4 Hz typically indicates an equatorial–axial relationship, whereas $^3J_{4,5}$ of 8 Hz confirms the axial–axial situation. The two similar values for $^3J_{5,6e}$ and $^3J_{5,6a}$ show that H-5 is placed somehow in between H-6a and H-6e with a similar dihedral angle to both.



Scheme 6.1-5

^{13}C Signals δ / ppm	Type of Carbon	Assignment	^{1}H Signals δ / ppm, J / Hz
172.9	C_q	C-7	
140.0	CH	C-2	6.83, $^3J_{2,3}$ = 4.2, $^4J_{2,6e}$ = 1.8, $^4J_{2,6a}$ =1.9
132.5	C_q	C-1	
73.8	CH	C-4	3.78, $^3J_{4,5}$ = 8.35
69.3	CH	C-5	4.04, $^3J_{5,6e}$ = 5.3, $^3J_{5,6a}$ = 6.45
68.5	CH	C-3	4.46, $^3J_{3,4}$ = 4.4, $^5J_{3,6e/a}$ = 1.25
33.1	CH_2	C-6	6e: 2.74, 6a: 2.23, $^2J_{6e,6a}$ = – 18.1

Table 6.1-1 NMR data for shikimic acid

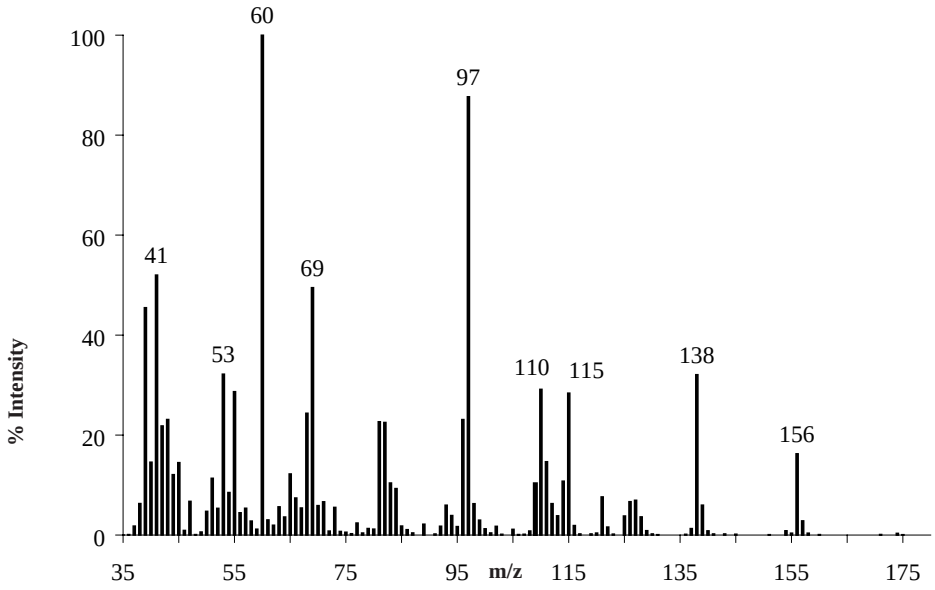
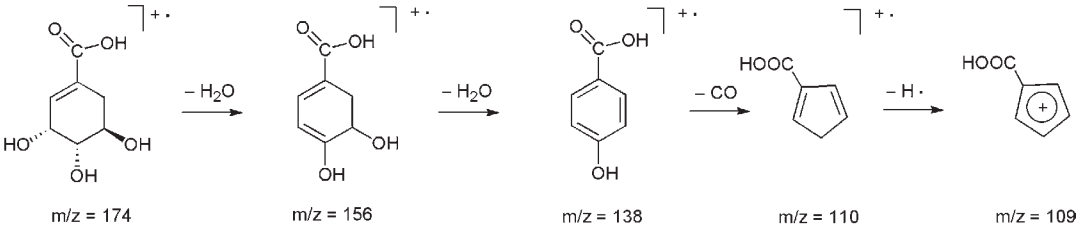


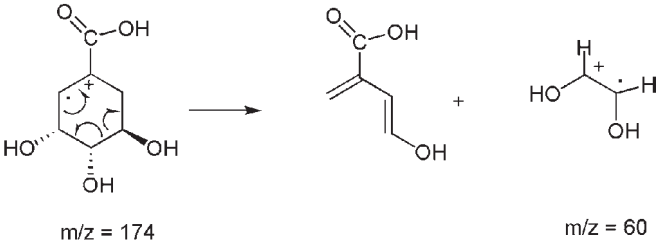
Fig. 6.1-11 Mass spectrum

The molecular ion cannot be seen in the EI mass spectrum; instead, the first signal appears at m/z = 156 caused by direct elimination of water. Another water elimination gives the signal at m/z = 138. Loss of CO will then lead to the peak of m/z =110. We might formulate this sequence as follows:



Scheme 6.1-6 Fragmentation of shikimic acid

The base peak of the mass spectrum at m/z = 60 is more difficult to explain. One suggestion might be:



Scheme 6.1-7 Base peak of shikimic acid

5. Questions

A. Compare the structures of shikimic acid and oseltamivir (Tamiflu) and give comments on (a) what is favourable in the structure of shikimic acid as a starting material and (b) what the basic problems to achieve the product are.

B. Often the shikimic acid pathway is stated to be the only natural process to yield aromatic rings. It is said that plants can do this and thus provide aromatic rings to animals by delivering them the amino acids tyrosine, phenylalanine and tryptophan within proteins of nutrition. However, on looking closer at this question it has to be stated that also the animal body is able "to produce an aromatic ring". This special transformation takes place within the group of sexual hormones. Which hormones are included and what is the name of the enzyme that is capable of bringing about this transformation?

C. Chiral polyhydroxy compounds of biochemical and/or biological function are not limited to sugars or shikimic acid. What is the trivial name of a hexahydroxycyclohexane which is synthesized in the human body in two steps from D-glucose-6-phosphate and in which group of organic compounds is it classified? How many stereoisomers are possible for the constitution of a hexahydroxy-substituted cyclohexane?

D. Compare the IR absorptions for the C=O and C=C bonds with the IR spectrum of crotonic acid, a standard substance for an α/β-unsaturated carboxylic acid.

E. Inspect the multiplets for the methylene group H-6 in the given expansion. Which feature points to the presence of two long-range spin couplings?

F. List all HMBC cross peaks for H-3, H-4 and H-5.

G. Explain the very strong peak in the mass spectrum at m/z = 97.

6. Own Observations

6.2 Aleuritic Acid

DL-*threo*-9,10,16-Trihydroxypalmitic acid

Isolated as a **chemical transformation product of shellac** obtained from excretions of *Kerria lacca* (Coccoidae) during work-up

$C_{16}H_{32}O_5$, MW 304.42

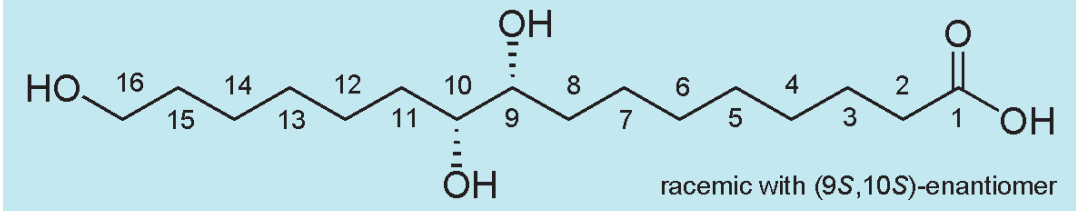
CAS RN 17941-34-3 (for racemic *threo*-form), 533-87-9 (for racemic *erythro*-form), BRN for racemic *threo*-form: 4675723, BRN for racemic *erythro*-form: 4675724

Colourless crystals, mp 96–97 °C (ref. [2] mp 104–105 °C for rac. *threo*-isomer and mp 124–125 °C for rac. *erythro*-isomer), $[\alpha]_D^{21} = 0$ ° (*c* 0.01 g/mL, ethyl acetate)

DL-*threo*-Aleuritic acid is commercially available.

Synonymous names:
For racemic *threo*-form: DL-*threo*-Aleuritic acid, β-Aleuritic acid, rac. *threo*-9,10,16-Trihydroxy-hexadecanoic acid.
For racemic *erythro*-form: DL-*erythro*-Aleuritic acid, α-Aleuritic acid, rac. *erythro* -9,10,16-Trihydroxy-hexadecanoic acid.

Level: easy



1. Background: How heavy! A brittle, circular story

Aleuritic acid belongs to the minority of natural products in this book that are isolated from an animal source: shellac. In India and China this natural resin has been known for about 4000 years, and it reached Europe about 300 years ago. Shellac is the only commercially used resin of animal origin. From its composition, shellac is quite different from other natural resins derived directly from plants; think of colophony. It consists of long-chain carboxylic acid esters, especially of hydroxy fatty acids such as aleuritic acid, and shellolic acid accompanied by some shellac wax.

The reason is that the shellac ingredients are made in biotransformations from plant cell constituents and excreted by tiny lac insects *Kerria lacca* (Kerriidae). The name of the initial scarlet resinous secretion is lac. It is the result of the secretions of hundreds of thousands of such female insects colonizing on branches of suitable host trees. The natural purpose for the insects is to secrete lac as a protective agent for their larvae. Interestingly, the word lac has a relation to the Hindi word *lakh*, that is also a unit in the Indian numbering system, and the Hindi word *crore* is another one. Whereas one lakh is equal to a hundred thousand (10^5), a crore is equal to one hundred lakhs or ten million (10^7). Used to counting with thousand, million and billion, it was impressive for the author to experience during a trip through India that there are other simple possibilities to calculate with big numbers: with lakh and crore. It was the advertising of a lottery that was his first contact with lakh. In this context, one should keep in mind that it was in this region of the world where the decimal system was invented a long time ago. So, probably, lakh is older than million. The relation of lakh to the lac insects is just with the meaning of very, very many of them. However, this is not the end of the story. In a lot of other languages trails of the word *lac* can be found. The reason is that alcoholic shellac solutions can be used for varnishing furniture or instruments such as violins and guitars (French polishing method). Shellac yields a coating of excellent hardness and durability and provides an excellent barrier against the penetration of water vapour into wood. Therefore, you can find *lac* adapted in English (lacquer), in Spanish (laca), in French (laque), in German (Lack) and in Italian (lacca), to name a few only.

To obtain 1 kg of shellac, the secretions of about 300 000 lac insects are necessary, i.e. 3 lakh of insects. Shellac is obtained in several steps. First, the resin-coated branches of host trees are cut off. This raw material is called *sticklac*. The secretions form hard layers on the branches and twigs. They are broken into small pieces, crushed and the wood is removed from the resin by mechanical selection. The crude resin is then ground and washed to remove further impurities such as insects parts and the scarlet colour (Lac Dye). The product obtained is about 95% pure in shellac ingredients and is called *seedlac*, after its pellet-like shape. However, to obtain *shellac* requires a final purification step. This can be done classically by heating it to about 140 °C and pouring it through a kind of filter fabric or by solvent extraction. A principal shellac constituent that may also be removed or not according to the

He rose to his feet and passed his hand over the top of the desk with the touch of a connoisseur.

"No," he said at last. "It ain't the same as Rifkin's. Rifkin's desk was a fine piece of Costa Rica mahogany without a flaw. I used to be in the furniture business once, you know, Mawruss, and so I can tell."

Abe flashed a triumphant grin on Morris, who frowned in reply.

"But ain't this here desk that-now-what-yer-call-it mahogany, too, Mr. Feigenbaum?" Morris asked.

"Well, it's Costa Rica mahogany, all right," Feigenbaum said, "but it's got a flaw into it."

"A flaw?" Morris and Abe exclaimed with one voice.

"Sure," Mr. Feigenbaum continued. "It looks to me like somebody laid a cigar on to it and burned a hole there. Then some cabinetmaker fixed it up yet with colored putty and shellac. Nobody would notice nothing except an expert like me, though."

Montague Glass (1877–1934)
Potash & Perlmutter

intended application is a natural wax (about 5%). Traditional home-made "Indian shellacs" do all contain this wax. They are avalaible in several colour grades (this may remind you of colophony) such as Ivory, Honey, Lemon and the like. The shellac that we used was dewaxed and belongs to the "dewaxed shellac flakes" quality (see the photograph in the margin). Shellac as a natural mixture of hydrophobic compounds has a lot of distinguishing properties, some of them being especially particular in their combination. Hence the main uses of shellac can be understood based upon its physicochemical description.

Fig. 6.2-1 Shellac powder

Shellac can be dissolved in lower alcohols but is resistant to hydrocarbon-based solvents, because it is too polar. It has excellent film-forming properties when its solutions are painted. It forms smooth and high-gloss films with excellent adhesion to many supports. Such a film can furthermore be polished. Shellac coatings are UV resistant. Of course, these properties make shellac a good varnish constituent.

Shellac is both thermoplastic and hard when at ambient temperature. Its melting point is about 90 °C. This makes shellac a constituent of sealing wax. Shellac has a low thermal conductivity and a low coefficient of expansion. At the same time, it has excellent dielectric properties, high dielectric strength, a low dielectric constant and a good tracking resistance. Correspondingly, shellac was used as a basis for making phonographic records for several decades. Another use of its thermoplastic properties was that in former times, shellac, as a natural plastic, was used as a moulding compound to make decorative medals. Nowadays, shellac is considered obsolete as a moulding compound, because the products are too brittle.

Shellac is physiologically harmless and not toxic. It has no odour or taste. It is insoluble in water but undergoes swelling when in contact with water, which means it is not waterproof. It is soluble in aqueous alkaline media and is biodegradable. Therefore, it is used in the pharmaceutical industry to coat pills and tablets, which sometimes have to release their ingredients at definite places such as the small intestine. In the food industry, some fruits such as apples lose their natural wax covering during cleaning operations prior to sale. In such cases, recoating with shellac is possible to equip such fruits or vegetables with a pleasant appearance. Also, chocolate sweets can be covered with a glossy shellac coating. The E-number 904 is the code for shellac allowed as a food additive in the European Union.

So much praise for the material – is there a drawback, too? Yes, in the author's eyes it is clearly the brittleness of shellac. In contrast to vinyl records, shellac records should be treated like eggs, so as not to break them. This property leads us to the story of gramophone records – their rise and decline as a part of the decades of analogue sound recording.

It began in 1877 with Thomas Alva Edison's invention of the phonograph, in which a vibrating pen recorded sound on a tinfoil cylinder. The machine was made for office dictation records. Some technical drawbacks of cylinder recording were overcome by lateral-cut disc records, invented by the German Emil Berliner in 1888. Their initial

use was for speaking toys and the sound quality was still rather poor. Always, one of the main problems was the material for recording: it should at the same time be mouldable during its production to receive the sound information and resistant enough to allow multiple playback. Before the time of synthetic polymers, that was a real technological challenge. One-way wax rollers (drawback: not multipliable) and hard rubber discs were early inventions for sound recording. In addition to his technical understanding, Berliner proved to be a businessman with vision because he was the first to recognize that it was the entertainment and not the office sector that promised the best profit for any sound recording technology. Driven by this insight, he spent all his efforts on finding a material able to fulfil the two demands mentioned above. In 1896 he pressed the first records from a press mass based on 25% shellac mixed with some fillers such as barium sulfate, powdered slate, soot and cotton flocks made by the Duranoid Co., Newark, NJ, USA.

Fig. 6.2-2 A typical album sleeve for a shellac-based gramophone record

The mass production of such records began in Hanover, Germany, in 1898. It was one of the major inventions that gave rise to about 60 years of making such shellac records. Both the sound quality and durability were clearly improved. Soon, a *gramophone* became a machine affordable by almost everybody. Its name was coined by E. Berliner in 1888. As a result of this breakthrough, the world's shellac production rose to some 50 000 tons per year (it is now still about 5000 tons). The photograph at the beginning of this article shows a typical 10 inch shellac gramophone record. It can be played with a rotational speed of 78 rpm and has a length of only 3 minutes. Compare it with a standard long-play (LP) record played at 33 rpm and a length of up to 45 minutes! Such vinyl records made of PVC [poly(vinylchloride)] were invented in 1948 and had two main improvements compared with shellac discs: the synthetic polymer allowed miniaturization of the sound engraving by a micro groove and, last but not least, "vinyls" are far less brittle than gramophone discs. We have taken a gramophone record (GR) and a vinyl LP, and here are some technical details for comparison: diameter: GR 10 inch = 25 cm, LP 12 inch = 30 cm; thickness: GR 2.2 mm, LP 1.6 mm; mass: GR 193.8 g, LP 125.4 g. In summary: if you have a shellac record in your hand, your first impression will be: how heavy! If one looks at album sleeves of that time, one finds everything charming: the writing, the illustration and the ideas born to convince the audience of the sound quality. Just look at the dog listening to "His Master's Voice" ("Die Stimme seines Herrn" in German)! Digital sound recording of today may record tones of unknown perfection – the classical advertising of our ancestors is incomparable.

Aleuritic acid has the systematic name of 9,10,16-trihydroxypalmitic acid and belongs to the saturated hydroxy fatty acids. It was given its name by A. Tschirch, a Swiss chemist focused on the investigation of plant secretions and resins, who recognized that sticklac found on plants is an animal natural raw material excreted by scale insects. It was described in 1899 that several trees are suitable as hosts for the *Kerria lacca* insects, with a tree named *Aleurites laccifera*, belonging to the family Euphorbiaceae, among them. Such trees grow in the tropical and sub-tropical regions of Asia and South America. The name of the

genus *Aleurites* has a Greek root and stands for the property "floury", which can be used to describe the feeling that arises on touching the underside of such a leaf. Therefore, Tschirch named the novel hydroxy fatty acid aleuritic acid [1]. The correct constitution was first described in 1922 [2] and it was found that aleuritic acid can be isolated from shellac in about 20% yield, although, as the ^{13}C and ^{1}H NMR spectra of shellac show, aleuritic acid as such is not contained in this material at all, but will be generated during the isolation method described below. This is, to the best of our knowledge, a hitherto unreported finding. A likely assumption is that shellac contains an epoxide precursor of the *threo*-diol which may be opened during work-up with formation of the racemic aleuritic acid (see question D).

It has two chiral centres. Therefore, in principle four stereoisomers are possible: the two *erythro*-forms = (9*R*,10*S*)- or L-*erythro*- and (9*S*,10*R*)- or D-*erythro*-form; and the two *threo*-forms = (9*R*,10*R*)- or D-*threo*- (used in the formula in this section) and (9*S*,10*S*)- or L-*threo*-form. Experimentally, the specific rotation found for our sample was 0°. This could result from several phenomena, either the occurrence as the racemic *erythro*-form or as the racemic *threo*-form or as a mixture of both of them.

Which of these possibilities is true was established by classical chemical means in 1968 [3]. In a sequence of reactions, the diol unit of aleuritic acid was transformed into a *trans*-olefinic unit, a typical feature for *threo*-diols. Additionally, starting from the isomeric synthetic *cis*-16-hydroxyhexadec-9-enoic acid by two different hydroxylation methods both the racemic *erythro*- and *threo*-9,10,16-trihydroxyhexadecanoic acid were prepared. The *threo*-isomer was shown to be identical with natural aleuritic acid. The authors reported that for the natural sample a very small optical rotation was observed. We could not find it. Our sample was optically inactive. Usually, this is distinct evidence for a racemate.

However, one peculiarity should be kept in mind when looking at the formula of aleuritic acid, namely that the site of chirality is embedded in the middle of the molecule surrounded on both sides by a rather large non-chiral zone of two longer alkyl chains substituted at the end. This situation works like a dilution of the chiroptical effect resulting in principle from the configuration "inside" and may, in the case of a single enantiomer, cause a small value for the specific rotation α.

As a chiral pool component, aleuritic acid can be used for the synthesis of macrocyclic compounds such as ambrettolide that are of commercial interest as fragrances [5,6].

2. Literature

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The best solvent for shellac, however, in the preparation of the stiffening and proofing mixture for hats, is probably wood spirit or methylated spirit. A solution of shellac in wood spirit is indeed used for the spirit-proofing of silk hats, and to some extent of felt hats, and on the whole the best work, I believe, is done with it. Moreover, borax is not a cheap agent, and being non-volatile it is all left behind in the proofed material, whereas wood spirit or methylated spirit is a volatile liquid, i.e. a liquid easily driven off in vapour, and after application to the felt it may be almost all recovered again for re-use.

Watson Smith
The Chemistry of Hat Manufacturing
Lectures Delivered Before the Hat Manufacturers' Association

Fig. 6.2-3 A gramophone in an antique shop

The apparatus was virtually a Leyden jar, the two coatings of which were the two spheres, with a thick and variable insulator between them. The amount of charge in each jar was determined by bringing a proof-plane into contact with its knob and measuring by a torsion balance the charge taken away. He first charged one of his instruments, and then dividing the charge with the other, found that when air intervened in both cases the charge was equally divided.
But when shellac, sulphur, or spermaceti was interposed between the two spheres of one jar, while air occupied this interval in the other, then he found that the instrument occupied by the 'solid dielectric' takes more than half the original charge. A portion of the charge was absorbed by the dielectric itself. The electricity took time to penetrate the dielectric. Immediately after the discharge of the apparatus, no trace of electricity was found upon its knob.

John Tyndall (1820−1893)
Faraday as a Discoverer

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3. Isolation

3.1 Principle

Shellac powder, wax-free, (lat. Kerria lacca, CAS 9000-59-3) was used for this preparation. Shellac contains the aleuritic acid precursor in the form of an ester among other constituents. Therefore, the acid is formed by saponification in anionic form. Acidification leads to precipitation of this acid together with other, coloured impurifications and resins. Colorations were removed by absorption on active carbon. A separation from the resins contained in the crude product is possible during a first crystallization step due to density differences between aleuritic acid and the resins which are deposited at the bottom of the flask. The crude acid contains other acid compounds which could be removed by a second recrystallization step in which only aleuritic acid is dissolved from the crude product. Pure aleuritic acid thus obtained forms colourless crystals with a sharp melting point.

3.2 Method

This is based on the method described in [7]. Shellac powder (20 g) is mixed with methanol (80 mL) with stirring and warming in a 250 mL round-bottomed flask. Initially, a gelatinous, pale yellow mass is obtained which transforms into a slightly cloudy yellow solution. A solution of 2.0 M aqueous KOH (100 mL) is added and the solution heated under reflux for 20 min. The colour of the solution turns to brown immediately, and later to dark brown and becomes clearer. Then,

75 mL of methanol are removed on a rotary evaporator. The remaining cloudy solution is neutralized with acetic acid (12 mL, c = 0.2 mol/L) and diluted with water (160 mL). Further slow addition of such dilute acetic acid with stirring causes turbidity and precipitation of aleuritic acid in colourless streaks, and eventually flakes are formed. During this operation, the pH does not change and remains at 5.5. Addition of acetic acid is stopped when no further precipitation is observed. Active carbon as grains (4 g) is added, the suspension heated to reflux for 2 min, filtered and the filtrate allowed to stand at room temperature for one day in a beaker. A thin layer of a reddish-brown oil deposits at the bottom. In the liquid above, the crude, colourless, fluffy aleuritic acid precipitates. This portion of the beaker contents is first decanted from the oil at the bottom and then filtered. The oil and the filtrate are discarded. To the colourless filter cake ethyl acetate (5 ml), active carbon (grains, 0.8 g) and Na_2SO_4 (2.0 g) are added. The mixture is heated to boiling and filtered while hot. Addition of three drops of *n*-hexane causes crystallization of a colourless solid on standing, which is filtered off. The melting range of this material is very broad (85–110 °C). Under the microscope, two kinds of crystalline material can be observed.

Voici toute la chose: Le jais blanc vient de Norvège, le jais noir vient d'Angleterre, la verroterie noire vient d'Allemagne. Le jais est plus léger, plus précieux, plus cher. On peut faire en France des imitations comme en Allemagne. Il faut une petite enclume de deux pouces carrés et une lampe à esprit de vin pour amollir la cire. La cire autrefois se faisait avec de la résine et du noir de fumée et coûtait quatre francs la livre. J'ai imaginé de la faire avec de la gomme laque et de la térébenthine. Elle ne coûte plus que trente sous, et elle est bien meilleure. Les boucles se font avec un verre violet qu'on colle au moyen de cette cire sur une petite membrure en fer noir. Le verre doit être violet pour les bijoux de fer et noir pour les bijoux d'or. L'Espagne en achète beaucoup. C'est le pays du jais...

Victor Hugo
Les Miserables, Vol. V, Book IX,
Chapt. 3

3.3 Purification

The colourless solid obtained above is suspended in hexane (50 mL) and ethyl acetate (65 mL) is added stepwise under reflux in a 250 mL round-bottomed flask. Some material remains undissolved. The hot suspension is filtered and the filter cake discarded. From the filtrate, colourless crystals of aleuritic acid deposit on standing.

Yield: 1.1 g, colourless crystals, mp 96–97 °C (Boetius micro hot-stage), $[\alpha]_D^{21} = 0\,°$ (*c* 0.01 g/mL, ethyl acetate).

4. Spectra and Comments

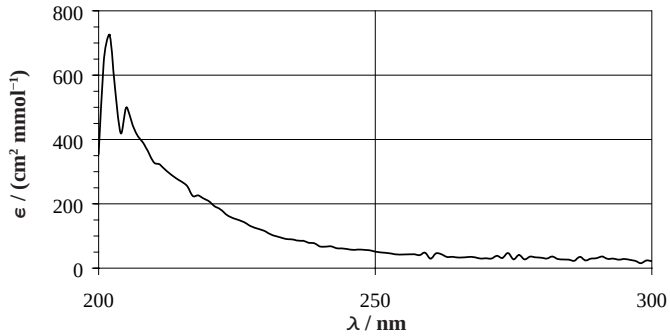
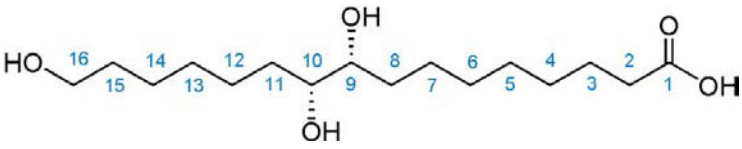


Fig. 6.2-4 UV spectrum in ethanol

As expected from the chemical formula, there is no significant UV absorption, since the carboxyl group as the single chromophore is isolated and the three OH groups are far away. For the same reason, we expect no CD spectrum, although the compound is chiral.



Scheme 6.2-1

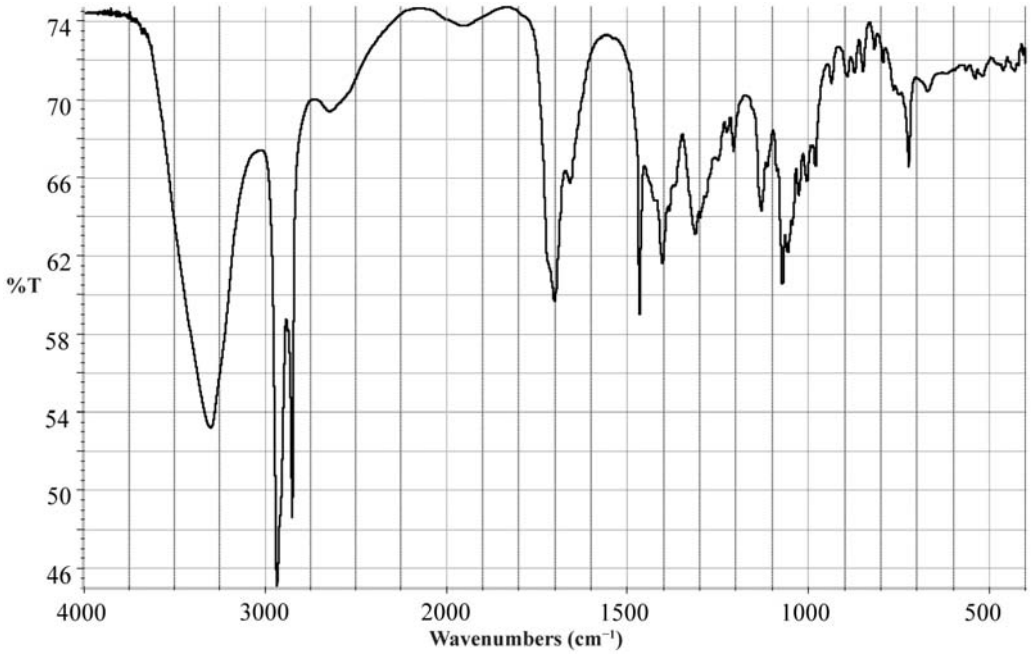


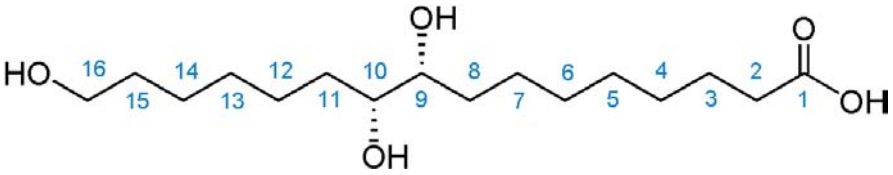
Fig. 6.2-5 IR spectrum in KBr

The IR spectrum displays the typical features of an aliphatic carboxylic acid, a broad OH valence vibration at 3250 cm^{-1} and the C=O band at 1700 cm^{-1}. The sp^3-CH valence bands are split and range down to 2900 cm^{-1}.

"The time has come," the Walrus said,
"To talk of many things: Of shoes − and ships − and sealing-wax −
Of cabbages − and kings −
And why the sea is boiling hot −
And whether pigs have wings."

Lewis Carroll (1832−1898)
Through The Looking Glass Chap. 4

Fig. 6.2-6 A shellac record



Scheme 6.2-2

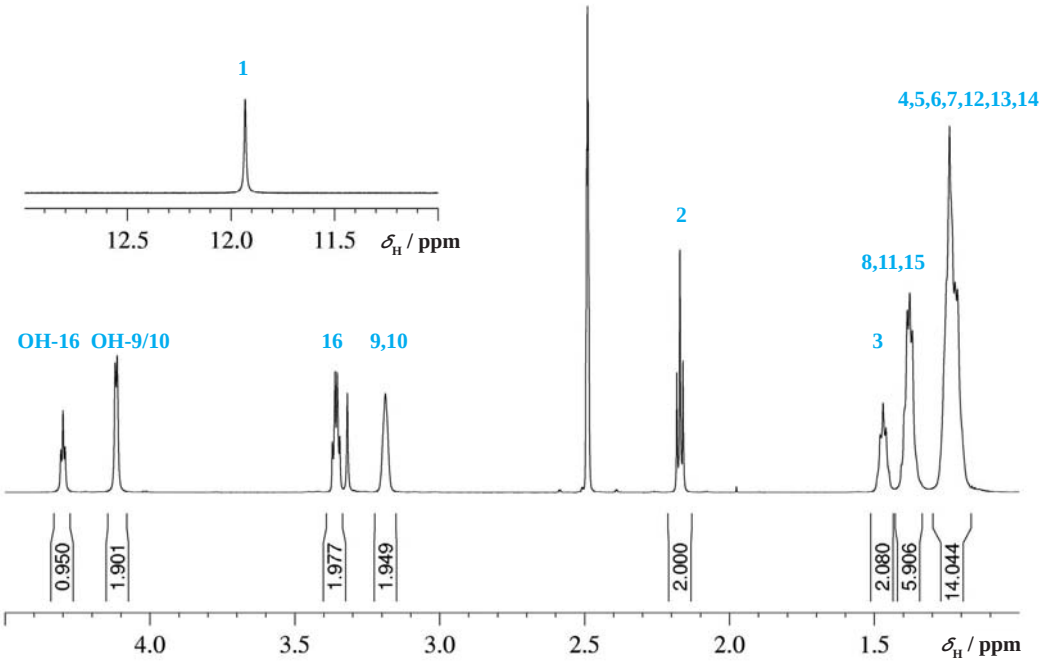


Fig. 6.2-7 ^{1}H NMR spectrum at 700 MHz in DMSO-d_6

Looking at the integrals of the displayed ^{1}H NMR spectrum, we find indeed the 32 hydrogen atoms required for this molecule, 22 hydrogen atoms are in the far aliphatic region and the signal at 2.17 ppm can be directly identified as belonging to H-2. In the CHO region, we find two signals, each of two hydrogen atoms, and these signals therefore will belong to H-9/10 (3.19 ppm) and to H-16 (3.36 ppm). Since the compound was measured in DMSO-d_6 we find three additional OH absorptions, at 4.1 ppm the OH groups at C-9/10, at 4.3 ppm the OH group at C-16 and at 12 ppm the carboxylic acid proton. A residual H_2O signal is present at 3.3 ppm.

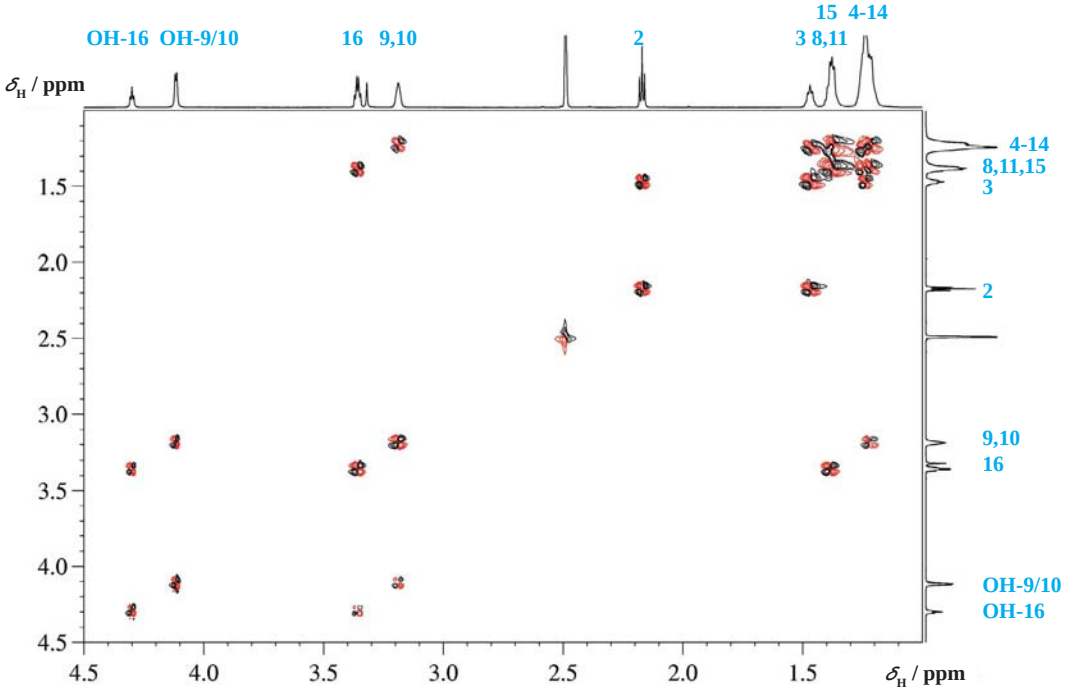


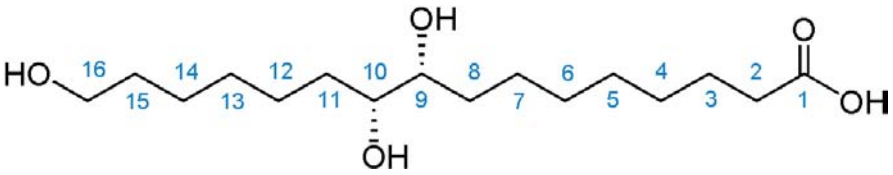
Fig. 6.2-8 Double quantum filtered COSY spectrum

We interpret the COSY spectrum starting from a secure entry point which is the triplet at 2.17 ppm belonging to H-2. This leads us to a signal of two protons at 1.47 ppm, which are therefore assigned to H-3. These protons are connected with the aliphatic bulk at 1.22 ppm, from which a cross peak leads us to both H-9/10 which apparently are not separated. Similarly, the aliphatic signal of six protons at 1.38 ppm is connected to the aliphatic bulk and has cross peaks to H-16 at 3.36 ppm. Since the spectrum was recorded in DMSO, we find cross peaks from H-16 to the OH group at C-16 and also from H-9/10 to the OH groups at C-9/10.

Fig. 6.2-9 Advertisement for gramophone records

He paid no attention to my explanations, and, playing with a stick of sealing-wax, repeated several times that the situation was 'very grave, very grave.' There were rumors that a very important station was in jeopardy, and its chief, Mr. Kurtz, was ill. Hoped it was not true. Mr. Kurtz was . . . I felt weary and irritable. Hang Kurtz, I thought. I interrupted him by saying I had heard of Mr. Kurtz on the coast. 'Ah! So they talk of him down there,' he murmured to himself. Then he began again, assuring me Mr. Kurtz was the best agent he had, an exceptional man, of the greatest importance to the Company; therefore I could understand his anxiety. He was, he said, 'very, very uneasy.' Certainly he fidgeted on his chair a good deal, exclaimed, 'Ah, Mr. Kurtz!' broke the stick of sealing-wax and seemed dumbfounded by the accident.

Joseph Conrad (1857−1924)
Heart of Darkness



Scheme 6.2-3

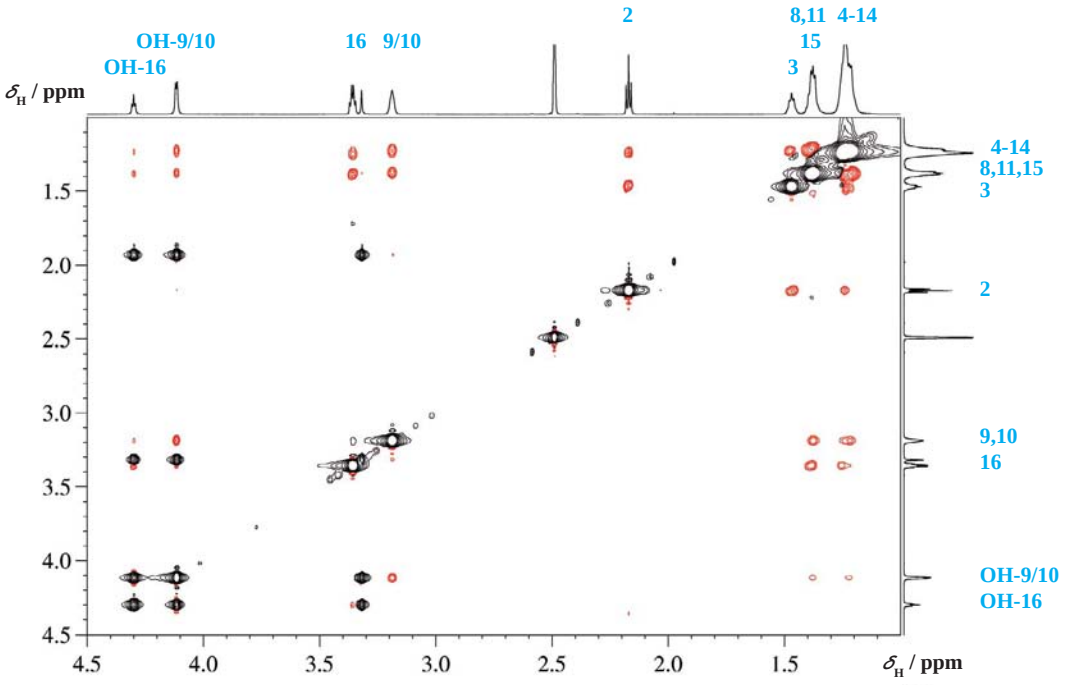


Fig. 6.2-10 NOESY spectrum

The NOESY spectrum displays NOE effects along the aliphatic chains of the molecules, starting from H-2 to the aliphatic bulk and from these groups to H-9/10 and also to H-16 and even to the OH groups. Furthermore, the spectrum shows − in black − exchange peaks between the OH groups and the signal of the residual water. The spectrum demonstrates the importance of phase-sensitive NOESY spectra to be able to distinguish between NOE cross peaks and exchange signals.

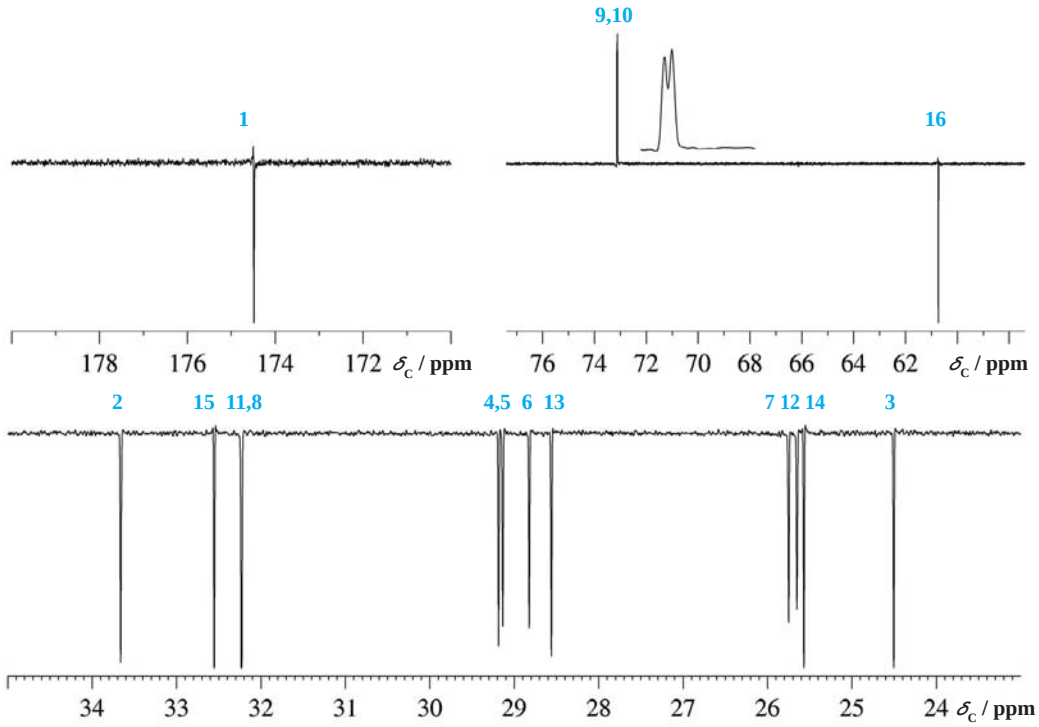


Fig. 6.2-11 ^{13}C NMR spectrum at 175 MHz in DMSO-d_6

The ^{13}C NMR spectrum shows only 15 of the required 16 signals and the signals of C-9 and C-10 are hardly separated (only 2.5 Hz on a 700 MHz spectrometer). There are three spectral regions, the carboxyl group at 174.5 ppm as expected, and three OH-bearing carbon atoms at 73.1 ppm for C-9/10 and at 60.7 ppm for C-16, as revealed by the phase of the APT spectrum. All the other signals of the aliphatic bulk appear between 24 and 34 ppm. These are all methylene groups and an individual assignment will therefore be rather difficult.

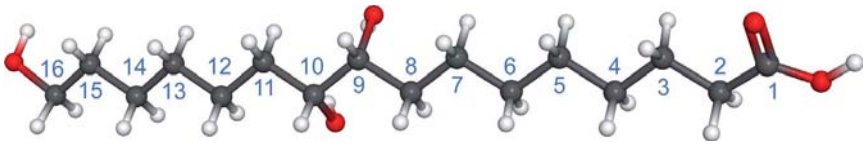


Fig. 6.2-12 Molecular model of aleuritic acid

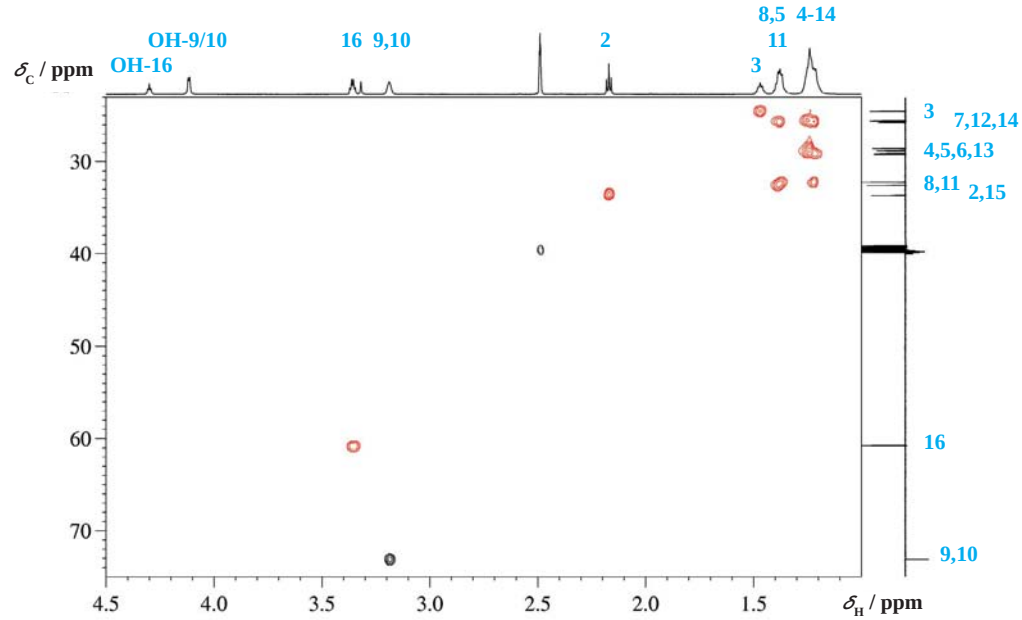


Fig. 6.2-13 Expansion of CH edited HSQC spectrum

The standard HSQC spectrum does not help much for the detailed assignment problem.
Whereas the assignment of C-9/10 and C-16 can be immediately corroborated, the assignment within the aliphatic bulk remains very difficult. In the expansion we can see a firm assignment for C-2 und C-3 due to their correlation peak with their respective protons; however, the other carbon atoms are resonating so closely together that an individual assignment will be insecure. Since their corresponding protons also overlap, the CH correlation would not help, even if the HSQC spectrum would be recorded at much higher resolution in the indirect dimension.

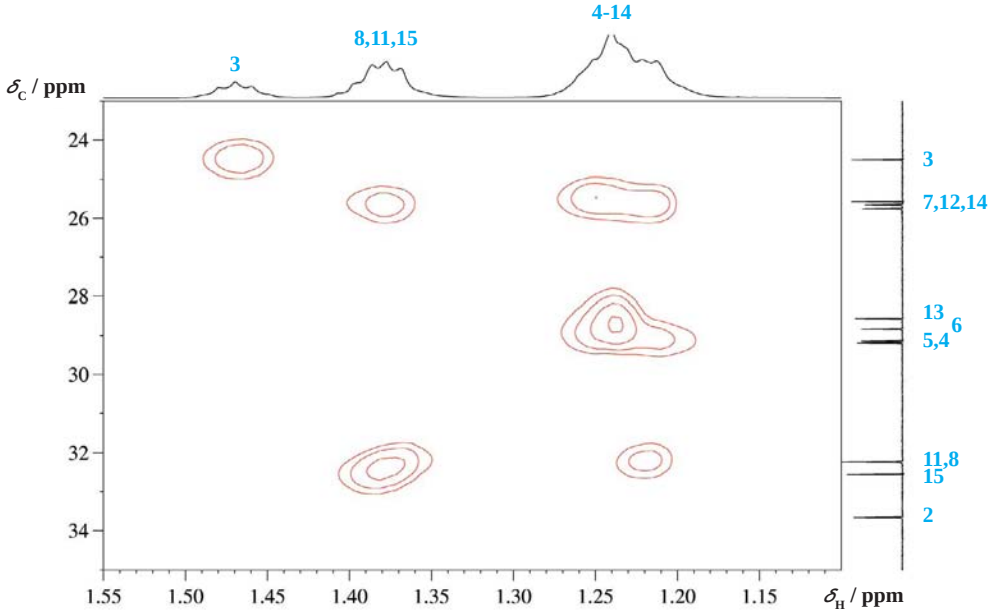


Fig. 6.2-14 Expansion of the HSQC spectrum in the aliphatic region

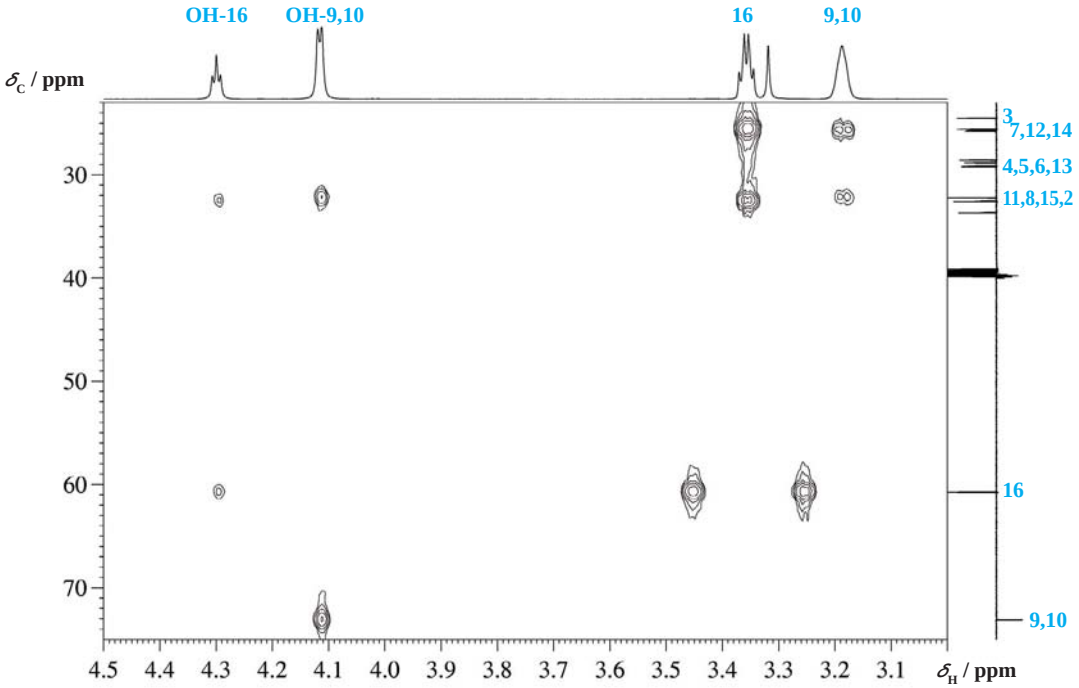


Fig. 6.2-15 Expansion of HMBC spectrum between 4.5 and 3 ppm

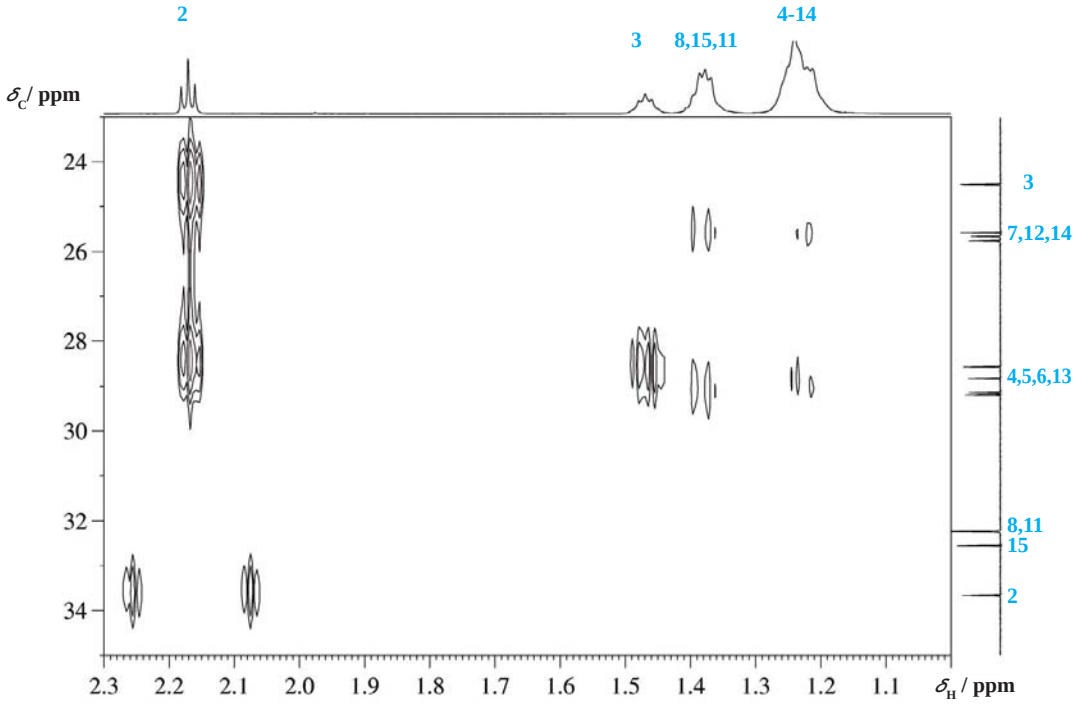
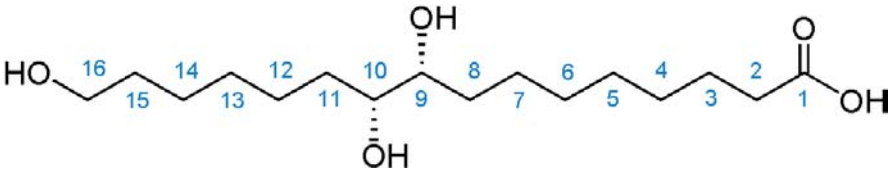


Fig. 6.2-16 Expansion of the HMBC spectrum between 2 and 1 ppm



Scheme 6.2-4

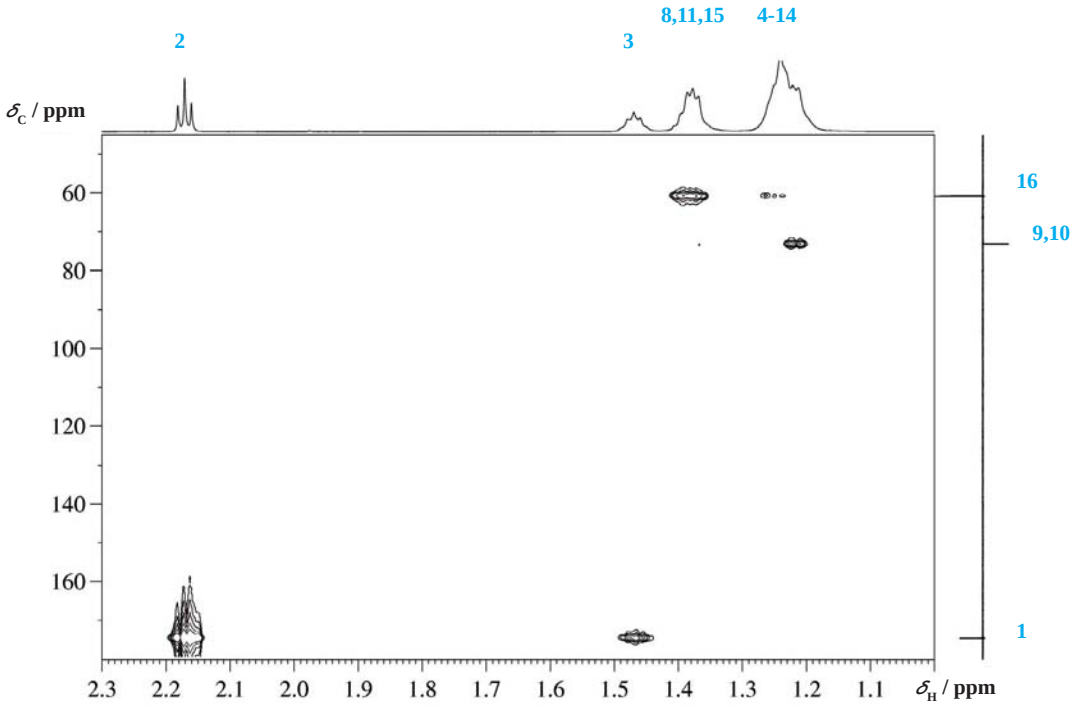


Fig. 6.2-17 Expansion of the HMBC spectrum for the hydroxylated carbon atoms

Similarly to the HSQC spectrum, the HMBC-spectrum also gives here no real new insights due to the limited resolution in the indirect dimension, the overlapping proton signals and the closely resonating carbon atoms. Nevertheless, the first expansion corroborates the assignment of the OH protons and it is reassuring that HMBC cross peaks from OH protons can be observed in DMSO. OH-16 “sees” C-16 and C-15, OH 9/10 see C-9/10 and C-11/8, H-16 is connected to C-14 and C-15, H-9/10 to C-7 and C-12 and C-11/8. In the second expansion, we see that H-2 and H-3 are connected to the carboxyl group C-1. Furthermore, this spectrum predicts that in the proton signal at 1.22 ppm must be protons that are connected to C-16 over two or three bonds and this is also true for the proton signal at 1.22 ppm with respect to carbon atoms C-9/10.

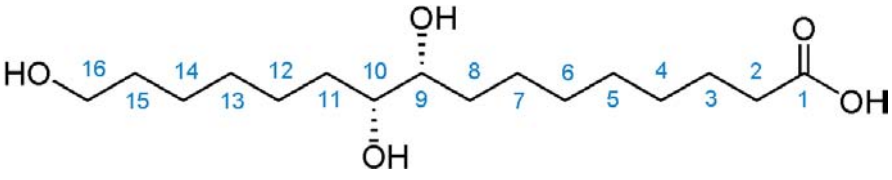
To fill the chemical shift table for this compound with numbers for individual assignments, we must rely on programs for carbon and proton chemical shift prediction, since there is, as discussed above, no real spectroscopic proof. For ^{13}C these programs predict three signals at about 25 ppm, namely C-7,12 and 14, four signals at about 29 ppm, namely C-4, 5, 6 and 13, and three signals at 32 ppm, namely C-8, 11 and 15. The corresponding ^{1}H chemical shifts are given in the table and the carbon chemical shifts obtained with these programs are marked.

I must tell you of my wedding present. When the chaplain and the sisters had left me alone with my husband − oh, Lucy, it is the first time I have written the words 'my husband' − left me alone with my husband, I took the book from under his pillow, and wrapped it up in white paper, and tied it with a little bit of pale blue ribbon which was round my neck, and sealed it over the knot with sealing wax, and for my seal I used my wedding ring. Then I kissed it and showed it to my husband, and told him that I would keep it so, and then it would be an outward and visible sign for us all our lives that we trusted each other, that I would never open it unless it were for his own dear sake or for the sake of some stern duty. Then he took my hand in his, and oh, Lucy, it was the first time he took his wife's hand, and said that it was the dearest thing in all the wide world, and that he would go through all the past again to win it, if need be.

Bram Stoker (1847−1912)
Dracula, Chap. 9

^{13}C Signals δ / ppm	Type of Carbon	Assignment (*,#,§,& = relative assignment insecure)	^{1}H Signals δ / ppm, J / Hz
174.5	C_q	C-1	
73.13	CH	C-9*	3.19
73.12	CH	C-10*	3.19
60.7	CH_2	C-16	3.36
33.7	CH_2	C-2	2.17
32.6	CH_2	C-15#	1.38
32.2	CH_2	C-11#	1.38
32.2	CH_2	C-8#	1.38
29.2	CH_2	C-13§	1.22
29.1	CH_2	C-6§	1.22
28.8	CH_2	C-5§	1.22
28.6	CH_2	C-4§	1.22
25.8	CH_2	C-7&	1.22
25.7	CH_2	C-12&	1.22
25.6	CH_2	C-14&	1.22
24.5	CH_2	C-3&	1.47
		COOH	11.9
		OH-16	4.30
		OH-9/10	4.11

Table 6.2-1 NMR data for aleuritic acid



Scheme 6.2-5

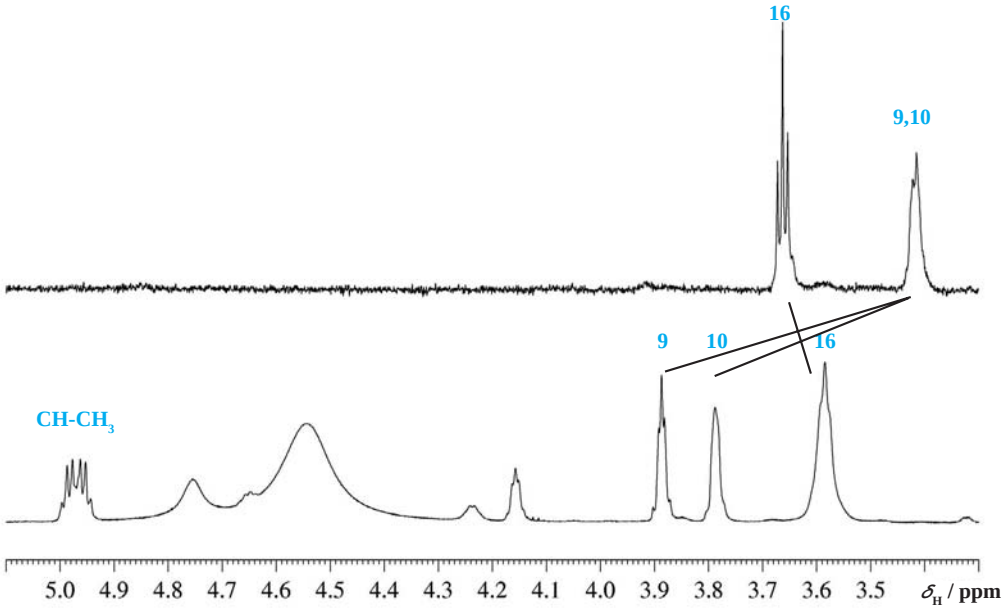
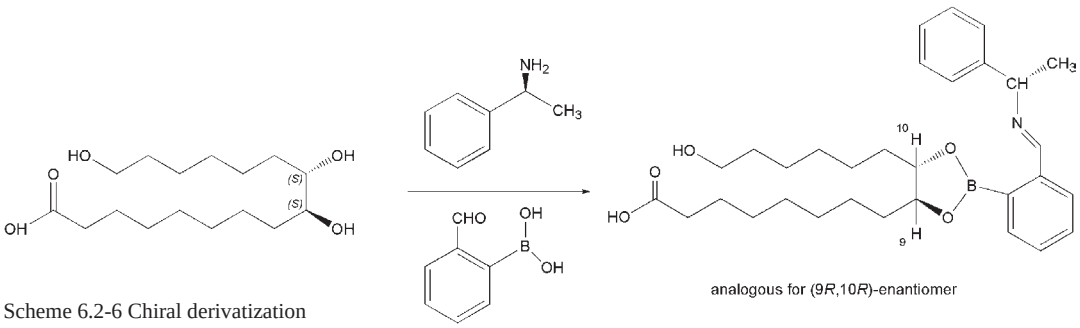


Fig. 6.2-18 Stereochemical assignment, upper trace: expansion of ^{1}H NMR of normal aleuritic acid, lower trace: after addition of chiral agent.

Chiral HPLC on an analytical Chiradex column in methanol yielded a separation of the two enantiomers present and showed a ratio of 45:54, indicating a racemate considering the error margin of the method. Recently a new protocol for the NMR analysis of enantiomeric diols was published [4]. It is based on a derivatization of a mixture of enantiomeric 1,2-diols with 2-formylboronic acid and (*S*)-α-methylbenzylamine to yield a mixture of diastereoisomeric imino boronate esters. The signals of selected protons of these derivatives showed definite chemical shift differences and allowed the enantiopurity of the diols investigated to be determined.



Scheme 6.2-6 Chiral derivatization

We applied this method to our isolated aleuritic acid and observed a 1:1 splitting of the signal for both H-9 and H-10, whereas the signals for H-16 and H-2 remained unaffected. Most important, we observed in addition a splitting of the benzylamine methine proton into two quadruplets and a splitting of the benzylamine methyl group into two doublets. This confirms the presence of a racemate.

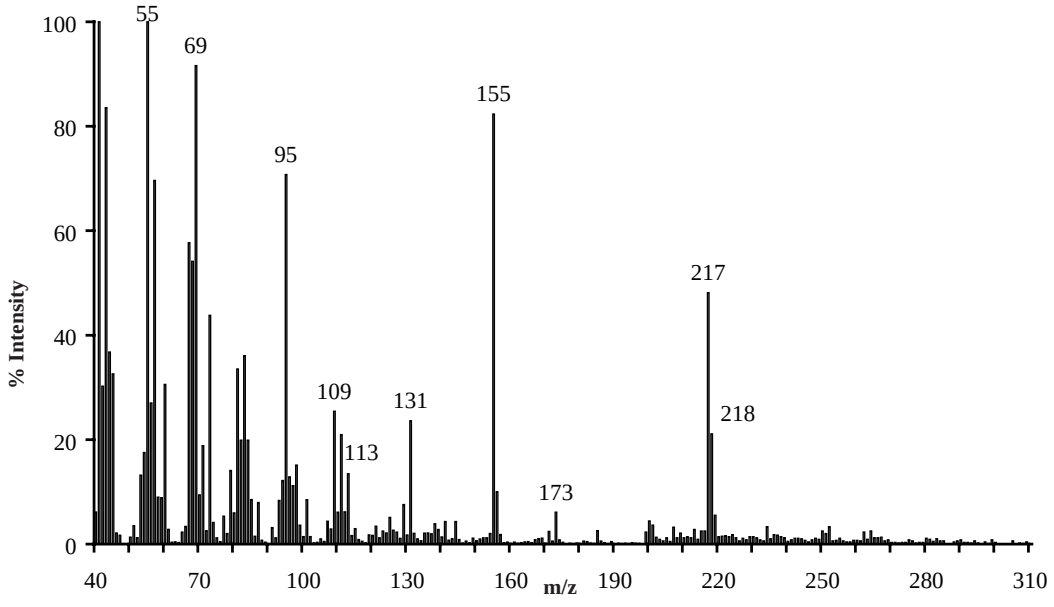
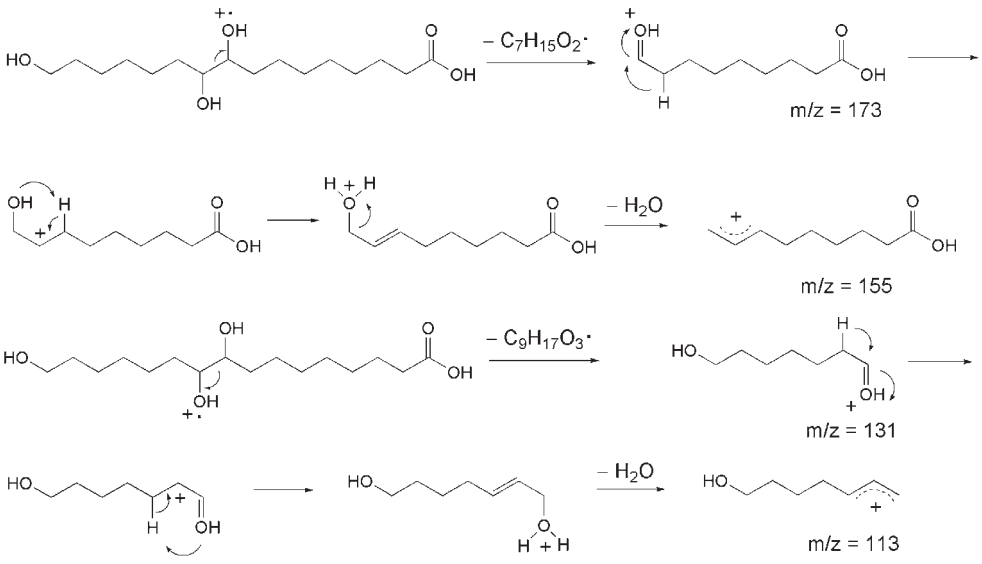


Fig. 6.2-19 Mass spectrum (EI)

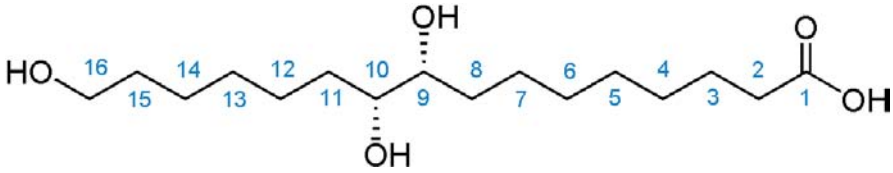
The EI mass spectrum first reveals that there is no molecular ion peak. Apparently the compound directly fragments in the ion source. The large signal at m/z = 155 can be rationalized by ionization at the OH group of C-9, a typical α-cleavage which produces the small peak at m/z = 173, and this ion loses water to form the signal at m/z = 155. Similarly, if we assume ionization at the OH group of C-10, α-cleavage will form an ion with m/z = 131, which also loses water to form the ion with m/z = 113.



Scheme 6.2-7 Fragmentation of aleuritic acid

В комнате, в которой лежал Федор Павлович, никакого особенного беспорядка не заметили, но за ширмами, у кровати его, подняли с полу большой, из толстой бумаги, канцелярских размеров конверт с надписью: “Гостинчик в три тысячи рублей ангелу моему Грушеньке, если захочет придти”, а внизу было приписано вероятно уже потом, самим Федором Павловичем: “и цыпленочку”. На конверте были три большие печати красного сургуча, но конверт был уже разорван и пуст: деньги были унесены. Нашли на полу и тоненькую розовую ленточку, которою был обвязан конверт. В показаниях Петра Ильича одно обстоятельство между прочими произвело чрезвычайное впечатление на прокурора и следователя, а именно: догадка о том, что Дмитрий Федорович непременно к рассвету застрелится, что он сам порешил это, сам говорил об этом Петру Ильичу, пистолет зарядил при нем, записочку написал, в карман положил и проч. и проч.

Fyodor Dostoevsky (1821−1881)
Братья Карамазовы, Book IX, Chap. 2



Scheme 6.2-8

Fig. 6.2-20 The beauty of an old gramophone

5. Questions

A. Define the term “fatty acid”.

B. Give the name of three common unsaturated fatty acids. What is the configuration at their double bonds? Which are the so-called two “essential” fatty acids? Explain the term.

C. The alkali metal salts of long chain fatty acids, such as sodium palmitate, are known to acts as soaps and have been obtained since ancient times by the saponification of fats and oils. As with all surfactants their main structural feature consists in being a hybrid of a large nonpolar part and a hydrophilic part. The amphiphilicity resulting reduces the surface tension of an aqueous surfactant solution and brings about a better wetting ability than pure water, easier spreading and allows emulsification of hydrophobic particles in water; overall it allows one to wash effectively. Make a comment on the influence of the three hydroxyl groups in aleuritic acid. Do they improve their surfactant properties or not? Explain your answer.

D. Make a suggestion on the biological precursor for the *threo*-diol part of aleuritic acid and another one on the fact that this natural product is isolated racemic.

E. Comment in the light of your answer to question D why NMR was unable to show if aleuritic acid has the relative configuration *threo* or *erythro*, even though it was possible to show by formation of diastereomeric derivatives that a racemate is present.

F. In the expansion of the CH edited HSQC spectrum, it can be seen that the carbon signal group at 25 ppm has correlations to both proton groups at 1.38 and 1.22 ppm. Similarly, this is true for the carbon signal group at 33 ppm. How can this be understood?

G. In the CH edited HSQC spectrum, the signal of DMSO reveals a black correlation. Explain.

H. The black exchange peaks in the NOESY spectrum are much stronger than the red NOE cross peaks. Why?

I. There are only 15 carbon signals from 16 carbon atoms. C-10 and C-9 are hardly separated. Which carbon atoms are also likely to coincide and why?

J. Give a mechanism for the formation of the peak at m/z = 217 in the mass spectrum.

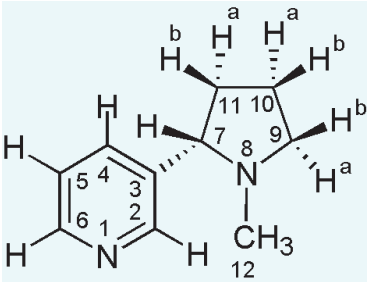
6. Own Observations

Answers to Questions and Translations

Chapter 1 Alkaloids

1.1 Nicotine

Answers



Scheme 1.1-6

A. The N-atom of the pyrrolidine ring is most basic. This ring is not aromatic, hence it is a tertiary cycloaliphatic amine, the basicity of which is much higher than that of the pyridine ring in which electron density from the N-atom is drawn off by the aromatic ring.

B. "Nor" in nomenclature means a compound with a lacking methyl group when compared with the reference compound. Therefore, nornicotine means a lack of the methyl group on the pyrrolidine ring. That is true for 3-[(*S*)-2-pyrrolidinyl]pyridine.

C. Nicotine has a strong anthelmintic activity (i.e. it kills parasitic worms) and insecticidal activity, both of which help the plant to survive in the biological fight with pests.

D. Tobacco juice has been used as an exterminator for plants in the house because it acts against pests such as aphids as described under answer C. Although definitely of high activity, a serious drawback of tobacco juice is its strong stench. Therefore, using tobacco juice has dropped out of fashion with the development of synthetic insecticides.

E. Because getting a single N-atom out of the soil in the form of an NH_4^+ ion is always a challenge for plants, this will be especially difficult for alkaloid producers. Therefore, a definite application of an N-fertilizer will be necessary. Otherwise, tobacco plants will soon leach out the soil.

F. Same absorption, however, with vibrational fine structure due to rigidity of pyridine

G. Both carbon atoms C-2 and C-6 show first a large doublet due to their own bound proton of 176 and 178 Hz. Each part is split into a long-range multiplet caused by three other protons. The fine analysis requires spin simulation. This spin simulation gave for C-6: $^1J(C_6,H_6)$ = 178.1 Hz, $^2J(C_6,H_5)$ = 3.4 Hz, $^3J(C_6,H_4)$ = 7.9 Hz, $^3J(C_6,H_2)$ = 10.9 Hz. For C-2, there is a $^4J(C_2,H_5)$ of approximately 0.5 Hz and an additional spin coupling $^3J(C_2,H_7)$ of 5.2 Hz.

H. ^2J(H,N) spin coupling constants are dependent on the dihedral angle with respect to the free electron pair at the nitrogen in a Karplus-type fashion.

I. Apparently one proton is located in the vicinity of the shielding region of the aromatic ring; also there is an influence of the free electron pair at the nitrogen atom.

J. The 3D structure minimized with the application of Chem Draw MM2 shows that the methyl group is located above the pyridine ring and therefore in its shielding region.

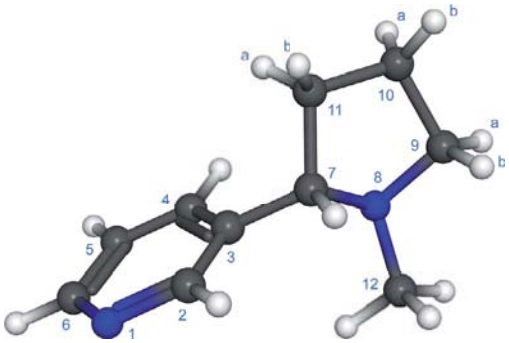
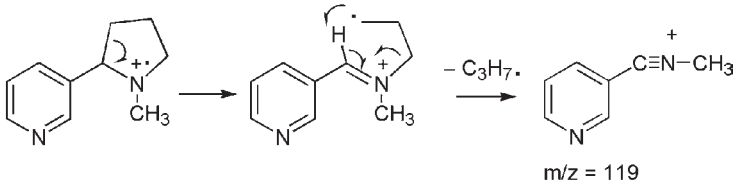


Fig. 1.1-32

K. One would need a chiral acid available in both enantiomers, which forms salts with nicotine. An analysis of the NMR spectra of both diastereomeric salts should lead to the desired configurational assignment.

L.



Scheme 1.1-7

Translations

Fig. 1.1-33

To create a chemical analysis of the herb nicotianum and to investigate more accurately the chemical nature of the principles which emanate from this herb and the powers which they have in the animal body. By this examination it shall be shown whether both effects of this herb, its sharpness and narcotic action, depend on one and the same principle or from diverse ones.

Scientific Competition, University of Heidelberg, 1827
Translated from the Latin by Franziska Berger

However, on the morning of the appointment, the young man had established himself in the small salon down-stairs. There, on a table, surrounded at some distance by a large and luxurious divan, every species of tobacco known, − from the yellow tobacco of Petersburg to the black of Sinai, and so on along the scale from Maryland and Porto-Rico, to Latakia − was exposed in pots of crackled earthenware of which the Dutch are so fond; beside them, in boxes of fragrant wood, were ranged, according to their size and quality, pueros, regalias, havanas, and manillas; and, in an open cabinet, a collection of German pipes, of chibouques, with their amber mouth-pieces ornamented with coral, and of narghiles, with their long tubes of morocco, awaiting the caprice or the sympathy of the smokers. Albert had himself presided at the arrangement, or, rather, the symmetrical derangement, which, after coffee, the guests at a breakfast of modern days love to contemplate through the vapour that escapes from their mouths, and ascends in long and fanciful wreaths to the ceiling.

Alexandre Dumas (1802–1870), *The Count of Monte-Cristo*, Chap. 39
Translator unknown

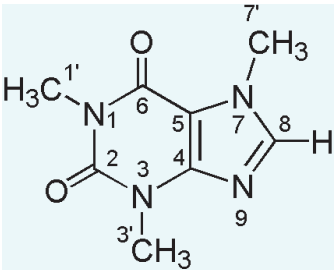
Vasili Andreevich meanwhile had unfastened his coat, and holding its skirts up for shelter, struck one sulfur match after another on the steel box. But his hands trembled, and one match after another either did not kindle or was blown out by the wind just as he was lifting it to the cigarette. At last a match did burn up, and its flame lit up for a moment the fur of his coat, his hand with the gold ring on the bent forefinger, and the snow-sprinkled oat-straw that stuck out from under the drugget. The cigarette lighted, he eagerly took a whiff or two, inhaled the smoke, let it out through his moustache, and would have inhaled again, but the wind tore off the burning tobacco and whirled it away as it had done the straw.

But even these few puffs had cheered him.

Lev Nikolayevich Tolstoy (1828–1910), *Master and Men*
Translator unknown

1.2 Caffeine

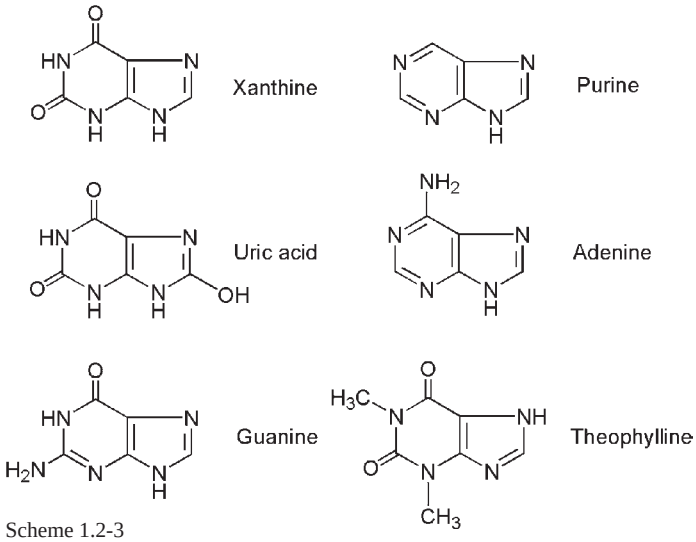
Answers



Scheme 1.2-2

A. The six-membered ring contains two amide groups. At the molecular level, the oxygen atoms of the carbonyl groups can act as acceptors for H-atoms from water in intermolecular hydrogen bonds, which leads to dissolution of the caffeine, macroscopically.

B.



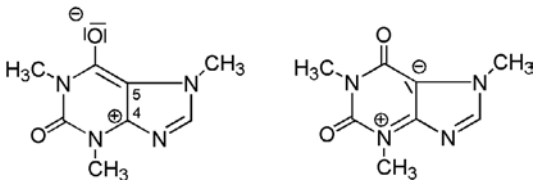
Scheme 1.2-3

C. A high uptake of proteins, as typical e.g. for sea birds which eat fish exclusively, causes the need for an efficient output of the excess nitrogen. Therefore, denitrification, but with a minimum loss of water, is the reason for this biochemical achievement of evolution.

D. Imidazole has a maximum at about 216 nm, but with significant lower ε-value, urea has no significant absorption above 200 nm.

E. The urea-like carbonyl group of C-2 should appear at lower wavenumbers.

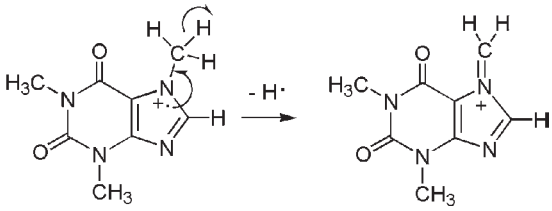
F. The positive charge can be placed mainly at the position of C-4, whereas a negative charge can be drawn at C-5



Scheme 1.2-4

G. The proton signals of the methyl group C-1' are connected to C-2 and C-6, the proton signals of the methyl group C-3' are connected also to C-2 and in addition to C-4, the proton signals of the methyl group C-7' are connected to C-5 and C-8. The most deshielded nitrogen is the “aromatic” nitrogen N-9. The subtle differences between N-1, N-3 and N-7 have to be considered after quantum mechanical calculations.

H. Most likely caffeine is ionized at the nitrogen atoms. Loss of methyl groups would require an α-cleavage but this would occur after ionization within the ring system, where the electron density is apparently less than that at the nitrogen atoms. Instead, α-cleavage leads to a loss of a hydrogen atom, which can be clearly seen in the mass spectrum.



Scheme 12-5

I. Most likely this is caused by a matrix effect, which causes a different kinetic release of the drug.

Translations

Fig. 1.2-31

It has been discovered that some shepherds of camels or, as other authors say, of goats, as it is a common Eastern custom with the monks of a monastery, in the region of Ayaman, which is situated in “felix Arabia” (= Yemen), once on the seventh day used not to watch their flock, but kept dancing through the whole night. The abbot of this monastery, intrigued by curiosity, thought this would be caused by the pasture ground. Considering this he went with a friend to the place where the camels or goats were pastured that night, where the dancing happened. He found small trees with fruits or better berries, from which they ate. He wanted to experience the properties of these fruits and put them into boiling water. After drinking he had to stay awake the whole night.

Faustus Naironius Banesius, 1671, *De Saluberrima Potione Cahve*
Translated from the Latin by Franziska Berger

Oh, how sweet is the taste of coffee
lovelier that a thousand kisses
milder than muscatel.
Coffee, coffee I must have
and if somebody will do me any good
Just pour me some coffee in.

Johann Sebastian Bach (1685−1750) *The Coffee Cantata*, BMV 211
Translated from the German by Dominika Berger

Goethe told me his uppermost consent and gave me a box with coffee beans, a precious gift from a Greek. “You may use this for your investigations” said Goethe. He was right, because soon enough I discovered therein the coffein which became famous by its high nitrogen content.

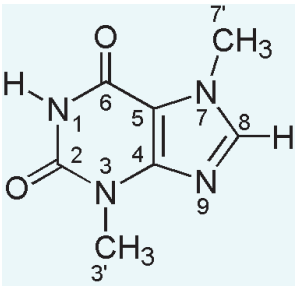
F. F. Runge (1794−1864), *Hauswirthschaftliche Briefe*
Translated from the German by Stefan Berger

Far from being harmful, coffee and tea, even when consumed in abundance, but not in excess (which excess is not harmful!), are salutary. The Germans at least owe them a considerable advantage, which in itself deserves to be acknowledged. In Germany, these beverages have tempered the vice of drunkenness more successfully than the lessons of the moralists and theologians and more than even the progress in science and education.

Honoré Gabriel Riqueti, Comte de Mirabeau (1749–1791)
De la Monarchie Prussienne, sous Frédéric le Grand
Translated from the French by Franziska Berger

1.3 Theobromine

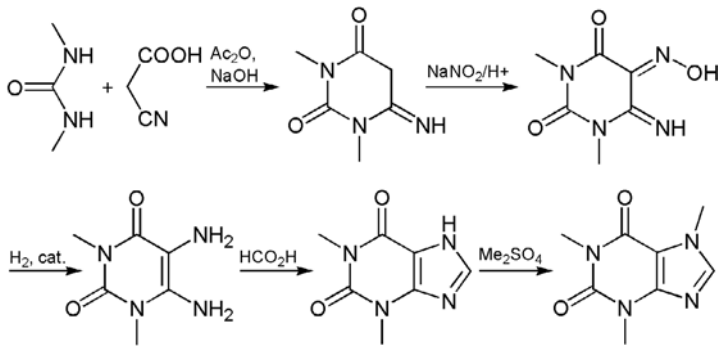
Answers



Scheme 1.3-5

A. In such a coffee shrub the purine synthesis would stop at the level of non-methylated xanthine (3,9-dihydro-1*H*-purine-2,6-dione). This coffee would "naturally" be free of caffeine, i.e. the current procedures for decaffeination (destraction) would no longer be necessary. In the poppy example, an opium would be accessible with a high natural codeine level. Morphine would not or only to a limited extent be contained. Codeine is pharmaceutically useful. It could then just be extracted and would not require a synthesis from morphine as a precursor, as currently. Growing opium poppy is now connected with its illegal abuse for making heroin from morphine, which gives a much higher profit than codeine.

B.



Scheme 1.3-6

C. Both are caused by three-bond couplings with a similar geometry (bond distances and bond angles) However, the spin coupling pathway to C-4 involves a double bond and therefore leads to a larger spin coupling constant.

D. The typical α-deshielding effect for a methyl group in ^{13}C NMR is about 7−9 ppm. Here we also find a deshielding, but only of 4 ppm.

E. Loss of HCN from the ion with m/z = 109.

Translations

Fig. 1.3-21

"You are not feeling very well, you have not slept, and chocolate will revive you."

And somewhat later: "And now, my dear child, I must tell you that chocolate no longer holds the place in my esteem that it used to do, fashion has influenced me, as it always does: those who used to praise chocolate now speak ill of it. They curse it and believe it to be the cause of all the ailments one suffers from, it is a source of vapours and palpitations; it flatters you for a time indeed but presently lights up a fever that continues, and at length carries you to the grave."

Marie de Rabutin-Chantal, Marquise de Sevigné (1626−1696), *Correspondance avec sa Fille*
Translated from the French by Dominika Berger

He sent me a big box of chocolates for my name-day, that was very nice and attentive of him. I forgot to tell you about it when I wrote, and I only remember now that you ask me about it. Chocolate, as I am sure you are aware, disappears straight away in this lodging house, almost as soon as you know somebody has given you chocolate it is gone.

Franz Kafka (1883−1924), *The Trial*, Chap. VI
Translated from the German by David Wyllie

The Duchessa, in desperation, risked going into the drawing-room where she found the Marchese Crescenzi, who was in waiting that day. On her return to Parma he had thanked her effusively for the place of Cavaliere d'Onore, to which, but for her, he would never have had any claim. Protestations of unbounded devotion had not been lacking on his part. The Duchessa appealed to him in these words: "Rassi is going to have Fabrizio, who is in the citadel, poisoned. Take in your pocket some chocolate and a bottle of water which I shall give you. Go up to the citadel, and save my life by saying to General Fabio Conti that you will break off your marriage with his daughter if he does not allow you to give the water and the chocolate to Fabrizio with your own hands."

Stendhal (1783−1842), *La Chartreuse de Parme*, XXV
Translated from the French by C. K. Scott-Moncrieff

Chicory is mixed in good coffee. Chicory, or some similarly cheap substance, is skilfully moulded into the form of the coffee berry, and is mixed with the bulk very liberally.... Cocoa is extensively adulterated with fine brown earth, wrought up with mutton fat, so as to amalgamate with portions of the real article.... The leaves of tea are mingled with sloe levies and other abominations. Used leaves are also re-dried, and re-coloured on hot copper plates, and sold as tea. Pepper is adulterated with dust from husks etc.; port wine is altogether manufactured (from spirits, dyes, etc.), it being notorious that more port wine is drunk in this country than is made in Portugal. Nasty things of all sorts are mixed with the weed tobacco in all its manufactured forms.

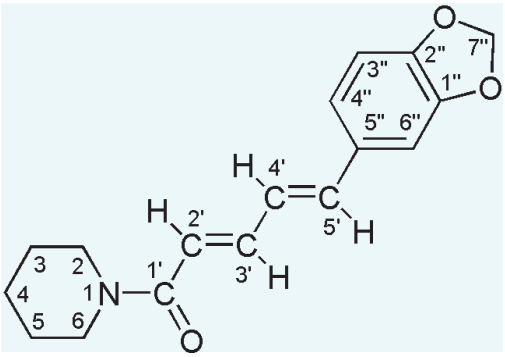
Friedrich Engels (1820−1895), *The Condition of the Working Class in England*
Translated from the German by Florence Kelly Wischnewetzky

We rise in the morning – by "we" I mean I and my master – we rise not too early, but not too late either; that is to say, on the stroke of eleven. I should mention, by the way, that I have a soft roomy couch made up for me not far from the Baron's bed, and we snore in such harmony that if I wake suddenly we can't tell which of us was snoring. The Baron pulls the bell and his manservant immediately appears, bringing a beaker of steaming chocolate for the Baron and a china disk of the best sweet coffee with cream for me, which I empty with as good an appetite as the Baron shows in draining his beaker. After breakfast we play together for half an hour; not only is the practical exercise good for our health, it also cheers our spirits.

E.T.A. Hoffmann (1776−1822), *The Life and Opinions of the Tomcat Murr*, IV
Translated from the German by Anthea Bell

1.4 Piperine

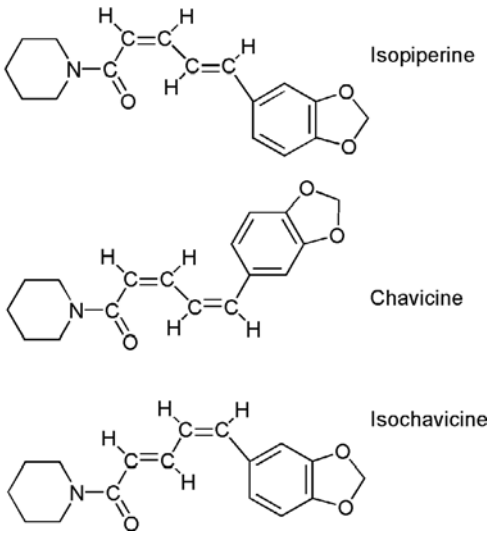
Answers



Scheme 1.4-4

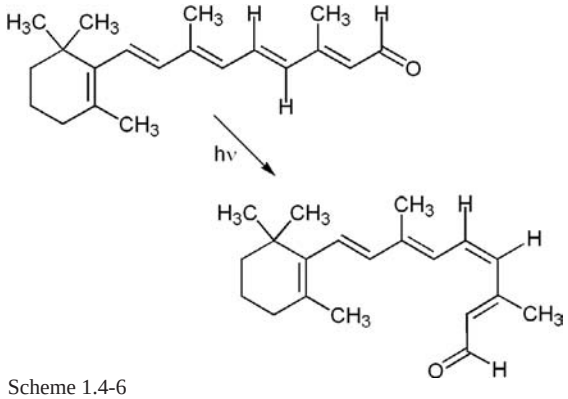
A. The amide bond is hydrolysed to yield piperidine and (2*E*,4*E*)-5-(1,3-benzodioxol-5-yl)-2,4-pentadienoic acid (trivial names: piperic acid, piperinic acid, piperonic acid).

B.



Scheme 1.4-5

C. Likely a 1,2,4-trisubstituted aromatic ring with two oxygen functions in the *ortho*-position and a chain-like substituent at the 4-position.



Scheme 1.4-6

E. A mesomeric resonance structure with a negative charge on the oxygen puts the positive charge on C-3', and this mesomeric effect is stronger than the inductive one.

F. Hindered rotation about the amide bond. Changing the temperature or the strength of the magnetic field (chemical shift separation in Hz) would alter the situation.

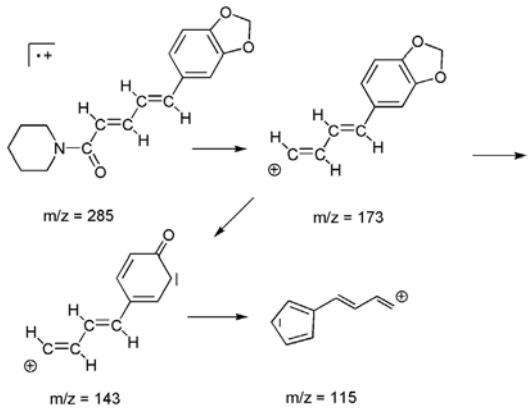
G. DEPT or similar. Anomeric carbon atoms are always CH moieties.

H. Breakthrough of the large $^{1}J_{CH}$ spin coupling constant for the signal of C-2'.

I. It can be seen that H-6" and H-4" have cross peaks to the carbon signals at δ= 138.1 ppm, whereas H-2' shows a cross peak to the carbon signal at δ= 125.4 ppm.

J. Change of the last coupling constant to about 10 Hz.

K. m/z = 173: loss of CO from 201 or cleavage of the piperidoyl moiety direct from the mother ion; m/z = 143: loss of formaldehyde from m/z = 173 and sigmatropic H-shift; m/z = 115: subsequent loss of CO from m/z = 143 to form $C_9H_7^+$:



Scheme 1.4-7

Translations

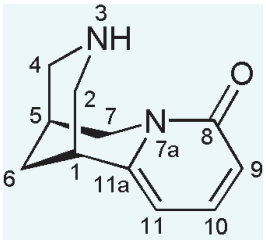
Fig. 1.4-20

Very similar in appearance to our junipers, although, indeed, it has been alleged by some authors that they only grow on the slopes of Caucasus which lie exposed to the sun. The seeds, however, differ from those of the juniper, in being enclosed in small pods similar to those which we see in the kidney-bean. These pods are picked before they open, and when dried in the sun, make what we call "long pepper". But if allowed to ripen, they will open gradually, and when arrived at maturity, discover the white pepper; if left exposed to the heat of the sun, this becomes wrinkled, and changes its colour.

Pliny the Elder, *Naturalis Historia Liber*, XII, 26
Translated from the Latin by John Bostock

1.5 Cytisine

Answers



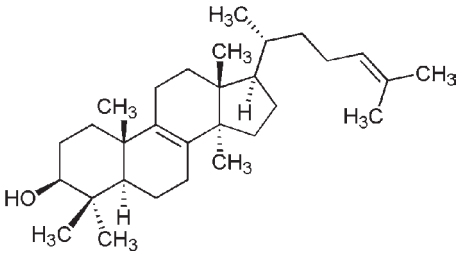
Scheme 1.5-9

A. Structural rigidity and restricted conformational flexibility reduce the ability for internal rotations or more generally movements within a molecule and hence increase the possibility of their definite association

within a crystal lattice. A feature that induces more rigidity is a ring. The macroscopic property in which this molecular pattern is mirrored is the level of the melting point. All mp differences above can be explained by comparing the structures in this respect.

(a) Nicotine {3-[(2*S*)-1-methyl-2pyrrolidinyl]pyridine} has two rings, but they can rotate versus each other, although there are two preferred conformations. In contrast, cytisine is a rigid scaffold that Nature obviously can easily stack in a crystal lattice. An additional advantage is that cytisine has both an H donor and an H acceptor for intermolecular hydrogen bonding, whereas there is an H donor lacking in nicotine. But see note (c).

(b) Lanosterol, $C_{30}H_{50}O$ (mp 140 °C), is the oxidized tetracyclic triterpenoid cyclization product of the open-chain triterpene squalene, $C_{30}H_{50}$, which is a liquid at ambient temperature. This example shows how much four cycles restrict the molecular flexibility. The secondary hydroxy group in lanosterol can also be regarded as helpful for intermolecular associations.



Scheme 1.5-10

(c) However, the simplest example shows the effect of a ring in the same manner. *n*-Hexane, a liquid, solidifies only at –95 °C, whereas cyclohexane crystallizes at 6 °C.

B. Chiral natural compounds are called members of the *chiral pool*. They are appreciated e.g. as starting materials for asymmetric synthesis and for the synthesis of other natural products to which they give a contribution due to their chirality. Working with *chiral pool* compounds means using the chiral store of Nature.

C. There is no exception. All alkaloids contain at least one N-atom.

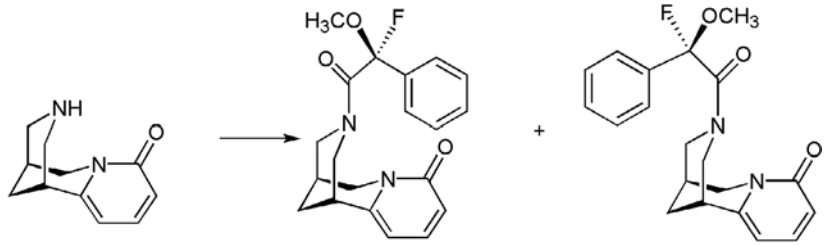
D. As with many other drugs, a frequent problem is that their solubility as neutral compounds is too low. Therefore, formation of a salt is a usual method to increase the solubility of the active principle. One of the acids used for such salt formations is tartaric acid.

E. The valence bond CH vibrations of N-CH_2 groups absorb in this region.

F. (1) The signal of H-11 at 6.0 ppm has a distinct NOESY cross peak to H-1 at 2.8 ppm. The signal of H-9 does not reveal a connection to the aliphatic part in the NOESY spectrum. (2) The signal of H-9 at 6.45 ppm shows a distinct cross peak to the carbonyl C-atom in the HMBC spectrum. (3) The signal of H-11 at 6.0 ppm shows a cross peak to the carbon atom C-11a, the only other quaternary olefinic carbon atom in the molecule. The carbon signals of C-9 and C-11 can be assigned using the HSQC spectrum.

G. This is due to the Karplus equation. H-7a forms a dihedral angle to C-6 across the C-7−C-5 bond close to 90° and therefore will not show an HMBC cross peak to C-6.

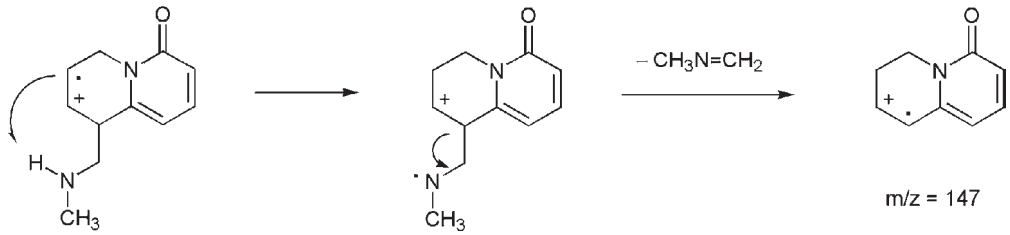
H. The reaction forms an amide with the secondary amine group of cytisine. The *cis/trans* isomerization around the new amide bond is slow on the NMR time scale.



Scheme 1.5-11

I. If the molecule is indeed an alkaloid, it must contain at least two or four nitrogen atoms, since with one nitrogen atom the molecular ion gives an uneven number.

J.



Scheme 1.5-12

Translations

Fig. 1.5-20

"Adieu, adieu, adieu," she said, without the soul communicating one single intelligent inflexion to the word. It was uttered impassively, as the bird sings his note. "She does not recognize me!" cried the colonel, in despair. "Stephanie! it is Philippe, thy Philippe, PHILIPPE!" And the poor soldier went to the acacia; but when he was a few steps from it, the countess looked at him, as if defying him, although a slight expression of fear seemed to flicker in her eye; then, with a single bound she sprang from the acacia to a laburnum, and thence to a Norway fir, where she darted from branch to branch with extraordinary agility.

Honoré de Balzac (1799−1850), *Adieu*

Translated from the French by Katharine Prescott Wormeley

A cold, sharp wind whistled through the leafless branches, and yet the drops fell from my forehead. I recollected that I was stabbed just as I was trampling the ground to fill up the hole; while doing so I had leaned against a laburnum; behind me was an artificial rockery, intended to serve as a resting-place for persons walking in the garden; in falling, my hand, relaxing its hold of the laburnum, felt the coldness of the stone. On my right I saw the tree, behind me the rock. I stood in the same attitude, and threw myself down. I rose, and again began digging and enlarging the hole; still I found nothing, nothing − the chest was no longer there!

Alexandre Dumas (1802−1870), *The Count of Monte Cristo*, Chap. 67
Translator unknown

In a garden
Beneath dark trees,
We wait for the spring night.

No star is yet shining.

From a window
Increasing,
The sound of a violin.

Laburnum glistens,
Lilacs scent the air,
In our hearts the moon rises.

Arno Holz (1863−1929), *Phantasus*
Translated from theGerman by Dominika Berger

And so, when laburnum and lilac just started to blossom, a rosy-cheeked girl stepped over the threshold of his house for the first time. Cousin Christian couldn't comprehend why even the old walls inside the house suddenly began to glow. It was only later that he thought to himself that it must have been the radiance of kindness arising from those young eyes. The great-aunt on her part shook her head over this very young housekeeper, and Cousin Christian would never reveal what it was that old Caroline shook.

Theodor Storm (1817−1888), *At Cousin Christian's*
Translated from the German by Dominika Berger

Do you hear the swans above the shining plain,
Their silvery course is soundless and arcane.
Do you see the boughs, they tremble in a stream
Of Golden Rain running down in silent gleam.
Do you hear the Nightingale, it sadly sings
in the Syringa-Tree, and feel the balmy wings
on which the shadows of visions or of things
are wandering over the blueing meadows.

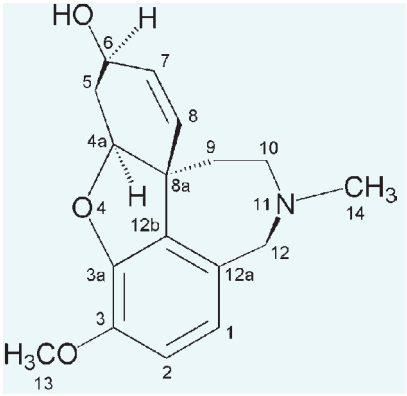
Max Dauthendey (1867−1918), *The Shine of Roses*
Translated from the German by Astrid Lohöfer

"Sure, Joan honey, that's all fine and dandy, but what are we supposed to do then? We've already seen the path. And the cemetery? Do you want to...?" "Sure I do. I've sort of got my feelings, especially on a day like this. And it's always a good thing to keep in mind that you've got to die. And when the lilac is in bloom like this..." "But Joan, the lilac isn't in bloom anymore, at best it's the laburnum, and that's already putting out seed pods. Goodness sakes, if you're so keen on cemeteries, why you can go see the one on Oranienstrasse every day. But I can see, there's no use talking to you. Zeuthen and cemeteries, that's nothing but nonsense! We'd rather stay here and not see a thing. Come on, little girl, give me your arm again."

Theodor Fontane (1819−1898), *Trials and Tribulations*, Chap. 13
Translated from the German by William L. Zwiebel, 1989

1.6 Galanthamine

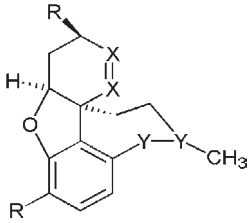
Answers



Scheme 1.6-7

A. (i) Both compounds have one aromatic ring, which in the case of strychnine is substituted with N and in the case of galanthamine with O. (ii) Both compounds contain a heterocyclic ring with a tertiary amine function. (iii) Both compounds contain an olefinic bond within a ring system. (iv) Both compounds contain an ether bridge.

B. Typical variations are known at positions X, Y and R; see ref. [9] in the main text.



C. The signals are broadened most likely to a slow inversion at the nitrogen atom which is already visible at the given NMR frequency.

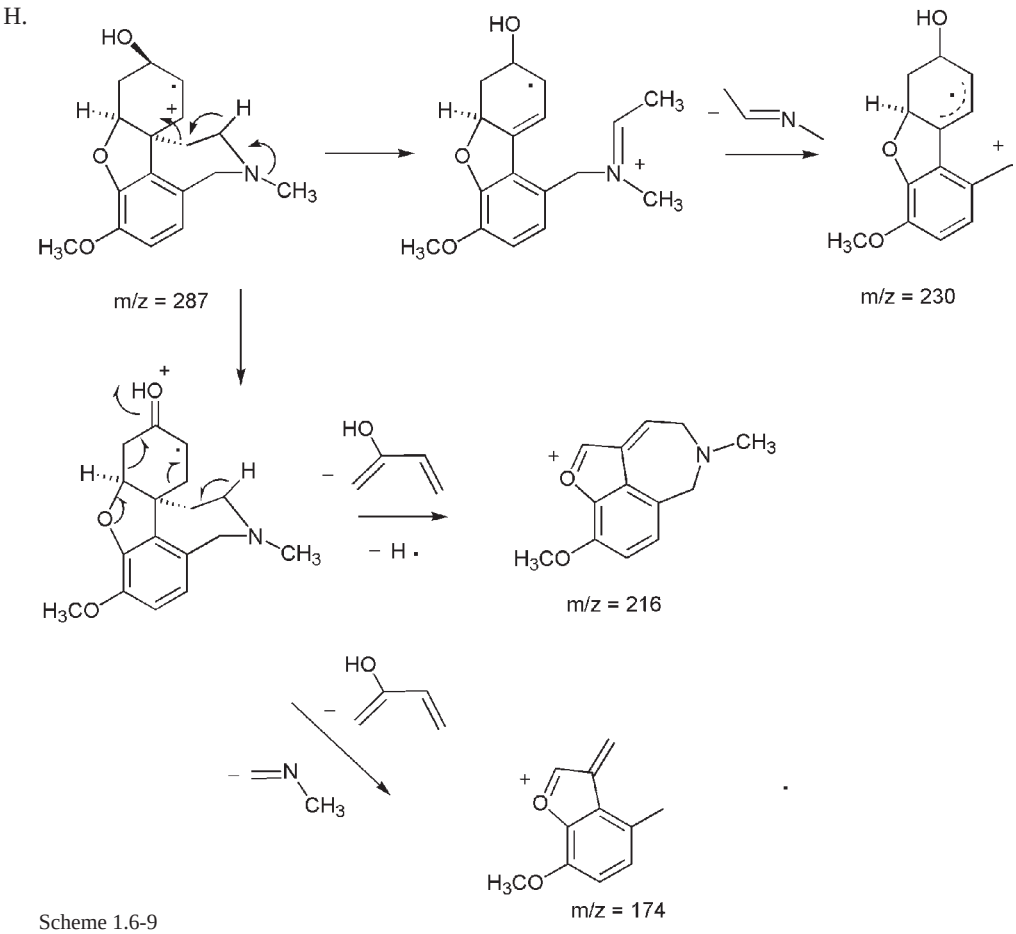
D. AA'XX'

E. Since galanthamine has a secondary hydroxy group at C-6, it should be easy to synthesize an ester with both enantiomers of the Mosher chloride. Comparison of the NMR chemical shift values of these two compounds should lead to a correct stereochemical assignment.

F. The signal of H-2 is coupled to the signal of C-12a and C-3a, H-1 to C-12, C-12b and C-3. H-8 is coupled to C-8a, C-6 and C-4a,; H-7 is coupled to C-5. The methylene protons H-12 are coupled to C-14, C-10, C-1 and C-12a. H-13 is connected to C-3.

G. This is a classical case of applying the Karplus curve for $^{3}J_{C,H}$ spin coupling constants. Inspect a molecular model.

H.



Scheme 1.6-9

Translations

Fig. 1.6-25

Those few who occasionally smiled at the abbot's simplicity were all the more enchanted by Narcissus, the wonder boy, the handsome youth with such elegant Greek, with the faultless aristocratic bearing, the quiet, penetrating thinker's gaze and the narrow, finely chiselled lips. The scholars loved him for his wonderful Greek. Almost everyone loved him for his nobility and refinement; many fell in love with him. Some resented his extreme quietness and self-control, his courtly manners.

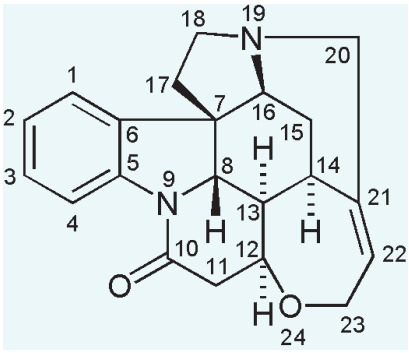
Herman Hesse (1877−1962), *Narzissus and Goldmund*
Translated from the German by Leila Vennewitz

Here Narcissus, tired
of hunting and the heated noon, lay down,
attracted by the peaceful solitudes
and by the glassy spring. There as he stooped
to quench his thirst another thirst increased.
While he is drinking he beholds himself
reflected in the mirrored pool − and loves;
loves an imagined body which contains
no substance, for he deems the mirrored shade
a thing of life to love. He cannot move,
for so he marvels at himself, and lies
with countenance unchanged, as if indeed
a statue carved of Parian marble. Long,
supine upon the bank, his gaze is fixed
on his own eyes, twin stars; his fingers shaped
as Bacchus might desire, his flowing hair
as glorious as Apollo's, and his cheeks
youthful and smooth; his ivory neck, his mouth
dreaming in sweetness,
his complexion fair
and blushing as the rose in snow-drift white.
All that is lovely in himself he loves,
and in his witless way he wants himself: −
he who approves is equally approved;
he seeks, is sought, he burns and he is burnt.

P. Ovidius Naso (43 BC–17 AD), *Metamorphoses III*, 413−426 (Narcissus)
Translated from the Latin by Brookes More

1.7 Strychnine

Answers

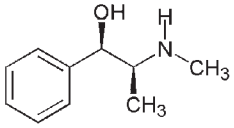


Scheme 1.7-8

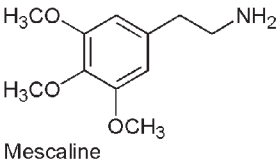
A. Alkaloids are classified according to two principles: (a) their occurrence in a certain biological species or (b) their chemical structure. That means the classification is either a biologically or a chemically based one.

B. Strychnine is a nonpeptidic alkaloid and thus not a suitable subject for digestion by proteases that can degrade peptides. Chemical degradation by reactions such as hydrolysis does not occur. What remains as a kind of reaction leading to degradation finally is microbial oxidation by soil bacteria and fungi. Such reactions do not, however, easily cleave one of the seven rings. Therefore, in a fashion, the polycyclic structure is the life insurance of this molecule.

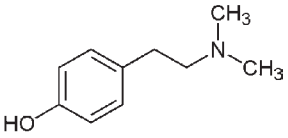
C. Some possible examples are ephedrine, mescaline, hordenine and colchicine.



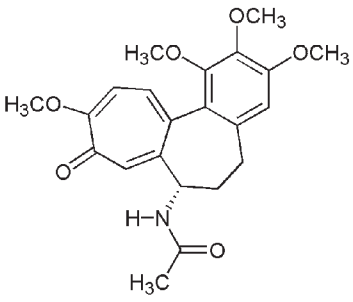
Ephedrine



Mescaline



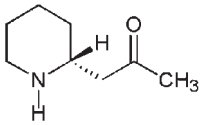
Hordenine



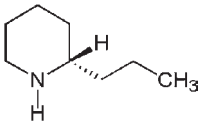
Colchicine

Scheme 1.7-9

D. *Pelletierine* is (–)-1-(2*R*)-2-piperidinyl-2-propanone and occurs in pomegranate bark (*Punica granatum* L.). The closely related alkaloid is coniine, a poisonous compound of poison hemlock (*Conium maculatum* L.). Its most famous victim is Socrates, who was condemned to death and given a potent solution of hemlock containing coniine, which is (+)-(2*S*)-2-propylpiperidine. Both alkaloids belong to the piperidine alkaloids.



Pelletierine



Coniine

Scheme 1.7-10

E. Many alkaloids are neurotoxic. From this property they would be a burden if not a danger for an animal. Therefore, evolution seems to have "abstained" from including this class of highly bioactive compounds in most animals. This fact in turn offers the possibility to use therapeutically many alkaloids that are "recognized" by different receptors in the body.

F. The molecule is very rigid and therefore vibrational losses do not occur.

G. Yes, the strong band at 760 cm^{-1} indicates four C–H moieties in a row.

H. H-15a displays cross peaks to C-14 and C-16 via two bonds and to C-7 and C-13 via three bonds. For H-15b the connectivities to C-16 and C-7 are much weaker. H-17 are strongly coupled to C-18, C-7, C-8 and C-16. H-13 shows connectivities to C-14 and C-8.

I. Yes. Since diastereotopic protons are situated on the same carbon atom, they show the identical correlation signals.

Translations

Fig. 1.7-24

All things are poison and nothing is without poison, only the dose permits something not to be poisonous.

Philippus Theophrastus Aureolus Bombastus von Hohenheim, named Paracelsus (1493−1541)

"Do you speak to me as a magistrate or as a friend?" asked Villefort.

"As a friend, and only as a friend, at this moment. The similarity in the symptoms of tetanus and poisoning by vegetable substances is so great, that were I obliged to affirm by oath what I have now stated, I should hesitate; I therefore repeat to you, I speak not to a magistrate, but to a friend. And to that friend I say. 'During the three-quarters of an hour that the struggle continued, I watched the convulsions and the death of Madame de Saint-Meran, and am thoroughly convinced that not only did her death proceed from poison, but I could also specify the poison.'"

"Can it be possible?"

"The symptoms are marked, do you see? − sleep broken by nervous spasms, excitation of the brain, torpor of the nerve centres. Madame de Saint-Meran succumbed to a powerful dose of brucine or of strychnine, which by some mistake, perhaps, has been given to her." Villefort seized the doctor's hand. "Oh, it is impossible," said he, "I must be dreaming! It is frightful to hear such things from such a man as you! Tell me, I entreat you, my dear doctor, that you may be deceived."

"Doubtless I may, but" −

"But?"

"But I do not think so."

Alexandre Dumas (1802−1870), *The Count of Monte Cristo*, Chap. 73
Translator unknown

The baron says to the servant – a new one, supposedly, a windbag with only one arm that the baron had taken in from the street, but who obeyed his orders like a dog – "Bring me the rhubarb tincture from my bedroom! It sits on the bureau, there is only one bottle, you cannot mistake it." And the young lady drinks her seltzer while the baron takes his medicine. He finishes it and says: "By Jove, that stuff tastes bitter! It could kill cats and dogs!" At this moment, the other servant, the old one who had already served the Baron's uncle, God rest his soul, comes running and cries: "For God's sake, Herr Baron, that was the strychnine for the foxes!" The baron only laughs and asks: "For how many foxes"? – "One hundred, I think!" Without batting an eye, the baron answers: "Then it will hopefully be enough for me as well". …Later, they tried to make him drink

milk and sent for a doctor. The doctor came, but as usual with the doctors one hour too late… Rumor has it though that the baroness of Bussardshof arrived at daybreak, and that it was her who closed his eyes. But they say he didn't send for her.

Johannes Richard zur Megede (1864−1906), *Modeste*
Translated from the German by Franziska and Dominika Berger

Told by the Pharmacist Stannebein in Meissen:

Among the Indians

"A very strange thing has happened to me among the Indians. One day, as our expedition is exploring a wild rocky valley, and as we three explorers, the brothers Humbold and I, had just hurried ahead of our soldiers and unsuspiciously stepped out of the narrow pass, hearken!, there are two troops of Indians cantering towards us in a sweeping gallop, to the left a troop of Sioux and to the right a troop of Iroquois – for I knew the brethren from their headgears – a volley of arrows is speeding towards us and − pronto! – two of the damn things are sticking in my left side.

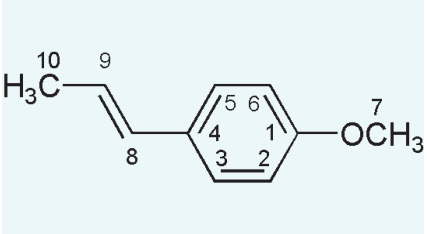
Now it is always good if a man has knowledge and keeps his eyes open. The arrows had come from the left, and on the left were the Sioux, and that they poison their arrows with strychnine, I already knew since elementary school. To pull them out was one thing. But what to do against the effects of the strychnine? Our first-aid-kit had been left behind with the soldiers.

Listen up! Then I luckily remembered that the Iroquois – who were shooting from the right – use curanine for their arrows, which is the antidote of strychnine. As soon as this thought soared through my mind, I already jumped forward to the right. But in the same moment, our soldiers appeared and advanced with a triple hooray, and the Indians retreated.

I, afraid that it would be too late for me, start chasing after one of the Iroquois, shouting continuously – in Iroquois of course – 'Shoot a single arrow at me! Only a single one! Please, won't you be so kind!' And the little blighter must have finally grasped it. For, he suddenly turns, and yuck! an arrow is sitting in my belly. I was saved – but it was high time, and for the next three days I was still sick from terror and anxiety."

Georg Bötticher (1849−1918), *A Story from Meissen*
Translated from the German by Franziska Berger

Chapter 2 Aromatic Compounds



Scheme 2.1-3

2.1 Anethole

Answers

A. Anethole is the more stable one because in this molecule the double bond is conjugated with the aromatic ring which diminishes the enthalpy of formation of the compound.

B. A methoxy group is biosynthesized in two steps: hydroxylation of the aromatic ring followed by methylation.

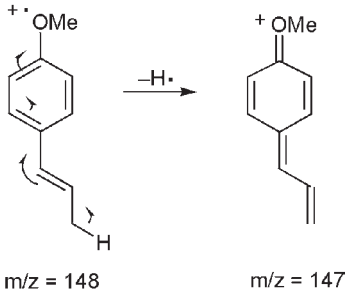
C. Anethole has in contrast to eugenol a direct conjugation of the olefinic π-system with the aromatic π-system, therefore a more extended chromophore and due to Bredt's rule the higher absorption.

D. This is sometimes called a "benzene finger" and the transitions are overtones or interferences between other vibrations.

E. There are many additional transitions of low intensity near the main signals.

F. Both protons H-9 and H-8 show an NOE contact to the methyl group protons, therefore the double bond has the *trans* configuration.

G. α-Cleavage of a hydrogen atom from the allylic methyl group.



Scheme 2.1-4

Translations

Fig. 2.1-16

Woe unto you, scribes and Pharisees, hypocrites! for ye pay tithe of mint and anise and cummin, and have omitted the weightier matters of the law, judgment, mercy, and faith: these ought ye to have done, and not to leave the other undone.

New Testament, Matthew, 23, 23

A woman who suffers from blocked menstruation and hence is in pain, shall take anise and centaury in equal proportions and mullein a little more than any one of these and heat them in open and running water, which has been exposed to sun and air, and then she should take bricks and put them into the fire and take a steam bath with the described water and the described herbs. When she enters that bath, she should put those warm herbs on a footstool, sit on it and further wind the same herbs around her genitals and up to her navel and all around the navel. If they have cooled down in the interim, she should warm them up again and wind them around the same spot and continue as long as she is sitting in this bath, so that, through the vapours of those herbs, her external skin and flesh and her internal matrix is softened and that her veins, which are closed, open. Because the heat of anis generates the vapours; the heat of centaury heals, and the heat of the mullein induces the flux.

Hildegard Bingensis (1098−1178), *Causae et Curae*, Lib. IV
Translated from the Latin by Franziska Berger

Anise, too, one of the comparatively small number of plants that have been commended by Pythagoras, is taken in wine, either raw or boiled, for the stings of scorpions. Both green and dried, it is held in high repute, as an ingredient in all seasonings and sauces, and we find it placed beneath the under-crust of bread. Pat with bitter-almonds into the cloth strainers for filtering wine, it imparts an agreeable flavour to the wine: it has the effect, also, of sweetening the breath, and removing all bad odours from the mouth, it chewed in the morning with smyrnion and a little honey, the mouth being then rinsed with wine.

This plant imparts a youthful look to the features; and if suspended to the pillow, so as to be smelt by a person when asleep, it will prevent all disagreeable dreams. It has the effect of promoting the appetite, also − for this, too, has been made by luxury one of the objects of art, ever since labour has ceased to stimulate it. It is for these various reasons that it has received the name of “anicetum,” given to it by some.

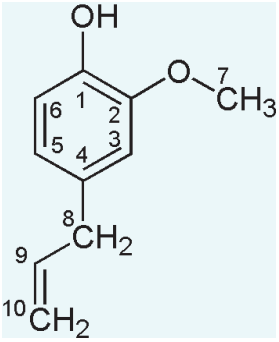
Pliny the Elder (23−79), *Naturalis Historia Liber* XX, 73
Translated from the Latin by John Bostock

Hamilcar’s kitchens being insufficient, the Council had sent them slaves, ware, and beds, and in the middle of the garden, as on a battle-field when they burn the dead, large bright fires might be seen, at which oxen were roasting. Anise-sprinkled loaves alternated with great cheeses heavier than discuses, crateras filled with wine, and cantharuses filled with water, together with baskets of gold filigree-work containing flowers. Every eye was dilated with the joy of being able at last to gorge at pleasure, and songs were beginning here and there.

Gustave Flaubert (1821−1880), *Salammbô*, Chap.1

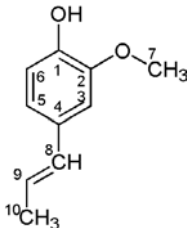
2.2 Eugenol

Answers



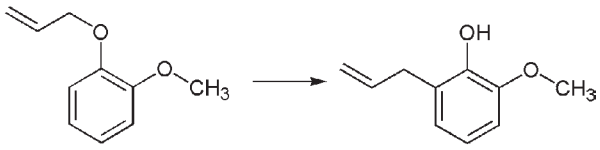
Scheme 2.2-1

A. In isoeugenol the side-chain is in full conjugation with the aromatic ring, which leads to a thermodynamic stabilization effect, i.e. the enthalpy of formation is slightly lower in isoeugenol than in eugenol.



Scheme 2.2-1

B. Guaiacol allyl ether, 1-allyloxy-2-methoxybenzene.



Scheme 2.2-3

C. $\pi \rightarrow \pi^*$ bands, UV of anisol: the UV pattern of anisol is very similar; however, ε is smaller by a factor of about 10.

D. Strong and broad OH valence band at 3300 cm^{-1}, sp^2 CH valence >3000 cm^{-1}, C=C at 1600 cm^{-1}.

E. The NOESY cross signal connects the aromatic multiplet of 2H at 6.68 ppm with H-8 and H-7, thus the single proton at 6.85 ppm must be H-6.

F. The line positions can be constructed as given in the diagram (shown without relative intensities). The large spin coupling of 17 Hz is the *trans*-olefinic coupling, the spin coupling of 10 Hz is the *cis*-olefinic coupling. The final triplets are due to the protons H-8.

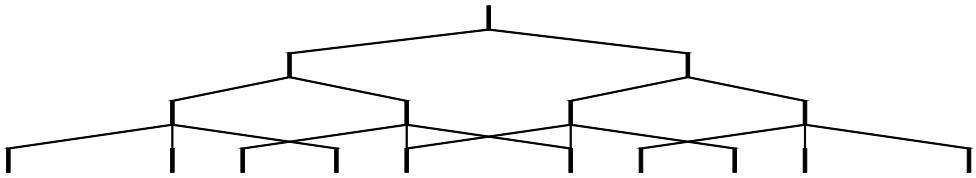
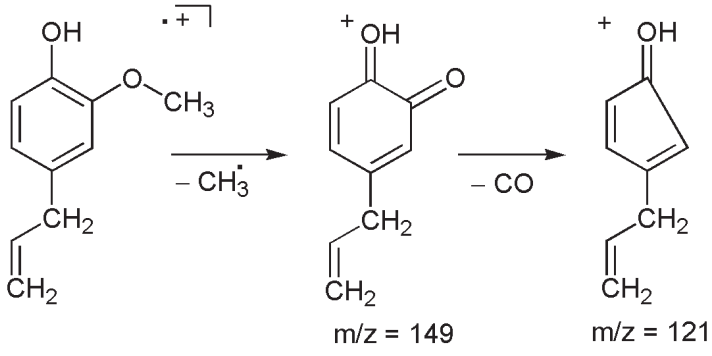


Fig. 2.2-18

G. C-3 situated in the β-position to the methoxy group must be more shielded than C-5.

H. H-6 does not show any NOE cross peak with H-7; missing HMBC correlation for C-1.

I. See: V. Kovacik, J. Skamla, “Mass spectrometry of some model substances of lignin. II” *Chemische Berichte* **1969**, *102*, 3623−3631.



Scheme 2.2-4

Translations

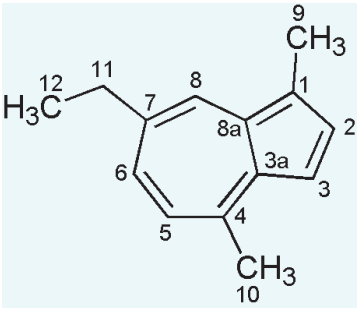
Fig. 2.2-19

Clover is very warm and has certain humidity in it, by which it pleasantly extends as the pleasant humidity of honey. And if somebody has a headache, that his head is whirling as if he would be deaf, he should often eat clover and this will diminish the whirling in his head.

Hildegard Bingensis (1098−1178), *Physica, Lib. I. de Plantis*, Chap. XXVII
Translated from the Latin by Franziska Berger

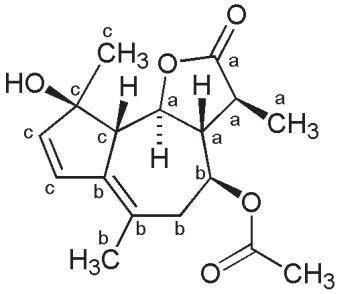
2.3 Chamazulene

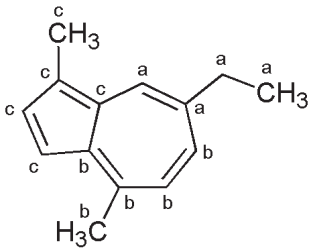
Answers



Scheme 2.3-5

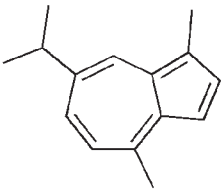
A. Decarboxylation removes the carbon atom of the lactone carbonyl group.



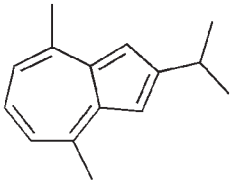


Scheme 2.3-6

B.



guaiazulene
1,4-dimethyl-7-isopropylazulene

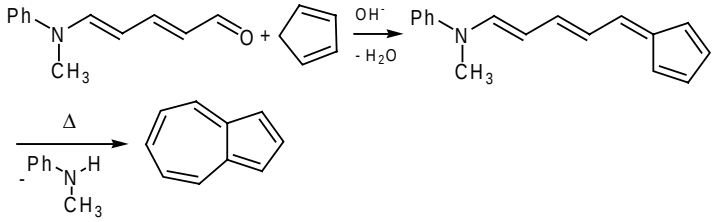


vetivazulene
4,8-dimethyl-2-isopropylazulene

Scheme 2.3-7

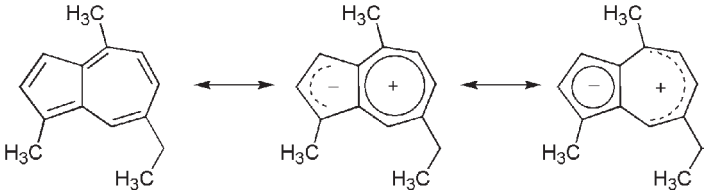
Guaiazulene is a constituent of oil of guaiac, an essential oil obtained by steam distillation of the wood of the palo santo tree (*Bulnesia sarmientoi*) growing in the Gran Chaco in South America. Guaiazulene is an approved cosmetic colour additive. Due to its physicochemical properties as a sesquiterpene hydrocarbon, $C_{15}H_{18}$, it is volatile and disappears by slow evaporation like a high-end perfume constituent. Therefore, a rare use is possible: it serves as a volatile dye to indicate optically the end of use for certain products such as insecticide strips. Vetivazulene is part of vetiver oil, an essential oil distilled from the roots of vetiver (*Chrysopogon zizanioides*), a perennial grass native to India. Due to its good fixative effect, vetiver is used in valuable perfumes.

C. It was Ziegler who had the idea that azulene formally can be regarded as a condensation product of cyclopentadiene and glutaconedialdehyde. That means that he saw azulene as the inner fulvene of a C_{10}-aldehyde. The main advantage of this retrosynthetic approach was that one could avoid a harsh dehydrogenation procedure because the proper unsaturation is already part of the building blocks. It was Hafner whom he asked to try it synthetically. Although it was impossible to accomplish the direct synthesis the latter found an elegant way to put into practice the new principle. Therefore, the so-called Zincke aldehyde was used. The closure of the seven-membered ring occurs as a result of a rare reaction, a 10π-electrocyclization.



Scheme 2.3-8

D. Our eyes see the complementary colour. If the red part of the spectrum is absorbed, mainly the blue part is left and we see this. This principle is called subtractive colour mixing.

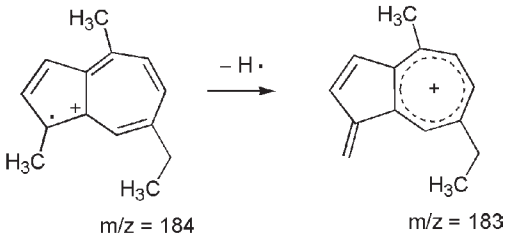


Scheme 2.3-9

E. One should not attempt to do this.

F. The charged resonance structure shown above suggests that the methyl group at the seven-membered ring is more deshielded; however, the opposite is the case!

G.



Scheme 2.3-10

Translations

Fig. 2.3-22

Julchen is as round as ball
Now without her napkins all.
Deep at night, the wife at rest,
Knopp is trying for the best.

Julchen in her cradle cries
Not a moment still she lies.
He remembers chamomile
and its tea which helps pains heal

In her uttermost dismay
Julchen roars: rabay, rabay!
O, dear me, what is the matter?
Is there something else for better?

Well we see it here in fiction:
Help he tries of all description.
But in vain is all assistance
Julchen goes on in resistance.

Maybe she wants to be laid
In her parents snuggy bed
Where they both are near and kind.

Wilhelm Busch (1832−1908), *Tobias Knopp*, Chap. 79
Translated from the German by Dora Fermina Hoffmann

Chamomile tea is used for the treatment of colds, especially those attended by fever, for the treatment of gripes, convulsions, strong congestions, etc. Warming chamomile cushions used for different indispositions are well known in every house, so that it seems unnecessary to say anything further about them.

Sebastian Kneipp (1821−1987), *Father Kneipp's Medicine Chest* (1886)
Translated from the German by Dominika Berger

"The animal is called Mashura (the Famous One). To call its attention, you have to say this name twice, after which you say the word "bubuna" (chamomile) three times. If you do this, it will speed up to a degree which will make the calm air seem like wind, and it won't stop before you say the work "yavash" (slowly, softly) three times as well. Since the camel exceeds the horse in speed, my Mashura, which is now yours, can hold out under "the secret" much longer than a horse, which can save you from great danger and which will render any pursuit pointless. Do you remember all these things well?"

"Yes. I thank you. But tell me, why have you chosen the word bubuna in particular?"

"Because this hedshin has a great and very curious liking for chamomiles. Therefore, whenever I ride on it, I carry some of these plants in my pocket. I crumble them in my hand until it smells of them, and then I caress Mashura's mouth and nose with that hand. If you do that, you will quickly gain its friendship and love. But you shouldn't let any one else know, otherwise someone else could use this method to gain its adherence. I am carrying chamomiles now as well, and I will give them to you. They have dried up, but their smell is still strong enough."

Karl May (1842−1912), *Am Jenseits, El Aschdar*
Translated from the German by Franziska Berger

All the parts of this plant are administered together, in doses of one drachma, for the stings of serpents of all kinds. Taken in drink, too, they bring away the dead fœtus, act as an emmenagogue and diuretic, and disperse calculi of the bladder. The anthemis is employed, also, for the cure of flatulency, affections of the liver, excessive secretions of the bile, and fistulas of the eye; chewed, it heals running sores. Of all the different varieties, the one that is most efficacious for the treatment of calculi is that with the purple flower, the leaves and stem of which are somewhat larger than those of the other kinds. Some persons, and with strict propriety, give to this last the name of "eranthemis".

Pliny the Elder (23−79), *The Natural History*, XXII, 54
Translated from the Latin by John Bostock

The blue flower

I am searching for the blue flower,
I'm searching but cannot succeed
My dreams whisper that in this flower,
True happiness I shall meet.

I'm playing my harp in each country,
in cities and towns all around
To see whether somewhere or other
The blue flower can yet be found

So long have I wandered,
Gone forward in hope and in trust
But alas, of the pretty blue flower
Nowhere a glimpse did I cast.

Joseph von Eichendorff (1788−1857)
Translated from the German by Franziska Berger

The chamomile: but some call it leucanthemon, others eranthemon, because it blossoms in spring, others chamaemelon, because of the similarity of its scent to apples, others melanthemon, others chrysocallia, and others callia. There are three kinds of this plant differing only in flowers; the sprays are a span tall, shrubby, and they have many axils; the leaves are small and delicate; the little flower heads are round and they have a small whitish and golden brilliancy in the centre. But outside, disposed in a circle, the little petals are either white or yellow or purple little petals the size of rue. It grows in rough terrains and by the roadside, and it is collected in the spring.

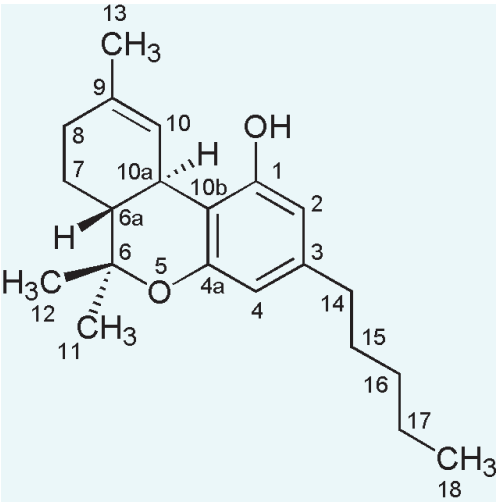
Pedanius Dioscorides (ca. 40–90), *De Materia Medica*, Book III, Chap.18
Translated from Ancient Greek by Lily Y. Beck

Many excellent medicines are prepared from it, beneath others a sky blue oil from the blossoms which first have been dried. This is best against pain in the bowels.

Joachim Camerarius (1534–1598), *Hortus Medicus et Philosophicus*
Translated from the Latin by Stefan Berger

2.4 Tetrahydrocannabinol

Answers



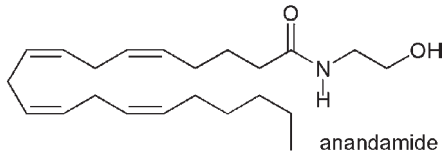
Scheme 2.4-11

A. Unlike alkaloids, THC is not basic and therefore basicity cannot be used as a tool in separation.

B. Their outstanding lipophilicity permits an easy extraction of cannabinoids from the plant source. Tetrahydrocannabinolic acids are carboxylic acids, whereas Δ^9-THC belongs to the phenols. The former are distinctly more acidic, dissolve as salts in a very weak base such as $NaHCO_3$ solution, a behaviour that is impossible for Δ^9-THC. Hence, the acids can be removed by hydrocarbonate extraction from Δ^9-THC.

C. Heating, such as occurs during smoking, will cause decarboxylation and thus increase the amount of Δ^9-THC available.

D. Hitherto, no endogenous cannabinoid was found with the structural skeleton of a tetrahydro-6*H*-dibenzo[*b*,*d*]pyran-1-ol. One of the endogenous keys created by evolution for this lock was given the name anandamide (arachidonoylethanolamide; see formula). The name of this neurotransmitter is an artificial creation combining the meaning of “delight” (in Sanskrit: ananda) with the word amide.



anandamide

Scheme 2.4-12

E. It is again their lipophilic nature that leads to storage in the tissue. For many other bioactive compounds this solubility property has to be assessed differently.

F. No, the polarimetry value is measured at the wavelenghth of the sodium D line and therefore has no connection with CD effects at different wavelengths.

G. The olefinic signal of H-10 is indeed deshielded by the ring current of the aromatic ring. H-10 is in a similar situation to the analogous hydrogen atom in phenanthrene. The two oxygen atoms in THC shield the protons H-4 and H-2 due to their +M effects.

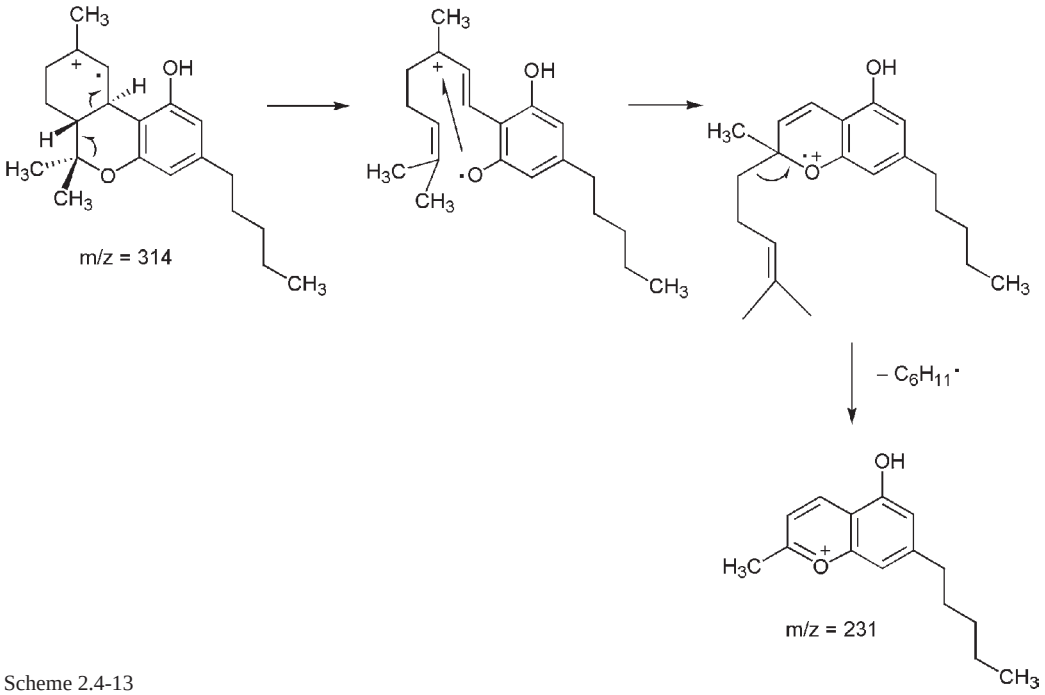
H. There are several possibilities, of which the most trivial would be a change of the solvent or using ^{13}C-depleted deuteriochloroform. Spectroscopically, one can use the CD spin coupling to give the $CDCl_3$ signal a different phase.

I. Yes, both partners are on the same side of the molecule.

J. H-10 is connected to C-13 and C-8, H-2 and H-4 are connected to C-14.

K. H-13 is connected to C-8, H-12 is connected to C-11 and C-6a, H-11 is connected to C-12 and C-6a, H-18 is connected to C-17 and C-16.

L. The literature [9] proposes a rather complicated rearrangement based on deuterium labelling studies. In the following scheme we give a simplified version. See also J. K. Terlouw, W. Heerma, P. C. Burgers, G. Dijkstra, A. Boon, H. F. Kramer, C. A. Salemink, "The use of metastable ion characteristics for the determination of ion structures of some isomeric cannabinoids" *Tetrahedron* **1974**, *30*, 4243–4248.



Scheme 2.4-13

Translations

Fig. 2.4-29

Concerning that hemp accordingly the Scythians, whenever they take hold of its seed, slip under the cloths and thereafter throw the seed on the glowing stones and it is burnt, when it is thrown on, and furnishes from itself so great a vapour that not even a single Greek vapour-bath could surpass it. Then the Scythians are pleased by the vapour-bath and howl; that for them is in place of a bath; for indeed they wash not for themselves with water entirely their body.

Herodot (480−425 BC), *The Histories*, Book IV
Translated from the Ancient Greek by A. D. Godley

Hemp originally grew in the forests, where it is found with a blacker and rougher leaf. Hempseed, it is said, renders men impotent: the juice of this seed will extract worms from the ears, or any insect which may have entered them, though at the cost of producing head-ache. The virtues of hemp, it is said, are so great, that an infusion of it in water will cause it to coagulate: hence it is, that if taken in water, it will arrest looseness in beasts of burden. A decoction of the root in water, relaxes contractions of the joints, and cures gout and similar maladies. It is applied raw to burns, but it must be frequently changed, so as not to let it dry.

Pliny the Elder (23−79), *Naturalis Historia Liber*, XX, 97
Translated from the Latin by John Bostock

Hemp is warm, and if the air is neither too hot nor too cold, hemp is growing and such is its nature. Its semen contains sanative power and it is wholesome to eat for healthy men. It is light and useful in their stomach because it can carry away somehow the slime from the stomach. It can easily be digested, it diminishes the bad liquors and strengthens the good ones.

Hildegard Bingensis (1098−1178), *Physica − Lib. I. de Plantis*, Chap. XI
Translated from the Mediaeval Latin by Stefan Berger

Upon their first experience with the drug, most novitiates complain of its slow action; they await its first sign with puerile impatience and, as the drug does not manifest its presence instantaneously, they utter incredulous cries of protest, much to the amusement of those who know the means by which hashish governs. The first gentle blows, like the warning winds of threatening storms, expand and multiply in the very midst of that incredulity. At first you are sized by an absurd, irresistible mirth. These demonstrations of excessive joy, of which you are almost ashamed, multiply in rapid succession, cutting into the stupor at intervals during which you earnestly struggle to collect yourself. The simplest words, the most trivial ideas, take on new, bizarre appearances; you are even amazed at having previously thought them so simple. Incongruous connections, coincidental resemblances, interminable puns, and comic sketches provide endless delight. The demon has invaded you. This gaiety, as irritating as a tickle, cannot be withstood. From time to time you laugh at

yourself, at your foolishness and folly. If your companions are with you, they join in the merriment, laughing at your condition and their own, but as their laughter is without malice, you take no offence.

Charles Baudelaire (1821−1867), *Artificial Paradises*
Translated from the French by Stacy Diamond

"Old Shatterhand has spoken well. We shall smoke the peace pipe with him."

We now sat down by the water. He took out his pipe whose vile aroma had offended my nose even at a distance and filled it with a mixture that seemed to consist of crushed red beets, hemp leaves, cut acorns, and sorrel, lit it, got up, inhaled, blew the smoke heavenward and toward the ground, and said:

"Up above, the Great Spirit lives, and here below grow the plants and animals he has set aside for the warriors of the Kiowas."

He inhaled four more times and, having blown the smoke towards the north, the south, the east and the west, he continued:

"In these regions, there live those Indians and white men who have unjustly kept these animals and plants for themselves. But we will search them out and take what belongs to us. I have spoken. Hogwh!"

Karl May (1842−1912), *Winnetou I*, Chap. 3
Translated from the German by Michael Shaw

After the third course Lysevitch said, turning to Anna Akimovna:

"The fin de siècle woman − I mean when she is young, and of course wealthy − must be independent, clever, elegant, intellectual, bold, and a little depraved. Depraved within limits, a little; for excess, you know, is wearisome. You ought not to vegetate, my dear; you ought not to live like every one else, but to get the full savour of life, and a slight flavour of depravity is the sauce of life. Revel among flowers of intoxicating fragrance, breathe the perfume of musk, eat hashish, and best of all, love, love, love . . . . To begin with, in your place I would set up seven lovers − one for each day of the week; and one I would call Monday, one Tuesday, the third Wednesday, and so on, so that each might know his day."

This conversation troubled Anna Akimovna; she ate nothing and only drank a glass of wine.

Anton Chekhov (1860−1904), *The Party and Other Stories*
Translated from the Russian by Constance Garnett

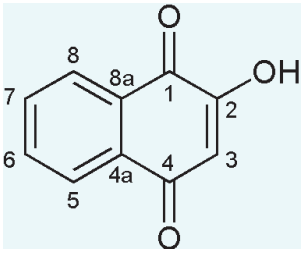
Rules of the House

No admission or sales to people under 18 years.
Sale and use of hard drugs forbidden.
Aggressive behaviour will not be tolerated.
No sales of stolen goods.
Dont't cause problems with the neighbourhood.
No gathering around the coffee shop.
Never buy hard or soft drugs on the street.
Transport of marihuana and hashish is forbidden.
Consumption is obligatory.

The Director

Translated from the Dutch by Stefan Berger

Chapter 3 Dyestuffs and Coloured Compounds



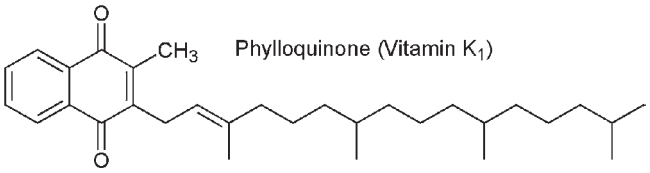
Scheme 3.1-8

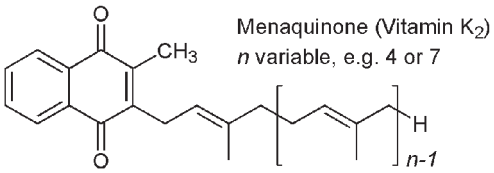
3.1 Lawsone

Answers

A. On deprotonation, lawsone forms an anion that is able to stabilize the anionic charge, as can be shown by mesomeric anionic forms with the charge distributed over the whole system of conjugated double bonds. Therefore, lawsone is even slightly more acidic than acetic acid, a carboxylic acid (pK_a 4.76). The trivial name hennotannic acid is an expression of this property.

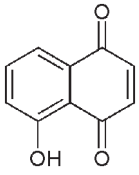
B. The *para*-benzoquinone unit present in lawsone is an oxidizing agent. On uptake of 2 H it can react to form a hydroquinone unit. This reaction is reversible and of biochemical significance. The structural motif of a *para*-benzoquinone is found in the highly lipophilic vitamins K_1 (phylloquinone) and K_2 (menaquinone), which are both 2-methyl-1,4-naphthoquinone derivatives. Both are necessary to accomplish the post-translational modification of proteins (carboxylation of glutamate residues) required for the blood coagulation in the vitamin K cycle.





Scheme 3.1-9

C. The compound is called juglone (5-hydroxy-1,4-naphthoquinone).

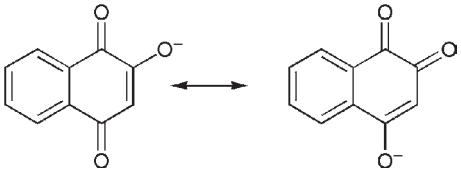


Scheme 3.1-10

D. HNQ stands for 2-hydroxy-1,4-naphthoquinone. It is a matter of debate whether the flooding of the chemical community with infinite amounts of acronyms, useful for the short expression of complex molecules such as DNA, is really progress in the world of small molecules.

E. Wool and silk are both protein fibres. From this point of view they are relatives of the protein keratin in human skin and hair. Like keratin, they can form covalent bonds to lawsone and hence undergo a fadeless coloration.

F. From the orthoquinoid structure shown, one would expect a UV band in the region of 450 nm.



Scheme 3.1-11

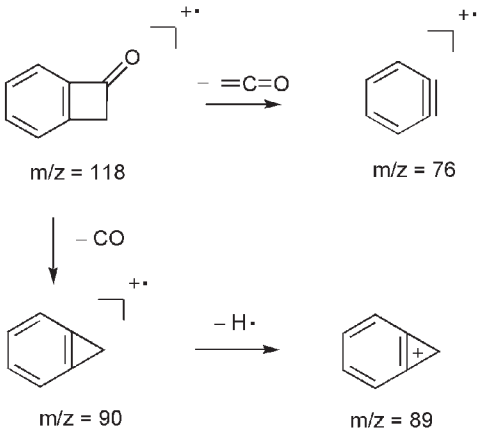
G. These overtone vibrations are often called “benzene finger” and indicate the presence of a benzene ring.

H. See answer F. In neutral solutions there is no band above 400 nm.

I. Olefinic or aromatic carbon atoms in the β-position to an oxygen substituent are considerably shielded.

J. C-4a has three hydrogen atoms, H-3, H-6 and H-8, situated via three bonds, hence the spin coupling multiplet will be complicated. C-8a has only two such hydrogens, H-5 and H-7, and therefore forms a triplet-like pattern.

K.



Scheme 3.1-12

Translations

Fig. 31-19

As for her eyes, though they did not justify what popular credulity said of them, they were at least wonderfully strange eyes; brown eyebrows, with extremities ending in points elegant as those of the arrows of Eros, and which were joined to each other by a streak of henna after the Asiatic fashion, and long fringes of silkily-shadowed eyelashes contrasted strikingly with the twin sapphire stars rolling in the heaven of dark silver which formed those eyes. The irises of those eyes, whose pupils were blacker than atrament, varied singularly in shades of shifting colour. From sapphire they changed to turquoise, from turquoise to beryl, from beryl to yellow amber, and sometimes, like a limpid lake whose bottom is strewn with jewels, they

offered, through their incalculable depths, glimpses of golden and diamond sands upon which green fibrils vibrated and twisted themselves into emerald serpents.

Theophile Gautier (1811−1872), *King Candaules*
Translated from the French by Lafcadio Hearn

Taanach came back to her; and after arranging two candelabra, the lights of which burned in crystal balls filled with water, she tinged the inside of her hands with Lawsonia, spread vermilion upon her cheeks, and antimony along the edge of her eyelids, and lengthened her eyebrows with a mixture of gum, musk, ebony, and crushed legs of flies.

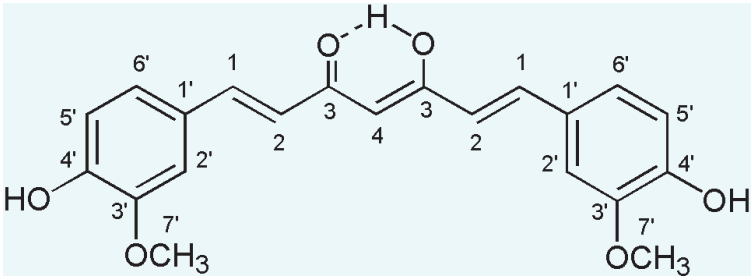
Salammbo seated on a chair with ivory uprights, gave herself up to the attentions of the slave. But the touchings, the odour of the aromatics, and the fasts that she had undergone, were enervating her. She became so pale that Taanach stopped.

"Go on!" said Salammbo, and bearing up against herself, she suddenly revived.

Gustave Flaubert (1821−1880), *Salammbo*
Translator unknown

3.2 Curcumin

Answers



Scheme 3.2-7

A. Two effects stabilize the enol form. First, in the enol form a fully conjugated system exists which is accompanied by the release of energy of delocalization. Second, an intramolecular hydrogen bonding is established which again releases bond energy. Neither effect exists at the 1,3-diketo unit.

B. Regioisomerism (constitutional isomerism). In the case that both tautomers exist in distinct amounts, a simple standard TLC on silica gel plates often shows a pair of spots with the enol spot in front, i.e. with the larger R_f value. This indicates that the enol form is the less polar one, which can be understood in terms of an internal "saturation of polar groups" by the hydrogen bonding.

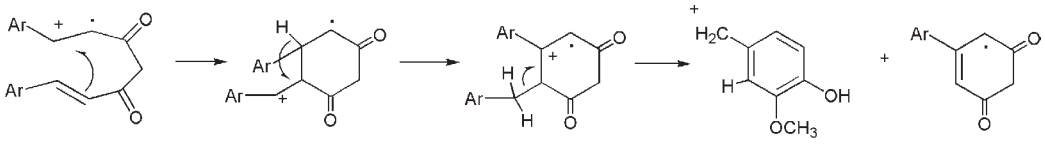
C. The enol form has an extended chromophore with 20 π-electrons in conjugation. The chromophore contains phenyl rings and alkene units as weak chromophores and one keto group is included as a strong chromophore. A bathochromic shift of the absorption maximum is caused by OH and OCH_3 groups as auxochromic substituents and also by the enolic OH group. It is not to assumed that the pure diketo form is coloured because the conjugation is interrupted there. If one cuts it into two units, a substituted cinnamic acid remains (ferulic acid) which has too small a conjugation to be as intensively coloured as the enol form.

D. At higher pH, two processes can occur: first the acidic enolic proton and then the phenolic hydrogen atoms dissociate. The observed red shift in the UV spectrum indicates a lower energy barrier of the charged π-system between the ground and first excited states.

E. Free rotation of the phenyl rings around the C-1−C-1' bonds with both conformers populated.

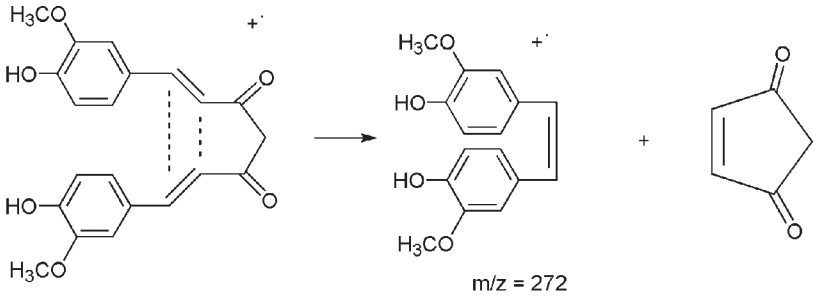
F. (a) H-1 sees the aromatic carbon atoms C-2', C-6' and C-1' over three and two bonds and C-3 via three bonds as expected.
(b) H-2' displays the expected three-bond connectivities within the aromatic ring to C-4' and C-6', a two-bond coupling to C-1' and a three-bond coupling to C-1.
(c) H-6' displays cross peaks to C-2' and C-4' within the aromatic ring and to C-1, all over three bonds.
(d) H-5' displays two three-bond connectivities to C-1' and C-3'.
(e) H-2 is connected to C-4, C-3 and C-1'.
(f) H-4 sees C-2 and C-3.

G. This ion is explained as a benzylic ion formed after ionization in the C-1–C-2-bond:



Scheme 3.2-8

H. A stilbene-type radical ion is formed:



Scheme 3.2-9

Translations

Fig. 3.2-16

The Crow

The crow is laughing because it knows
what's really true about scarecrows
and that a crow doesn't taste as nice
as a dish of curry chicken with rice.

Joachim Ringelnatz (1883–1934)
Translated from the German by Dominika Berger

"You see?" she said, sprinkling some curry powder into the hot pan. Then with a knife she sliced some veal sausage into it, adding "Weisswurst, horrible, and then sweet mustard! It's enough to turn your stomach, isn't it?" She gave herself an exaggerated shudder. "Brr," then plopped some ketchup into the pan, stirred, shook a bit more black pepper over it, and finally pushed the sausage slices into the crimped paper plate. "This is the genuine article. Has something to do with the wind. Believe me. With a cold wind you need hot stuff".

Her stand really was set up on a windy corner. The plastic sheet was torn where it was fastened to the stand, and now and again a strong gust would tip over one of the large plastic cone-shaped tables, advertising ice cream, at which you could stand as you ate your meatballs and, of course, that absolutely unique curried sausage.

"I am going to close the stand, for good".

Uwe Timm (1940–), *The Invention of the Curried Sausage*
Translated from the German by Leila Vennewitz

Currywurst

When you're going into town
what fills you up
a currywurst
When you're coming off your shift
there's nothing more beautiful
than currywurst...
With some French fries
You know what – make it two times
currywurst

When you've seen all through it
you need something chewy
a currywurst

Willi, come along
I really feel like having
A currywurst

I feel like I'm starving
And my buddy here also wants a
currywurst

Willi, ain't it nice,
The two of us standing here
With currywurst

Willi what about you?
You want another beer
with your currywurst?

Woah, this sausage is hot
Boy, I need a drink after
the currywurst!

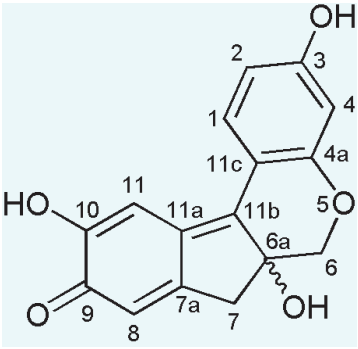
As soon as you're really drunk
your mind goes pretty blank
because of the currywurst

Walking home with it
the thing slips
damn currywurst!

On the shirt, on the jacket
Man, what a …
everything full of currywurst!

Come Willi
please, please come home with me
listen, I'm getting trouble
coming home like that
Willi, Willi please
you're the guy of my taste
Willi, Willi, come with me please, Willi

Herbert Grönemeyer (1956–)
Translated from the German by Dominika Berger

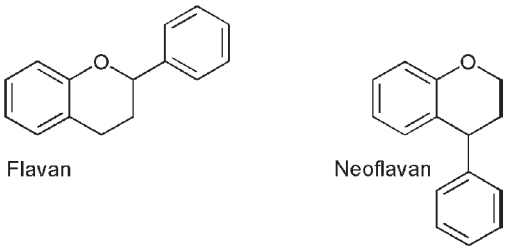


Scheme 3.3-6

3.3 Brazileine

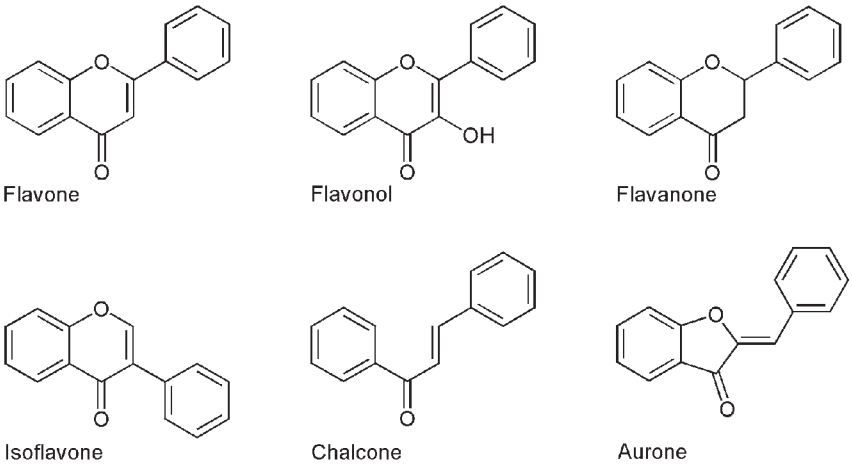
Answers

A. Flavan is 2-phenylchroman or 3,4-dihydro-2-phenyl-2*H*-1-benzopyran. Neoflavan is 4-phenylchroman or 3,4-dihydro-4-phenyl-2*H*-1-benzopyran, i.e. neoflavan is a regioisomer of flavan.



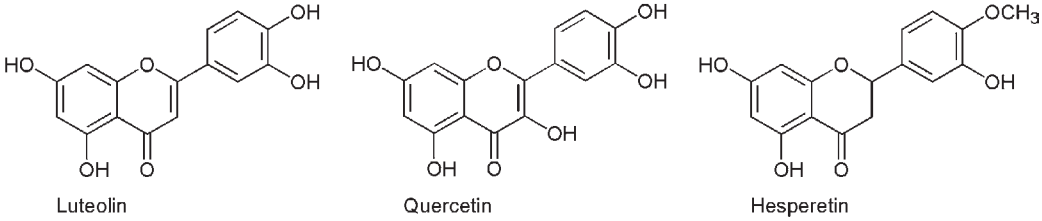
Scheme 3.3-7

The skeletons of the members of the flavanoid community are as follows:



Scheme 3.3-8

Luteolin belongs to the flavones, quercitin to the flavonols and hesperetin to the flavanones.



Scheme 3.3-9

B. By mordant dyeing, three chemical structures combine to form a coloured textile: the fibre of the fabric, e.g. cotton, wool or silk, the mordant and the dyestuff. The mordant acts as a link between fibre and dyestuff. The mordant is a solution of a polyvalent metal ion, such as Fe^{2+}, Al^{3+}, Cu^{2+}, Cr^{6+} in $Cr_2O_7^{2-}$ and Sn^{2+}. The ion of the mordant offers the possibilities for complexation both to the fibre and to the dyestuff. In other words, the fibre and the dyestuff need to have functional groups that can act as ligands. The catechol unit in quercetin is an example. An example of a long-known and esteemed mordant is alum [$KAl(SO_4)_2 \cdot 12H_2O$)], which is very useful for wool dyeing.

C. Although the position of chirality is surrounded by two methylene units, none of the possible experiments with chiral environments will be helpful in deciding whether C-6a has *R* or *S* configuration, because in any case one of the protons of each CH_2 unit will answer and the rest of the molecule on both sides is not helpful because they are flat and do also not contain another chiral centre.

In principle, a decision is possible when a chiral reagent is attached to C-6a. This is valid for the formation of a Mosher ester with both enantiomers of the Mosher reagent. This would lead to a decision as to which diastereomer of the two esters is present because the rules for the chemical shift differences in these diastereomers are known from analogues in general. The experimental problem with brazilein is that, prior to a Mosher esterification, the enolic and phenolic OH groups have to be protected, selectively. This should be possible because they are distinctly more acidic than the tertiary alcohol.

D. In both cases one can take the spin coupling constant from the difference in the line positions of the two sub-doublets; this yields for the AX spin system a value of $J_{7\alpha,7\beta} = -11.1$ Hz and for the AB system $J_{7\alpha,7\beta} = -18.1$ Hz. For the chemical shifts one can take with good approximation the centre of the two doublets in the AX spin system. For the AB spin system, however, this is not allowed and one has to calculate the shift values either using a spin simulation program or by hand using the formulae given in good NMR textbooks. It turns out that the chemical shifts of 7α and 7β are displaced by plus and minus 8.75 Hz from the centre of the AB system. Thus, the chemical shift of 7α and 7β is not in the centre of the two sub-doublets.

E. H-1 should be able to see via three bonds C-3, C-4a and C-11b, via two bonds C-2 and C-11c. Experimentally, we observe four correlations to quaternary carbon atoms, one of which is very weak. We assign this therefore to C-11c, because this signal also has a strong correlation to H-2. C-3 is found at 162.1 ppm, C-4a must be in the same chemical shift region as C-3 and we therefore assign the correlation signal at 157.7 ppm to C-4a. The signal at 151.5 ppm was already assigned to C-11b.

H-2: the dd signal at 6.55 ppm has correlation signals to C-4 and C-11c.

H-4 forms a doublet with 2.3 Hz at 6.35 ppm. In the HMBC spectrum it is connected with three carbon signals, C-11c at 110.8 ppm, C-4a at 157.7 ppm and C-3 at 162.1 ppm.

F. H-8: the singlet at 6.31 ppm shows connectivities to C-7 at 39.2 ppm, to C-7a at 126.0 ppm and to C-10 at 152.2 ppm. H-11: the singlet at 7.09 ppm has four correlation signals, a weak one to C-7a at 126 ppm and three rather strong signals to C-11a at 158.9 ppm, to C-10 at 152.2 ppm and of course to C-9 at 179.9 ppm.

Translations

Fig. 3.3-22

Next, the sailors left to the Naus, leaving two banished on shore and on the following day, 2nd of May, the fleet left towards Cabo da Boa Esperança, one ship having returned to the kingdom, with Gaspar de Lemos as captain, to bring to the King the good news of the official recognition of Brazil and of its belonging to the Portuguese Crown.

To this Land, which was known by the name of Parrot's Land, and which Cabral named Vera Cruz, D. Manuel, in 1502, gave the name Santa Cruz, which was to be replaced later by the name Brazil, due to the great commerce of the Pau Brazil that it produced.

When reporting, in a letter, to the King of Spain about the recognition of Brazil made by Cabral, D. Manuel said: “the captain left there two banished to the mercy of God”. One of the pilots of the fleet explained later that these banished started to cry and that the natives started to cheer them up, taking pity on them.

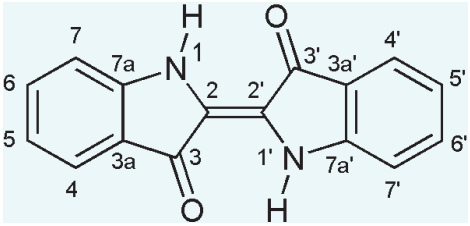
Manuel Ferreira Garcia Redondo (1854−1916), O *Descobrimento do Brazil*
Translated from the Portuguese by Eurico Cabrita

How to work with brasilium – Take a copper bowl, and scrape as much brasilium into in as you see fit. Then fill it with urine, sprinkle alum on top and let it stand thus for one night. On the morrow, set it on coals, have it boil up once or twice, and remove the bowl from the fire. Put in a little quicklime with brasilium and alum, mix it, and leave it like this until it thickens and water swims on top. Throw this out and let the remainder dry in the sun, and store as much of it as you want. You can use this colour both on wood and on walls, but most miraculously on parchment.

Heraclius (8th−10th Century), *On the Roman Colours and Arts*
Translated from the Latin by Franziska Berger

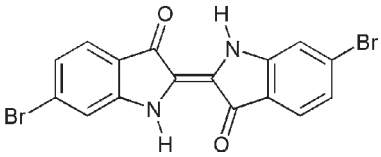
3.4 Indigo

Answers



Scheme 3.4-9

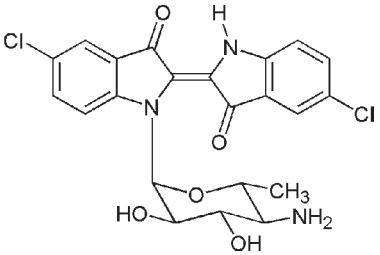
A.



Scheme 3.4-10

Tyrian purple has been isolated from purple snails occurring in the Mediterranian Sea. The centre of its isolation was Tyre, an ancient Phoenician city, today a part of Lebanon. It was found a century ago that 10 000 of such snails (different species form the dye) supply only ca. 1 g of the dye. It is said that one can still find them today. Therefore, Tyrian purple was a luxury good, e.g. in the Roman Empire, used to dye the clothes of high officials. Similarly, the name Cardinal's purple shows how the use was restricted to dignitaries. Interestingly, purple was also used to undercoat the parchment pages of old handwritten books made in monasteries. A look at the structure shows that this will probably be a marine natural product, due to the two bromine atoms present. Bromine as a heteroatom is not available to radical plants, similarly to iodine. However, sea water is a suitable source.

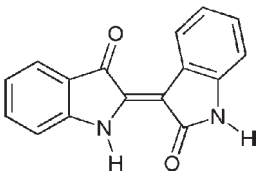
B. Akashin A:



Scheme 3.4-11

Citation: H. Laatsch et al., "Akashins A, B, and C: novel chlorinated indigoglycosides from Streptomyces sp. GW 48/1497" *Angew. Chem. Int. Ed.* **2002**, *41*, 597−599.

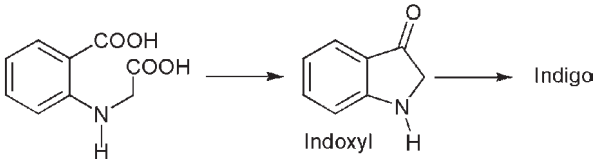
C.



Scheme 3.4-12

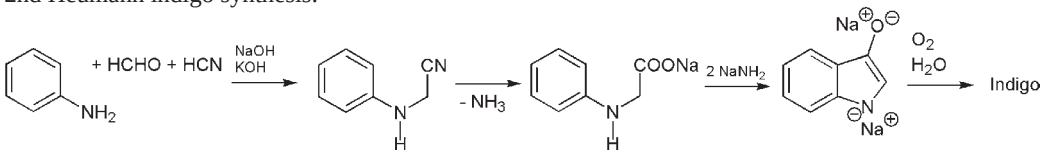
D. The extraordinarily high melting point, which from the viewpoint of application makes indigo a pigment, i.e. to a both coloured and insoluble compound.

E.



Scheme 3.4-13

2nd Heumann indigo synthesis:



Scheme 3.4-14

This is a contemporary indigo synthesis.

F. Indanthrene dyes.

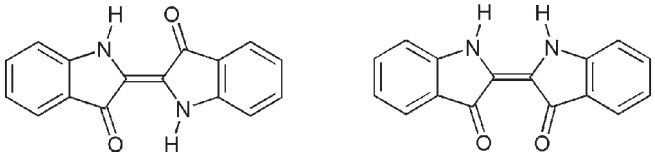
G. By the molar extinction coefficient ε_{max}, which is a measure of the ability of a coloured compound to absorb part of the irradiation in the visible part of the spectrum. For real dyestuffs the value of ε_{max} is between 10^4 and 10^5, i.e. much higher than for compounds showing a weak coloration only, such as a nitrophenol.

H. These three compounds are from living things that are very different from each other, a plant, a snail and a microorganism. It seems that indole can be regarded as an early hit in evolution, a suggestion to which its simple structure would fit. Think of the many other bioactive derivatives in plants and animals that contain indole moieties as a substructure.

I. H-5 is in a *para*-position to the NH group and therefore will be sensitive to the +M effect of this group. At the same time H-5 is in a *meta*-position to the carbonyl group and will therefore not be much influenced by its electron-withdrawing effect. For H-6 just the opposite is true.

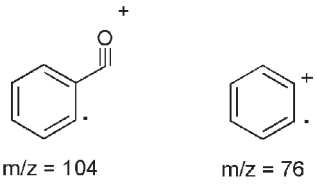
J. Comparing the pairs C-4 and C-7 with H-4 and H-7, or C-5 and C-6 with H-5 and H-6, we find that both ^{13}C chemical and proton chemical shifts are influenced by the same electronic principles, as discussed in answer I; thus, the chemical shift changes are parallel to each other. Comparing C-4 and C-5 with H-4 and H-5 or C-7 and C-6 with H-7 and H-6, however, reveals that the carbon chemical shifts of C-4 and C-7 are dominated by another influence and this is the β-substitution by C-3 with respect to the nitrogen atom. Thus, the two *ortho*-carbon atoms C-4 and C-7 experience a downfield shift by this substitution effect which is not valid for their protons.

K. This is a very difficult task. One could try two approaches which require, however, specific labelling.
   1. If one were to label both carbonyl atoms and both central carbon atoms C-2/2' with ^{13}C, these would form a ^{13}C AA'XX' spin system. In such a spin system, all spin couplings would be visible despite the symmetry of the molecule. From the values of the $^3J_{CO,CO}$ spin coupling, one could decide, whether these are on a *trans*- or a *cis*-double bond.
   2. A similar approach would label both nitrogens with ^{15}N and then the two NH groups would form an AA'XX' spin system. The $^3J_{N,N}$ spin coupling again could decide about the *cis*- or *trans*-situation. The problem here, however, might be the lack of suitable reference data and whether a $^4J_{NH}$ is present in this system.



Scheme 3.4-15

L.



Scheme 3.4-16

Translations

Fig. 3.4-26

All the Britons, indeed, dye themselves with woad, which occasions a bluish color, and thereby have a more terrible appearance in fight. They wear their hair long, and have every part of their body shaved except their head and upper lip.

Gaius Julius Caesar (100–44 BC), *The Gallic Wars*, V, 14
Translated from the Latin by W. A. McDevitte

Dying Dark Blue

Place one talent woad into a pit which is standing in the sun and which can hold not less than fifteen measures and cover it well. Add urine until the liquid stands over the woad, and let it warm in the sun. On the following day, mix the woad by stamping it with your feet in the sun, until it is wet very well. This has to be done for three days.

Papyrus Graecus Holmiensis (ca. 300–400 AD, from a grave in Thebes)
Translated from the Ancient Greek by Stefan Berger

The train left at the regular hour. Among the passengers were a number of officers, Government officials, and opium and indigo merchants, whose business called them to the eastern coast.

Jules Verne (1828–1905), *Le Tour du Monde en Quatre-Vingts Jours*, XI
Translator unknown

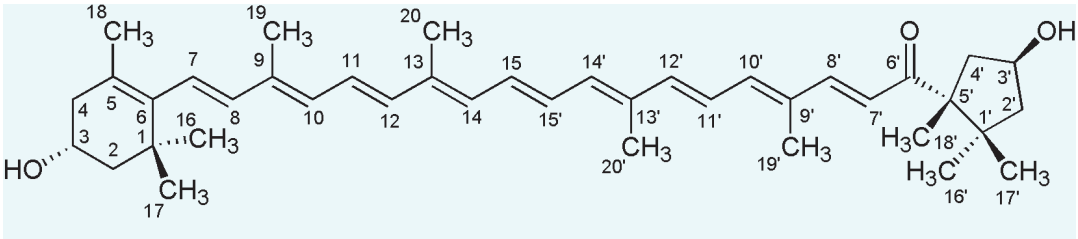
The theatre was beginning to fill; opera-glasses were taken from their cases, and the subscribers, catching sight of one another, were bowing. They came to seek relaxation in the fine arts after the anxieties of business; but "business" was not forgotten; they still talked cottons, spirits of wine, or indigo. The heads of old men were to be seen, inexpressive and peaceful, with their hair and complexions looking like silver medals tarnished by steam of lead.

Gustave Flaubert (1821–1880), *Madame Bovary*, XXIV
Translated from the French by Eleanor Marx (1855–1898)

She heard a splashing sound behind her, as if someone had come out of the water onto the shore. A shudder ran down her spine as she felt that she wasn't alone anymore. Abruptly, she turned around. A tall young man was standing there, between her and the water, stocky and strong. At first sight, it might have been the Dyer with his square stature, the broad forehead and the curly black hair. He was wearing a garment of a marvelous blue, not as if a white cloth had been laid into a vat filled with the combined strength of indigo and woad, but rather as if the ocean's blue had been taken away to be draped around his body.

Hugo von Hofmannsthal (1874−1929), *The Woman Without Shadow*
Translated from the German by Dominika Berger

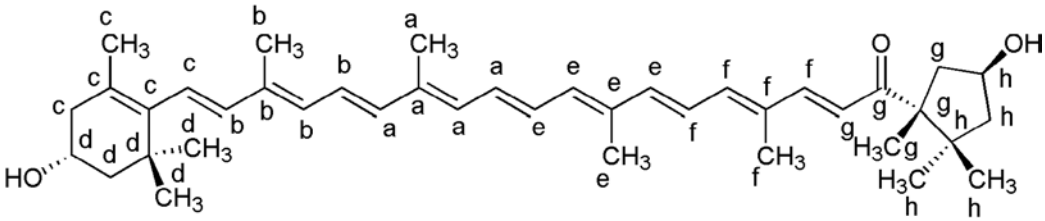
3.5 Capsanthin



Scheme 3.5-10

Answers

A.



B. For capsorubin, a bathochromic shift is observed compared with capsanthin. The structural reason can be found in the difference in the two chromophoric systems. In capsanthin, the chromophoric system is made up of 10 conjugated C=C double bonds and one C=O group attached. In capsorubin, this system consists of nine conjugated C=C double bonds and two C=O groups. Whereas a C=C double bond is regarded as a weak chromophore, a C=O double belongs to the strong chromophores. Hence, capsanthin and capsorubin represent a pair of compounds which illustrate how large this difference between C=C and C=O bonds is. Think also of diacetyl, $H_3C{-}CO{-}CO{-}CH_3$, a liquid diketone, responsible for the natural flavour of butter, which is yellow due simply to the conjugation of two C=O groups.

C. A well-known effect is that usually, *trans*-configured alkenes are for steric reasons thermodynamically more stable than their *cis*-isomers. However, the mp difference mentioned here does not give a hint about this thermodynamic difference, although it seems so. It just reflects another effect. Obviously, *trans*-forms can be "better" arranged into a crystal lattice than *cis*-forms, with "better" meaning "with a higher release of bond energy" caused by a tighter intermolecular bond arrangement. This is what an increase in the melting point shows.

D. The particularities of tetraterpenoid hydrocarbons, sometimes in different oxidized states, have led to a special nomenclature with the stem carotene. Formally, such molecules are cut into two halves with 20 C-atoms in each with the interface between C-15 and C-15' (see above). Starting from this double bond, a unit of four conjugated double bonds to both sides is the feature of all carotenoids, then. Their difference is found in both end groups, which can be a cyclic (as in capsanthin) or an open-chain structure containing

nine C-atoms. For the different patterns found in Nature, therefore, a list exists in which these groups are named with Greek characters. The end groups of capsanthin are labelled as β (six-membered unit) and κ (five-membered unit). Two such β groups are found in β-carotene, therefore the term β,β-carotene is really exact. ψ,ψ-Carotene is the synonymous name for lycopene, the open-chain red hydrocarbon, $C_{40}H_{56}$, from tomatoes, which is regarded as the stem compound for all carotenoids.

E. A group of Dutch scientists found that in the living lobster two molecules of astaxanthin are fixed by a kind of protein cage which leads to such a close proximity that by exciton coupling the light absorption is dramatically changed and is strong over nearly the entire visible region, leading to a "blue−black" colour. Boiling destroys the protein cage and releases single orderless carotenoids, which show a different light absorption behaviour, and to our eyes they are then red (compare F. Buda *et al*., *J. Am. Chem. Soc*. **2005**, *127*, 1438−1445).

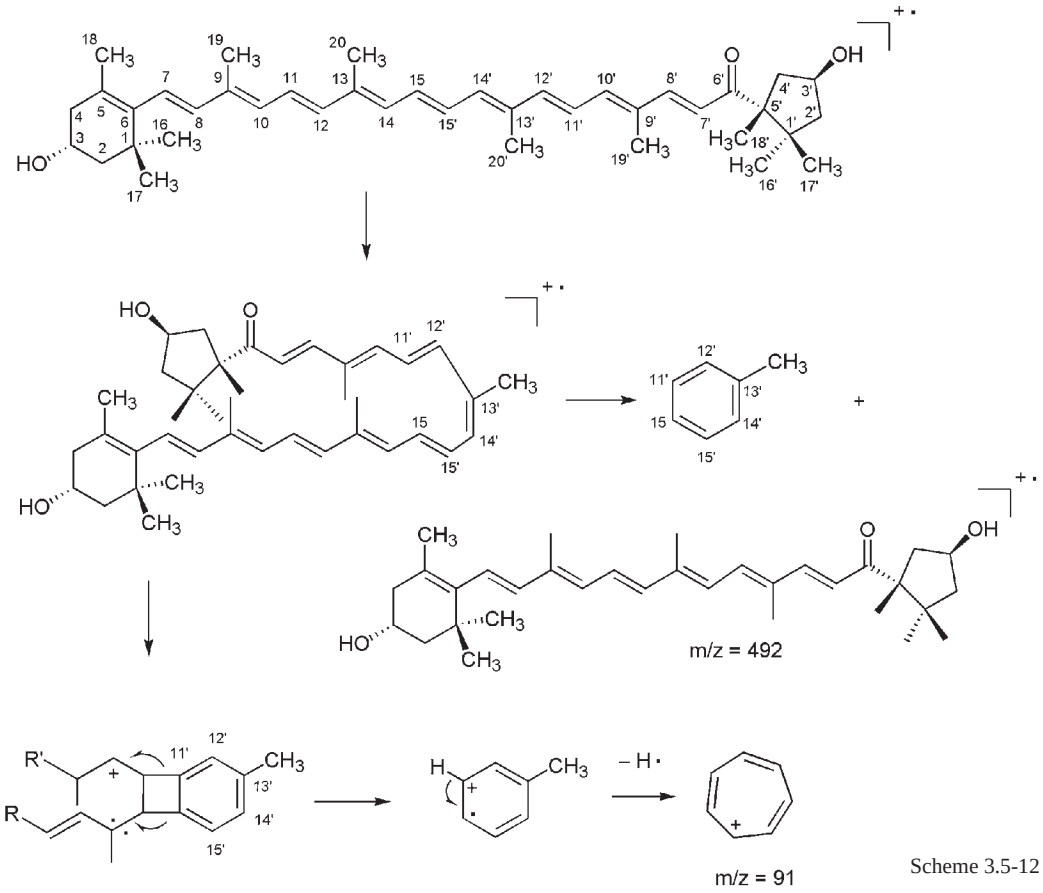
F. Due to the Karplus equation, the axial−axial spin couplings in a cyclohexane ring are larger than those in a five-membered ring.

G. The proton at 2.958 ppm has a much stronger NOE effect to H-3'.

H. The proton at 2.385 ppm has a much stronger NOE effect to H-3.

I. Connectivities over two or three bonds to C-18', C-1', C-2', C-5' and C-3'.

J. Very similar to the xylene formation as described in the main text, also toluene and the benzyl cation with respect to the tropylium ion can be formed. Isomerization at the C-13'−C-14' double bond leads to ring closure between C-11' and C-15, as shown in the scheme below:



Scheme 3.5-12

Fig. 3.5-33

Translations

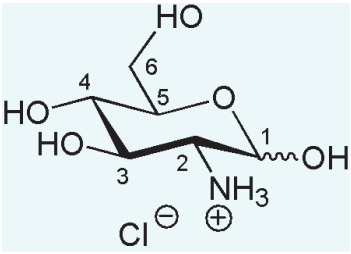
Carotene can in principle be called what I have detected in this sap, it stains, is purple, forms beautiful crystals, is completely soluble in fatty essential oils, and it resembles the consistency of hard resin or myricin. I reckon that the operating principle is the essential oil together with the sugar.

H. G. F. Wackenroder (1798−1854)
Translated from the Latin by Stefan Berger

Chapter 4 Carbohydrates

4.1 Glucosamine

Answers



Scheme 41-8

A. Such sugars undergo mutarotation and form an α- and a β-form in solution. A representation with an open-chain formula may be not an image of the predominant form in solution but it is definite. It does not have the drawback of a cyclic presentation that there are two possibilities: α- or β-anomer. Furthermore, it may be unclear whether a reference can be clearly assigned to α or β. Showing a reference under one formula avoids all these problems.

B. Citrus pectin consists of polygalacturonic acid, i.e. of D-galacturonic acid units linked via 1,4-α-glycosidic bonds. Some other fruit pectins occur with partial esterification of the –COOH units to methyl esters. The main use of citrus pectin, isolated from citrus peel on an industrial scale, is as a hydrocolloid former (gelling agent) in the manufacture of jam. Therefore, it is part of preserving sugar. It is likely that the presence of many free –COOH groups is responsible for the strong swelling effect; this would be similar to hyaluronic acid. Pectin is not digested and acts as a roughage.

C. Only a few marine bacteria are able to degrade agar (agar). Terrestrial microorganisms are not adapted to the decomposition of this heteropolysaccharidic mixture from a marine alga. Humans are also not able to digest agar (agar). Therefore, it can be used in dietetics as a soaking agent to increase the volume of food preparations.

D. The electronegative ammonium group in the direct neighbourhood of the anomeric carbon atom is responsible for easier ring opening.

E. This is due to the Karplus equation. Molecular mechanics calculations show that, for instance, H-1α has a dihedral angle of nearly 180° to C-5, whereas H-1β has a dihedral angle of only 73°. Hence, for the latter the spin coupling constant should be near zero.

F. One might argue that the anomeric proton in the β-form is shielded by the electron cloud of the amino group in the vicinity; however, these stereoelectronic effects are very subtle and prone to erroneous assumptions.

Translations

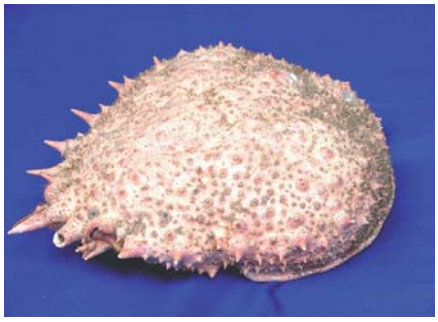


Fig. 4.1-22

One morning, as Gregor Samsa was waking up from anxious dreams, he discovered that in bed he had been changed into a monstrous verminous bug. He lay on his armour-hard back and saw, as he lifted his head up a little, his brown, arched abdomen divided up into rigid bow-like sections. From this height the blanket, just

about ready to slide off completely, could hardly stay in place. His numerous legs, pitifully thin in comparison with the rest of his circumference, flickered helplessly before his eyes.

Franz Kafka (1883−1924), *The Metamorphosis*
Translated from the German by Ian Johnston

The Gazkar were descendants of insects. Their chitin armour was black, and their compound eyes were glimmering with prismatic colours. They reached a body height of about 5.3 feet. From birth on, they had 17 chitin spikes on their heads. When a Gazkar was promoted, one of those spikes was burned off his head. When something happened that would lead to a degradation of any kind, the Gazkar concerned was expected to honourably commit suicide. For clothes, the Gazkar wore only different kinds of belts, which they used to transport laser weapons. They also possessed a self-unfolding escape capsule enabling them to quickly leave their space shuttle in case of an emergency.

Perry Rhodan (1996), *Warrior of Gazkar*
Translated from the German by Dominika Berger

He heaved a deep sigh, downed another glass of vodka and laughed hysterically: “I can tell you that I learned a lot about entomology in those six months. I still remember the Latin words for ladybirds, for poplar leaf beetles and for alder leaf beetles, both the blue and the green variety. On a single day, a Friday, fifty, no, fifty-two letters with crushed ladybirds arrived, thirty with poplar leaf beetles and at least twenty with alder leaf beetles. The clerks almost got cramps in their right arms and the secretary showed signs of entomological persecutory delusions. For my part, I had a feeling as if all tables and chairs had six legs and four wings, two made out of chitin and two membranous wings.”

Herrmann Löns (1866−1914), *The Appropriate Meyer and Other Stories*, Chap. 21
Translated from the German by Dominika Berger

Blushing and a little shy, the young girl said nothing, she was disturbed by the presence of this man whose thoughts she feared.

When the lobster was served, Caesar declared: “This is a fellow whose acquaintance I would gladly make.” Lesable, smiling, said a writer had once called lobsters the “Cardinals of the sea”, not knowing that these animals are black before being cooked.

Cachelin laughed with all his might and repeated constantly: “Ah! Ah! This one is funny”.

Guy de Maupassant (1850−1893), *The Heritage*
Translated from the French by Dominika Berger

“You ought to go on a holiday to the sea and do some bathing. It’s an excellent thing. And above all, eat shellfish, lots of shellfish. Nothing but shellfish.”

Monsieur Chabre’s hopes rose.

“Shellfish, doctor?” he asked eagerly. “Do you think that shellfish….?”

“Yes, I do indeed! There’s strong evidence of the success of that treatment. So you must understand, every day you eat oysters, mussels, calms, sea-urchins, not forgetting crayfish and lobsters!”

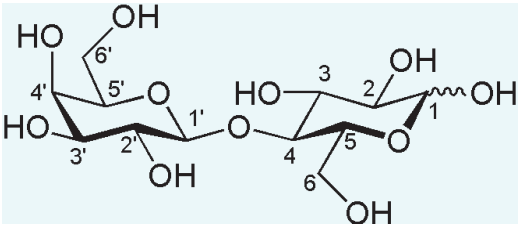
Then, just as he was standing in the doorway ready to leave, he added casually:

"Don't bury yourself in some out-of-the-way place. Madame Chabre is young and needs entertainment… Go to Trouville, it's full of ozone."

Emile Zola (1840−1902), *Shellfish for Monsieur Chabre*
Translated from the French by Douglas Parmée

4.2 Lactose

Answers

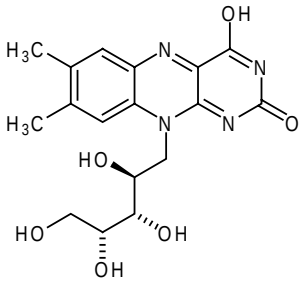


Scheme 42-3

A. Probably it is of polyolic nature. Some examples are glycerol, sugar alcohols (e.g. sorbitol, mannitol, galactitol, xylitol), monosaccharides, disaccharides, some glycosides, such as stevioside, or the polysaccharide maltodextrin.

B. The reason seems to lie in the thermodynamic stability. Only these sugars are able to form conformations with at most one axial OH group and all others in an equatorial position. That they are formed in the D- and not in the enantiomeric L-series (which would have the same enthalpy of formation) is due to the chirality of the enzymes making them and does not influence this statement.

C. Riboflavin has a strong *para*-quinoid chromophore, responsible for the light absorption in the visible region. It is the central component of the cofactors FAD and FMN and as such required by all flavoproteins. Furthermore, it can be regarded as an N-glycoside, namely an N-riboside. This feature makes it water soluble.



Scheme 4.2-4 Riboflavin

D. Very unlikely.

E. Doubling of the reaction rate.

F. The α-form due to the preference for the equatorial position of the methyl group.

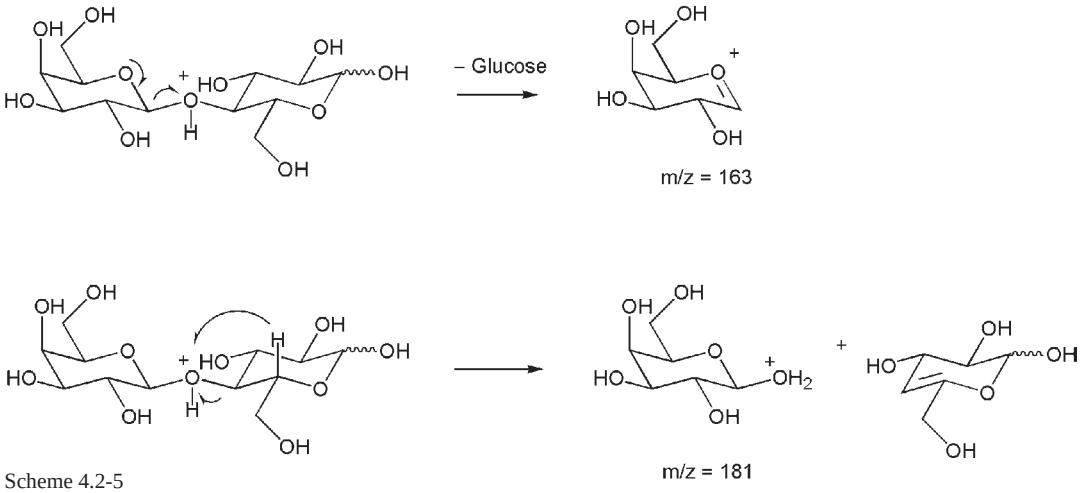
G. No, CD spectra need chirality and a chromophore.

H. Small coupling between H-3 and H-4, and between H-4 and H-5, therefore no effective transmission.

I. The diagonal signal at 3.52 ppm comes from H-2' of the galactose unit, the diagonal signal connected via a positive cross peak at 3.64 ppm stems from H-4 of the α- and β-glucose units. These are in equilibrium, but still interchanging, and thus the NOE cross peak H-2'−H-4 is exchange modulated and hence positive.

J. Signal breakthrough from $^{1}J_{C,H}$ due to the large spin coupling constant of anomeric carbon atoms.

K. Assuming protonation at the bridging oxygen, one can envisage the formation of a stable oxonium ion and of protonated galactose.



Scheme 4.2-5

Translations

Fig. 4.2-26

For cheese (since we used it as an example) does not prove equally injurious to all men, for there are some who can take it to satiety without being hurt by it in the least, but, on the contrary, it is wonderful what strength it imparts to those it agrees with; but there are some who do not bear it well, their constitutions are different, and they differ in this respect, that what in their body is incompatible with cheese, is roused and put in commotion by such a thing; and those in whose bodies such a humour happens to prevail in greater quantity and intensity, are likely to suffer the more from it. But if the thing had been pernicious to man, it would have hurt all. Whoever knows these things will not suffer from it.

Hippocrates (460–370 BC), *Ancient Medicine*, 20
Translated from the Ancient Greek by Franziska Berger

About milk. When a woman has conceived the semen of a man, and if this is growing in her, then, by a natural power, the blood of the women will be drawn up to her breasts. What from food and drink should have become blood, will be turned into milk. From this the child will be nourished who grows in her belly. As the child grows in the uterus of the mother, the milk in her breasts will be augmented that from this the child will be nourished.

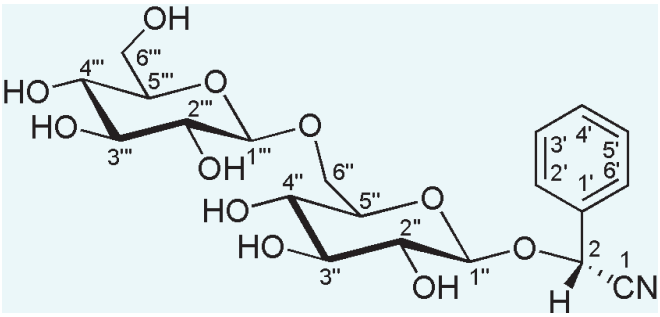
Hildegard Bingensis, (1098−1178), *Causae et Curae*, Lib. II
Translated from the Latin by Franziska Berger

I lived a quiet, harmless life – My shaft
Was only aimed at forest animals,
My thoughts were absolutely free of murder –
Thou hast aroused me from my peaceful state,
Into a seething dragon's poison hast
Thou turned the milk of my good disposition,
Thou hast accustomed me to monstrous things –
Who took aim at the head of his own child,
Can just as well strike at the heart o' th' foe.

Friedrich Schiller (1759–1805), *Wilhelm Tell*, IV, 3
Translated from the German by William F. Wertz, Jr.

4.3 Amygdalin

Answers



Scheme 4.3-7

A. Phenylalanine

B. Emulsin first cleaves the disaccharide into the monosaccharide prunasin, which is then cleaved to the cyanohydrin of benzaldehyde.

C. The second glucose acts as a protecting group as typically used in organic synthesis. Thus HCN can only be set free at the final storage location.

D. Only the benzene–acetonitrile moiety contains π-electrons which act as UV chromophores.

E. The two enantiomers of the aglucone benzaldehyde cyanohydrin would give mirror image-like CD spectra. However, the determination of the absolute configuration from one spectrum alone will be difficult. (2*R*)- and (2*S*)-amygdalin, however, would behave like diastereomers, and their spectra would not form perfect mirror images. Due to the consequences from answer D, it is nevertheless to be expected that in this case at least Cotton effects should be at opposite sides of the baseline.

F. –C≡C–, –C=C=O–, –C=C=C–.

G. Exchange with residual humidity.

H. For all other carbon atoms the substitution in the first and second binding shells is very similar. Only C-6''' has a hydrogen instead of a carbon atom in β-position.

I. One of the protons at C-6'' becomes coaxial with H-3'' and therefore an NOE contact is to be expected.

J. The protons at C-6'' are in a distinctly different stereochemical situation due to the second glucose unit, whereas the two protons at C-6''' both "look" more freely to the solvent.

Translations

Fig. 4.3-19

It was inevitable: the scent of bitter almonds always reminded him of the fate of unrequited love. Dr. Juvenal Urbino noticed it as soon as he entered the still darkened house where he had hurried on an urgent call to attend a case that for him had lost all urgency many years before. The Antillean refugee Jeremiah de Saint-Amour, disabled war veteran, photographer of children, and his most sympathetic opponent in chess, had escaped the torments of memory with the aromatic fumes of gold cyanide.

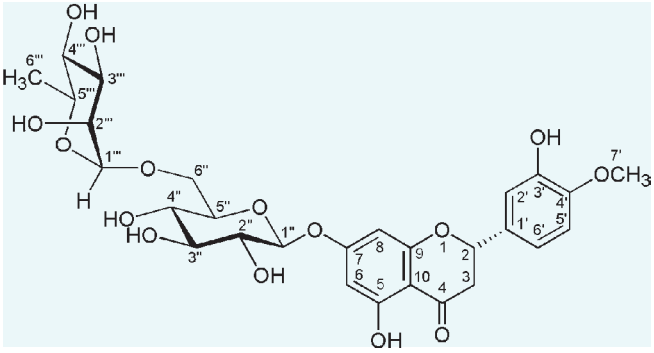
Gabriel García Márquez (1928−), *Love in the Time of Cholera* (1985)
Translated from the Spanish by Edith Grossman

The behaviour of amygdalin and of the white cheese-like part of the almonds raises great interest if one remembers that the presence of amygdalin in almonds is dependent on the accidental location of the tree. Botanists have not found differences between two trees from which one produces sweet and the other bitter almonds. Cases are known, where the relocation of a tree caused it to produce sweet almonds although it earlier gave bitter almonds. This is an interesting example of the influence which certain parts of the ground exert to the life processes of plants.

Justus von Liebig (1803−1873), *Chemical Letters* XVIII
Translated from the German by Stefan Berger

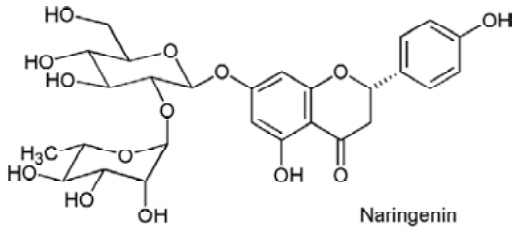
4.4 Hesperidin

Answers

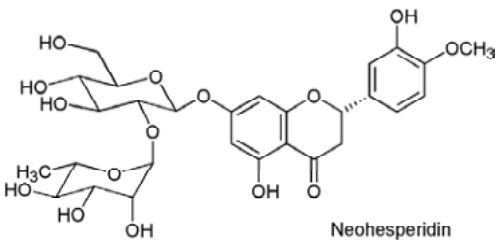


Scheme 4.4-7

A.



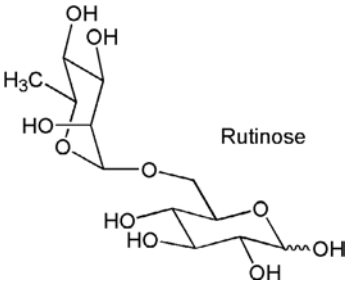
Naringenin



Neohesperidin

Scheme 4.4-8

B. Yes, rutinose [6-*O*-(α-L-rhamnopyranosyl)-D-glucose] is a reducing disaccharide because it contains a cyclic hemiacetal in the glucose moiety (see formula), which can open to a reducing hydroxyaldehyde form as a structural feature.



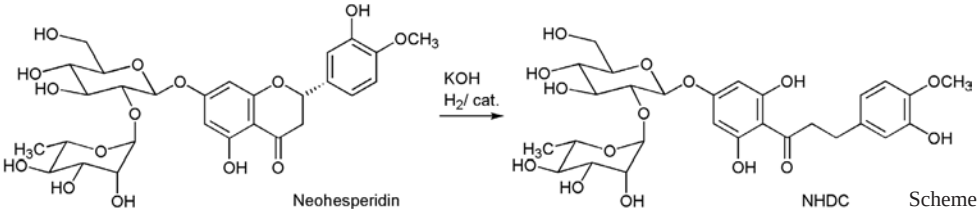
Scheme 4.4-9

C. Cellobiose: D-glucose + D-glucose; as β-D-glucopyranosyl-(1→4)-D-glucopyranose.
Maltose: D-glucose + D-glucose; as α-D-glucopyranosyl-(1→4)-D-glucopyranose.
Saccharose (sucrose): D-glucose + D-fructose; as α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside.
Lactose: D-galactose + D-glucose; as β-D-galactopyranosyl-(1→4)-D-glucopyranose.
Trehalose: D-glucose + D-glucose; as α-D-glucopyranosyl-(1→1) α-D-glucopyranoside.

D. The reducing ones are cellobiose, maltose and lactose for the hemiacetal feature mentioned above. In saccharose (sucrose) and trehalose, two hemiacetals have been linked together, a reaction whereby the reducing feature disappears and an acetal is formed.

E. Cellobiose and maltose are diastereomers. The only difference is that cellobiose has a β-link whereas maltose has an α-link in the D-glucopyranosyl-(1→4)-D-glucopyranose unit.

F. The 3-hydroxy-4-methoxyphenyl substituent attached to C-2 is part of a (substituted) benzyl ether subunit including the ring O atom. In analogy with a benzyl ether protecting group, it is cleaved on hydrogenation. Here, as a dihydro chalcone structure NHDC is the result.



Scheme 4.4-10

G. Phenol-type π→π* transition.

H. Positive signal from undeuterated DMSO; due to the isotope effect, the deuterated DMSO is more shielded than the parent DMSO.

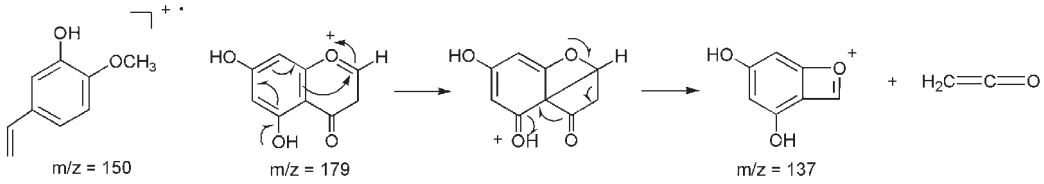
I. The proton at 4.426 ppm displays a small NOE correlation signal to H-4", therefore it should be placed towards this proton.

J. Both experience the shielding β-effect of an aromatic carbon atom in the β-position to the oxygen substituent.

K. The axial−equatorial spin coupling constant is typically about 3 Hz, hence it may not necessarily be detected in a standard COSY spectrum; the distance of the vicinal axial−equatorial relationship is sufficient for an NOE interaction.

L. One needs a chiral recognition reagent. We propose to hydrolyse the glycosidic bond and then to esterify OH-7 with the Mosher reagent.

M.



Scheme 4.4-11

Translation

Knowest thou where the lemon blossom grows,
In foliage dark the orange golden glows,
A gentle breeze blows from the azure sky,
Still stands the myrtle, and the laurel, high?
Dost know it well?
'Tis there! 'Tis there
Would I with thee, oh my beloved, fare.

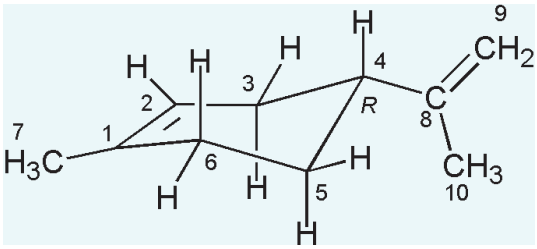
Johann Wolfgang Goethe (1749−1832), *Mignon*
Translated from the German by Walter Meyer

Fig. 4.4-26

Chapter 5 Terpenoids

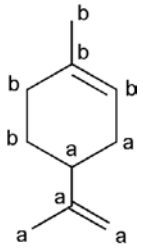
5.1 Limonene

Answers



Scheme 5.1-6

A.



Scheme 5.1-7

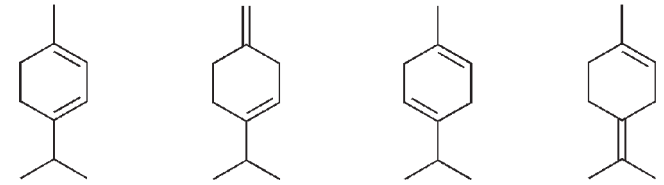
B. It is a laboratory experience that unsaturation and/or a cyclic structure change the dissolution abilities of hydrocarbons appreciably compared with the corresponding *n*-alkanes. Thus, cyclohexane is a better solvent for slightly polar compounds than hexane. Benzene is a distinctly better solvent for many polar compounds than hexane or cyclohexane. However, it was removed from daily use owing to its high toxicity. Alkenes such as dipentene are in the middle. Clearly, dipentene ($C_{10}H_{16}$) will be a better solvent than decane ($C_{10}H_{22}$). Double bonds introduce a dipole moment and polarizability into the molecule and this is connected with its solvation properties.

C. Rubber made from rubber latex of the Pará rubber tree (*Hevea brasiliensis*) and gutta-percha from the latex of Palaquium gutta trees are two natural polyisoprenes. Structurally, rubber consists of *cis*-1,4-polyisoprene with a molecular weight of about 2 MDa. In contrast, gutta-percha is *trans*-1,4-polyisoprene with a much smaller molecular weight. Due to the different structures, the properties of the two materials are also different. It is notable that it was gutta-percha which, due to its more rigid properties, was used as an isolator for the first transatlantic submarine telegraph cable that was laid in 1858 between Ireland and Newfoundland.

D. Mineral oil distillates such as cleaner's naphtha are rich in alkanes whereas dipentene is a diene. For microbial degradation, which is of course possible in both cases, the demand is much greater with alkane mixtures. The structural reason is that at the beginning of metabolism rather unreactive and stable C–H bonds have to be activated to obtain polar biodegradable derivatives. In the case of dipentene, two double bonds exist which are places of higher reactivity due to the comparatively weak π-bonds in this molecule. Microbial degradation follows a different pathway in this case.

E. It is likely that hydrophobic components of the skin's cell membranes are dissolved by dipentene in the same manner as one expects from the intrinsic cleaning operation intended, e.g. in household use. Disturbance of this biological structure may cause skin irritation or even sensitization, especially with long-term use.

F.



α-Terpinene β-Terpinene γ-Terpinene Terpinolene Scheme 5.1-8

α-Terpinene is most stable due to the release of energy on formation of the conjugated 1,3-diene system. In the other three isomers the double bonds are isolated and hence unstabilized.

G. CH deformation (bending motion).

H. We do not have a simple answer to this question!

I. MM2 in ChemDraw places the methyl group coaxial with H-3a and H-5a.

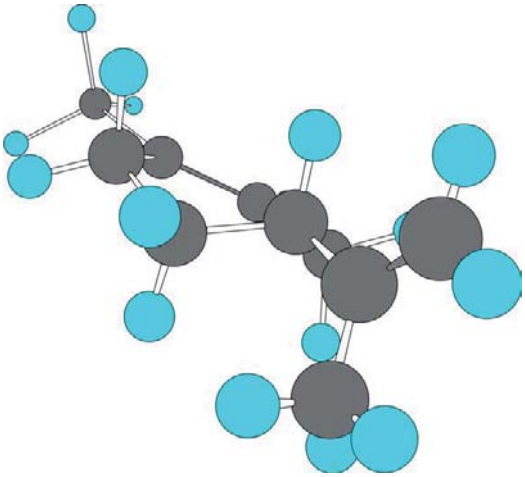
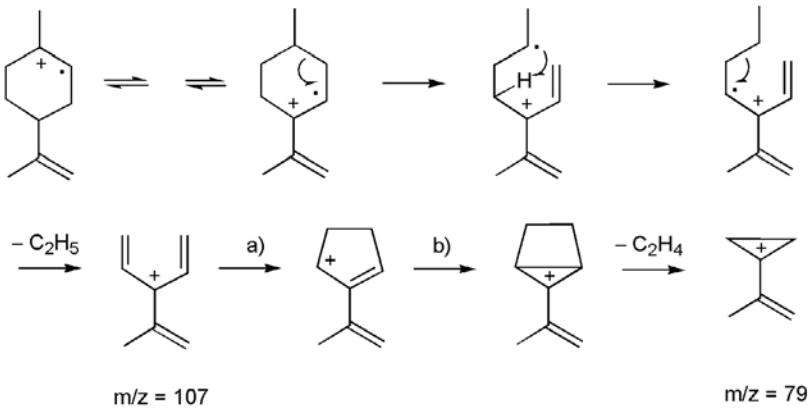


Fig. 5.1-22

J.



Scheme 5.1-9

(a) Allowed conrotatory electrocyclization of a pentadienyl cation.
(b) Allowed disrotatory electrocyclization of an allyl cation.

Translations

Fig. 5.1-23

Media yields
The bitter juices and slow-lingering taste
Of the blest citron-fruit, than which no aid
'Comes timelier, when fierce step-dames drug the cup
With simples mixed and spells of baneful power,
To drive the deadly poison from the limbs
Large the tree's self in semblance like a bay,
And, showered it not a different scent abroad,
A bay it had been; for no wind of heaven
Its foliage falls; the flower, none faster, clings;
With it the Medes for sweetness lave the lips,
And ease the panting breathlessness of age.

P. Vergilius Maro (70–19), *Georgica*, II, 126−131
Translated from the Latin by J. B. Greenough

The citron tree, called the Assyrian, and by some the Median apple, is an antidote against poisons.The leaf is similar to that of the arbute, except that it has small prickles running across it. As to the fruit, it is never eaten, but it is remarkable for its extremely powerful smell, which is the case, also, with the leaves; indeed, the odour is so strong, that it will penetrate clothes, when they are once impregnated with it, and hence it is very useful in repelling the attacks of noxious insects. The tree bears fruit at all seasons of the year; while some is falling off, other fruit is ripening, and other, again, just bursting into birth. Various nations have attempted to naturalize this tree among them, for the sake of its medical properties, by planting it in pots of clay, with holes drilled in them, for the purpose of introducing the air to the roots; and I would here remark, once for all, that it is as well to remember that the best plan is to pack all slips of trees that have to be carried to any distance, as close together as they can possibly be placed.

Pliny the Elder (23−79), *Naturalis Historia Liber*, XII
Translated from the Latin by John Bostock

At sunset on the shores of gulfs to breathe in the perfume of lemon trees; then in the evening on the villa-terraces above, hand in hand to look at the stars, making plans for the future. It seemed to her that certain places on Earth must bring happiness, as a plant peculiar to the soil, and that cannot thrive elsewhere.

Gustave Flaubert (1821−1880), *Madame Bovary*, Chap. 7
Translated from the French by Eleanor Marx Aveling

From blossoms
released
by the moonlight,
from an
aroma of exasperated
love,
steeped in fragrance,
yellowness
drifted from the lemon tree,
and from its plantarium
lemons descended to the Earth.

Tender yield!
The coasts,
the markets glowed
with light, with
unrefined gold;
we opened
two halves
of a miracle,
congealed acid
trickled
from the hemispheres
of a star,
the most intense liqueur
of nature,
unique, vivid,
concentrated,
born of the cool, fresh
lemon,
of its fragrant house,
its acid, secret symmetry.

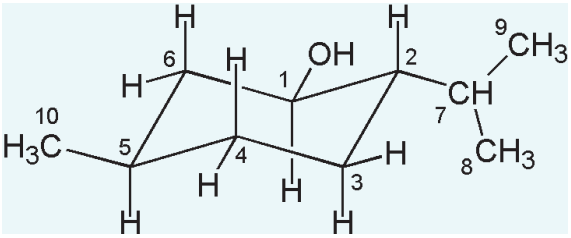
Knives
sliced a small
cathedral
in the lemon,
the concealed apse, opened,
revealed acid stained glass,
drops
oozed topaz,
altars,
cool architecture.

So, when you hold
the hemisphere
of a cut lemon
above your plate,
you spill
a universe of gold,
a yellow goblet
of miracles,
a fragrant nipple
of the Earth's breast,
a ray of light that was made fruit,
the minute fire of a planet.

Pablo Neruda (1904–1973) *Oda al Limón*
Translated from the Spanish by M. S. Peden

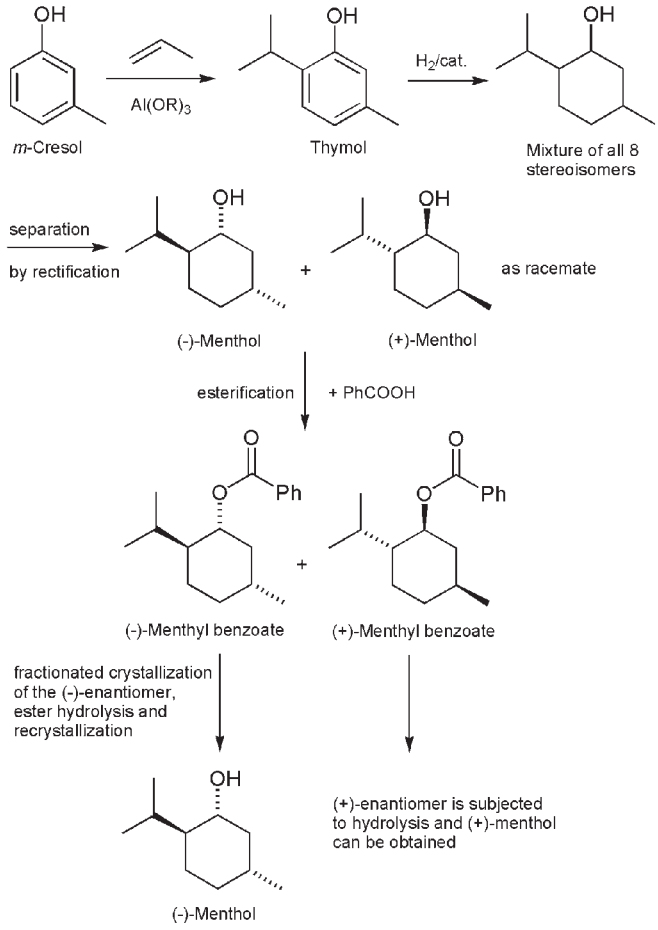
5.2 Menthol

Answers



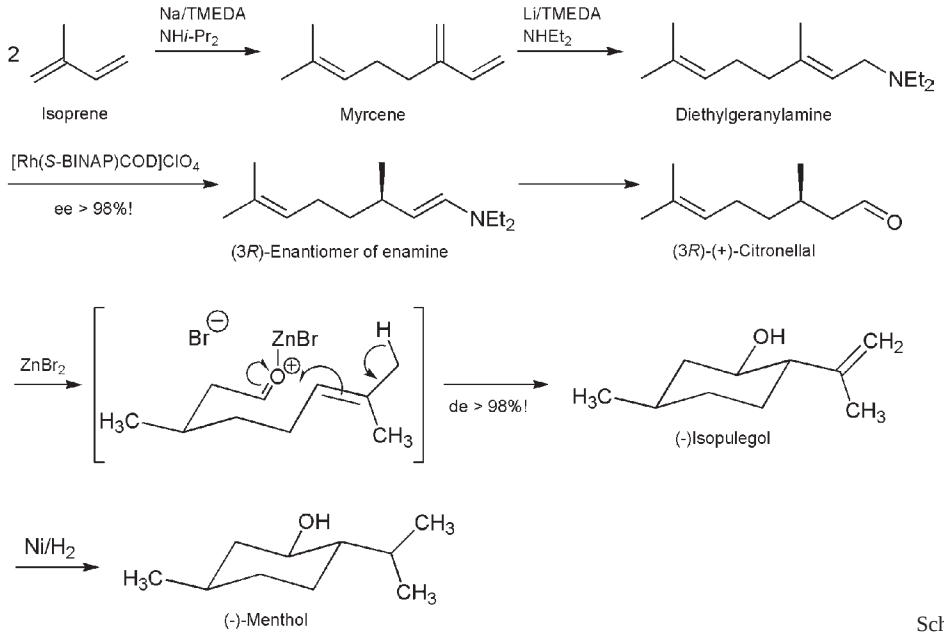
Scheme 5.2-7

A.



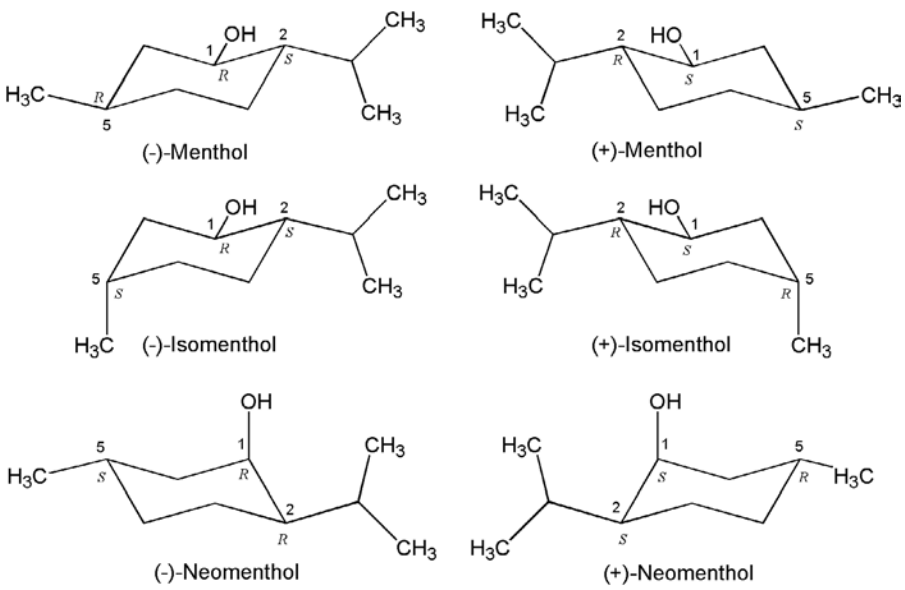
Scheme 5.2-8

B.



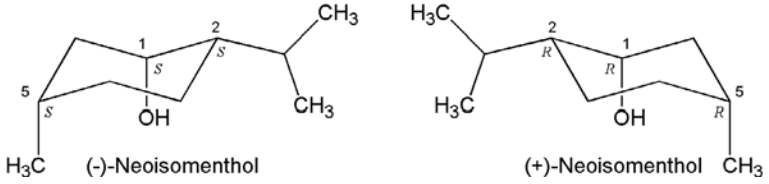
Scheme 5.2-9

C.



Scheme 5.2-10

alternative chair conformations for both Neomenthols governed by ring anchor effect of the i-Pr group



Scheme 5.2-11

D. (–)-Menthol: (1*R*,2*S*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexanol.
(+)-Menthol: (1*S*,2*R*,5*S*)-5-methyl-2-(1-methylethyl)cyclohexanol.
(–)-Isomenthol: (1*R*,2*S*,5*S*)-5-methyl-2-(1-methylethyl)cyclohexanol.
(+)-Isomenthol: (1*S*,2*R*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexanol.
(–)-Neomenthol: (1*R*,2*R*,5*S*)-5-methyl-2-(1-methylethyl)cyclohexanol.
(+)-Neomenthol: (1*S*,2*S*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexanol.
(–)-Neoisomenthol: (1*S*,2*S*,5*S*)-5-methyl-2-(1-methylethyl)cyclohexanol.
(+)-Neoisomenthol: (1*R*,2*R*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexanol.

E. By polarimetry, only. In no case can this information be taken from a formula drawing or configurational description.

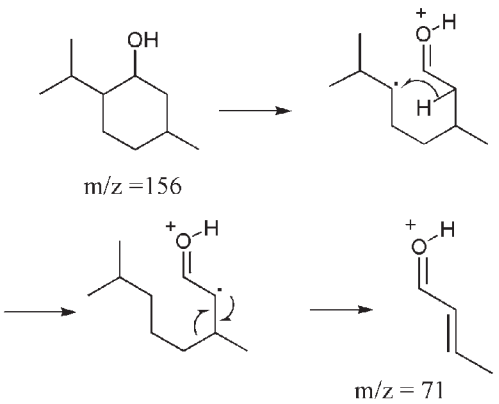
F. Biological receptors are inherently chiral. The interaction with different enantiomers of a chiral compound is therefore a diastereomorphic situation and hence leads to different type of biological signalling.

G. $^{3}J_{\text{H-2,H-7}}$ is a relatively small spin coupling constant and both proton signals form broad multiplets. Therefore, the intensity for a normal COSY plot is not sufficient, but by enhancement the cross peak can be made visible.

H. H-1 shows ^{2}J correlations to C-6 and C-7, ^{3}J correlations to C-3, C-7 and C-5; H-7 shows ^{2}J correlations to C-8, C-9 and C-2, ^{3}J correlations to C-1 and C-3; H-6e shows ^{2}J correlations to C-1 and C-5, ^{3}J correlations to C-2 and C-4; H-3e shows ^{2}J correlations to C-2 and C-4, ^{3}J correlations to C-1 and C-5; H-5 shows ^{2}J correlations to C-4, C-6 and C-10; H-2 shows ^{2}J correlations to C-1, C-3 and C-7, ^{3}J correlations to C-8 and C-9.

I. Menthol is chiral, but lacks a significant chromophore in the UV spectrum. Therefore, an interpretable CD spectrum is not expected.

J.



Scheme 5.2-12

Translations

Fig. 5.2-28

Woe to you Pharisees, because you give God a tenth of your mint, rue and all other kinds of garden herbs, but you neglect justice and the love of God. You should have practiced the latter without leaving the former undone.

New Testament, Luke 11,42

Now as the Gods reclined, the good old dame,
whose skirts were tucked up, moving carefully,
for so she tottered with her many years,
fetched a clean table for the ready meal −
but one leg of the table was too short,
and so she wedged it with a potsherd − so
made firm, she cleanly scoured it with fresh mint.

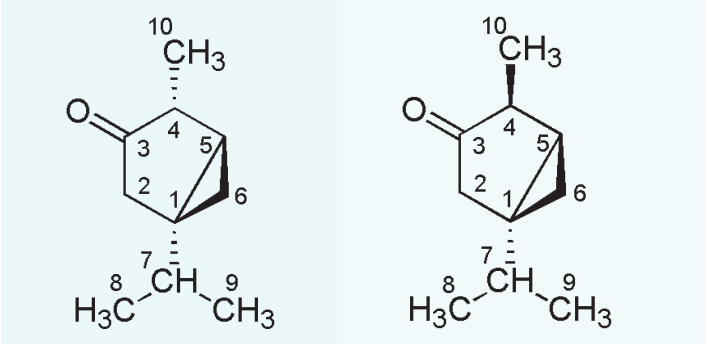
P. Ovidius Naso (43 BC–17 AD), *Metamorphoses* VIII, 660 (Philemon and Baucis)
Translated from the Latin by Arthur Golding

I have therefore experimented myself with peppermint. I distilled the fresh plant in the previous year. The distilled water was very aromatic, there was plenty of the etheric oil, but nothing of solid campher. I have separated the oil from the water and stored both the oil and the saturated water, each in own vials, on a cool place for a full year in the hope that campher would become solid in one of them. However, the hope failed, nothing crystalline appeared. I hardly find any other reason for this, if I had not used the green plant. It is by chance also a fact that no Chemist whom I know, recollects having obtained campher by distillation from this species of mint, although the water and the essence of it has been often used on many places in recent years for medical purpose.

H. D. Gaudius, *Adversariorum Varii Argumenti*
Liber unus, Caput VII, Leiden 1771 (Original found in the University Library, Leipzig)
Translated from the Latin by Stefan Berger

5.3 Thujones

Answers



Scheme 5.3-5

A. (i) Chemical shift of H-4: the higher shielding in **S** suggests the *endo*-situation bond, supported by the shielding effect of the cyclopropane ring.

(ii) Molecular modelling predicts a dihedral angle between proton H-4 and H-5 near 90° in **S**, thus no additional spin coupling in **S**.

(iii) ^{13}C chemical shifts of C-10 and C-6: higher shielding means higher steric congestion, which is present in **W**.

(iv) *endo*-H-6 displays an NOE cross peak to methyl group signal H-10 in **W** but not in **S**.

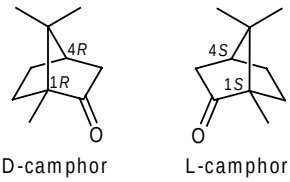
(v) In contrast to **S**, an NOE cross peak between H-5 and H-4 is present in **W**.

The sum of these arguments decides **S** = α-thujone and **W** = β-thujone.

B. In principle $2^3 = 8$. However, for reasons of ring strain, the cyclopropane ring cannot exist in other configurations than 1*S*,5*R* or 1*R*,5*S*. This gives rise to two pairs of enantiomers, i.e. the natural products discussed here and their enantiomers. The four compounds with configurations 1*S*,5*S* or 1*R*,5*R* cannot exist.

C. The protons attached to C-2 and C-4 are the most acidic ones in these molecule due to the adjacent carbonyl group. In principle, enolization can take place in both directions. Re-formation of the keto form leads to the initial product if enolization has taken place with the CH_2 unit regardless of α- or β-thujone as starting material. However, if enolization has affected the proton H-4 there are two possibilities for re-formation of a keto form, one leads to the initial thujone and the other to its diastereomer.

D.



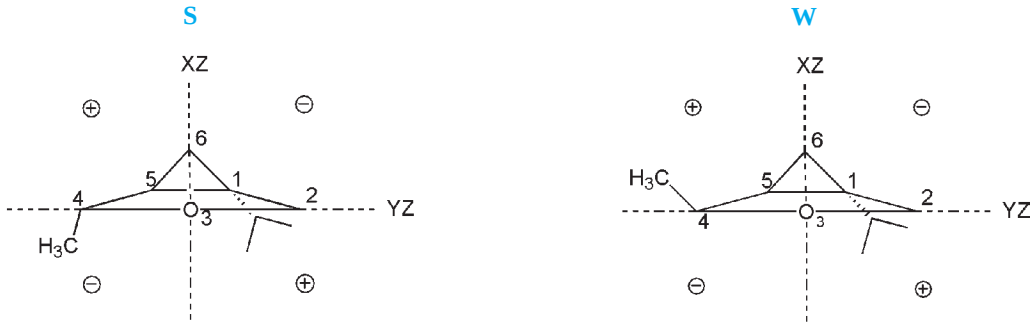
D-camphor L-camphor

Scheme 5.3-6

E. For two non-miscible compounds a and b, the partial vapor pressures p_a and p_b are additive. If compound b is water, its vapor pressure at about 100 °C is equal to the atmospheric pressure p_{atm}. Thus, compound a will also distil, since $p_a + p_b = p_{atm}$, which leads to a kind of vacuum distillation at moderate temperature, but without vacuum.

F. The octant rule is valid for the n→π* transitions of saturated ketones. Substituents far away from the C=O group have only a minor influence. In the examples given, the isopropyl group placed in the β-position

near the central plane will have for both isomers the same rather small influence. The methyl group in the α-position to the carbonyl will contribute to a negative CD effect in **S** and to a positive CD effect in **W**.



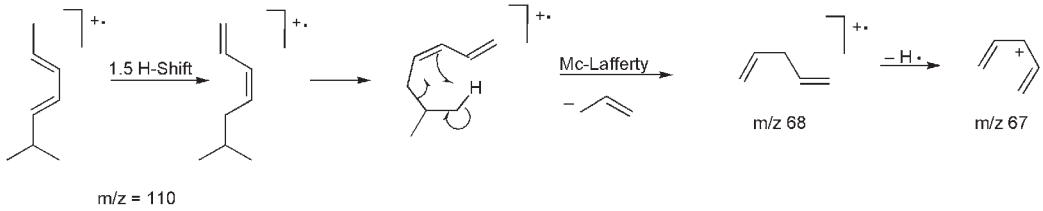
Scheme 5.3-7

G. In both compounds the methyl group signals H-9 show a stronger NOE effect to H-6_{exo}, whereas the methyl group signals H-8 show a stronger NOE effect to H-2_{exo}.

H. In both compounds the dihedral angle between H-2_{endo} via C-2, C-1 to C-5 was calculated by molecular modelling to be about −130°, whereas that of H-2_{exo} is close to 100°. According to the Karplus equation, a spin coupling $^3J_{\text{H-2}exo,\ \text{C-5}}$ is therefore less likely. The same is true for $^3J_{\text{H-2}exo\ \text{or H-2}endo\ \text{to C-8}}$. In both compounds the d*ih*edral angle H-2_{exo} to C8 is −37° but the dihedral angle H-2_{endo} to C-8 is close to 90°.

I. After an MM2 optimization, the dihedral angle H-4−C-4−C-5−H-5 is shown to be 88° in **S**, but 45° in **W**, thus due to the Karplus equation no $^3J_{\text{H-4, H-5}}$ is expected for **S**.

J. Minor loss of a methyl group and of CO.



Scheme 5.3-8

Translations

Fig. 5.3-27

The third angel blew his trumpet, and a great star fell from heaven, blazing like a torch, and it fell on a third of the rivers and on the springs of water. The name of the star is Wormwood. A third of the waters became wormwood, and many people died from the water, because it had been made bitter.

New Testament, Book of Revelation, 8, 10−11

Is not without a reasonable ground:
But as physicians, when they seek to give
Young boys the nauseous wormwood, first do touch
The brim around the cup with the sweet juice
And yellow of the honey, in order that
The thoughtless age of boyhood be cajoled
As far as the lips, and meanwhile swallow down
The wormwood's bitter draught, and, though befooled,
Be yet not merely duped, but rather thus
Grow strong again with recreated health.

Titus Lucretius Carus (99–55), *De Rerum Natura*, I, 935–950

Why should a man die, in whose garden sage is growing?

Coat of Arms, Medical School of Salerno (1100–1300)

Sage

There in the front glows sage, sweetly scented.
It deserves to grow green for ever, enjoying perpetual youth;
For it is rich in virtue and good to mix in a potion,
Of proven use for many a human ailment.
But within itself is the germ of civil war;
For unless the new growth is cut away, it turns
Savagely on its parent and chokes to death
The older stems in bitter jealousy.

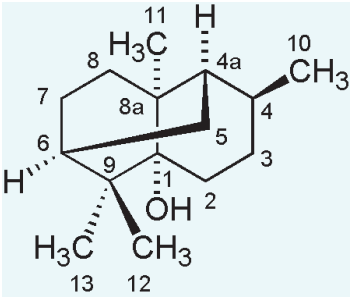
Walahfrid Strabo (808–849), *Hortulus*,
Translated from the Latin by R. Payne

But man doth not live by bread alone, but
also by the flesh of good lambs, of which I have two:
These shall we slaughter quickly, and cook spicily with sage: it is so
that I like them. And there is also no lack of roots and fruits, good
enough even for the fastidious and dainty, nor of nuts and other riddles
for cracking.

Friedrich Nietzsche (1844–1900), *Thus Spoke Zarathustra*, LXXII, The Supper
Translated from the German by Thomas Common

As to its general utility, a plant so commonly found and applied to such numerous uses, people are universally agreed; but with the Romans more particularly it has been always held in the highest esteem, from the fact of its being employed in their religious ceremonials. Thus, for instance, upon the Latin Festival, it is the custom to have a race of four-horsed chariots in the Capital, and for the conqueror to be presented with a draught of wormwood; from the circumstance, no doubt, that our forefathers were of opinion that good health was the most valuable reward they could bestow upon his skill. This plant is very strengthening to the stomach, and hence it is that wines are flavoured with it, as already stated.

Pliny the Elder (23–79), *Naturalis Historia Liber XXVII*, 28
Translated from the Latin by John Bostock

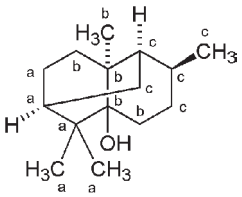


Scheme 5.4-6

5.4 Patchouli Alcohol

Answers

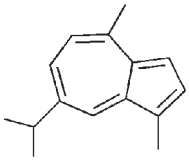
A. C_5-isoprene units are marked with small letters:



Scheme 5.4-7

B. A short answer is: its nature as a sesquiterpene. Therefrom it has 15 C-atoms. This ensures a boiling point that is high enough, in other words a volatility that exists but low enough to last for hours. The OH group present increases the boiling point compared with a pure C_{15} hydrocarbon such as α-patchoulene, $C_{15}H_{24}$. A variety of other esteemed perfume ingredients fulfil the same volatility requirement just by a similar molecular formula, with muscone [(*R*)-3-methylcyclopentadecanone, $C_{16}H_{30}O$] as a prominent example.

C. In contrast to their precursors, azulenes are stabilized by a system of conjugated double bonds. They belong to the class aromatic hydrocarbons although they are non-benzenoid, because they consist of a five-membered ring fused to a seven-membered ring.



Guaiazulene

Scheme 5.4-8

D. Azulene consists of a fused cycloheptatriene and cyclopentadiene ring. Azulene has a 10 p-electron system similar to cyclodecapentaene and therefore has aromatic properties. An azulene molecule can also be considered as a fusion product of a 6 π-electron tropylium cation (aromatic!) with a 6 π-electron cyclopentadienyl anion (also aromatic!). However, both substructures are charged, in contrast to the two benzene rings that result from a similar inspection of naphthalene. Hence the blue colour is explained by a charge transfer between the two rings in azulenes.

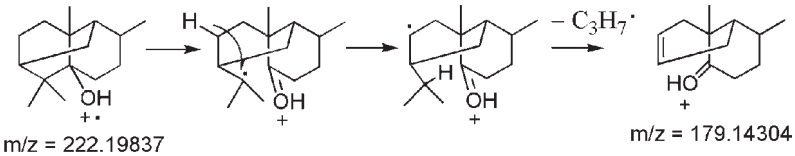
E. The gain in stability from aromaticity in azulene is half that of naphthalene. Therefore, blue azulene rearranges into colourless naphthalene on long-term standing. At the end of the experiment, one has two flasks with naphthalene. An experiment such as this has not been undertaken intentionally, its result has just been observed. The point is not to ignore strange findings such as this because they do not seem to fit in an existing framework of knowledge!

F. A molecular model shows that one of the H-8 protons is nearly exactly parallel with and in close vicinity to the C−O bond, whereas the other one points outwards from the surface of the molecule.

G. The H-2 proton at 1.50 ppm displays a strong NOE cross peak to H-13 and hence points towards the geminal methyl groups. The multiplet at 1.29 ppm, from which the other H-2 proton is a part, displays an NOE cross peak to H-10.

H. (a) 24.6 ppm caused by $^{2}J_{\text{H-8, C-7}}$ or by $^{3}J_{\text{H-5, C-7}}$.
(b) 37.7 ppm caused by $^{2}J_{\text{H-8, C-8a}}$ or $^{3}J_{\text{H-5, C-8a}}$.
(c) 43.8 ppm caused by $^{3}J_{\text{H-8, C-4a}}$ or by $^{2}J_{\text{H-5, C-4a}}$.
(d) 75.7 ppm caused by $^{3}J_{\text{H-8, C-1}}$.

I. The elemental composition of the ion at m/z = 179 was confirmed to be $C_{12}H_{19}O$ = 179.14304 by high-resolution mass spectrometry. A possible mechanism is given below:



Scheme 5.4-9

Translations

Fig. 5.4-17

Here he stopped, gathering his forces, and smelled. He had it. He had hold of it tight. The odour came rolling down the rue de Seine like a ribbon, unmistakably clear, and yet as before very delicate and very fine. Grenouille felt his heart pounding, and he knew that it was not the exertion of running that had set it pounding, but rather his excited helplessness in the presence of this scent. He tried to recall something comparable, but had to discard all comparisons. This scent had a freshness, but not the freshness of limes or pomegranates, nor the freshness of myrrh or cinnamon bark or curly mint or birch or campher or pine needles, nor that of a May rain or a frosty wind or of well water… and at the same time it had a warmth.

Patrick Süskind (1949−), *Perfume, The Story of a Murderer*, Part I
Translated from the German by John E. Woods

I'm watching Adrienne Septmance. She sings, bustles about, runs out onto the street, and laughs aloud, fictitiously. I sense around her the smell of this common perfume one buys here at Maumond's, the hairdresser's, that perfume one senses, it seems, with the tonsils, which makes you think of sweetish horse urine, drying on the streets…

Adrienne, you smell of patchouli!, declared my mother, who never knew what patchouli really was…

Finally, in the kitchen, I meet a young man, black under his white straw hat, sitting against the wall, silently like someone who is there for all the right motives. I exult, while my mother's mood darkens.

Sidonie-Gabrielle Colette (1873−1954), *My Mother's House*, The Wedding
Translated from the French by Dominika Berger

What is Adam? The kingdom of Eve. No 89 for Eve. There has been the royal sceptre surmounted by a fleur-de-lys, there has been the imperial sceptre surmounted by a globe, there has been the sceptre of Charlemagne, which was of iron, there has been the sceptre of Louis the Great, which was of gold, − the revolution twisted them between its thumb and forefinger, ha'penny straws; it is done with, it is broken, it lies on the earth, there is no longer any sceptre, but make me a revolution against that little embroidered handkerchief, which smells of patchouli! I should like to see you do it. Try. Why is it so solid? Because it is a gewgaw. Ah! you are the nineteenth century? Well, what then? And we have been as foolish as you. Do not imagine that you have effected much change in the universe, because your trip-gallant is called the cholera-morbus, and because your pourree is called the cachuca. In fact, the women must always be loved. I defy you to escape from that.

Victor Hugo (1802−1885), *Les Miserables*, Tome V Jean Valjean, Livre VI, Chapitre II
Translated from the French by Isabel F. Hapgood

On the toilet table the bouquets − roses, lilacs and hyacinths − appeared like a very ruin of flowers. Their perfume was strong and penetrating, while through the dampish air of the place, which was full of the spoiled exhalations of the washstand, came occasional whiffs of a more pungent scent, the scent of some grains or dry patchouli ground to fine powder at the bottom of a cup. And as she gathered herself together and drew up her dressing jacket, which had been ill fastened, Nana had all the appearance of having been surprised at her toilet: her skin was still damp; she smiled and looked quite startled amid her frills and laces.

Emile Zola (1840−1902), *Nana*, Chap. II
Translator unknown

Calyste arranged a great Gothic chair for her near the window, and opened one of the sashes. Camille Maupin, who shared the oriental taste of her illustrious sister-author, took a magnificent Persian hookah, given to her by an ambassador. She filled the nipple with patchouli, cleaned the bochettino, perfumed the goose-quill, which she attached to the mouthpiece and used only once, set fire to the yellow leaves, placing the vase with its long neck enamelled in blue and gold at some distance from her, and rang the bell for tea.

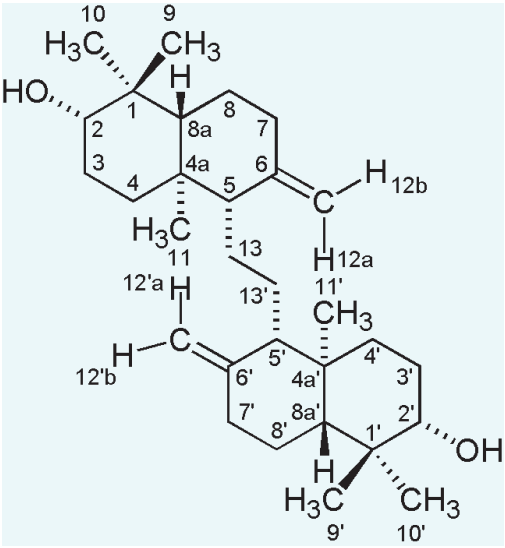
Honoré de Balzac (1799−1850), *Beatrix*, VII
Translated from the French by Katharine Prescott Wormeley

The first thing that struck him as he went into the entrance hall was a scent of patchouli, always distasteful to him; there were some high travelling-trunks standing there. The face of his groom, who ran out to meet him, seemed strange to him. Not stopping to analyse his impressions, he crossed the threshold of the drawing room.... On his entrance there rose from the sofa a lady in a black silk dress with flounces, who, raising a cambric handkerchief to her pale face, made a few paces forward, bent her carefully dressed, perfumed head, and fell at his feet.... Then, only, he recognised her: this lady was his wife!

Ivan Turgenev (1818−1883), *A House of Gentlefolk*, Chap. XXXVI
Translated from the Russian by Constance Garnett

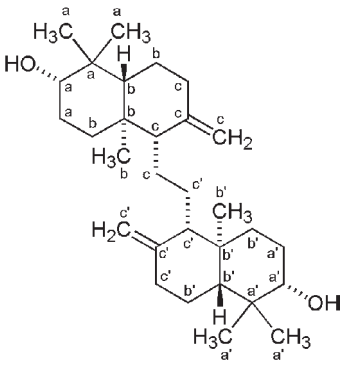
5.5 Onocerin

Answers



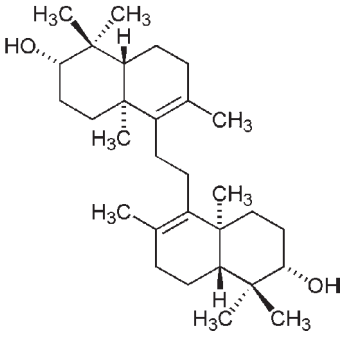
Scheme 5.5-7

A.



Scheme 5.5-8

B.



Scheme 5.5-9

The difference between the double bonds in α-onocerin and β-onocerin is that in the case of an *exo*-methylene group we have two substituents whereas in the latter case there is a tetrasubstitution of the double bond. The thermodynamic stabilization that obviously is possible on rearrangement of the α- to the β-form can be understood with the term hyperconjugation that was introduced by Mulliken. It means that a slight but definite release of energy is possible by interaction of the π-electrons of a double bond with the electrons in the σ-bond of an attached C−H bond. In β-onocerin three of the four substituents attached to the double bond contain such C−H bonds, whereas in α-onocerin their number is only two. This feature makes the difference.

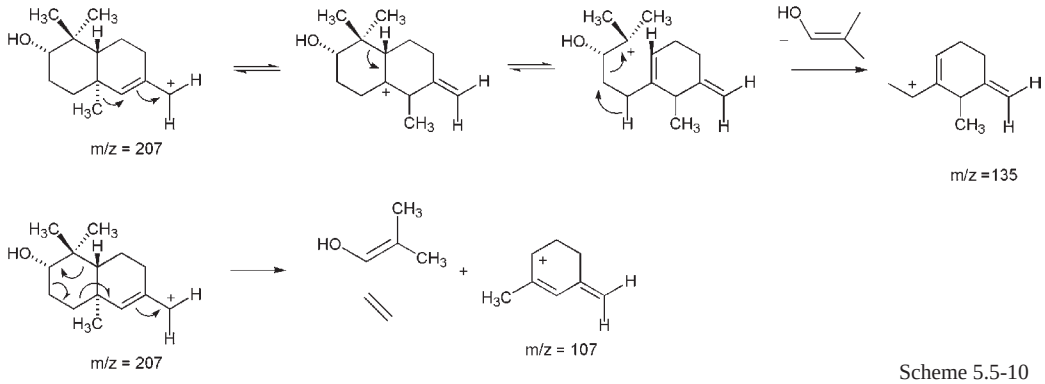
C. Addition of bromine to a solution of each onocerin regioisomer will lead to immediate decoloration.

D. The methyl groups C-11 and C-10 are in a 1,3 relationship on a cyclohexane ring. Their respective proton signals show strong NOE cross peaks. Therefore, they must be in a cisoid position to each other.

E. The protons of the methyl group C-9 do show significant NOE cross peaks to H-2. Therefore, H-2 and H-9 should be in a cisoid position to each other and hence the OH group is transoid to the methyl group.

F. In the HMBC spectrum, one finds from both diastereotopic protons H-13 long-range correlation peaks to C-13, which is according to the HSQC spectrum their own carbon atom. This is only possible if C-13 is doubly present and the observed correlation signals are of the $^{2}J_{HC}$ type.

G.



Scheme 5.5-10

As suggested by Prof. K. P. Zeller, University of Tübingen, the ion with m/z = 207 can rearrange via several steps and finally eliminate C_4H_8O to form the ion at m/z = 135 ($C_{10}H_{15}$ = 135.11683, confirmed by high-resolution MS) as depicted. At the same time, the ion with m/z = 207 can also eliminate C_4H_8O and ethene simultaneously to form the ion with m/z = 107.

Translations

Fig. 5.5-18

The rest harrow, but some call it ononis. The sprays are a span long or even longer, shrubby, and highly articulated; they have many axils, round heads, thin and small leaves resembling closely the leaves of rue or of the lotos that grows in meadows, somewhat hairy, and they are aromatic. It is steeped in brine before it has put out thorns and it is very pleasant. Its branches have strong and sharp thorns that are pointed like pales. It has a warming and white root whose skin removes the urine, shatters stones, and breaks off scabs all around when taken in a drink. Boiled in sour wine, mixed with water, and used as a mouthwash, it assuages toothaches.

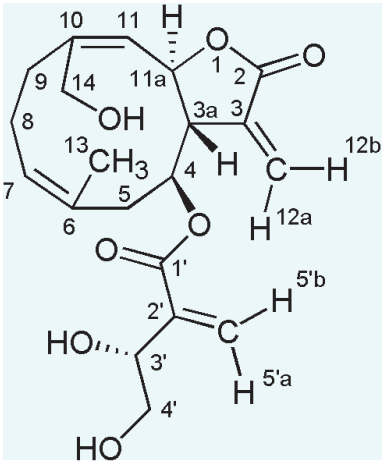
Pedanius Dioscorides (ca. 40–90), *De Materia Medica*, Book III, Chap. 18
Translated from the Ancient Greek by Lily Y. Beck

Tell us, young girl Trandafilya:
Why do you keep getting engaged without ever marrying?
Six rings in three years!
− Oh, my disbelieving friends,
Why won't you believe me?
A dragon has been in love with me for three years now,
I can't get away from him.
− Listen, young girl Trandafilya:
Go to the garden and gather restharrows,
Go to the new market and buy a new pot,
Boil them on the eve of Wednesday,
Drink them on the eve of Friday,
And you will be free of the dragon.

Dimitar Miladinov (1810−1862) and Konstantin Miladinov (1830−1862)
Translated from the Bulgarian by Dominika Berger

5.6 Cnicin

Answers

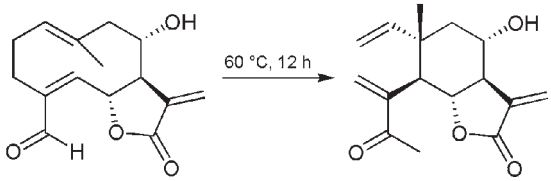


Scheme 5.6-8

A. Limonin, humulon and absinthin are terpenoids, quinine is an alkaloid. Limonin occurs in the kernels of lemons and oranges and is a taste problem when making orange juice. Humulon is a hop constituent necessary for making beer, absinthin is the bitter principle of wormwood, and quinine occurs in cinchona bark and is a classical anti-malaria agent. However, the most widespread application is its use as a bitter compound in tonic waters.

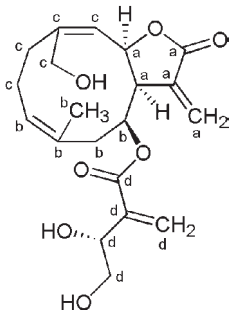
B. The five basic taste sensations are sweet, salty, sour, bitter and the so-called umami (savoriness). Sweetness is caused by sugars or sweeteners, saltiness by minerals such as sodium chloride, sourness by a low pH value as characteristic for acidic solutions such as lemon juice or vinegar, bitterness by bitter substances and savoriness, a taste of meat, by monosodium glutamate, a flavour enhancer.

C.



Scheme 5.6-9

D.



Scheme 5.6-10

E. Lactones usually absorb at higher wavenumbers, which may be due to the smaller bond angle.

F. With the substituents in α, β and γ positions being rather similar, and negligible ring strain within the lactone ring, we feel that the main difference is the cisoid attachment of the terminal methylene group in the lactone and the transoid in the side chain. The transoid arrangement in the side chain will be more apt for electron delocalization and therefore more likely to be somewhat shielded compared with the lactone.

G. Both protons H-9 and H-8 are allylic protons and a certain amount of diastereotopicity is expected from both. In addition, H-9a is, as a molecular model shows, coplanar with the C-14−oxygen bond and this should cause considerable deshielding.

H. The distinction is possible via the NOESY spectrum. The more deshielded resonance of H-14 at 4.07 ppm has a strong NOE contact to H-11a, which is below the ring plane, whereas the more shielded resonance at 3.87 ppm has strong contacts to H-13 and H-9a, which are above the ring plane.

Translations

Fig. 5.6-19

Idleness is the enemy of the soul; and therefore the brethren ought to be employed in manual labour at certain times, at others, in devout reading. Hence, we believe that the time for each will be properly ordered by the following arrangement; namely, that from Easter till the calends of October, they go out in the morning from the first till about the fourth hour, to do the necessary work, but that from the fourth till about the sixth hour they devote to reading. After the sixth hour, however, when they have risen from table, let them rest in their beds in complete silence; or if, perhaps, anyone desireth to read for himself, let him so read that he doth not disturb others. Let None be said somewhat earlier, about the middle of the eighth hour; and then let them work again at what is necessary until Vespers.

If, however, the needs of the place, or poverty should require that they do the work of gathering the harvest themselves, let them not be downcast, for then are they monks in truth, if they live by the work of their hands, as did also our forefathers and the Apostles.

Benedict of Nursia (480−547), *Rule of St. Benedict*, Chap. 48
Translated from the Latin by Boniface Verheyen, OSB

On Blessed Thistle

Description

There is a noble and famous herb called Carduus benedictus, or Blessed Thistle, for the sake of its considerable healing power. It grows in many a garden. It is very hirsute and thick. Its stems are similar to those of the common sow-thistle or of Hare's Colwort. The stems creep along the ground because they are very soft and tender. Those stems produce round woolly flower heads which blossom in a pale yellow. When the blooming period is over, long and pale yellow seeds can be found in the closed flower heads, covered in white hair or wool. The roots also grow rather long, they are tender and succulent. The entire plant is very bitter.

Places of growth

In Insula Lemno, Bellonius describes that Blessed Thistle frequently grows in flat fields. He calls it Garderacantha.

Natural power and effect

Despite its bitterness, Blessed Thistle is of a warm and dry nature. It is proven to be an excellent herb against pestilence and poisoning. It can be used in many different ways, both internally and externally.

Pietro Andrea Mattioli (1501−1577). Herbal book written by the learned and renowned scholar Pietro Andrea Mattioli. With three well-structured and useful indices of the herbs, their Latin and German names, and the medicaments for which they can be used. 1586. University Library, Leipzig
Translated from the German by Franzika and Dominika Berger

The trough was full, and faithful Tray
Came out to drink one sultry day;
He wagg'd his tail, and wet his lip,
When cruel Fred snatch'd up a whip,
And whipp'd poor Tray till he was sore,
And kick'd and whipp'd him more and more;

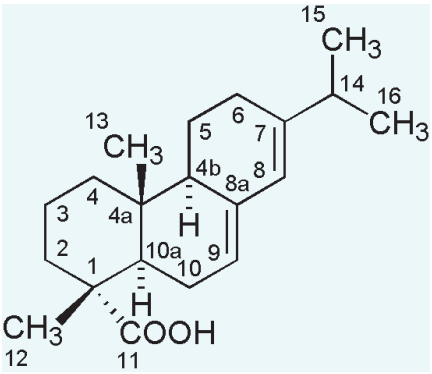
At this, good Tray grew very red,
And growl'd and bit him till he bled;
Then you should only have been by,
To see how Fred did scream and cry!

So Frederick had to go to bed;
His leg was very sore and red!
The Doctor came and shook his head,
And made a very great to-do,
And gave him bitter physic too.

Heinrich Hoffmann (1809−1894), *Struwwelpeter, The Story of Cruel Frederick*
Translator from the German unknown

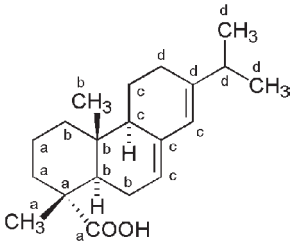
5.7 Abietic Acid

Answers



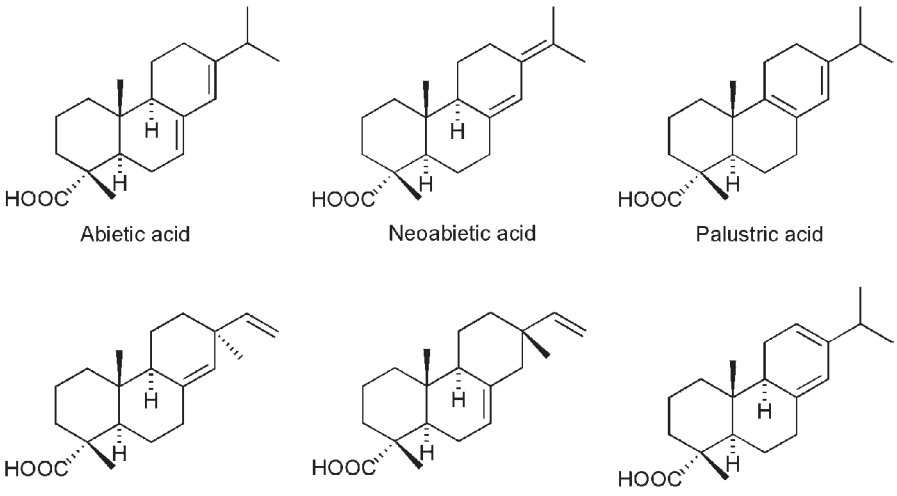
Scheme 5.7-11

A.



Scheme 5.7-12

B.



Scheme 5.7-13

Pimaric acid and isopimaric acid should have the highest enthalpy of formation due to the unconjugated double bonds. For the other isomers a conjugation of two double bonds is found in each case which releases bond energy. It is hard to ascertain which of the four isomers will be the most stable. A method to do this is a molecular modelling calculation (for the gas phase) or an equilibration experiment (as described under Isolation 3.2). The experiment shows that abietic acid is the most stable, although it is not the only resin acid remaining, obviously. As expected, there is a driving force to aromatize the ring with the isopropyl group and thus distinctly stabilize the skeleton; this compound is called dehydroabietic acid.

C. Abietic-type resin acids will be thermodynamically more stable due the conjugation of the two double bonds that releases additional bond energy in comparison with the pimaric-type acids.

D. A surfactant is an amphiphilic structure with a hydrophilic part (may be ionic or neutral as supplied from a sugar moiety in a saponine) and a hydrophobic part with at least ca. 12 C-atoms to bring about the surface-active effect. Resin soaps fulfil this demand. In the human body, the liver synthesizes bile acids, e.g. cholic acid by the oxidation of cholesterol (daily synthesis by the body about 25 g). They are stored in the gallbladder and secreted into the small intestine. Their task is to emulsify macroscopic particles of lipids (fats, oils) and thus ensure their uptake and digestion. Similarly, they allow the body access to all fat-soluble vitamins.

E. Whereas colophony is readily soluble in ethanol, amber is slightly soluble or insoluble therein. This indicates a bonding transformation that had produced large and therefore insoluble macromolecules: polymers. A process that seems possible when looking at the rosin constituents is a slow radical polymerization, with crosslinking as a side reaction. The necessary prerequisite, double bonds, is part of the resin acids structure and other diterpenes of the labdane group. However, it cannot be expected that amber has a regular structure like other natural polyterpenes from plants [caoutchouc (rubber), gutta-percha], because the monomeric starting material for amber is manyfold and in an amorphous glassy state. Finally, the long time for the slow formation of amber is an expression for the low reaction rate, which in turn results from the very high viscosity that prevents efficient diffusion of molecules. Occasional inclusions of prehistoric insects or needles evidence the natural source of amber and are interesting objects to study the biological process of evolution. A material that from its age is “on the way to become amber” is copal, a fossil, but a much younger resinous substance which is used e.g. as incense.

F. The Greek name for amber is ἤλεκτρον (electron). It was connected to the sun god, who was seen as *elector* or *awakener*. This denomination is close to the appeal of light resulting from a pale colour that some amber pieces have when polished. On rubbing, amber shows the strange phenomenon of electrostatic charging (negatively) and is able to attract other small objects. With the understanding of what a negative elementary charge is, its subatomically small carrier acquired the name electron at the end of the 19th century.

G. The most famous place to find amber is in Samland on the southeastern shore of the Baltic Sea, a peninsula in the Kaliningrad region of Russia. The Lithuanian name of the place is Semba. Although amber appears at a few other places in Europe, it was a rare and typically “northern” good for the civilizations in the south, e.g. on the coasts of the Mediterranean Sea and Black Sea. Amber was used to make emblazonments. For the people from the north, amber was a bestseller which could be exchanged against any other valuable thing from the south. A further advantage was that not much capacity for transport was necessary because even small amounts of excellent pieces had a comparably high value.

H. It is best do copy the page of the book and draw the appropriate rectangles with a pencil.

I. H-5 at 1.2 ppm shows an NOE effect to the methyl group H-13; it is therefore the axial proton.

J. These protons apparently are slowly exchanging due to conformational processes in the six-membered rings.

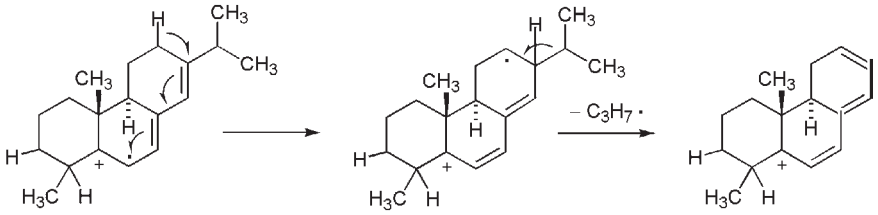
K. The molecular model shows a dihedral angle of about 90° to one of the H-10 protons, hence from the Karplus equation we will not expect a spin coupling.

L. It must be axial, and the cross peak arises from 1,3-diaxial interaction.

M. Both the methine proton of the isopropyl group and at least one methyl group have strong NOE cross peaks to H-8, but not to H-6. It can be assumed, therefore, that H-14 is mainly in the same plane and directed towards H-8.

N. C-10 + C-9 = 146.1, C-10 + C-10a = 70.5, C-6 + C-5 = 50.0, C-6 + C-7 = 172.8, C-10a + C-1 = 91.2, C-10a + C-4a = 79.2. All signals are present at their correct places, which confirms the assignment.

O.



m/z = 256 m/z = 213 Scheme 5.7-14

Fig. 5.7-29

Translations

And they remained there in our land a full year, and got by trade much substance in their hollow ship. But when their hollow ship was laden for their return, then they sent a messenger to bear tidings to the woman. There came a man, well versed in guile, to my father's house with a necklace of gold, and with amber beads was it strung between. This the maidens in the hall and my honoured mother were handling, and were gazing on it, and were offering him their price; but he nodded to the woman in silence. Then verily when he had nodded to her, he went his way to the hollow ship, but she took me by the hand, and led me forth from the house.

Homer (8th Century BC), *Odyssey*, 15−455−465
Translated from the Ancient Greek by William Butler

Nay, they even search the deep, and of all the rest are the only people who gather amber. They call it glasing, and find it amongst the shallows and upon the very shore. But, according to the ordinary incuriosity and ignorance of Barbarians, they have neither learnt, nor do they inquire, what is its nature, or from what cause it is produced. In truth it lay long neglected amongst the other gross discharges of the sea; till from our luxury, it gained a name and value. To themselves it is of no use: they gather it rough, they expose it in pieces coarse and unpolished, and for it receive a price with wonder. You would however conceive it to be a liquor issuing from trees, for that in the transparent substance are often seen birds and other animals, such as at first stuck in the soft gum, and by it, as it hardened, became quite enclosed. I am apt to believe that, as in the recesses of the East are found woods and groves dropping frankincense and balms, so in the isles and continent of the West such gums are extracted by the force and proximity of the sun; at first liquid and flowing into the next sea, then thrown by winds and waves upon the opposite shore. If you try the nature of amber by the application of fire, it kindles like a torch; and feeds a thick and unctuous flame very high scented, and presently becomes glutinous like pitch or rosin.

Publius Cornelius Tacitus (55−115), *Germania*, 45
Translated from the Latin by Thomas Gordon

"Spare me! O mother spare me; in the tree
my flesh is torn! farewell! farewell! farewell!"
And as they spoke the bark enclosed their lips.
Their tears flow forth, and from the new-formed
boughs
amber distils and slowly hardens in the sun;
and far from there upon the waves is borne
to deck the Latin women.

Publius Ovidius Naso (43−18), *Metamorphoses II*, 361−366.
Translated from the Latin by Brookes More

Matter and Form unmingled and conjoined
Came into being that had no defect,
E'en as three arrows from a three-stringed bow.

And as in glass, in amber, or in crystal
A sunbeam flashes so, that from its coming
To its full being is no interval,

So from its Lord did the triform effect
Ray forth into its being all together,
Without discrimination of beginning.

Dante Alighieri (1265−1321), *Divine Comedy, Paradise*, XXIX, 9
Translated from the Italian by Henry Wadsworth Longfellow

One of them was a sallow, clean-shaven civilian with a thin and wrinkled face, already growing old, though he was dressed like a most fashionable young man. He sat with his legs up on the sofa as if quite at home and, having stuck an amber mouthpiece far into his mouth, was inhaling the smoke spasmodically and screwing up his eyes. This was an old bachelor, Shinshin, a cousin of the countess, a man with "a sharp tongue" as they said in Moscow society.

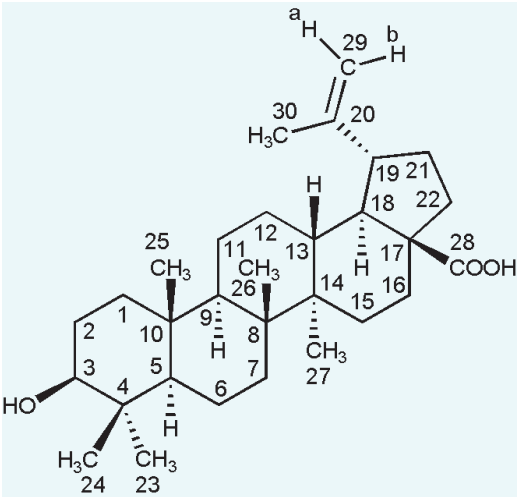
Lev Nikolayevich Tolstoy (1828 –1910), *War and Piece*, Book I, Chap. 18
Translator unknown

Thereupon she herself opened the glass cabinet in which objects made of amber were kept. Sicilian amber differs from the northern type in that it ranges from transparent and opaque honey colours through all the shadings of a saturated yellow up to the most beautiful hyacinthine red. Urns, goblets, and other things were carved out of it, so that in some cases we imagined that the original chunks of material must have been impressively large. The lady took a special delight in these objects, as well in the carved shells made in Trapani and in choice works out of ivory; she had many an entertaining story to tell about them. The prince drew our attention to more important objects, and so several hours passed in a pleasant and instructive manner.

Johann Wolfgang Goethe (1749−1832), *Italian Journey, Catania*, 3.5.1787
Translated from the German by R. R. Heitner

5.8 Betulinic Acid

Answers

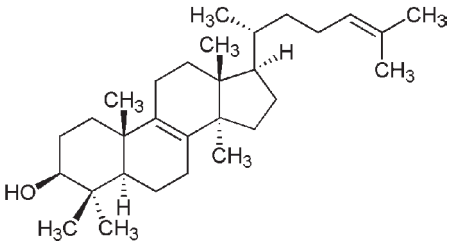


Scheme 5.8-5

A. Isoprene (2-methyl-1,3-butadiene). In the 1950s it was discovered that the main pathway for terpene synthesis is based upon two forms of "active isoprene": isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAP).

B. Squalene, or better all- *trans*-squalene [2,6,10,15,19,23-hexamethyl-(2*E*,6*E*,10*E*,14*E*,18*E*)-2,6,10,14,18,22-tetracosahexaene]. Squalene was isolated in 1916 from Japanese shark liver oil; cf. M. Tsujimoto, "An unsaturated hydrocarbon in shark liver oil", *J. Chem. Ind. Japan*, **1916**, *19*, 277−281, cited in SciFinder under CAN: 10:8379.

C. The compound's name is lanosterin (3-β-lanosta-8,24-dien-3-ol). The structure is:



Scheme 5.8-6

D. H-5 is in fact the most aliphatic proton of the molecule. It is surrounded by a "fence" of methyl groups, which probably due to their +I effect shield this proton.

E. According to the model it should show NOE contacts to H-23, and H-9, which can clearly be identified.

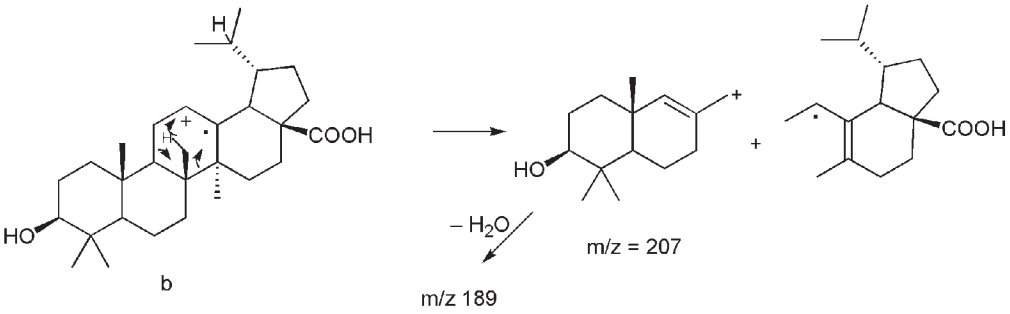
F. In addition to three α-increments there is three times a β-effect of the three methyl groups.

G. Those which are on the same side as the carboxyl group.

H. Probably the conformation of the C-17−C-28 bond.

I. NOE from H-29a, H-29b and of H-30 to either H-21 and H-13.

J. The ion b shown in the main text can undergo an additional hydrogen transfer. After breaking the C-8−C-14 and the C-9−C-11 bonds, the charge remains in the left part of the molecule. This ion loses water to form the species with m/z = 189.



Scheme 5.8-7

Translations

Fig. 5.8-27

Let me have a rich mellow vintage dating back to one of our elder consuls. It is only after many years that the plane tree affords a shelter from the scorching sun, and fields but newly reaped hurt the naked foot.

P. Ovidius Naso (43 BC–17 AD), *Ars Armatoria Lib*. II, 695
Translated from the Latin by Christopher Marlowe

Therefore I put several logs of young birches with white bark upward, close to a calm fire, until the wood started to evaporate steam and the bark became brown. After about 10 minutes flocks appeared which I collected with a paper. When no more flocks appeared I took other logs of wood and so on. In this way I sampled on one day an open glass of half a lit; the flocks, however, weighted only less than a gram.

J. T. Lowitz (1757−1804), *Chemische Annalen*
Translated from the German by Stefan Berger

Inclined you are, tall
Sycamore, and you are nude,
As white as a Scythian youth,
And yet your purity is
kept, your feet are glued
To the landscape's powerful
truth.

The sounding shadow
where the same heavenly blue
Stands still which
carries you away,
The dark black mother
always must your pure foot screw
The winds ignore your
forehead tired of leisure,
The earth, both your
friend and your trap,
O Sycamore, never
allows you the pleasure
Which lies in taking a step.

Paul Valery (1871−1945), *Au Platane*
Translated from the French by Astrid Lohöfer

Socrates: But let me ask you, friend: have we not reached the plane-tree to which you were conducting us?

Phaedrus: Yes, this is the tree.

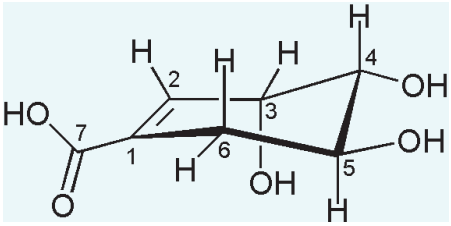
Socrates: By here, a fair resting-place, full of summer sounds and scents. Here is this lofty and spreading plane-tree, and the agnus cast us high and clustering, in the fullest blossom and the greatest fragrance; and the stream which flows beneath the plane-tree is deliciously cold to the feet. Judging from the ornaments and images, this must be a spot sacred to Achelous and the Nymphs. How delightful is the breeze: so very sweet; and there is a sound in the air shrill and summerlike which makes answer to the chorus of the cicadae. But the greatest charm of all is the grass, like a pillow gently sloping to the head. My dear Phaedrus, you have been an admirable guide.

Plato (428–348 BC), *Phaedrus*
Translated from the Ancient Greek by Harold N. Fowler

Chapter 6 Miscellaneous

6.1 Shikimic Acid

Answers



Scheme 6.1-8

A. It is advantageous that the constitution of the C-skeleton is already correct. The main challenge consists in the substitution of the two heteroatoms at C-4 and C-5 (O-atoms) by two N-atoms with inversion of the configuration at both centres at the same time. Furthermore, correct substituents have to be attached to all three heteroatoms at C-3, C-4 and C-5.

B. The transformation to form an aromatic ring takes places when testosterone is converted into estradiol and on conversion of androstenedione to estrone. The enzyme responsible is called aromatase (EC 1.14.14.1) and belongs to the cytochrome P450 superfamily.

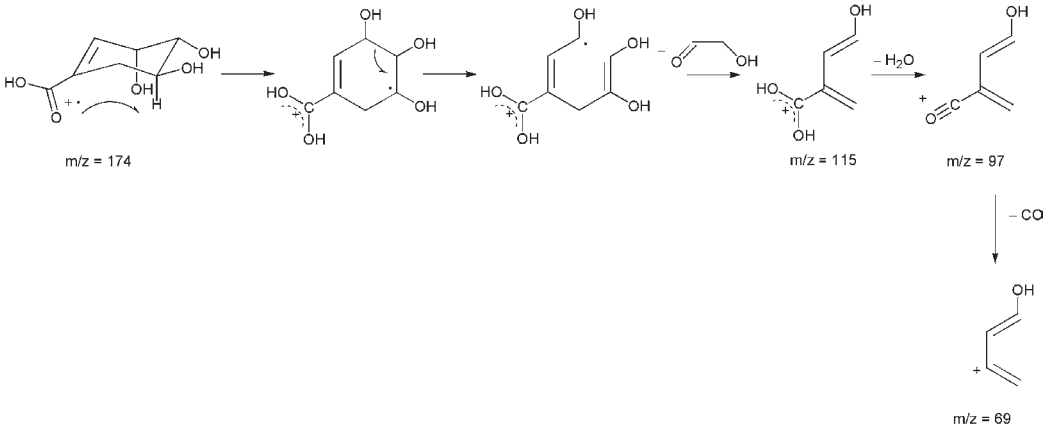
C. This compound is called *myo*-inositol (*cis*-1,2,3,5-*trans*-4,6-cyclohexanehexol). All OH groups except that at C-2 are in the equatorial position in the most stable conformation. *myo*-Inositol is an important precursor for a number of secondary messengers. It belongs to the group of so-called cyclitols. Nine stereoisomers are possible.

D. The spectrum shows nearly an identical pattern in the C=O/C=C region.

E. The sharp triplets seen can be only explained by two long-range spin couplings of similar magnitude. One coupling is obviously present in the signal of H-2 and is easily recognized as allylic coupling. The other partner over four bonds would be H-4; however, this signal does not show a further splitting.

F. H-3 shows cross peaks to C-1, C-2, C-4 and C-5; H-4 shows cross peaks to C-2, and C-5; H-5 shows cross peaks to C-1, C-4 and C-3.

G.



Scheme 6.1-9

Fig. 6.1-12

Translations

Slumber Song

Some day, if I should ever lose you,
will you be able then to go to sleep
without me softly whispering above you
like night air stirring in the linden tree?

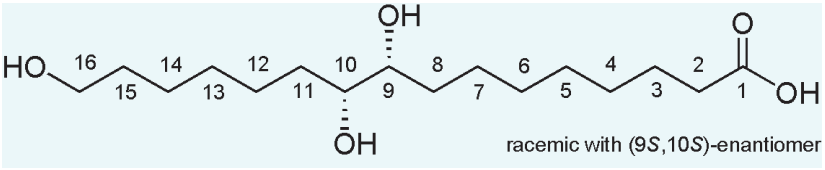
Without my waking here and watching
and saying words as tender as eyelids
that come to rest weightlessly upon your breast,
upon your sleeping limbs, upon your lips?

Without my touching you and leaving you
alone with what is yours, like a summer garden
that is overflowing with masses
of melissa and star-anise?

Rainer Maria Rilke (1875−1926)
Translated from the German by Albert Ernest Flemming

6.2 Aleuritic Acid

Answers



Scheme 6.2-9

A. A fatty acid is an unbranched monocarboxylic acid with an even number of C-atoms. This is due to the biosynthetic origin of the C_2-units of acetyl-CoA. A long chain is not an imperative for a fatty acid. Butyric acid with four C-atoms is the smallest fatty acid. Its name is derived from its occurrence in the fat of butter.

B. Oleic acid [(9*Z*)-octadec-9-enoic acid], linoleic acid [(9*Z*,12*Z*)-octadecadienoic acid], linolenic acid [(9*Z*,12*Z*,15*Z*)-octadecatrienoic acid]. As the rational names show, the configuration at the double bond is always *cis*. Essential fatty acids are absolutely necessary for the human metabolism and they have to be taken up with the food because the body itself cannot produce them. They are required as precursors of arachidonic acid (all-*cis*-5,8,11,14-eicosatetraenoic acid) that in turn is a key precursor for several

so-called eicosanoids such as leukotrienes, thromboxanes, prostaglandins and prostacyclins that act as signalling molecules in the tissue.

C. From the chain length, 15 C atoms in the chain at the carboxylate of an aleuritate ion are more than necessary to obtain a good surfactants effect. However, any hydroxyl groups in this molecule part will weaken the effect due to their hydrophilicity.

D. It seems likely that a *cis*-double bond has been present in the precursor. Enzymatic oxidation could then be assumed to lead first to an epoxide. A feature of such an epoxide would be that the substituents at the two ring carbons are equally substituted at the next six (!) spheres (six CH_2-units at each carbon) before a difference occurs (OH vs. CH_2–COOH). This means that a differentiating influence of the two end groups at the reactive centre epoxide is excluded due to their distance from it. Hence, for a hydrolytic ring opening, both courses of reaction are possible with the same rate: attack at C-9 or attack at C-10. As a result, a racemate is obtained, even though in principle both substituents are unequal.

E. The structural similarity feature mentioned above can be regarded as a kind of molecular wrapping of the information on the diol configuration. The similarity of both substituents at C-9 and C-10 is so extraordinary that the signals for H-9 and H-10 do not differ. Thus, the identity of these signals which prevent the NMR assignment whether the relative configuration is *threo* or *erythro* is also a sign of the concealment of the chiral part in the hiding place within the middle of a long alkyl chain as the occurrence of aleuritic acid as racemate.

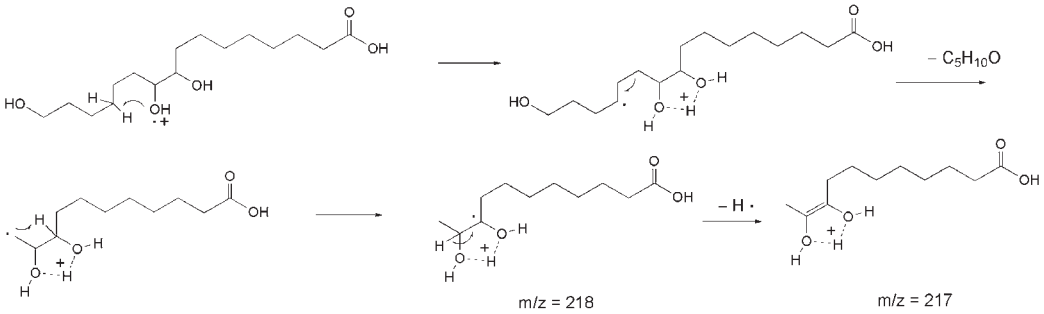
F. There must be a diastereotopicity for the proton signals of the corresponding methylene groups with one proton resonating at 1.22 and the other at 1.38 ppm.

G. The signal stems from the residual CHD_2–SO–CD_3 component in the solvent.

H. True NOE correlation signals are usually well below 5% of the intensity of the diagonal, since the cross relaxation is not that effective. Exchange peaks mean that magnetization is directly transferred due to chemical exchange and therefore these exchange peaks can be very strong.

I. The carbon atoms C-8 and C-11 have over four bonds the same environment “looking” to the right and left sides within the molecule.

J. The ions at m/z = 218 and 217 can probably be understood by assuming ionization at the OH group of C-10, and a subsequent McLafferty-type rearrangement involving a β-cleavage between C-11 and C-12. The resulting ion will stabilize by loss of a hydrogen atom to form the ion with m/z = 217:



Scheme 6.2-10

Translations

Fig. 62-23

Here is the whole matter: white jet comes from Norway, black jet comes from England, black glass jewellery comes from Germany. Jet is the lightest, the most precious, the most costly. Imitations can be made in France and also in Germany. What is needed is a little anvil two inches square, and a lamp burning spirits of wine to soften the wax. The wax was formerly made with resin and lampblack, and cost four livres the pound. I invented a way of making it with gum shellac and turpentine. It does not cost more than thirty sous, and is much better. Buckles are made with a violet glass which is stuck fast, by means of this wax, to a little framework of black iron. The glass must be violet for iron jewellery, and black for gold jewellery. Spain buys a great deal of it. It is the country of jet . . .

Victor Hugo (1802−1885), *Les Miserables*, Vol. V, Book IX, Chap. 3
Translated from the French by Isabel F. Hapgood

There were no signs of disturbance in the room where Fyodor Pavlovitch was lying. But by the bed, behind the screen, they picked up from the floor a big and thick envelope with the inscription: “A present of three thousand roubles for my angel Grushenka, if she is willing to come.” And below had been added by Fyodor Pavlovitch, “For my little chicken.” There were three seals of red sealing-wax on the envelope, but it had been torn open and was empty: the money had been removed. They found also on the floor a piece of narrow pink ribbon, with which the envelope had been tied up. One piece of Pyotr Ilyitch’s evidence made a great impression on the prosecutor and the investigating magistrate, namely, his idea that Dmitri Fyodorovitch would shoot himself before daybreak, that he had resolved to do so, had spoken of it to Ilyitch, had taken the pistols, loaded them before him, written a letter, put it in his pocket, etc.

Fyodor Dostoevsky (1821−1881), *The Brothers Karamazov*, Book IX, Chap. 2
Translated from the Russian by Constanze Garnett

Appendix

Spectroscopic Experiments

1. Polarimetric experiments were performed at the given concentrations and with the mentioned solvents at the stated temperature with a Polartronic MHZ-8 automatic polarimeter from Schmidt & Haensch (Berlin, Germany).
2. UV/vis spectra were all been recorded at room temperature on a UV1 Thermo Spectronic UV/vis instrument in quartz cuvettes from 1 mm to 1 cm. The solvents used were measured in an identical cuvette simultaneously as the reference and were of UV grade. The velocity of the scan was 2000 nm/min, with 2 nm for each datapoint. The digital data were transferred to an Excel data sheet and plotted using Excel graphics.
3. CD spectra were measured at 25 °C on a JASCO-715 CD spectropolarimeter. All CD spectra were recorded with the standard sensitivity, a bandwidth of 2.0 nm, a data pitch of 0.5 nm and a scan velocity of 20 nm/min. The solvent was always recorded on the same day as the analytical sample. Evaluation of the data was performed with the program "Spectra Analysis" of the JASCO-715 instrument. The digital data were transferred to an Excel data sheet and plotted using Excel graphics. Prior to use, the CD spectrometer was calibrated using (1R)-(−)-10-camphorsulfonic acid and (*R*)-(−)-pantolactone according to J. Miles et al.[1].
4. IR spectra were recorded on a Thermo-Nicolet Avatar 360 FT-IR instrument either in KBr or as film. Typically the data were baseline corrected. The data were obtained as WMF files and used as such.
5. All NMR spectra were recorded at room temperature on Bruker DRX-400, DRX-600 and Avance-700 instruments using either inverse probe heads or, in the case of the 700 MHz instrument, a cryo probe head. The experimental and the processing conditions were very similar to those experiments 3.1, 6.4, 12.3, 12.5, 12.9, 12.18 and 12.19 in ref. [2]. The data were extracted as .png files from the Bruker Topspin plot program.
6. Mass spectra were recorded in low- and high-resolution mode mainly on a Finnigan-MAT 8230 or on a VG Analytics instrument VG ZAB HSQ with 70 eV excitation. The data were digitally transferred to an Excel data sheet, recalculated and plotted using Excel graphics. The ESI spectra were recorded on a Bruker FT ICR APEX-II mass spectrometer.

[1] A. J. Miles, F. Wien, J. G. Lees, A. Rodger, R. W. Janes, B. A. Wallace, "Calibration and standardisation of synchrotron radiation circular dichroism and conventional circular dichroism spectrophotometers" *Spectroscopy* **2003**, *17*, 653−661.

[2] S. Berger, S. Braun, "200 and More NMR Experiments", Wiley-VCH Verlag GmbH, Weinheim, **2004**.

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Fig.1.3-1, 1.3-3, 1.3-4, 1.3-5, 1.3-10, 1.3-13, 1.3-22, 1.4-6, 1.4-12, 1.7-3, 1.7-9, 2.2-17, 2.4-3, 2.4-4, 2.4-7, 2.4-14, 3.1-4, 3.1-17, 3.1-19, 3.2-3, 3.2-10, 5.4-1, 5.4-7 by Mona Knop, Botanic Garden University Bonn

Page 1	„Coffeeshop in the desert“ taken from ref. 1, page 28.
Page 189	“Indigo production Bengal” taken from ref. 1, page 28.
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