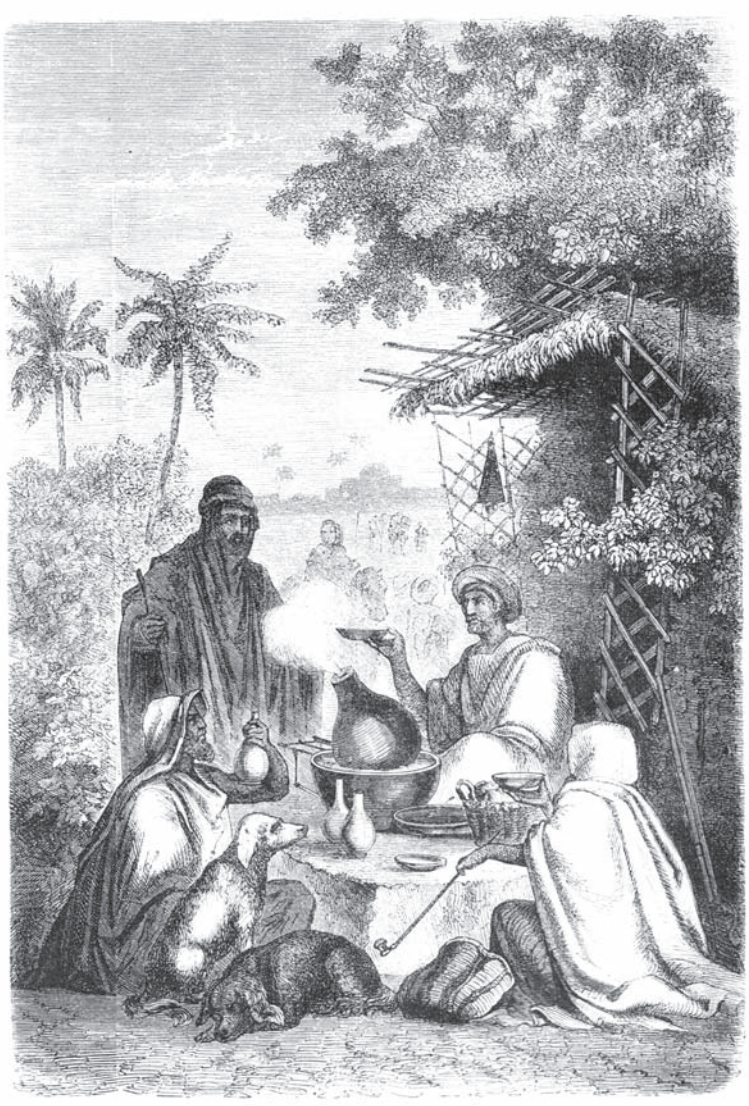
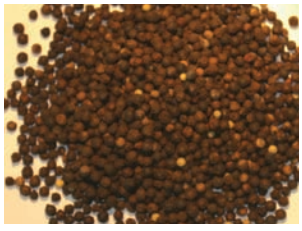
Chapter 1 Alkaloids





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

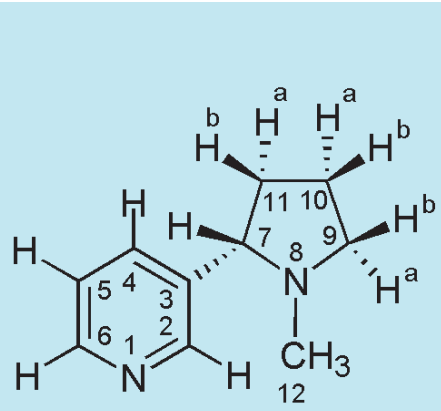
$[\alpha]_D^{24} = -168.5°$ (*c* 0.0465 g/mL, acetone)

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.

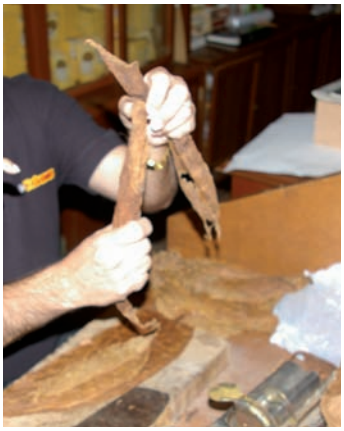
Therefore, it has been used in the form of tobacco broth as one of the first means of pest control when this technique arose in the 19th century. This natural product is very effective against aphids and the like and was used at that time in day-to-day life. In households, tobacco broth was made as an 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.

2. Literature

[1] W. Posselt, L. Reimann, "Chemische Untersuchungen des Tabaks und Darstellung des eigenthümlichen wirksamen Prinzips dieser Pflanze" [Chemical investigation of tobacco and isolation of the peculiar active principle of this plant] *Geigers Magazin der Pharmazie* **1828**, *24*, 138−141.

[2] A. Pinner, "Ueber Nicotin. Die Constitution des Alkaloids" [On nicotine. The constitution of the alkaloid] *Ber. Dtsch. Chem. Ges.* **1893**, *26*, 292−305.

[3] W. Meissner, "Über ein neues Pflanzenalkali (Alkaloid)" [On a new plant alkali (alkaloid)] *J. Chem. Phys. (Schweiger)* **1819**, *25*, 379.

[4] M. Hesse, "Alkaloids – Nature's Curse or Blessing?", Verlag Helvetica Chimica Acta, Zürich and Wiley-VCH Verlag GmbH, Weinheim, **2002**, 5 ISBN 3-906390-24-1.

[5] J. F. Seeman, "Using basic principles to understand complex science: nicotine smoke chemistry and literature analogies" *J. Chem. Educ.* **2005**, *82*, 1577−1583.

[6] D. Yildiz, "Nicotine, its metabolism and an overview of its biological effects" *Toxicon* **2004**, *43*, 619−632.

[7] S. K. Das, "Harmful health effects of cigarette smoking" *Mol. Cell. Biochem*. **2003**, *253*, 159−165.

[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

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

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

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, тегодну 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

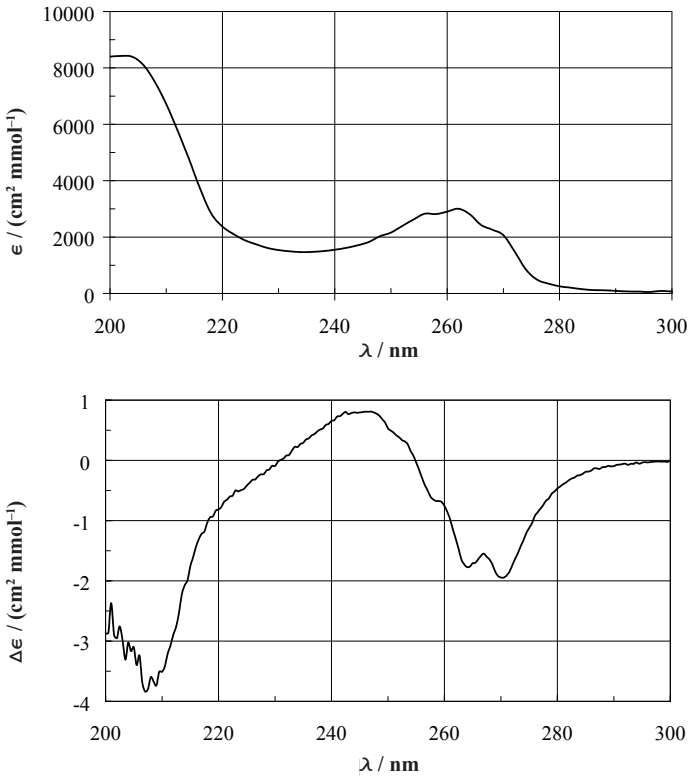
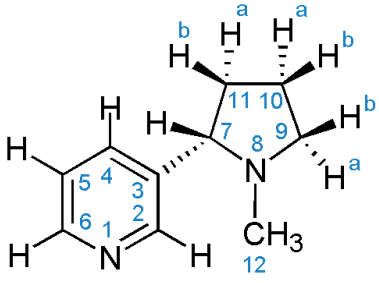


Fig. 1.1-10 UV and CD spectra in ethanol

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

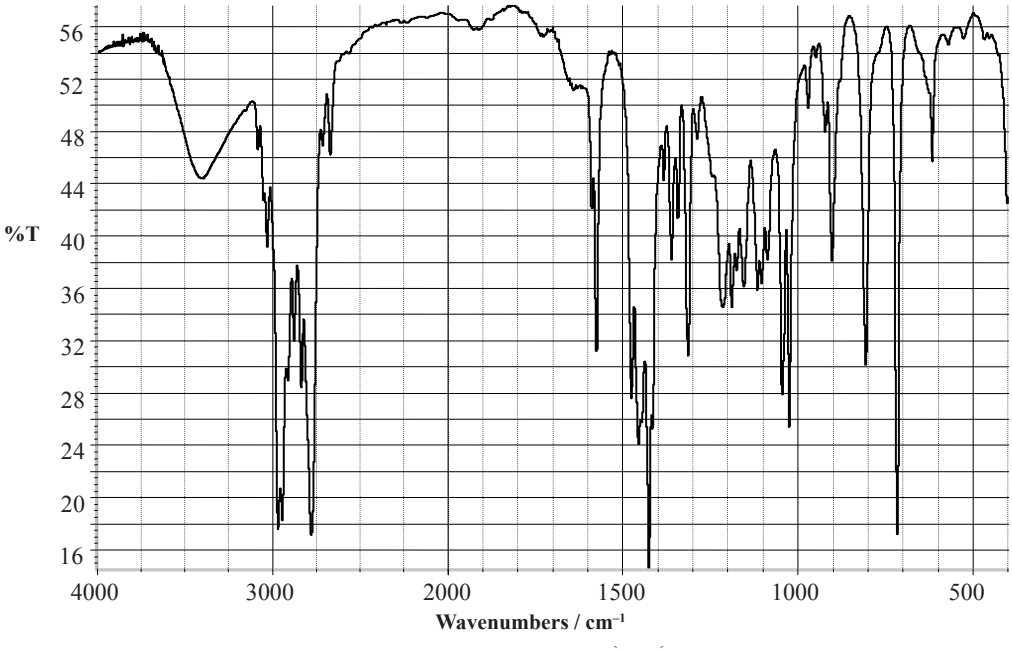
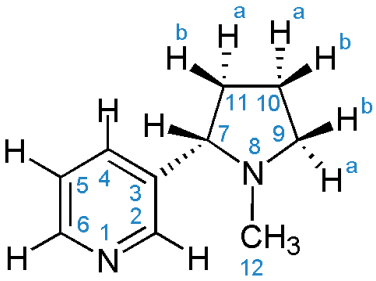


Fig. 1.1-13 IR spectrum as film

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

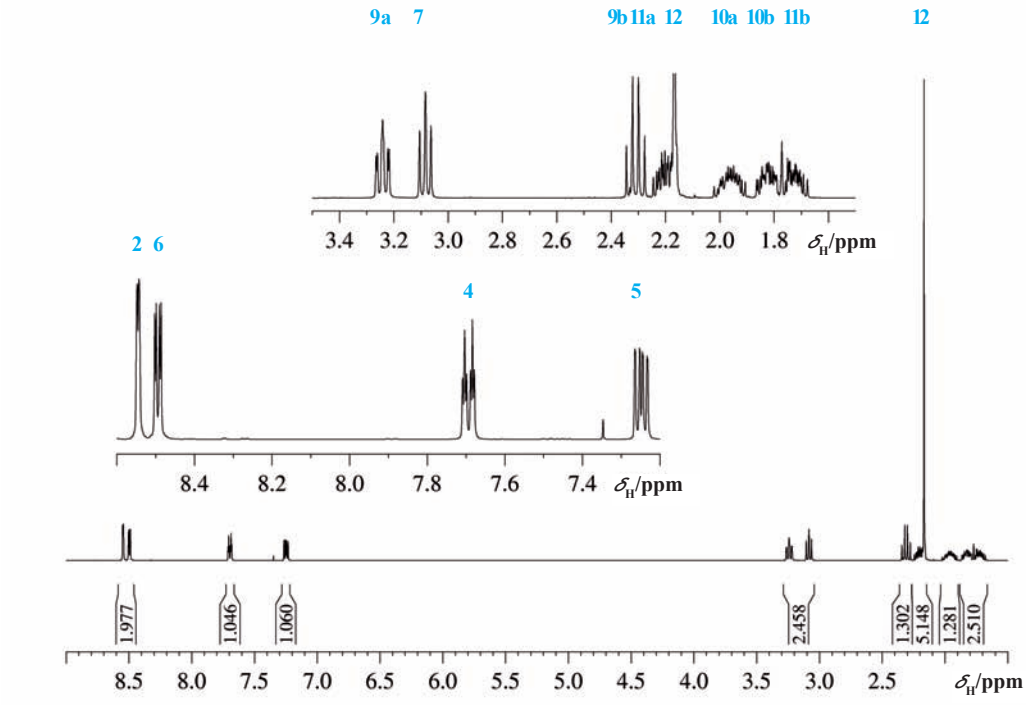


Fig. 1.1-14 ^{1}H NMR spectrum at 400 MHz in $CDCl_3$

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.

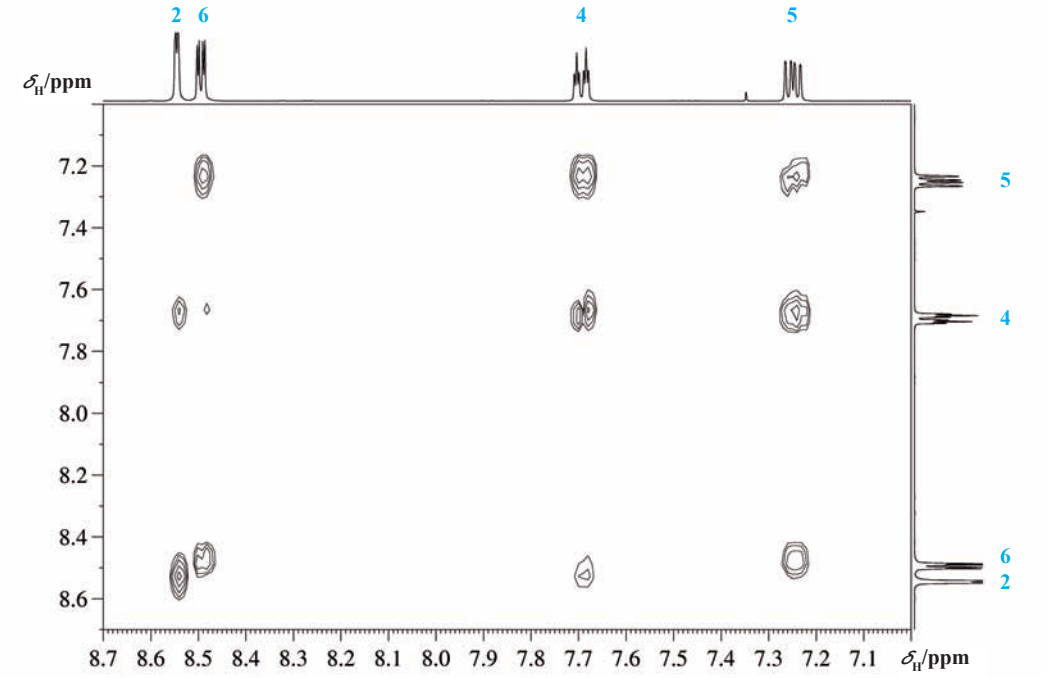


Fig. 1.1-15 Expansion of the COSY spectrum in the aromatic region

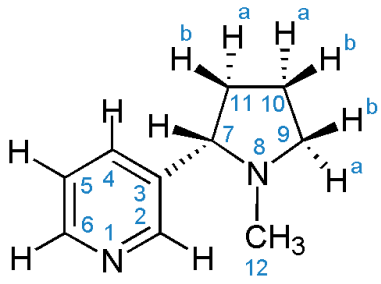
The expansion of the COSY spectrum in the aromatic region is a good example of the assignment of an aromatic four-spin system.The cross peaks starting from H-2 and H-6 give a firm assignment for H-5 and H-5, which, of course, couple to each other in turn.

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

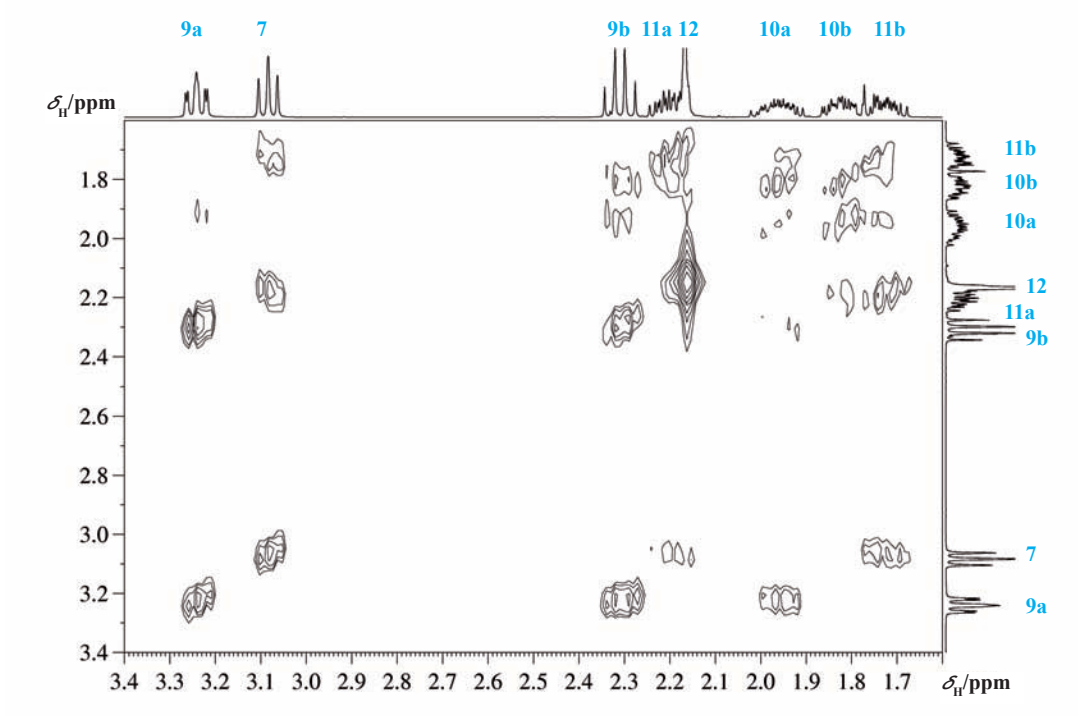


Fig. 1.1-18 Expansion of the COSY spectrum in the aliphatic region

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.

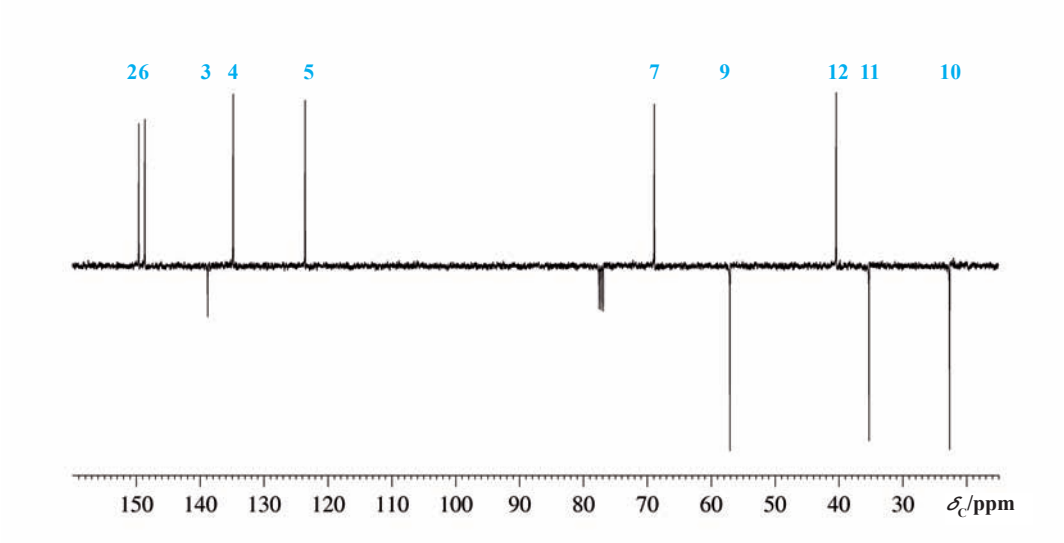


Fig. 1.1-19 APT ^{13}C NMR spectrum at 100 MHz in $CDCl_3$

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.

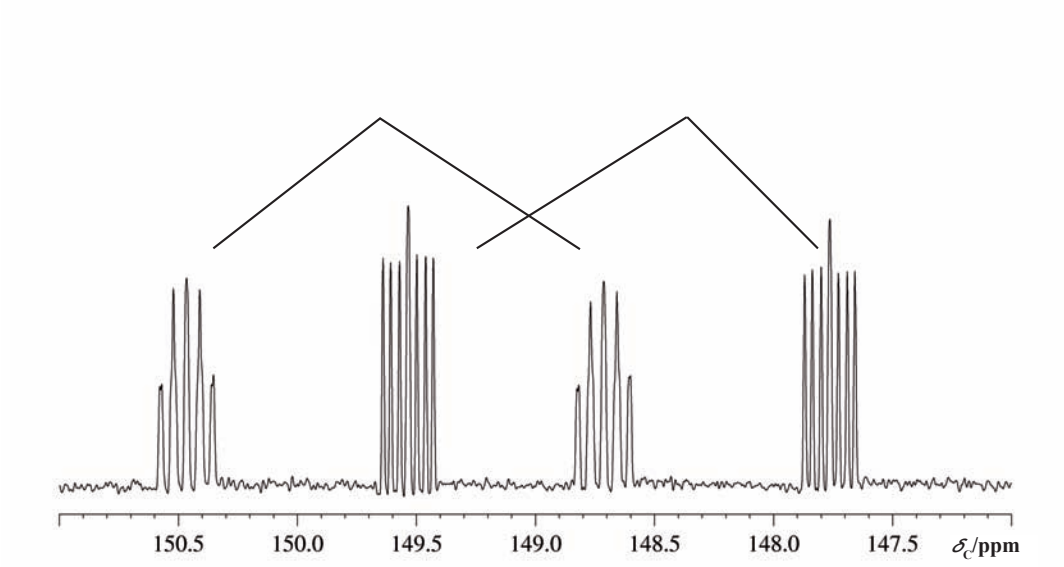


Fig. 1.1-20 Expansion of the gated decoupled ^{13}C NMR spectrum at 100 MHz in $CDCl_3$ in the aromatic region

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.

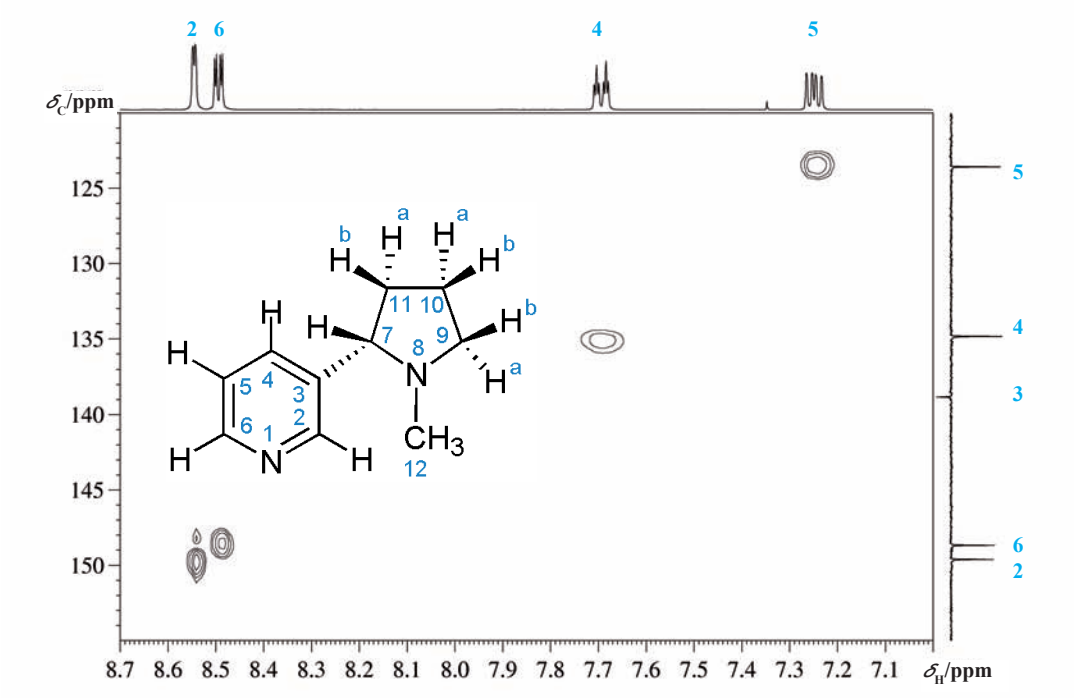
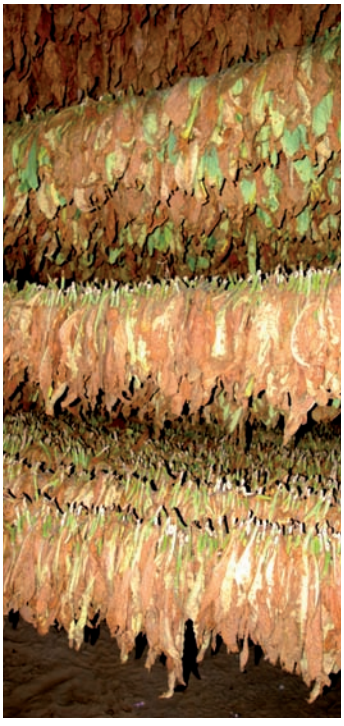


Fig. 1.1-21 Expansion of the HSQC spectrum in the aromatic region

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

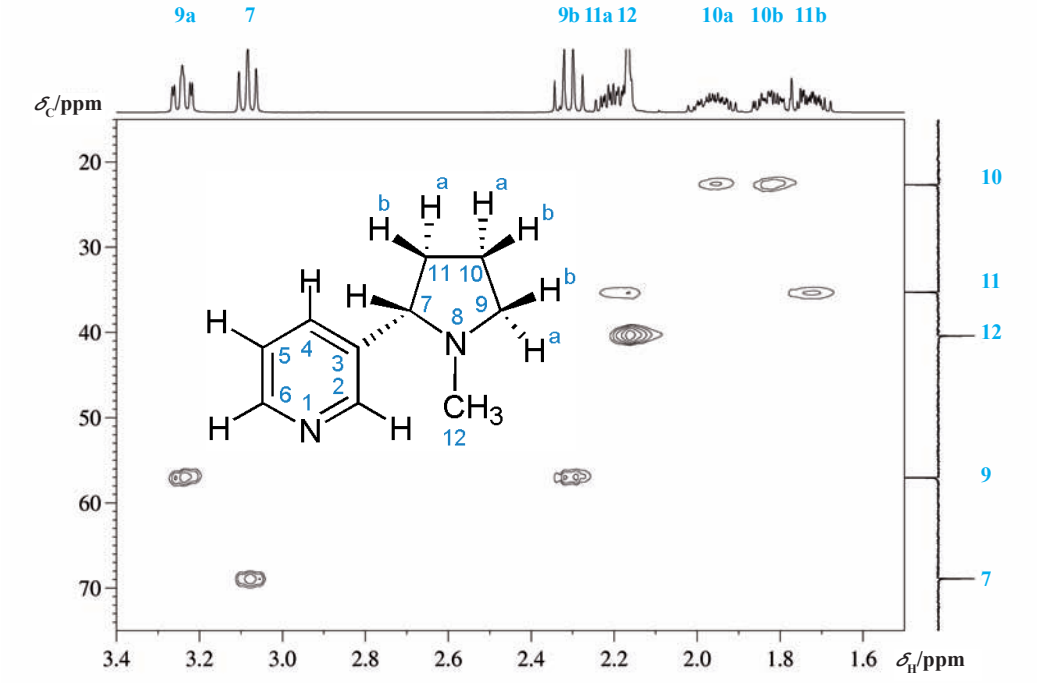


Fig. 1.1-23 Expansion of the HSQC spectrum in the aliphatic region

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.

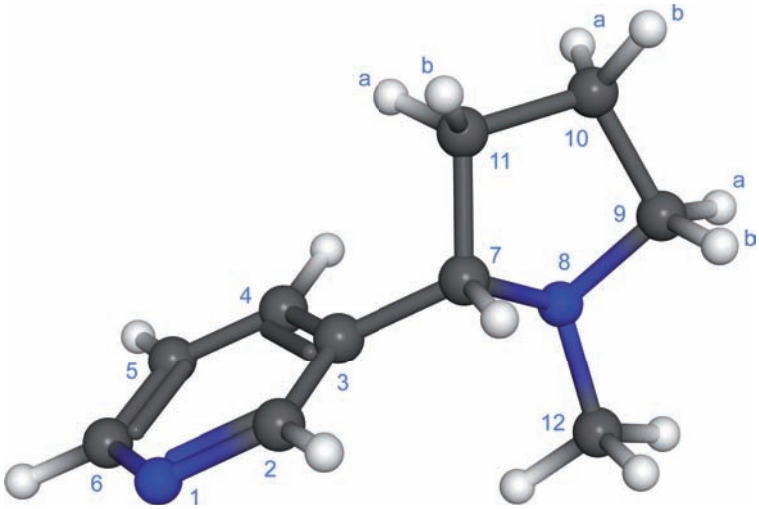


Fig. 1.1-24 Molecular model of nicotine

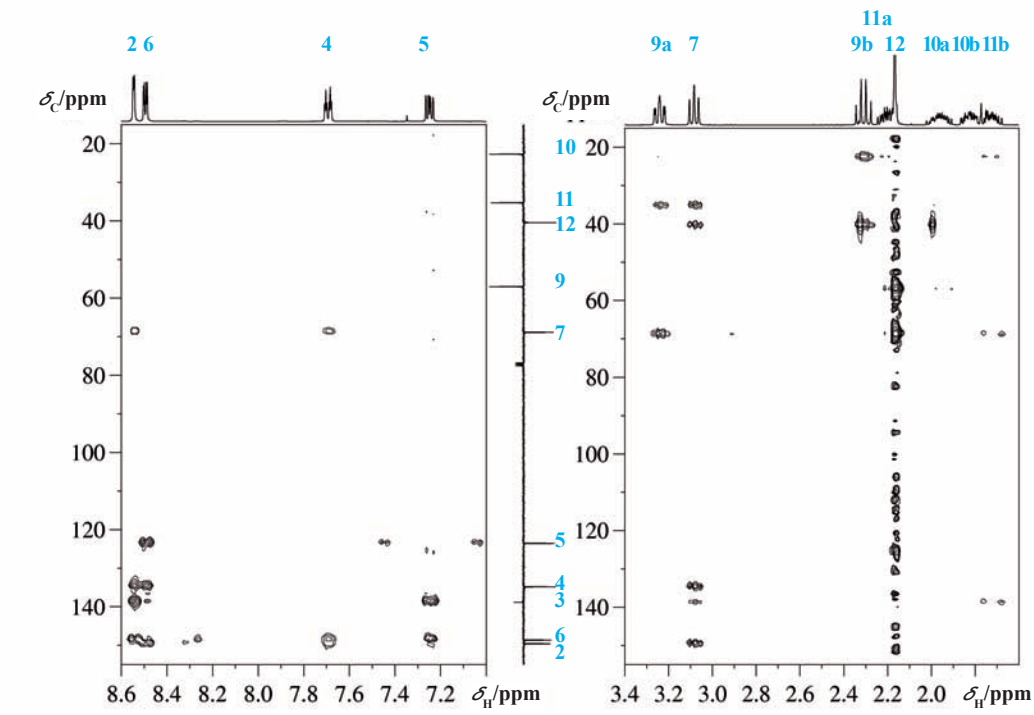


Fig. 1.1-25 HMBC spectra

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.

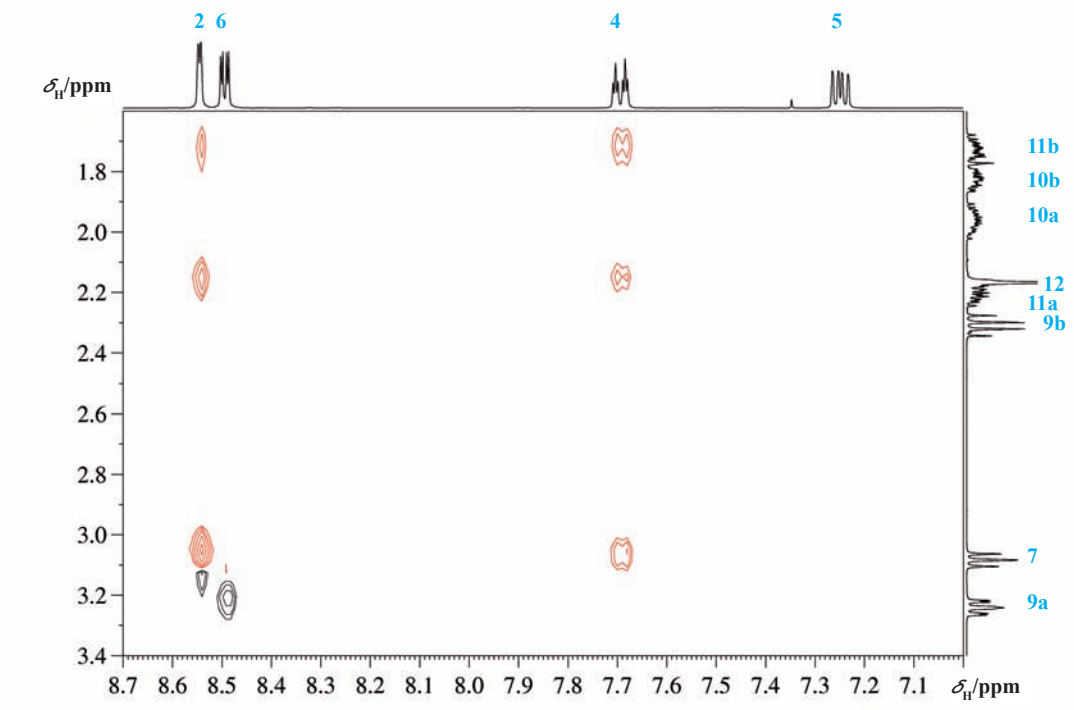


Fig. 1.1-26 NOESY spectrum connecting the aromatic with the aliphatic region

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

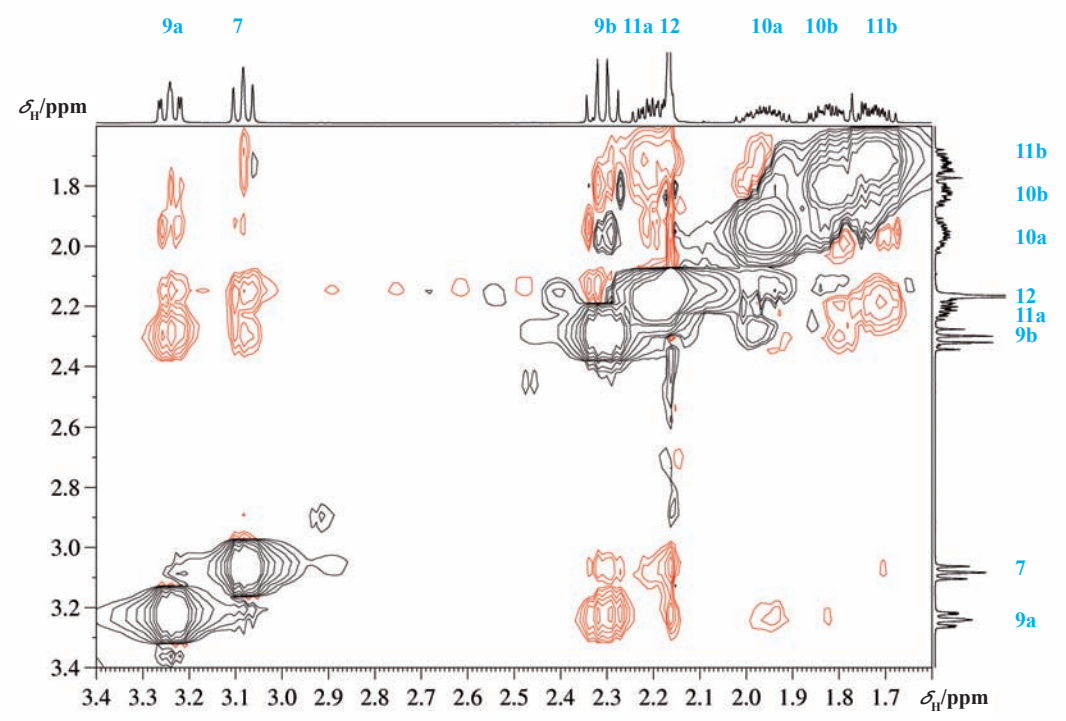


Fig.1.1-28 Expansion of the aliphatic region of the NOESY spectrum

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

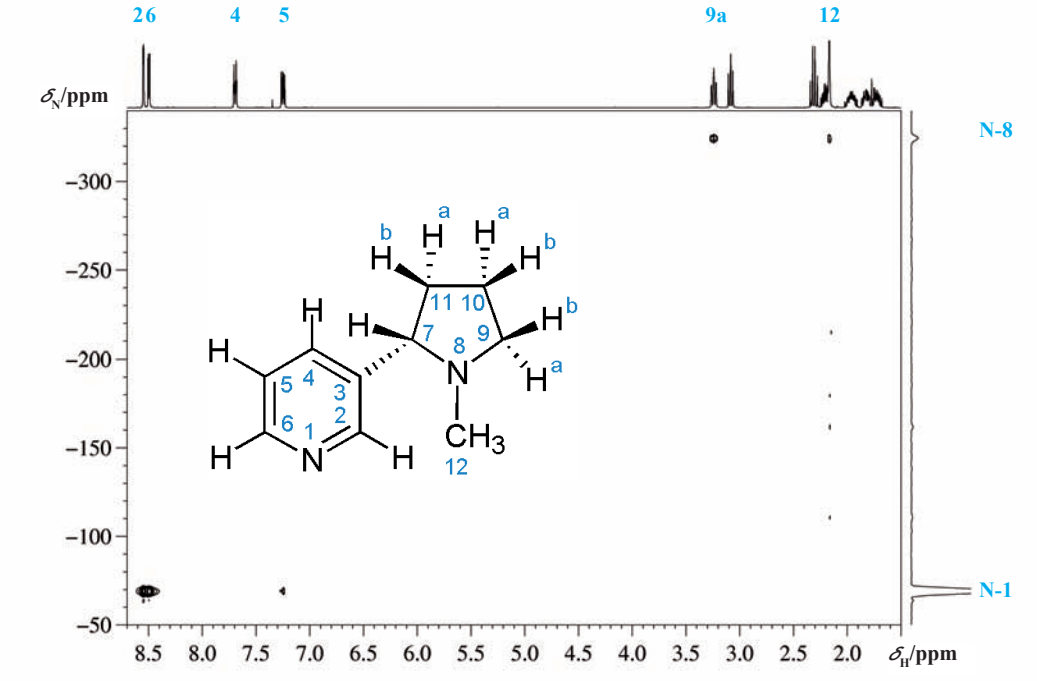


Fig. 1.1-30 $^{1}H^{15}N$ HSQC spectrum

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.

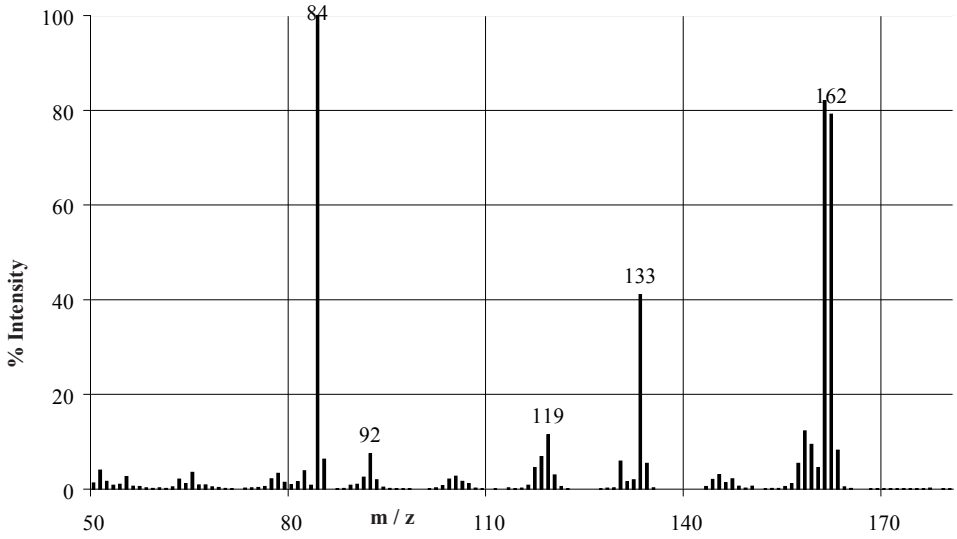
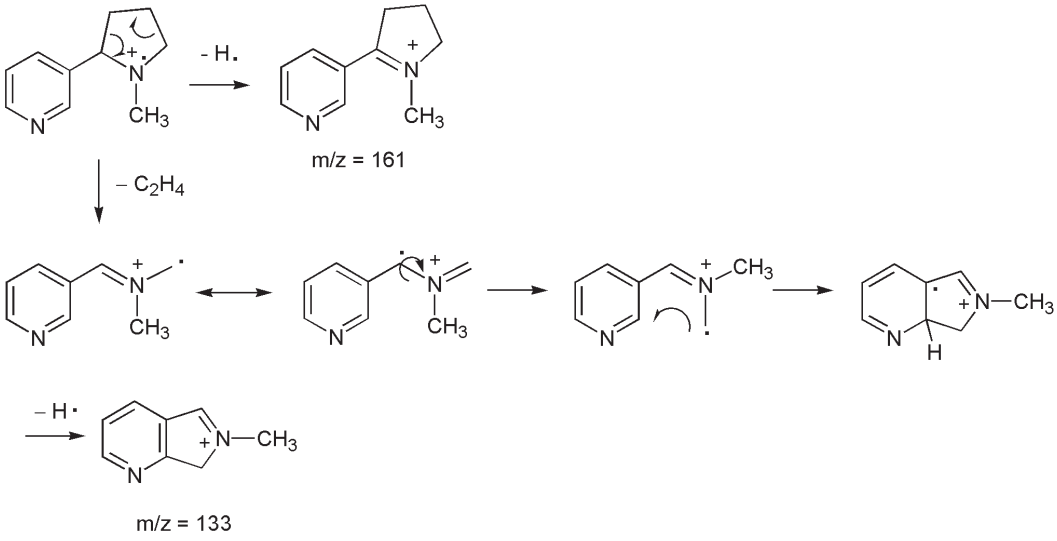


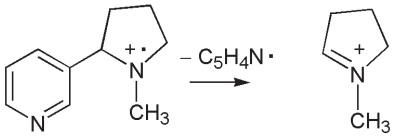
Fig. 1.1-31 Mass spectrum (EI)

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.



m/z = 84

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

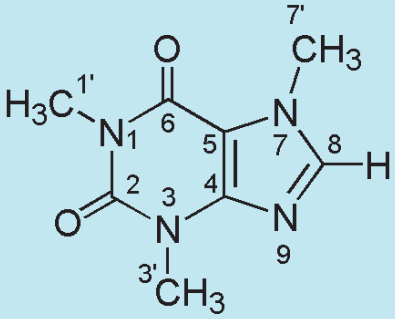


Fig. 1.2-1 Japanese green tea

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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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.

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

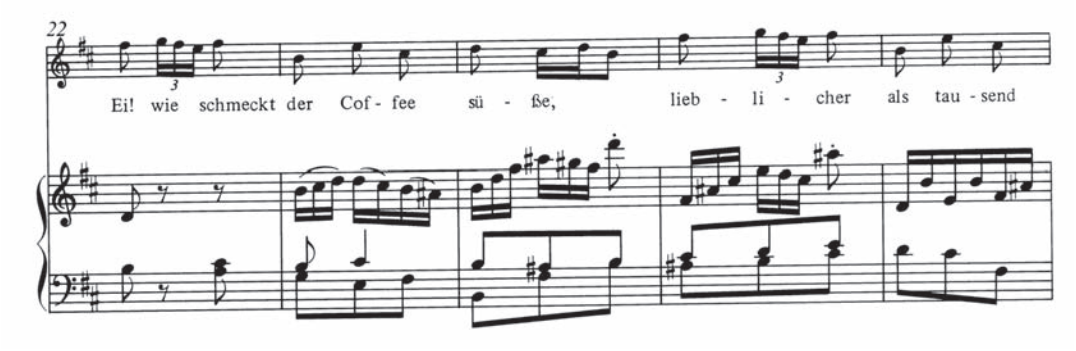


Fig. 1.2-11 The Coffee Cantata

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

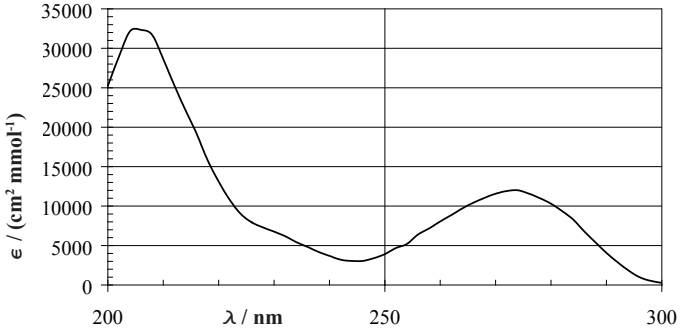


Fig. 1.2-16 UV spectrum in ethanol

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

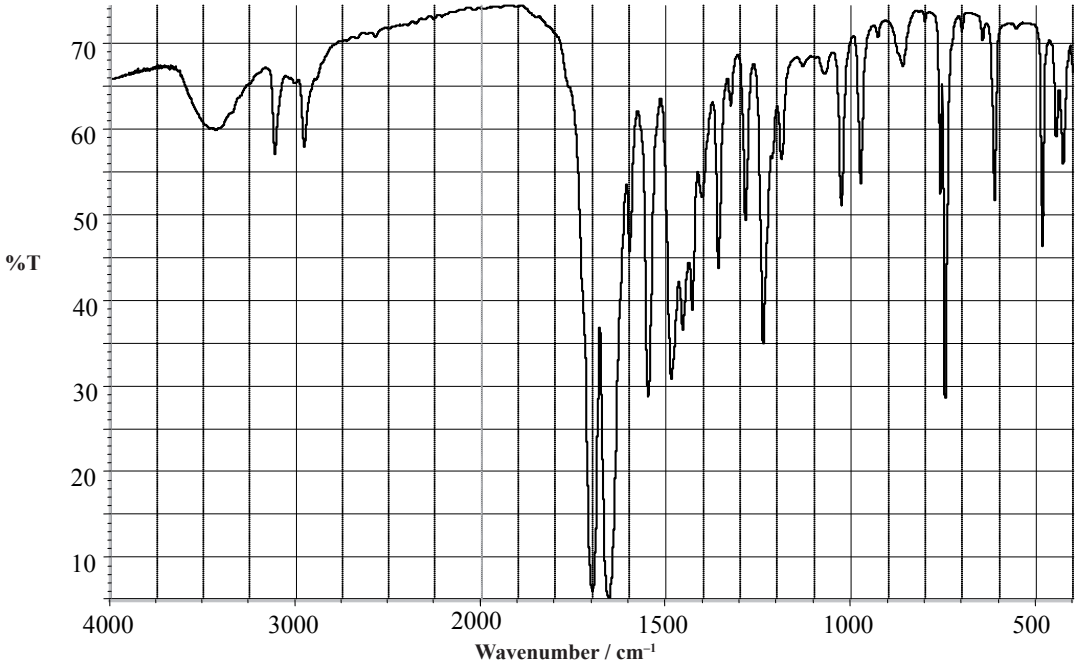


Fig. 1.2-17 IR spectrum in KBr

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.

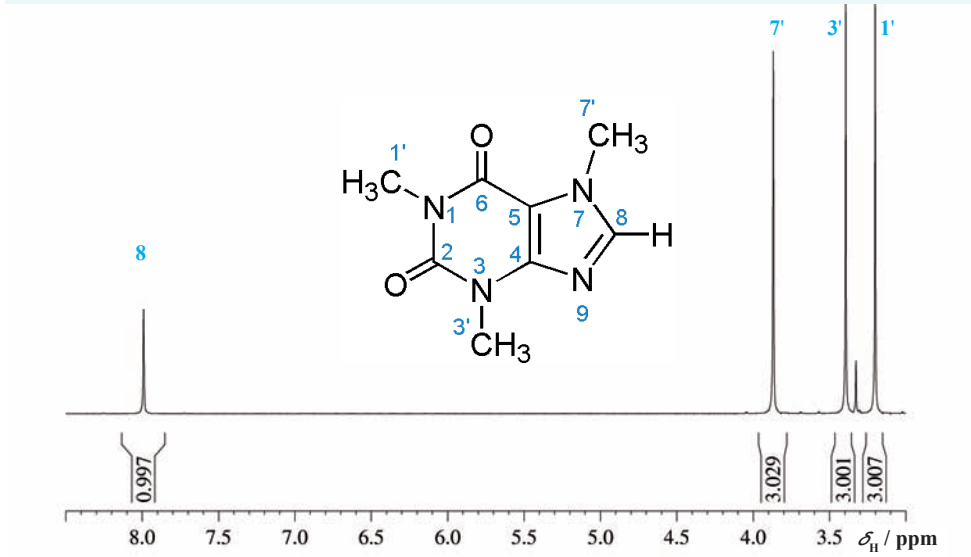


Fig. 1.2-18 ^{1}H NMR spectrum at 400 MHz in DMSO-d$_6$

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.

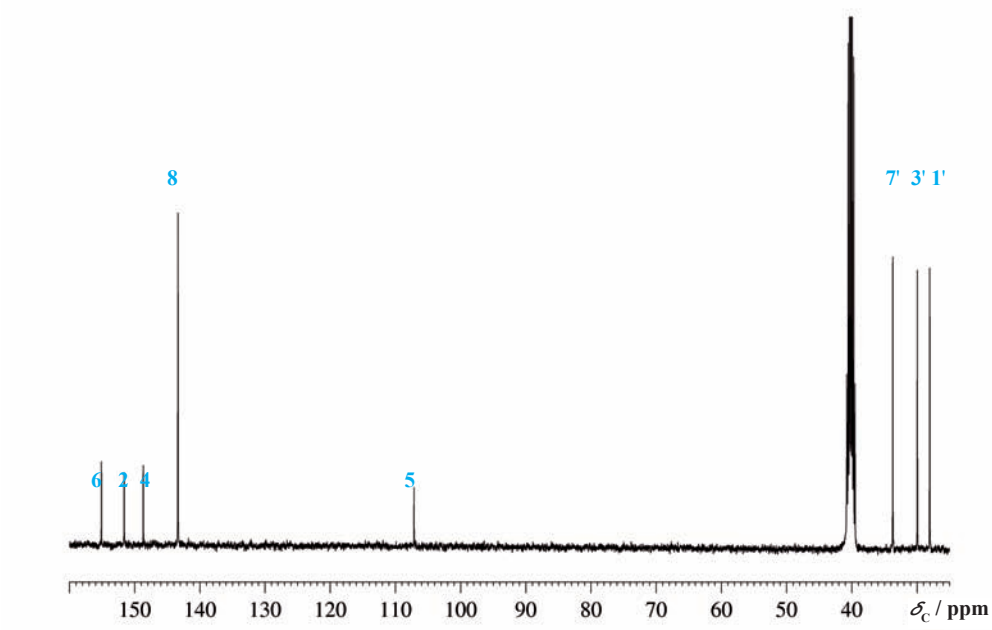


Fig. 1.2-19 ^{13}C NMR spectrum at 100 MHz in DMSO-d_6

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.

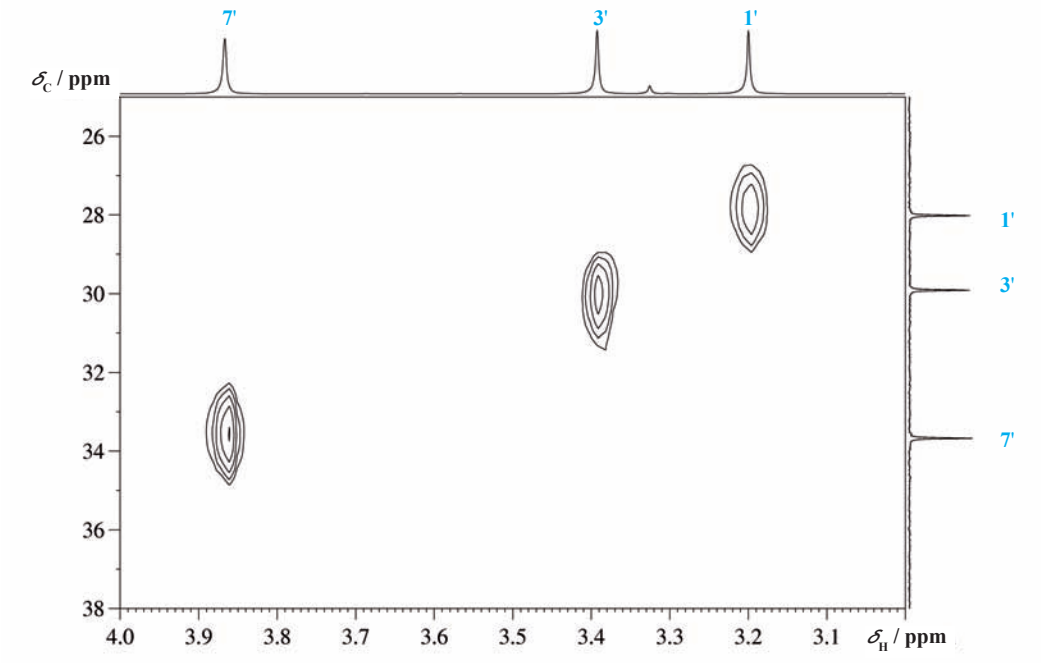


Fig. 1.2-20 Expansion of the HSQC spectrum in the aliphatic region

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.

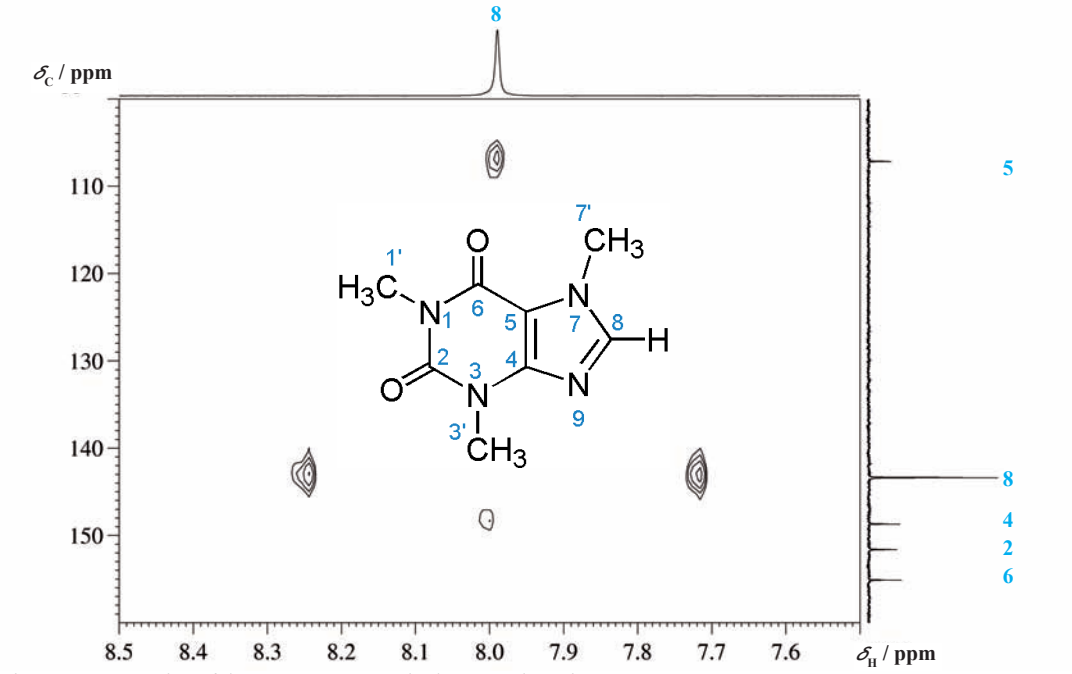


Fig. 1.2-21 Expansion of the HMBC spectrum in the aromatic region

In the first expansion of the HMBC spectrum we see a breakthrough of the ^{1}J(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

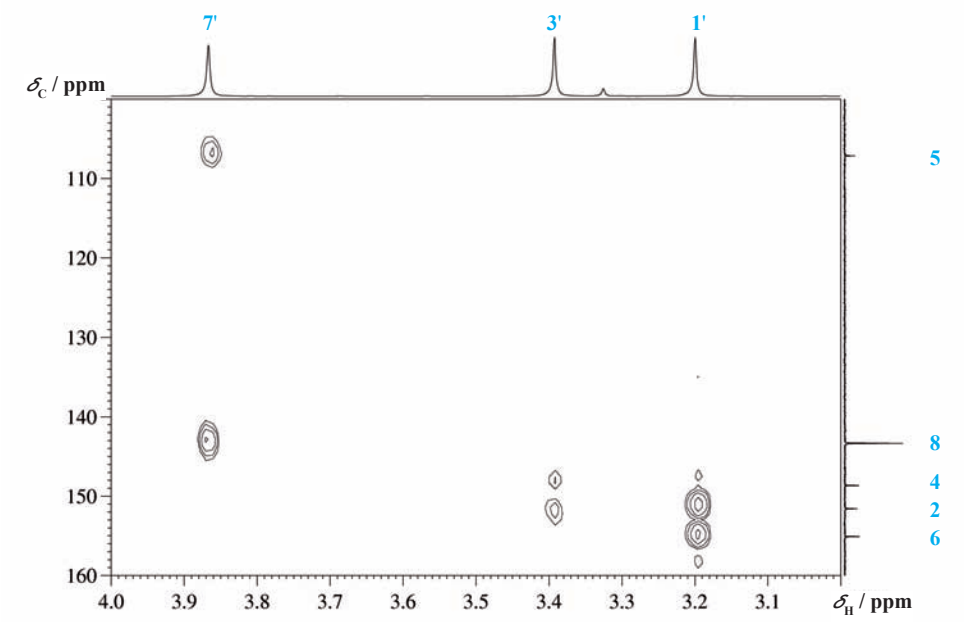


Fig. 1.2-25 Expansion of the HMBC spectrum in the carbonyl region

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.

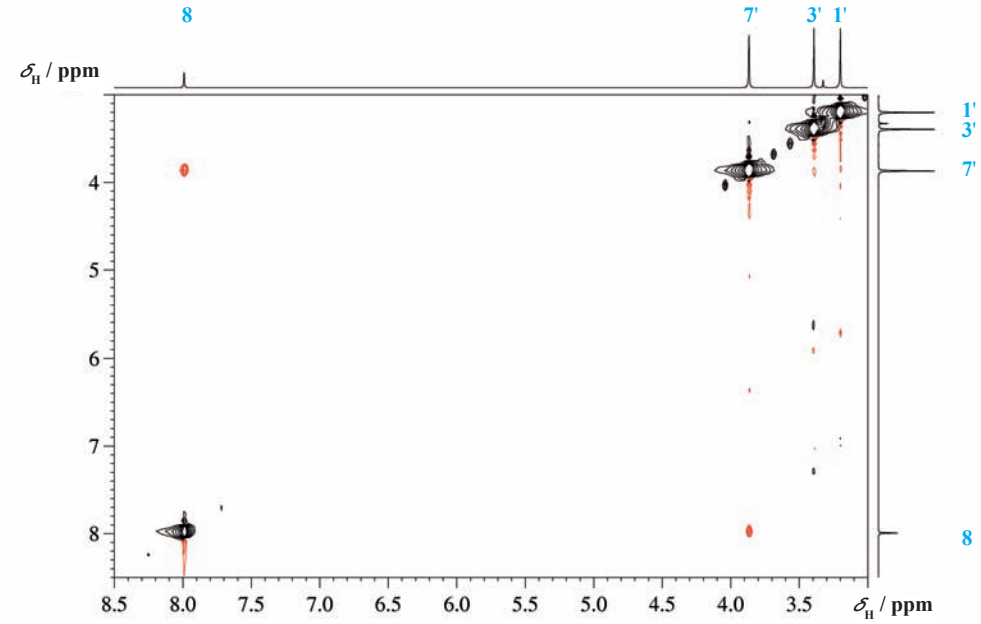


Fig. 1.2-26 NOESY spectrum connecting the aromatic with the aliphatic region

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.

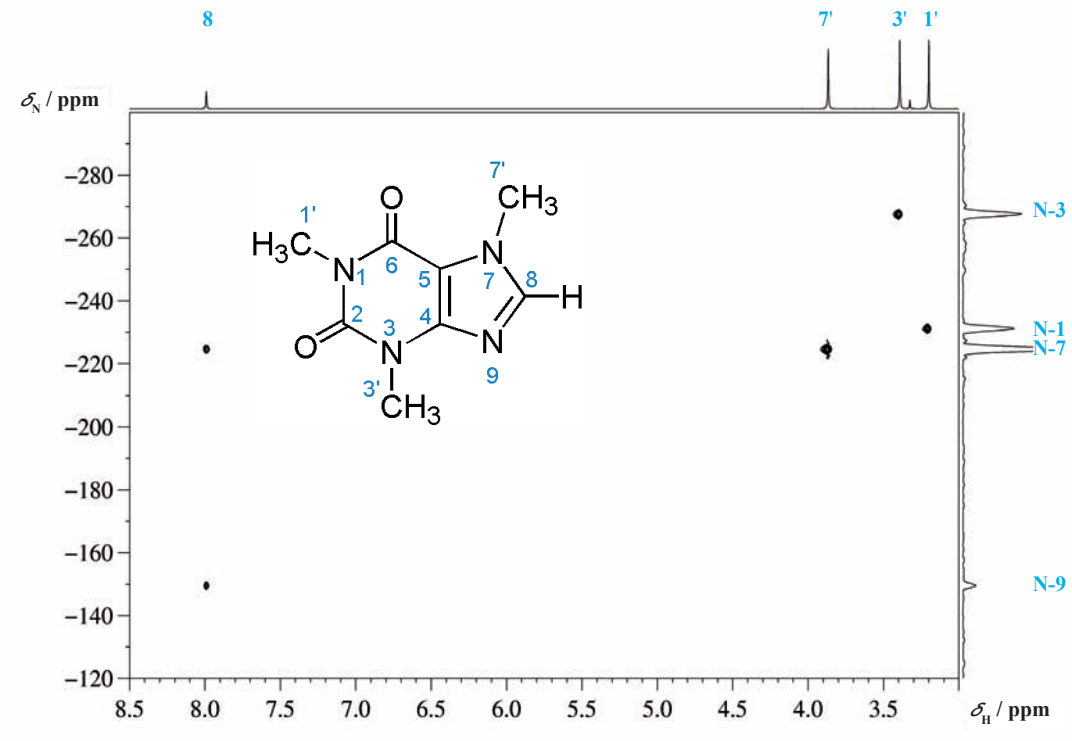


Fig. 1.2-27 ^{1}H ^{15}N HMQC spectrum

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

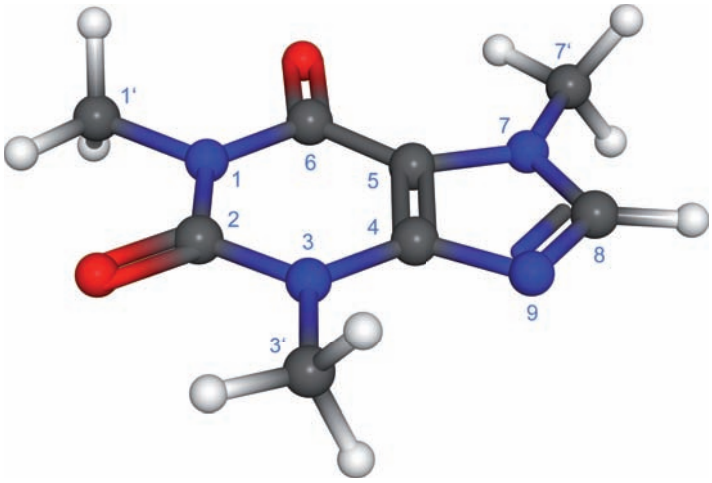


Fig. 1.2-28 Molecular model of caffeine

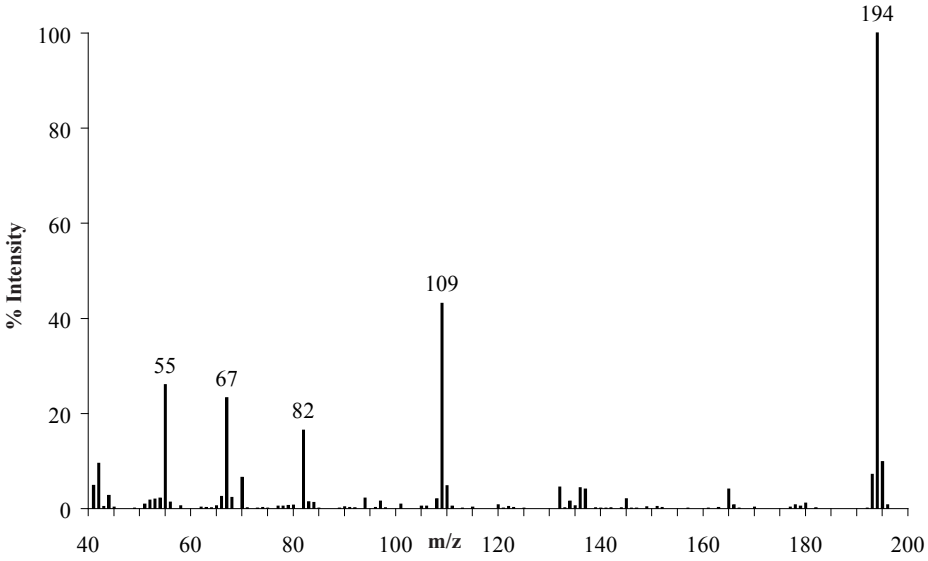
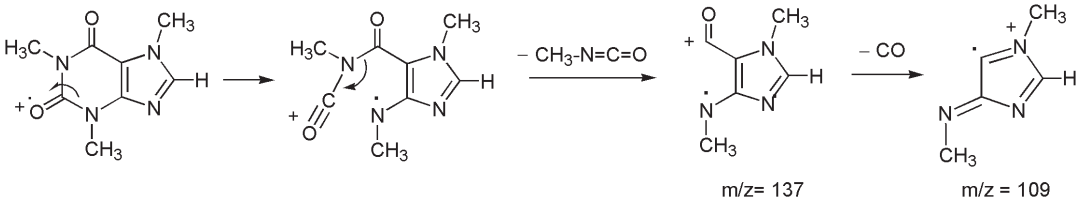


Fig. 1.2-29 Mass spectrum (EI)

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

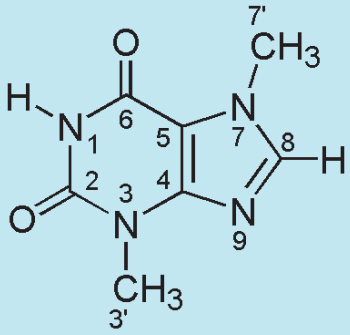
CAS RN 83-67-0, BRN 16464

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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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

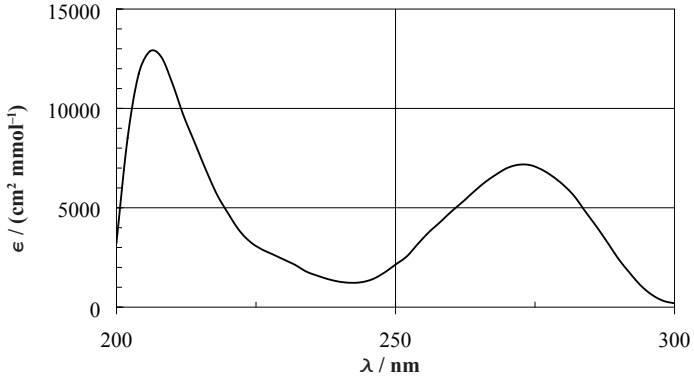
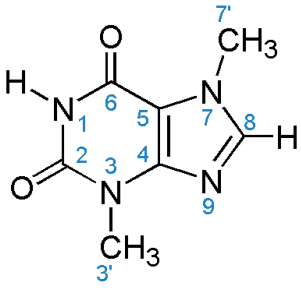


Fig. 1.3-7 UV spectrum in ethanol

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

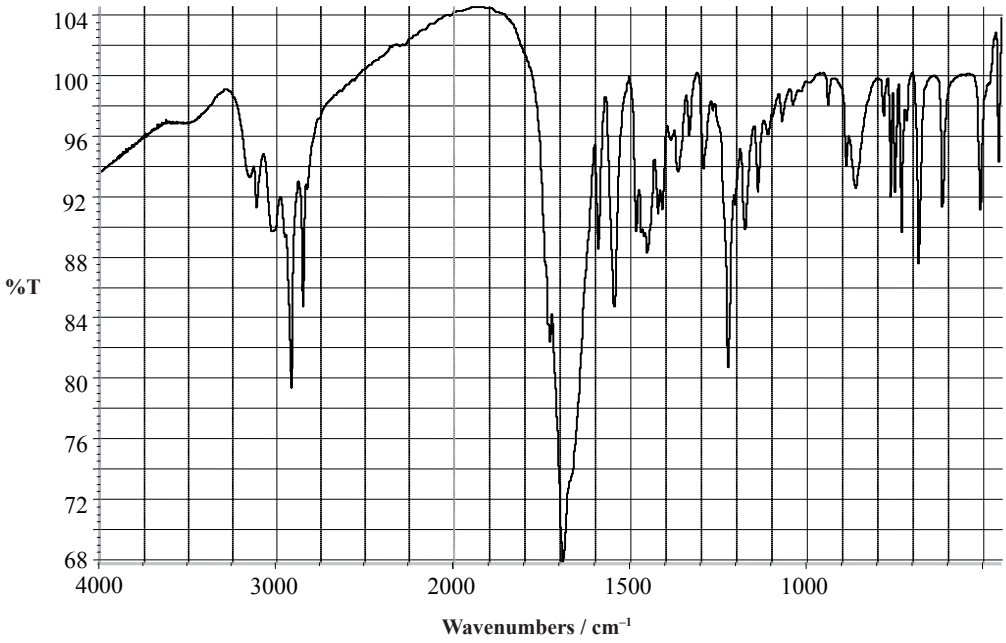
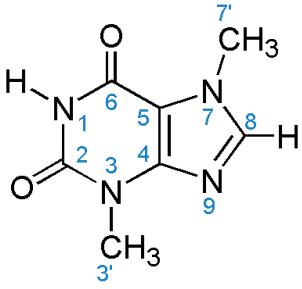


Fig. 1.3-10 IR spectrum in KBr

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

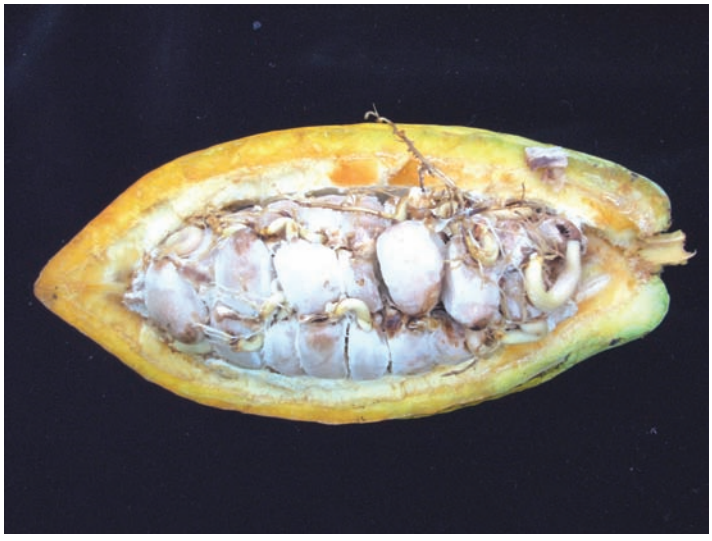


Fig. 1.3-11 Cocoa pod with germinating beans. The size is ca. 60% of the original

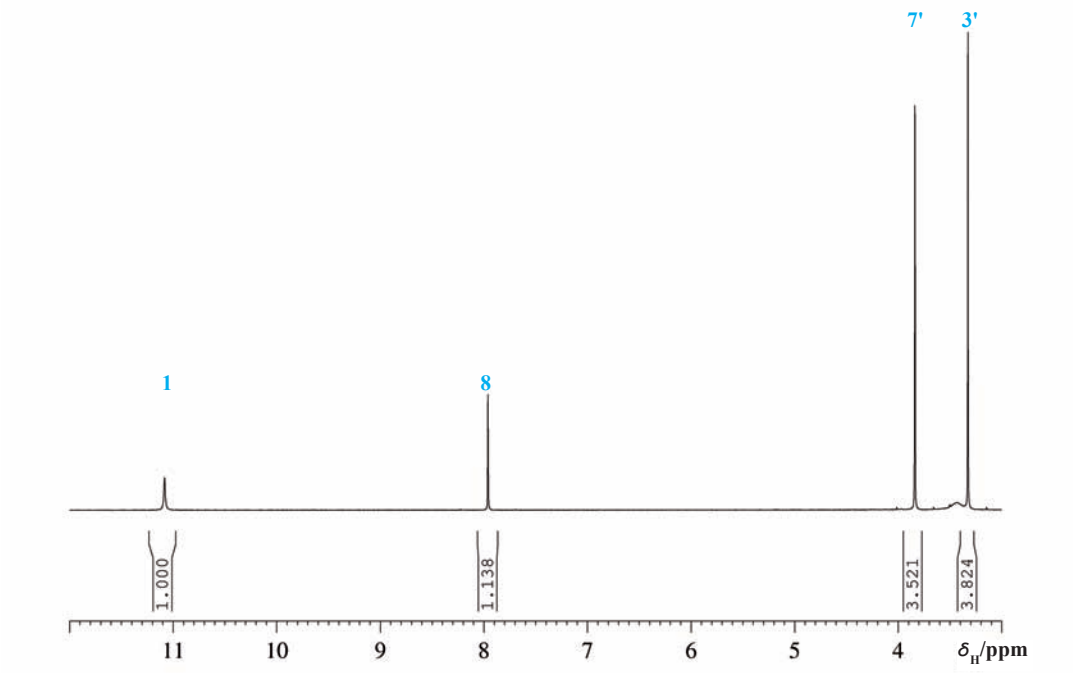


Fig. 1.3-12 ^{1}H NMR spectrum at 400 MHz in DMSO-d_6

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.

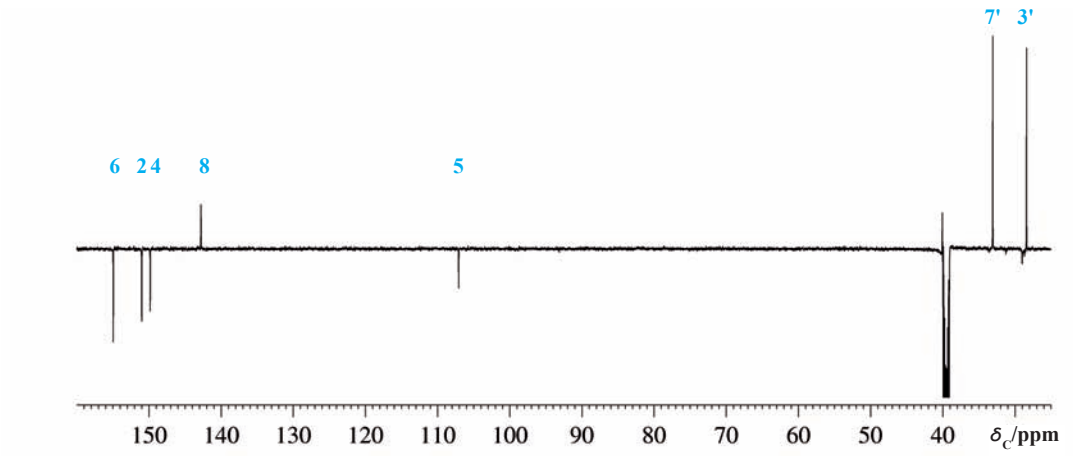


Fig. 1.3-13 APT ^{13}C NMR spectrum in DMSO-d_6

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.

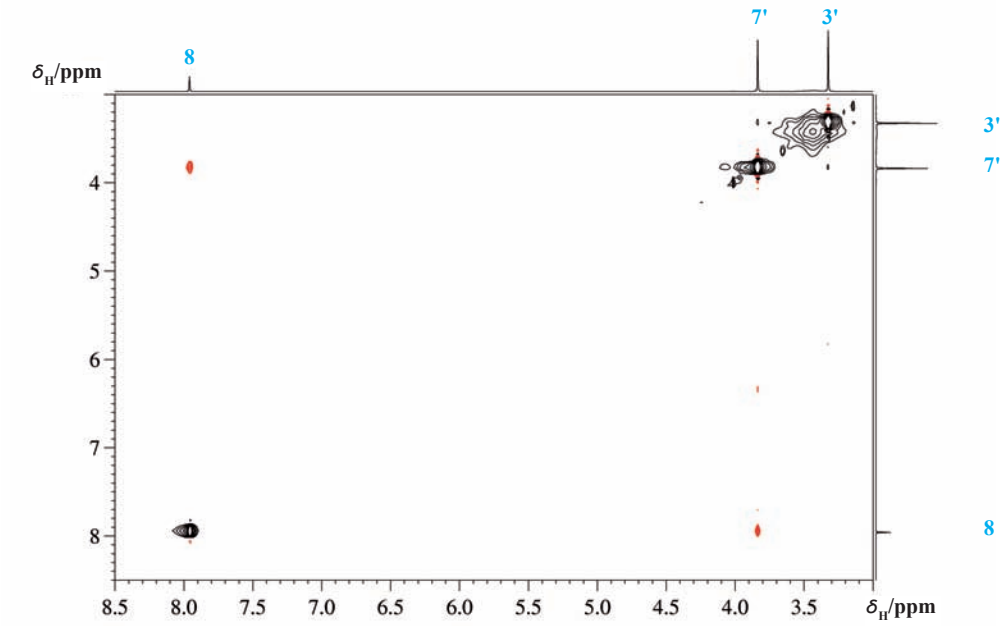


Fig. 1.3-14 NOESY spectrum connecting the aromatic with the aliphatic region

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.

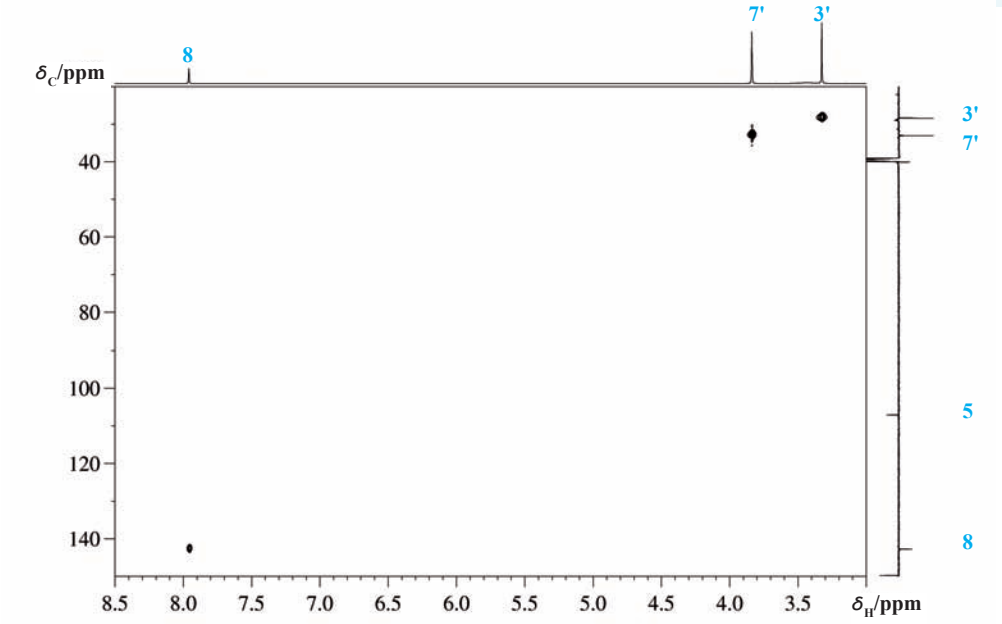


Fig. 1.3-15 HSQC spectrum

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.

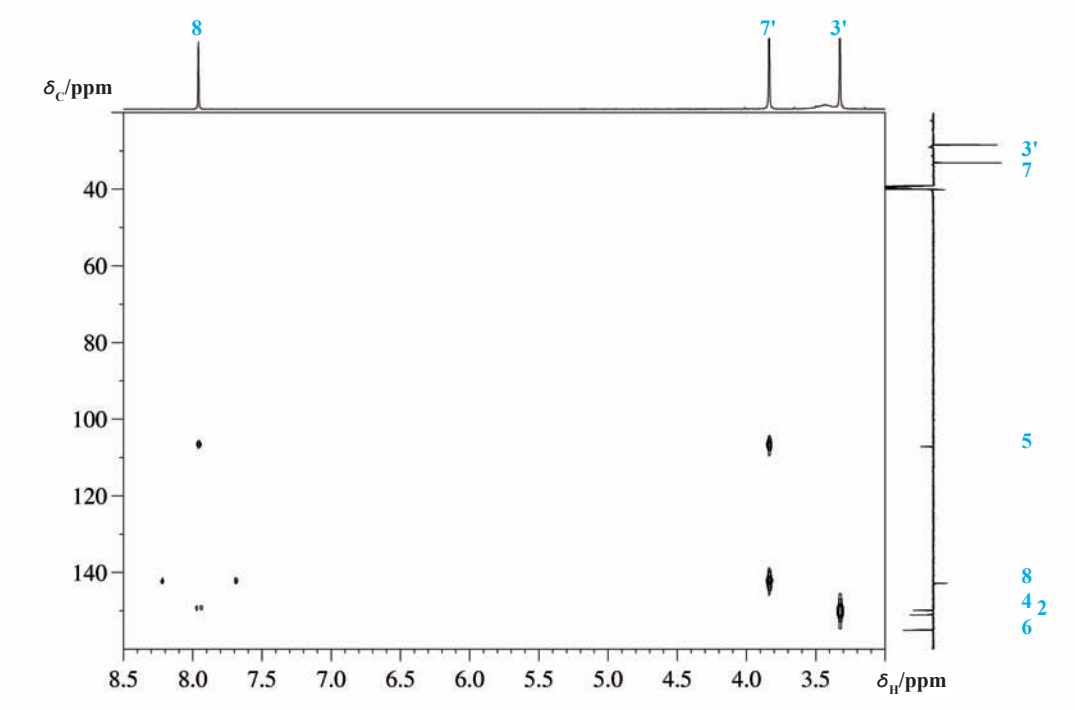


Fig. 1.3-16 HMBC spectrum

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.

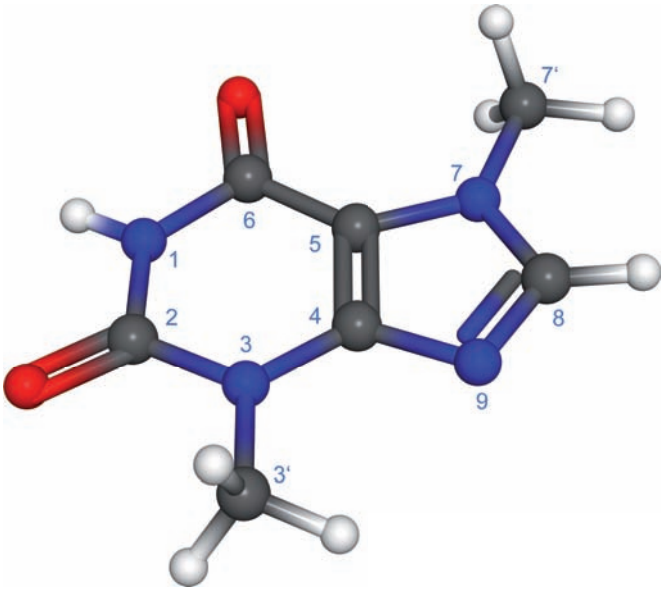


Fig. 1.3-17 Molecular model of theobromine

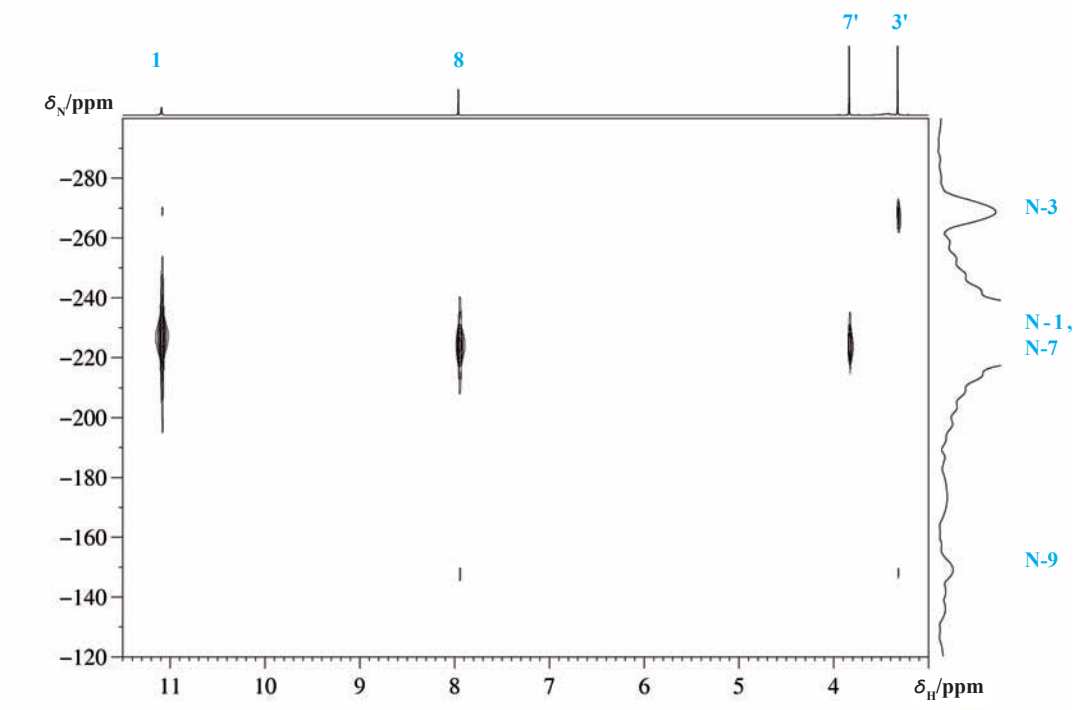
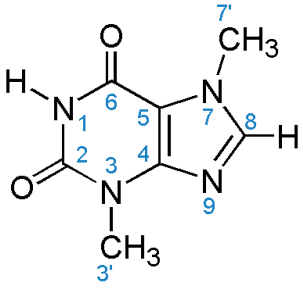


Fig. 1.3-18 ^{1}H ^{15}N HMQC spectrum

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

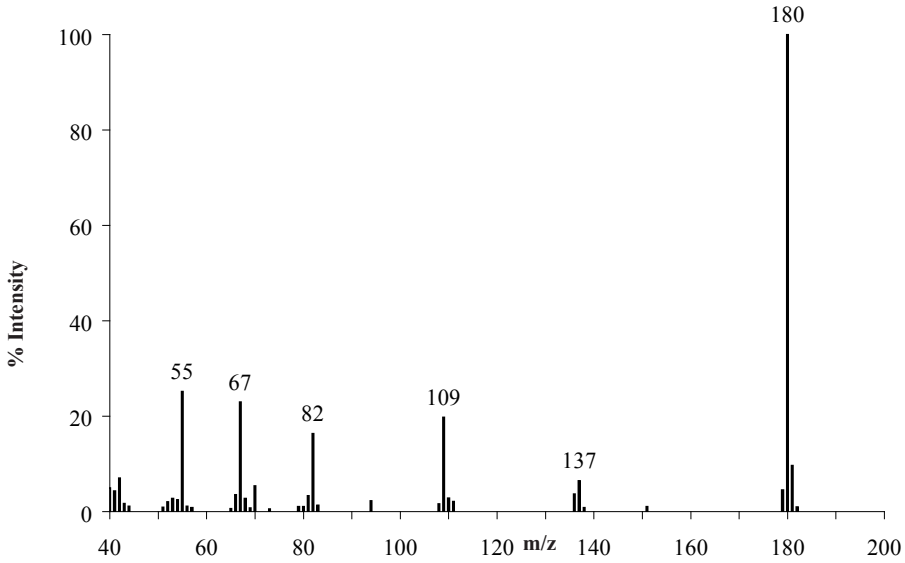
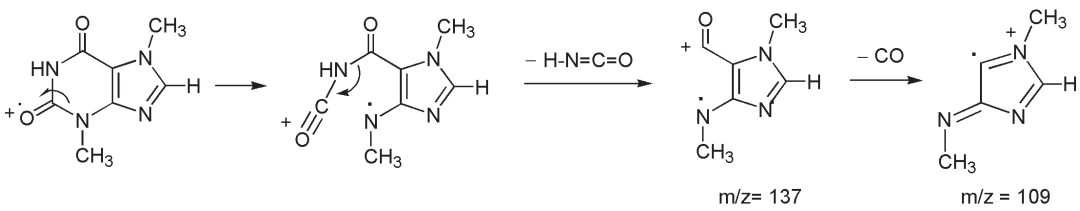


Fig. 1.3-19 Mass spectrum (EI)

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

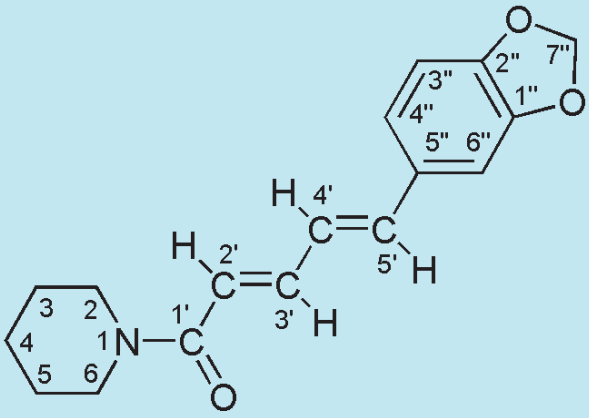
CAS RN 94-62-2, BRN 90741

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].

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.

Fig. 1.4-2 Peppercorns

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

2. Literature

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[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

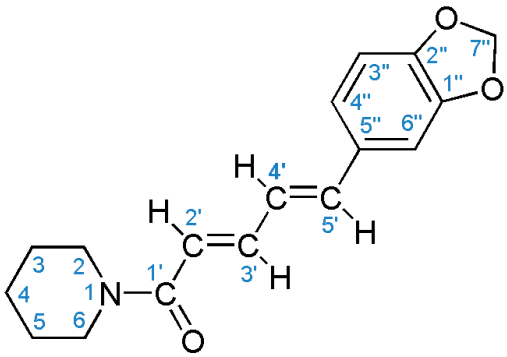
Fig. 1.4-4 Green pepper

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

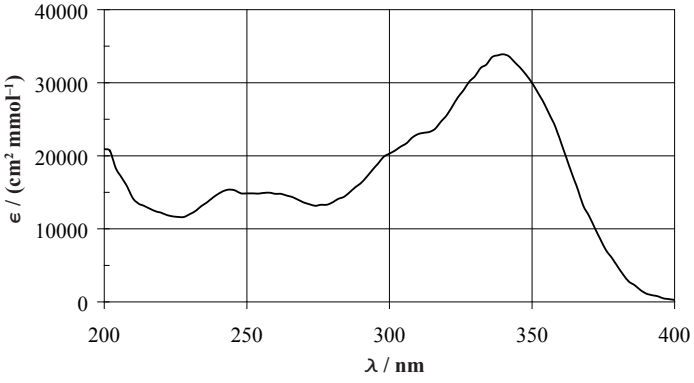


Fig. 1.4-7 UV spectrum in ethanol

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.

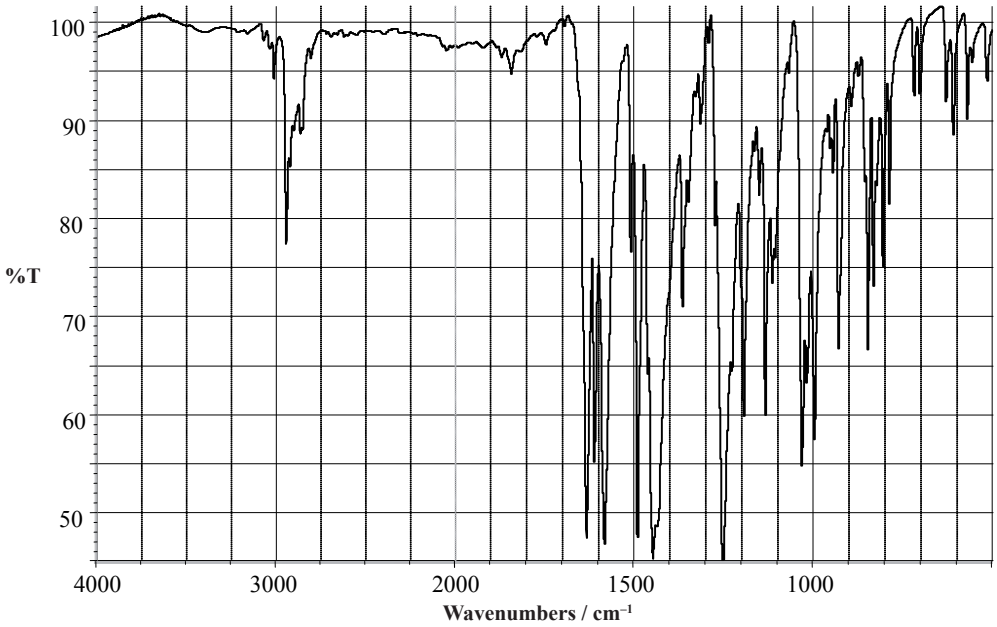


Fig. 1.4-8 IR spectrum in KBr

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.

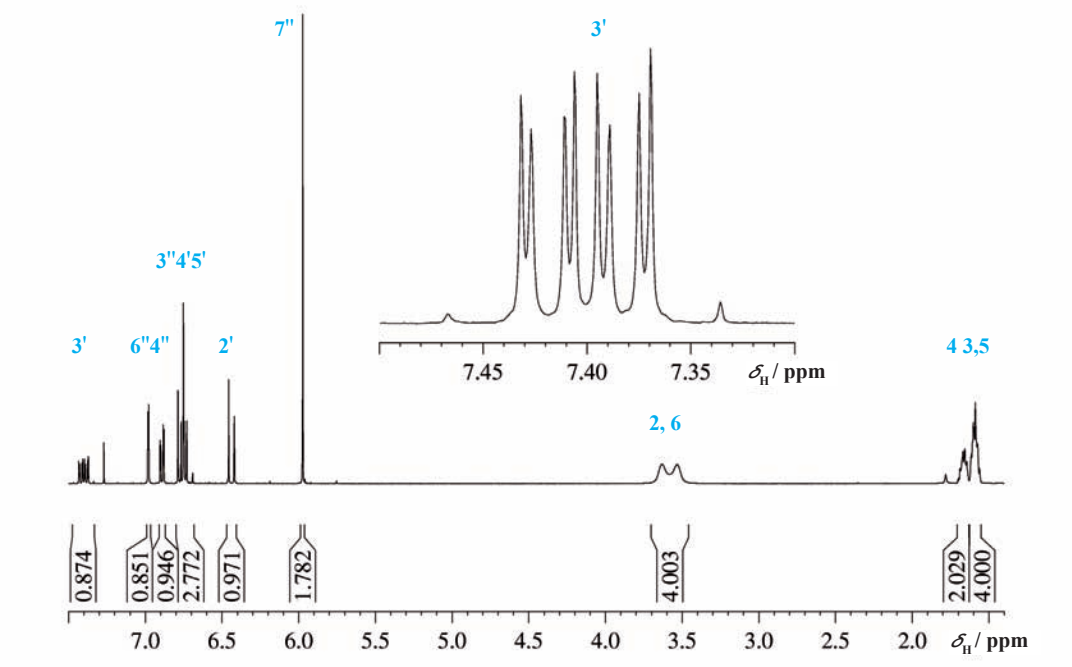
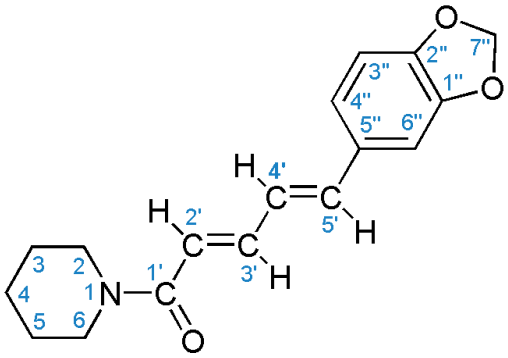


Fig. 1.4-9 ^{1}H NMR spectrum at 400 MHz in $CDCl_3$

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

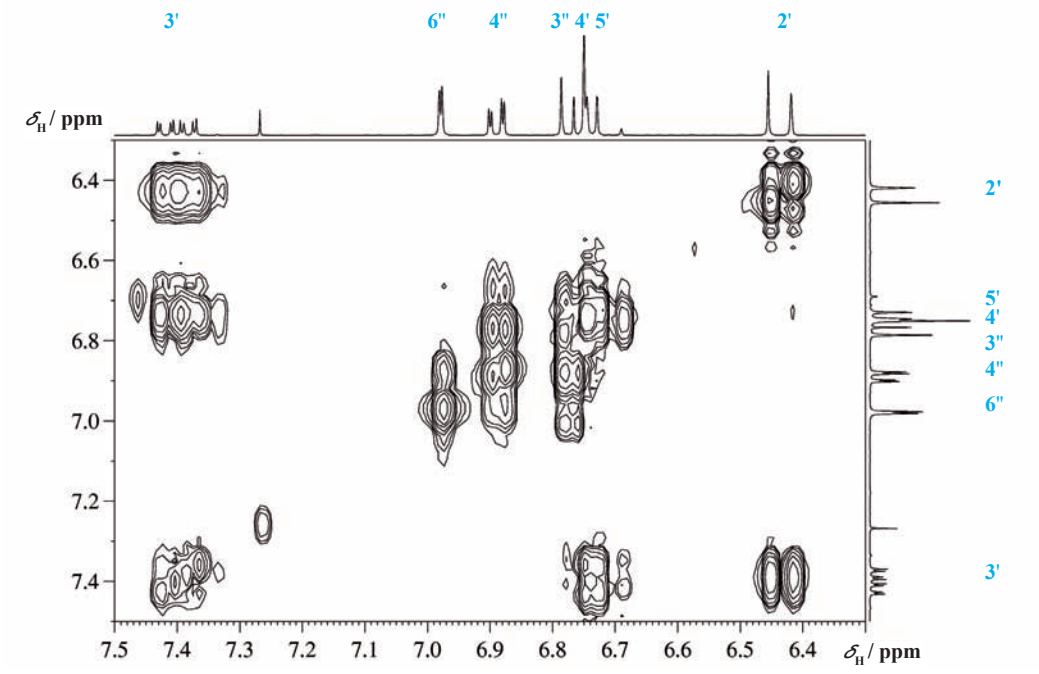


Fig. 1.4-12 Expansion of the COSY spectrum in the olefinic/aromatic region

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.

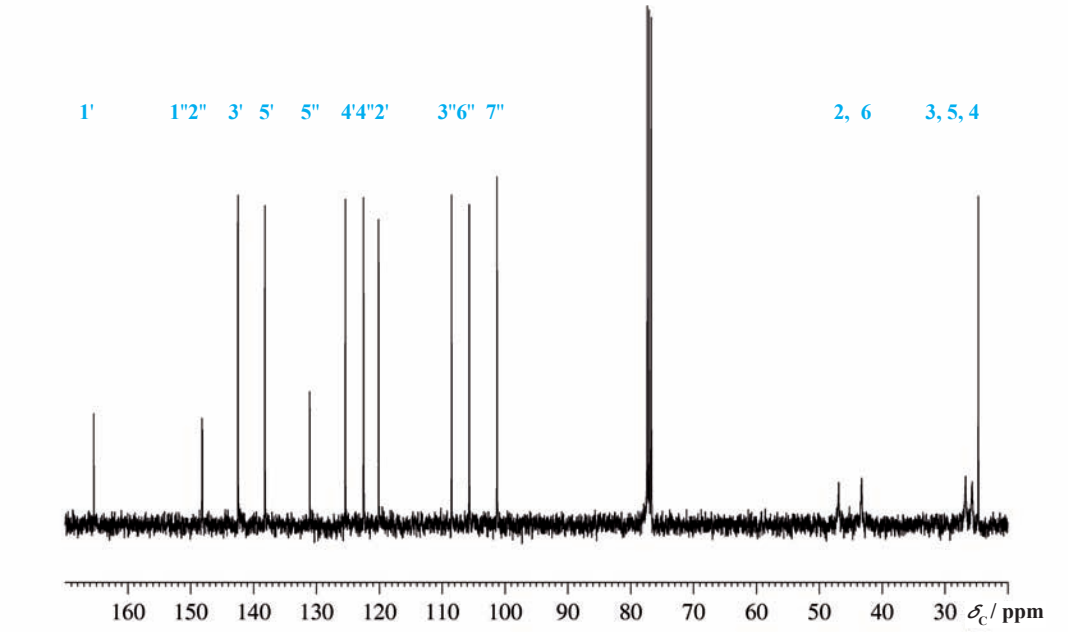


Fig. 1.4-13 ^{13}C NMR spectrum at 100 MHz in $CDCl_3$

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.

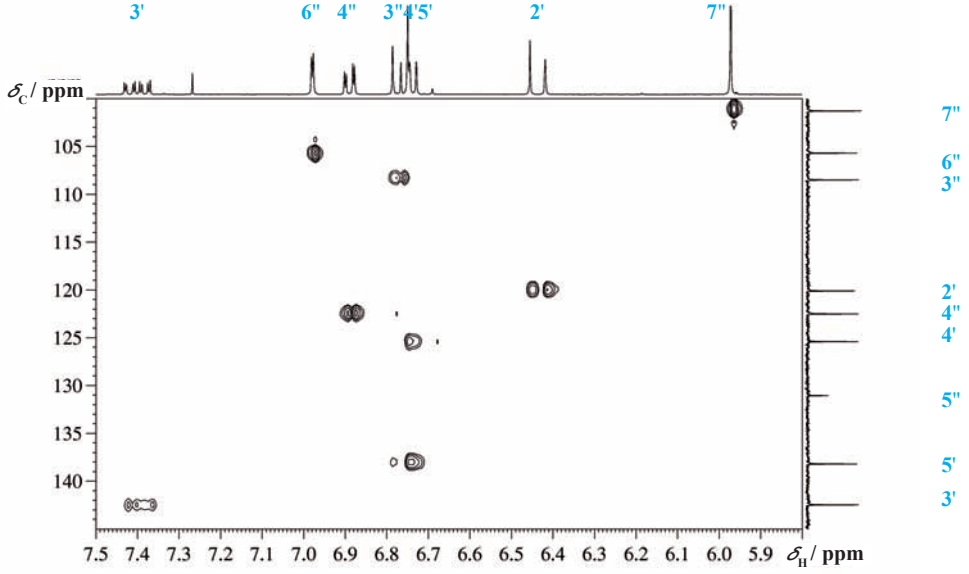


Fig. 1.4-14 Expansion of the HSQC spectrum in the olefinic and aromatic region

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.

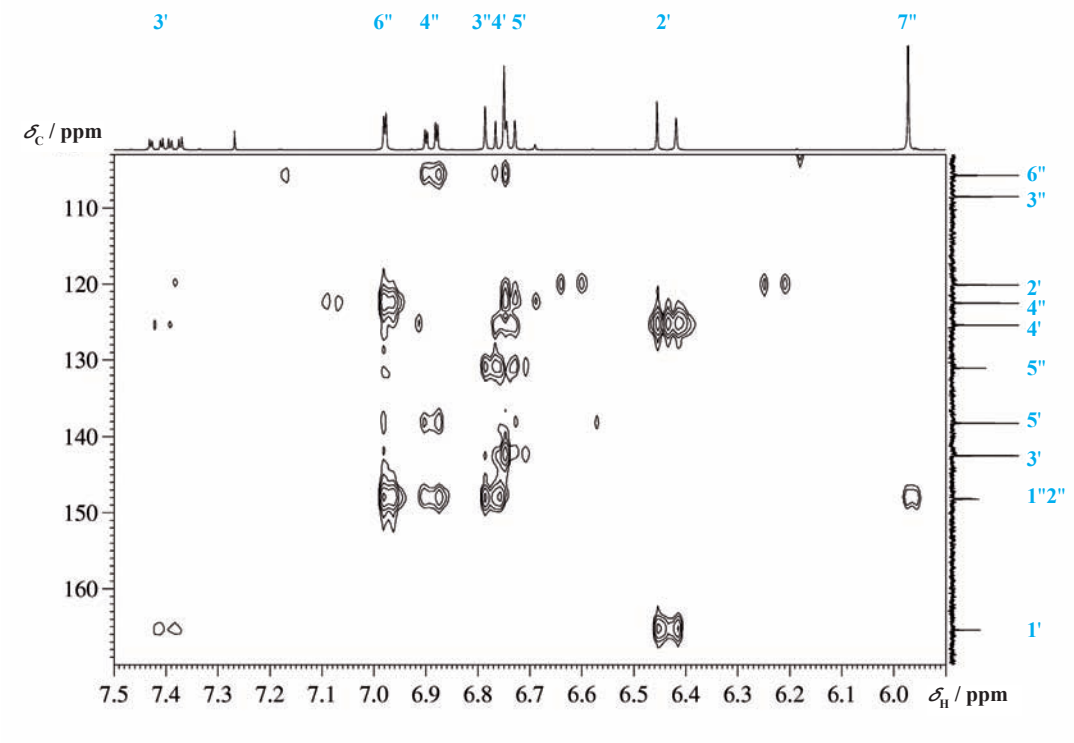
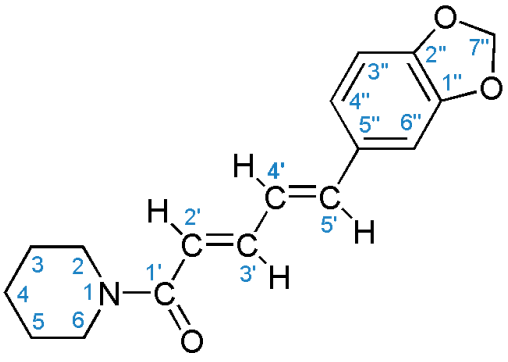


Fig. 1.4-15 Expansion of the HMBC spectrum in the aromatic region

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

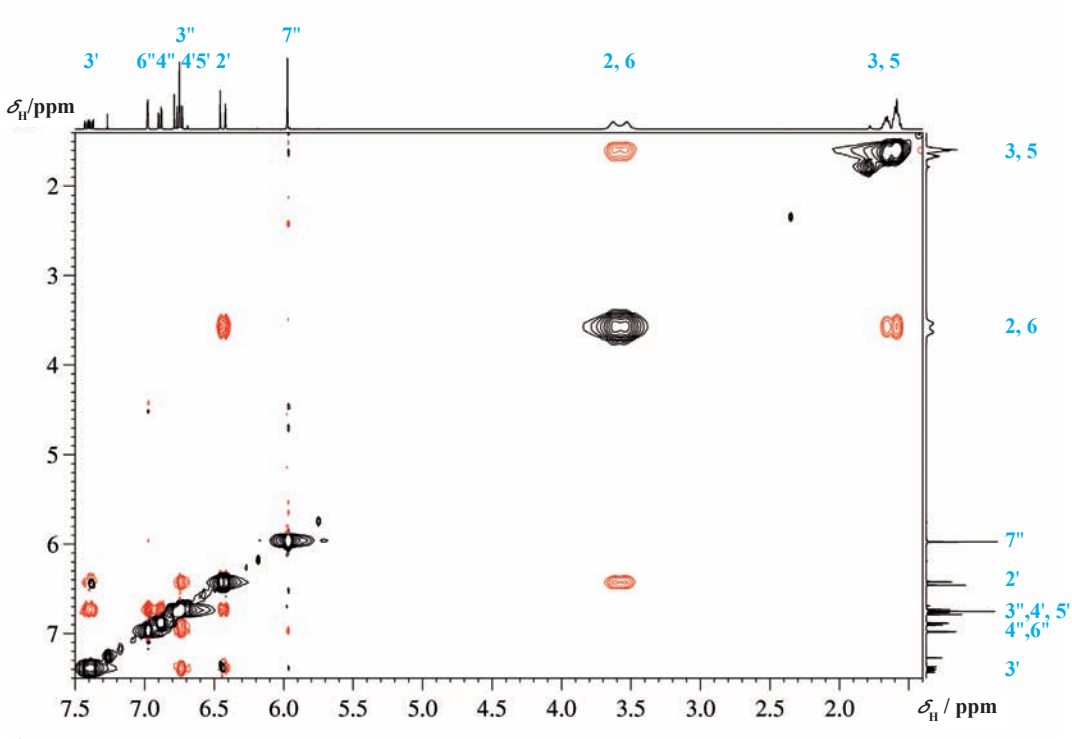


Fig. 1.4-16 NOESY spectrum

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'.

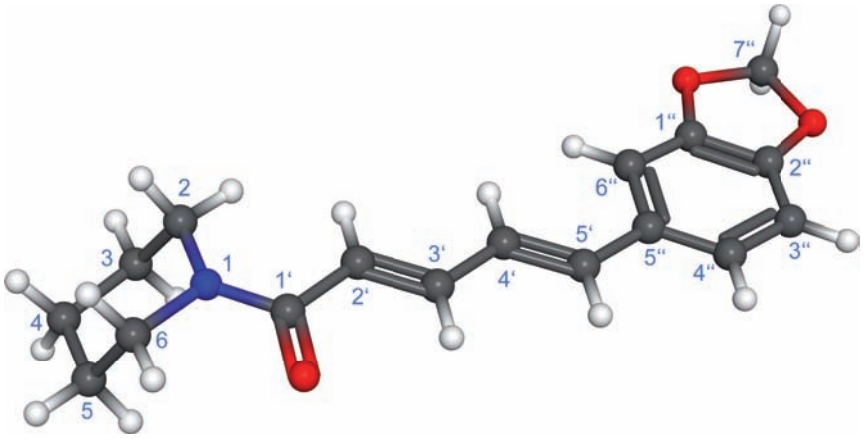


Fig. 1.4-17 Molecular model of piperine

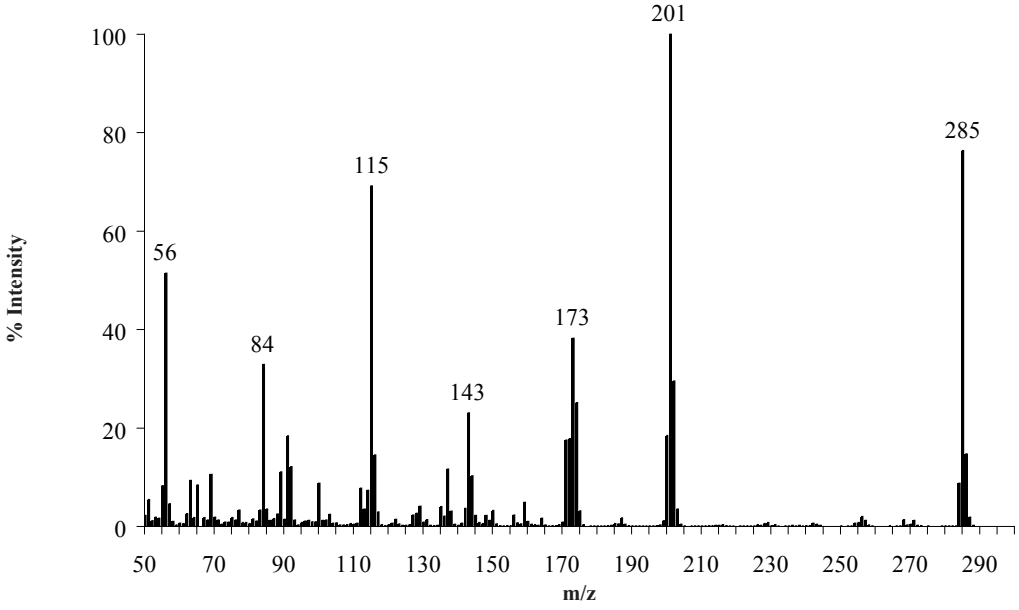


Fig. 1.4-18 Mass spectrum (EI)

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.

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)

^{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

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

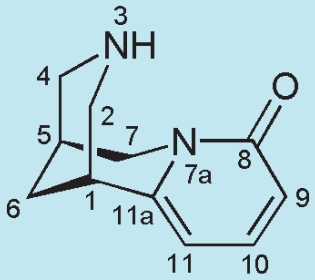
$[\alpha]_D^{22}$ −108.1° (*c* 0.0198 g/mL, ethanol)

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

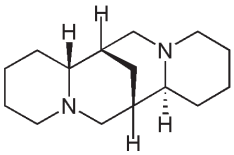
seems understandable when comparing the two structures. This leads to the pharmacological effect of cross-tolerance between the compounds. Cytisine intoxication is similar to nicotine poisoning. The central nervous system is first stimulated and then paralysed. The symptoms, which set in rapidly within 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

[1] J.-F. Etter, R. J. Lukas, N. L. Benowitz, R. West, C. M. Dresler, "Cytisine for smoking cessation: a research agenda" *Drug and Alcohol Dependence* **2008**, *92*, 3−8.

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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

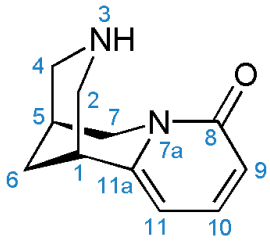
The filter cake is washed with dichloromethane until a colourless filtrate is obtained. The filtrate is transferred into a separating funnel and shaken with 3.3 M HCl (ca. 140 mL). The contents are left in the funnel for 2 h and shaken repeatedly to ensure good mixing and passing over of the alkaloid 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.

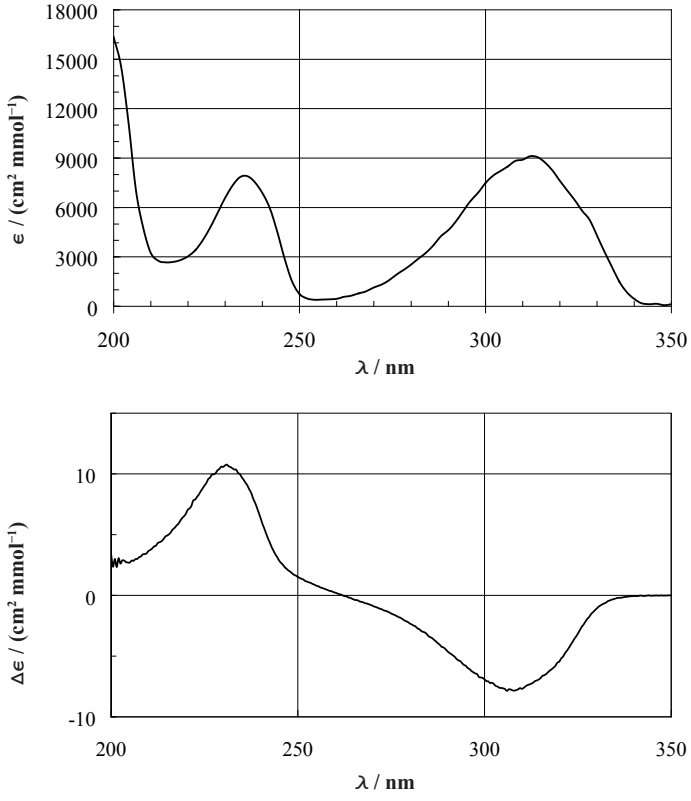
$[\alpha]_D^{22}$ −108.1° (*c* 0.0198 g/mL, ethanol).

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



Scheme 1.5-2

4. Spectra and Comments

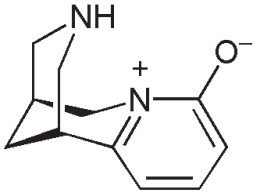


In einem Garten
unter dunklen Bäumen
erwarten wir die Frühlingsnacht.
Noch glänzt kein Stern.
Aus einem Fenster,
schwellend,
die Töne einer Geige. . . .
Der Goldregen blinkt,
der Flieder duftet,
in unsern Herzen geht der Mond auf!

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.

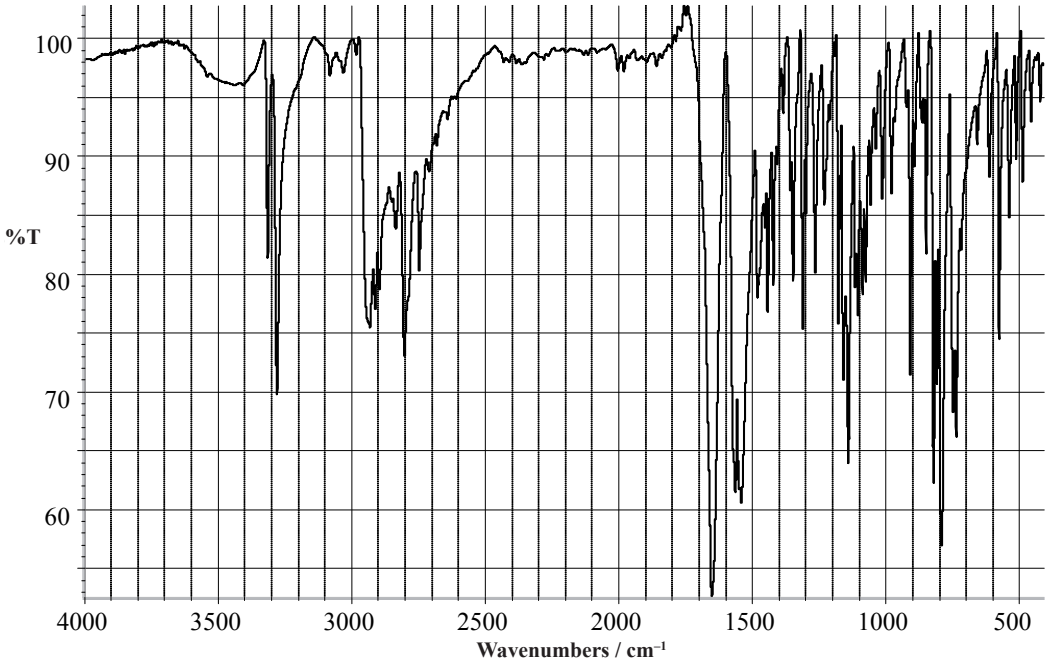


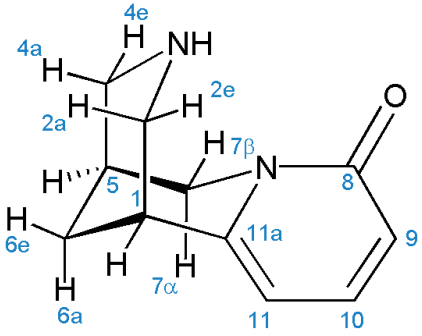
Fig. 1.5-5 IR spectrum in KBr

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

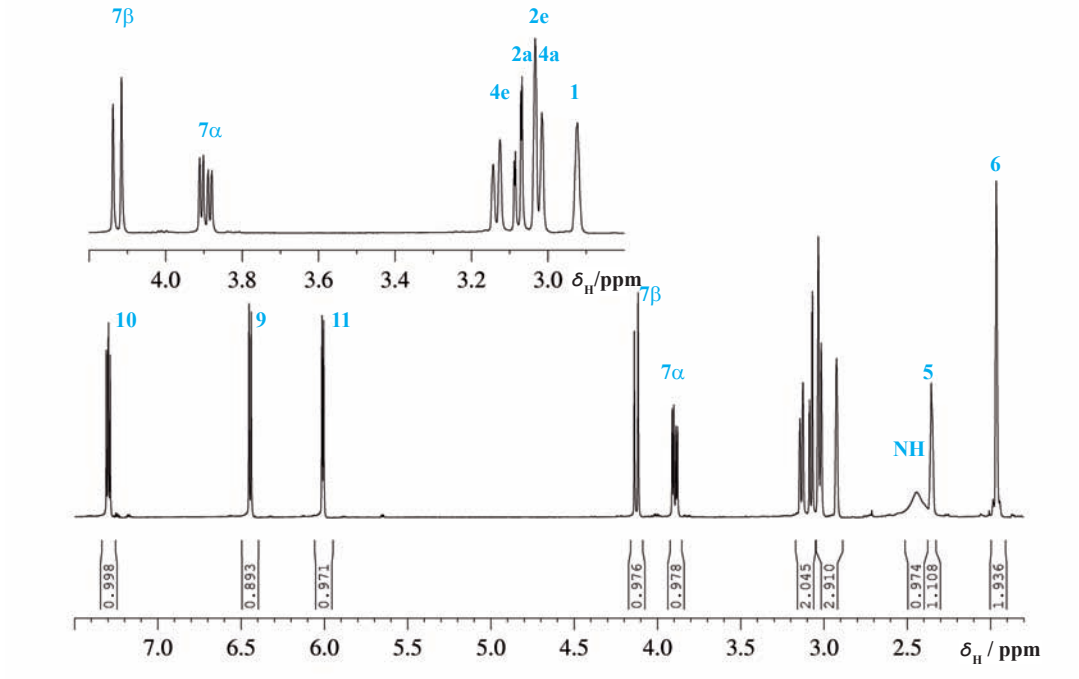


Fig. 1.5-7 ^{1}H NMR spectrum at 700 MHz in $CDCl_3$

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.

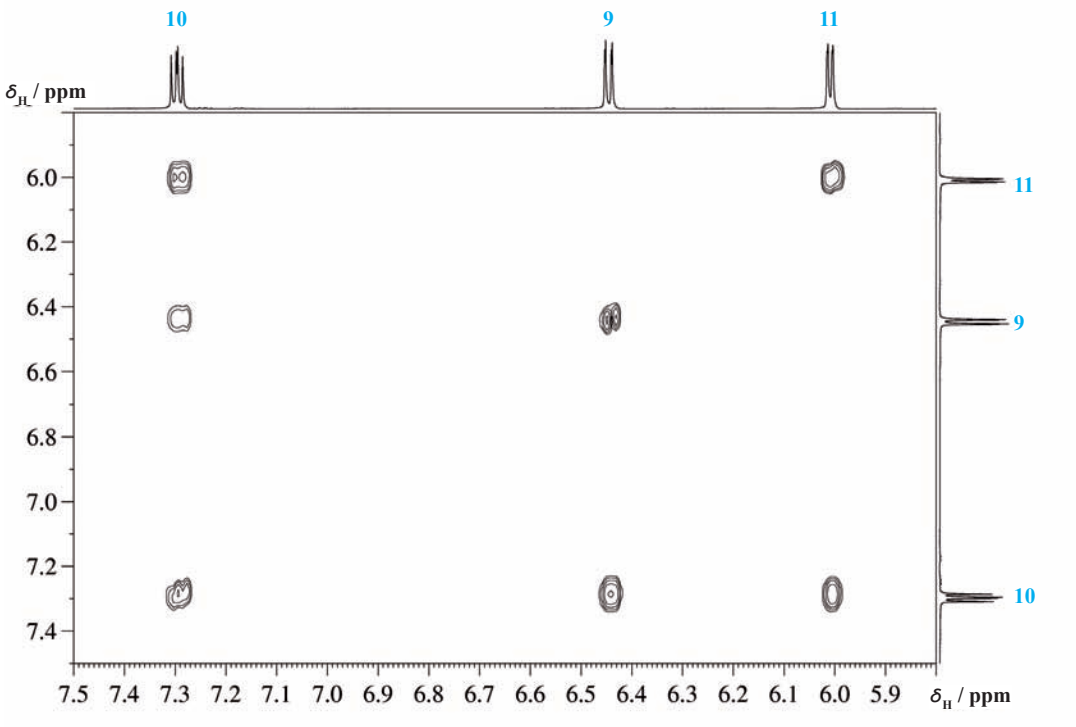


Fig. 1.5-8 Expansion of the COSY spectrum in the aromatic region

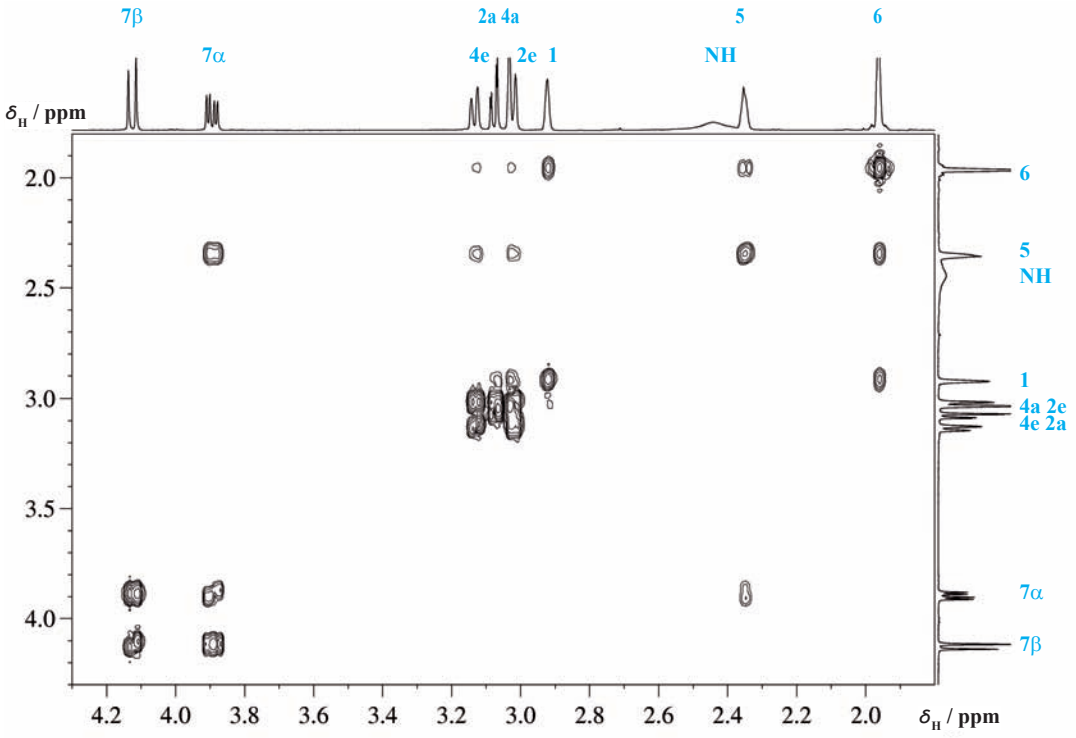
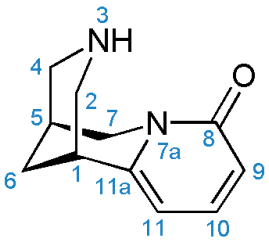


Fig. 1.5-9 Expansion of the COSY spectrum in the aliphatic region

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

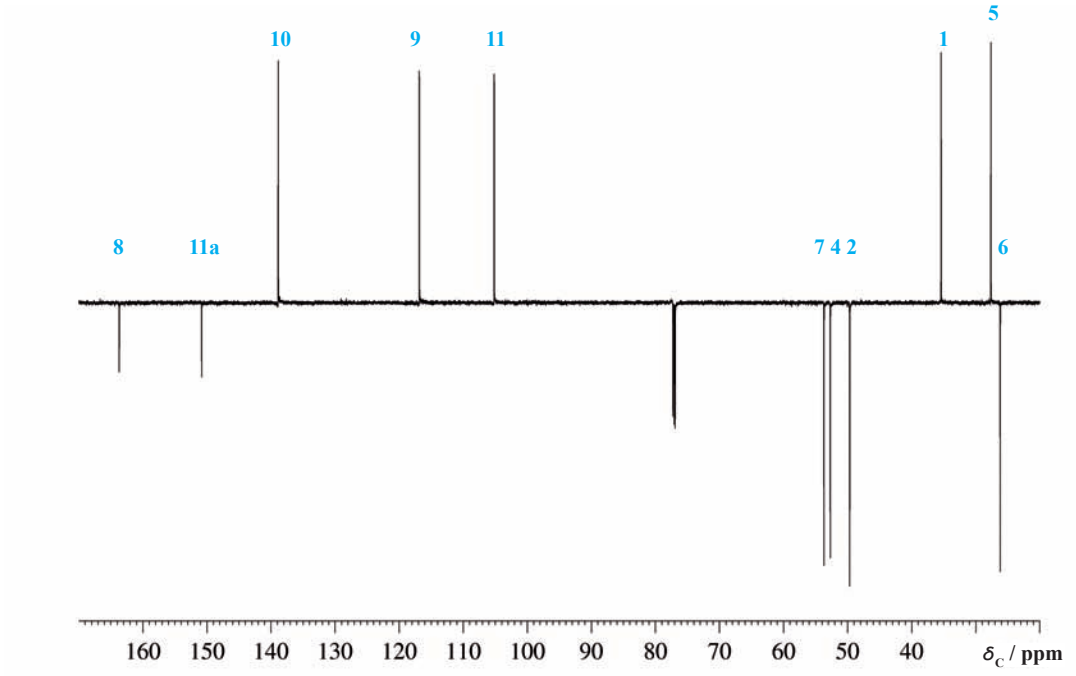


Fig. 1.5-10 APT ^{13}C NMR spectrum

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.

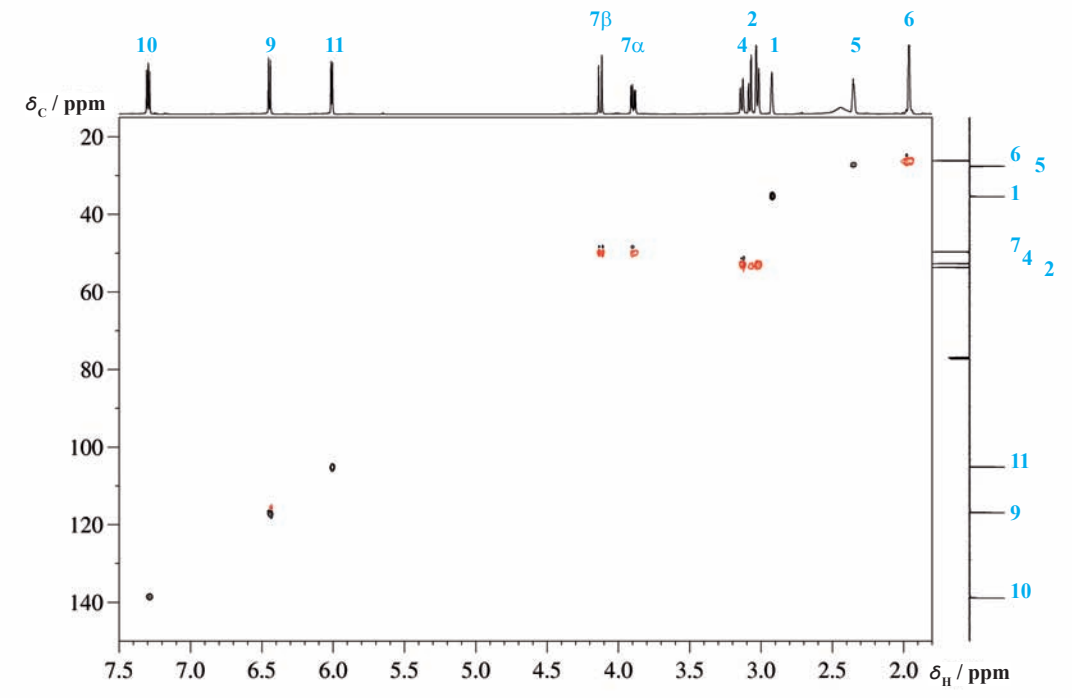
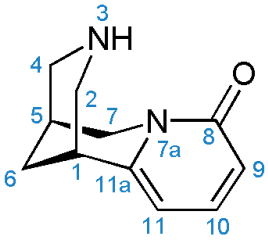


Fig. 1.5-11 HSQC spectrum

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.

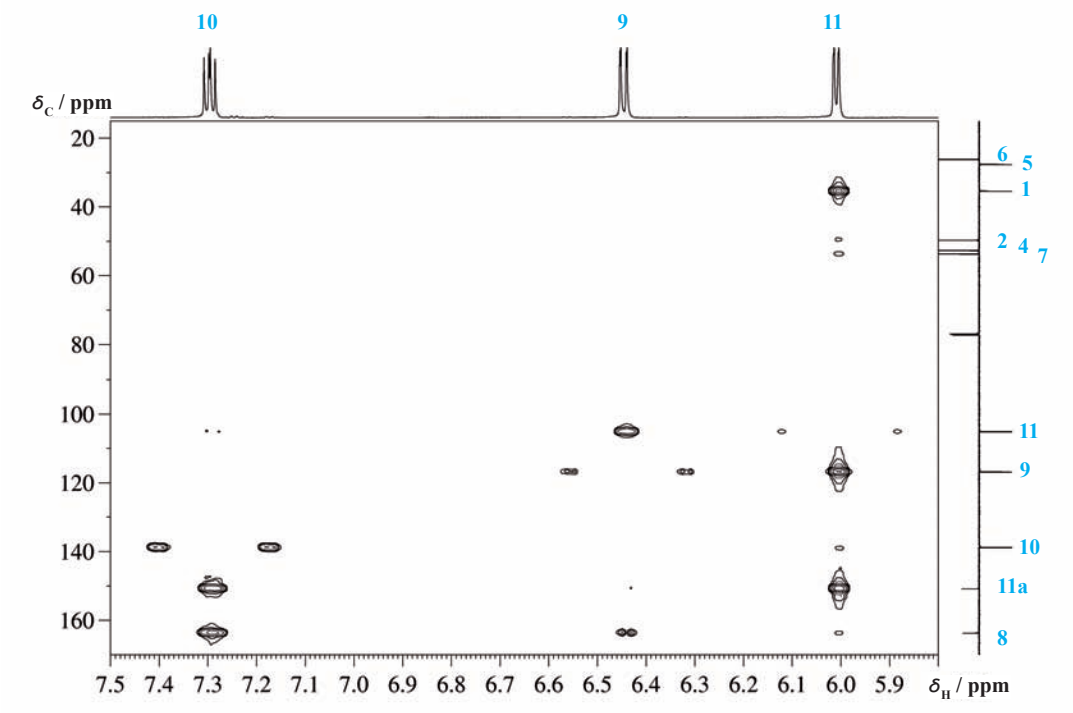


Fig. 1.5-12 Expansion of the HMBC spectrum in the aliphatic region

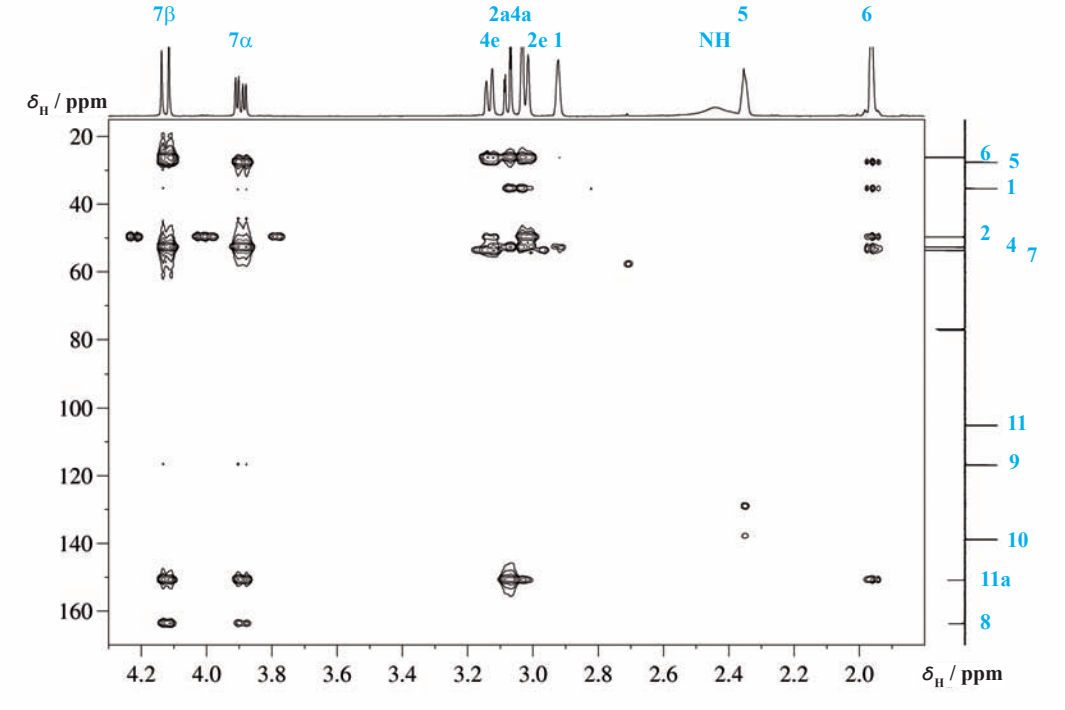


Fig. 1.5-13 Expansion of the HMBC spectrum in the aromatic region

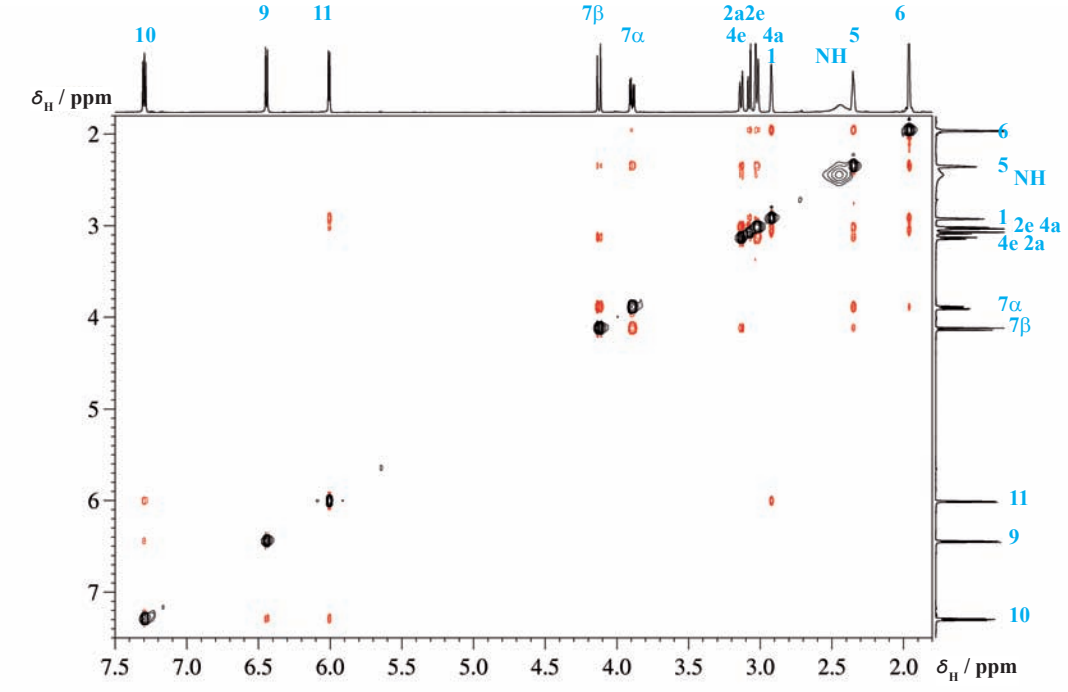


Fig. 1.5-14 NOESY spectrum

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.

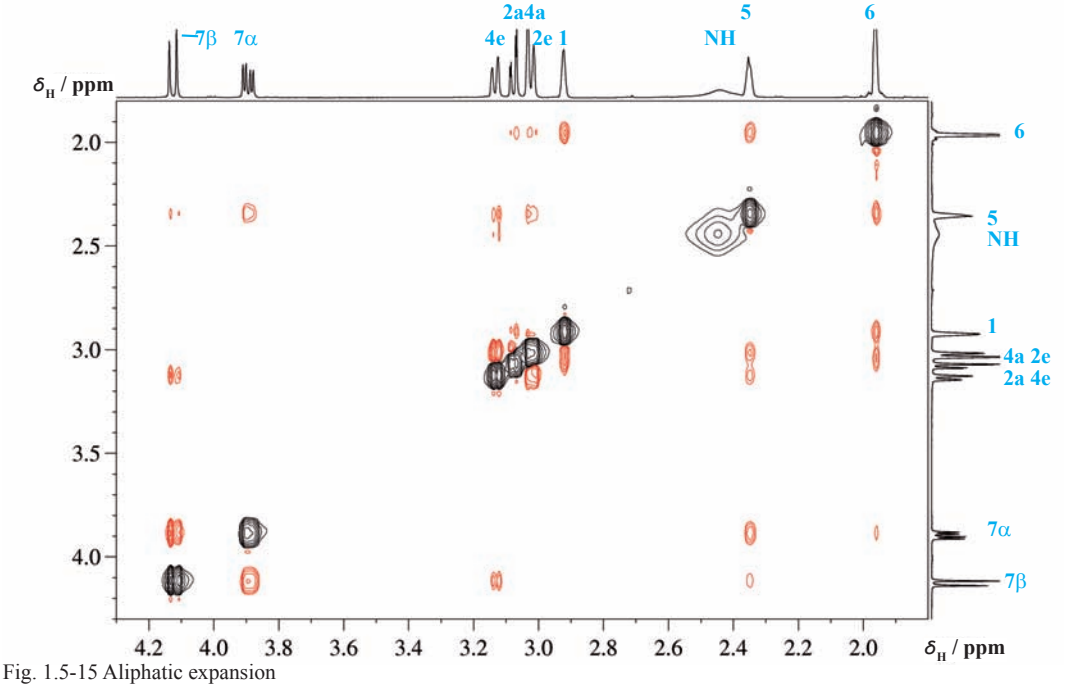


Fig. 1.5-15 Aliphatic expansion

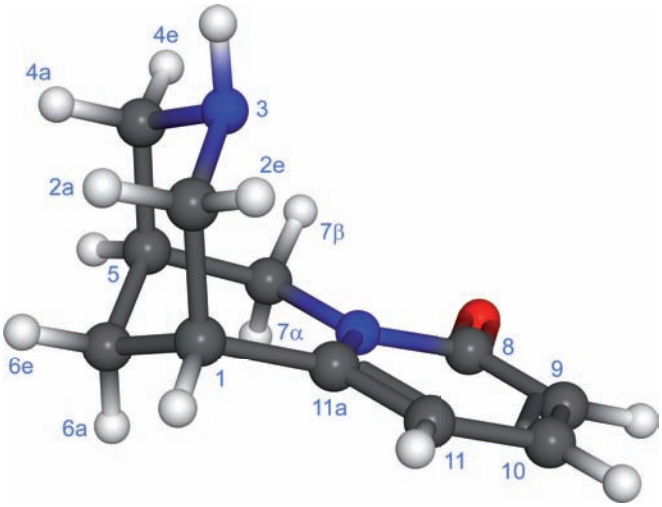


Fig. 1.5-16 Molecular model of cytisine

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

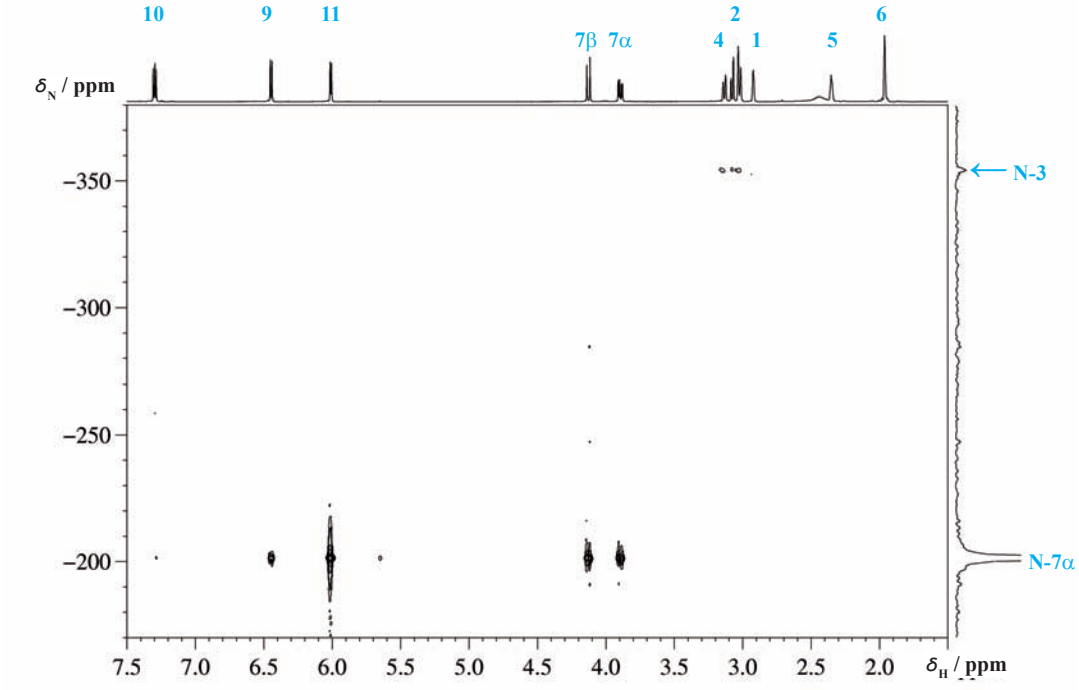
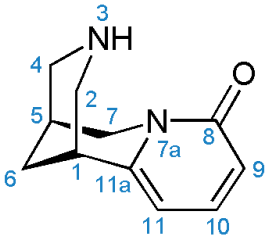


Fig. 1.5-17 $^{1}H^{15}N$ HMQC spectrum

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

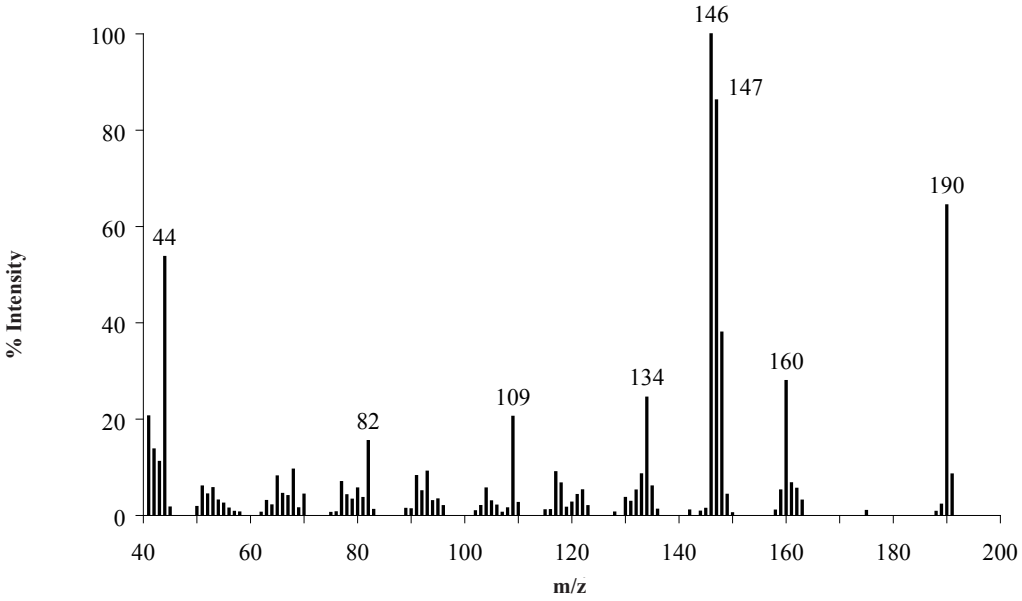
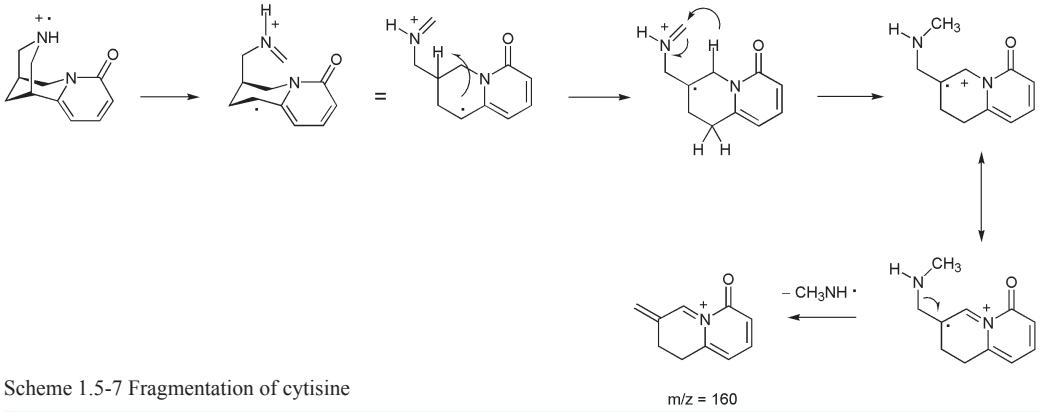


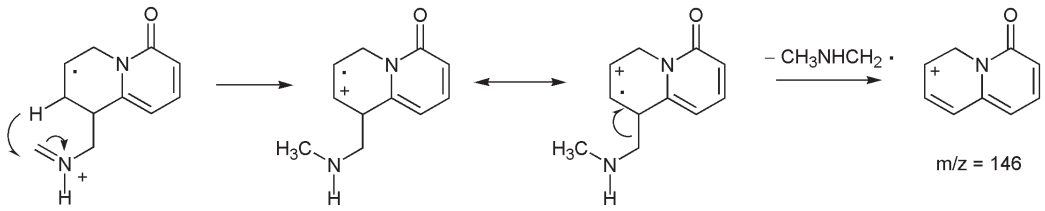
Fig. 1.5-19 Mass spectrum

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

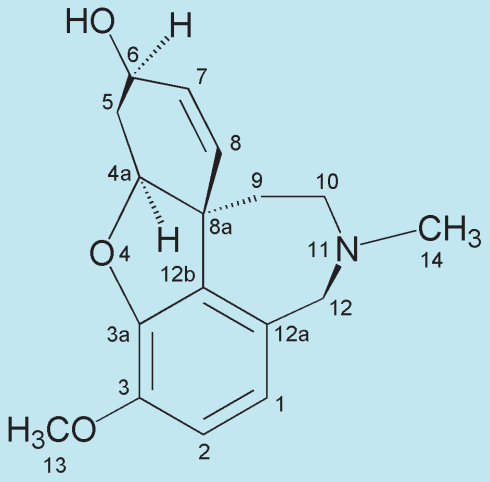
Colourless crystals, mp 128 °C (database value)

$[\alpha]_D^{22}$ –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.

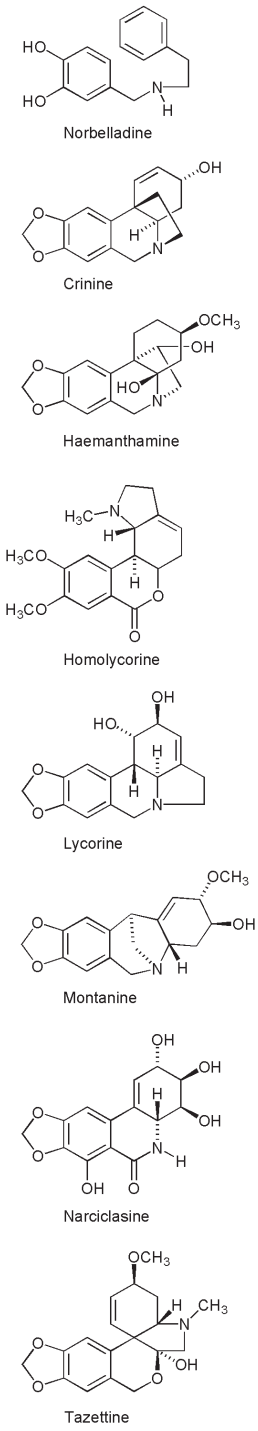
Early, its acetylcholinesterase-inhibiting property was recognized and the ability to act as an antagonist of curare's action. In fact, galanthamine enhances the cholinergic function by increasing the level of the neurotransmitter acetylcholine in the brain. Furthermore, galanthamine is also capable 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

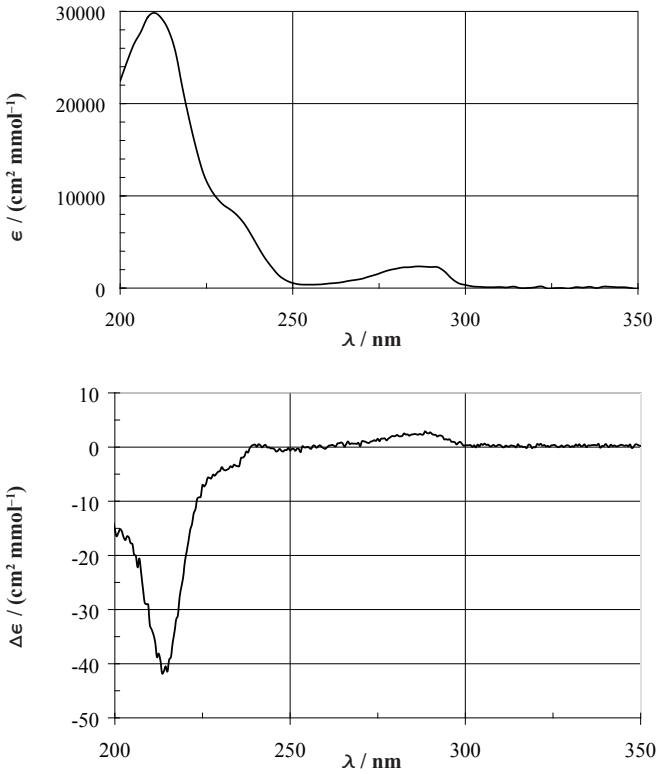
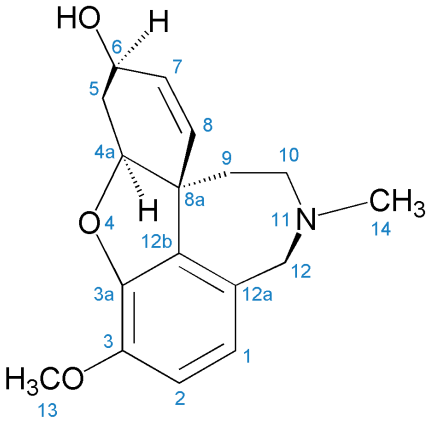


Fig. 1.6-12 UV and CD spectra in ethanol

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

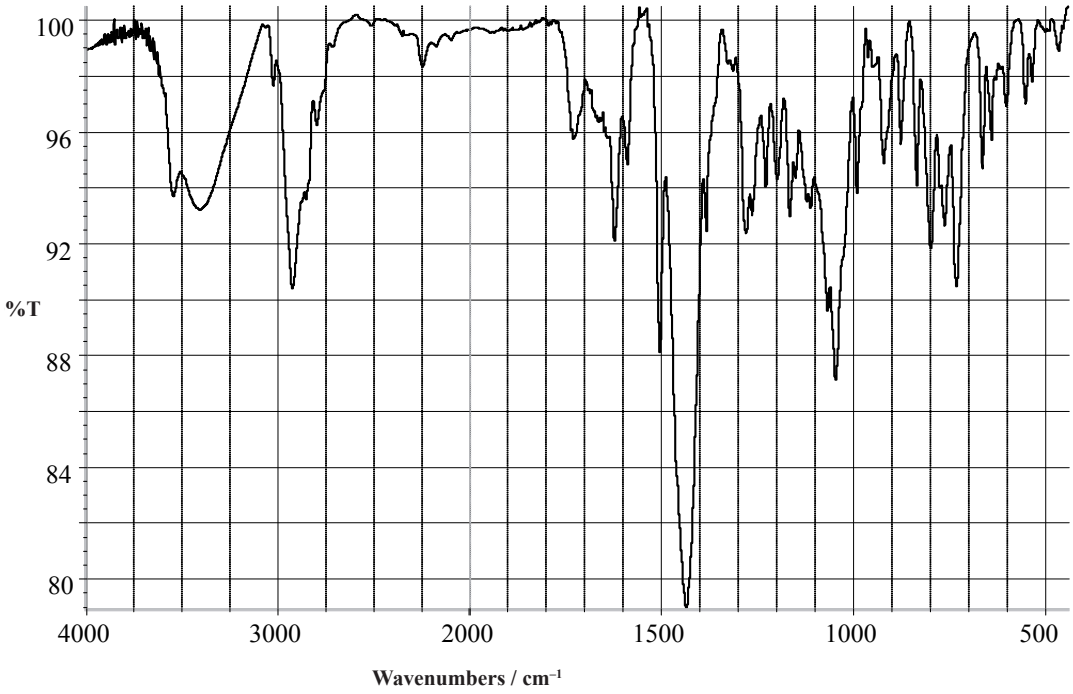


Fig. 1.6-14 IR spectrum as film

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.

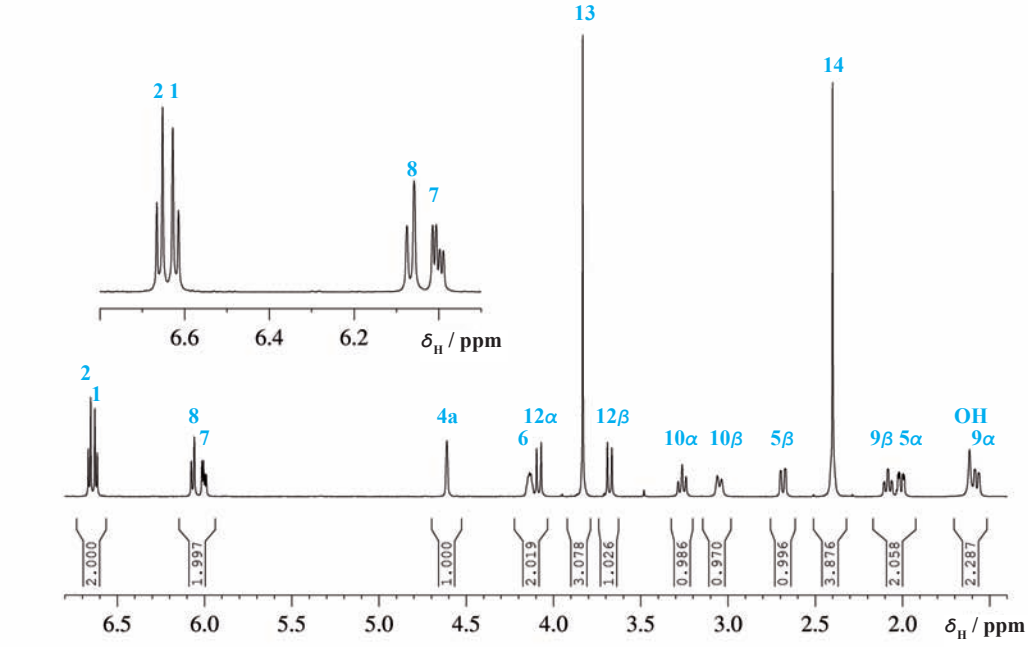


Fig. 1.6-15 ^{1}H NMR spectrum at 600 MHz in $CDCl_3$

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.

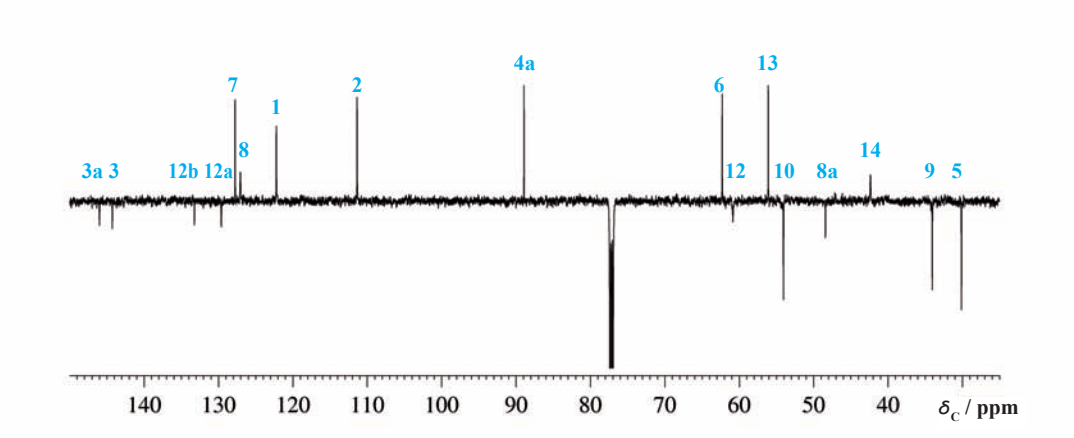
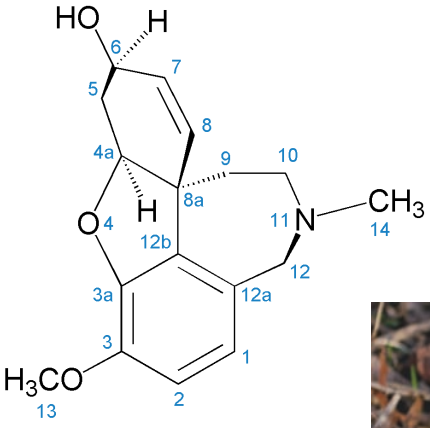


Fig. 1.6-16 APT ^{13}C NMR spectrum at 150 MHz in $CDCl_3$

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.

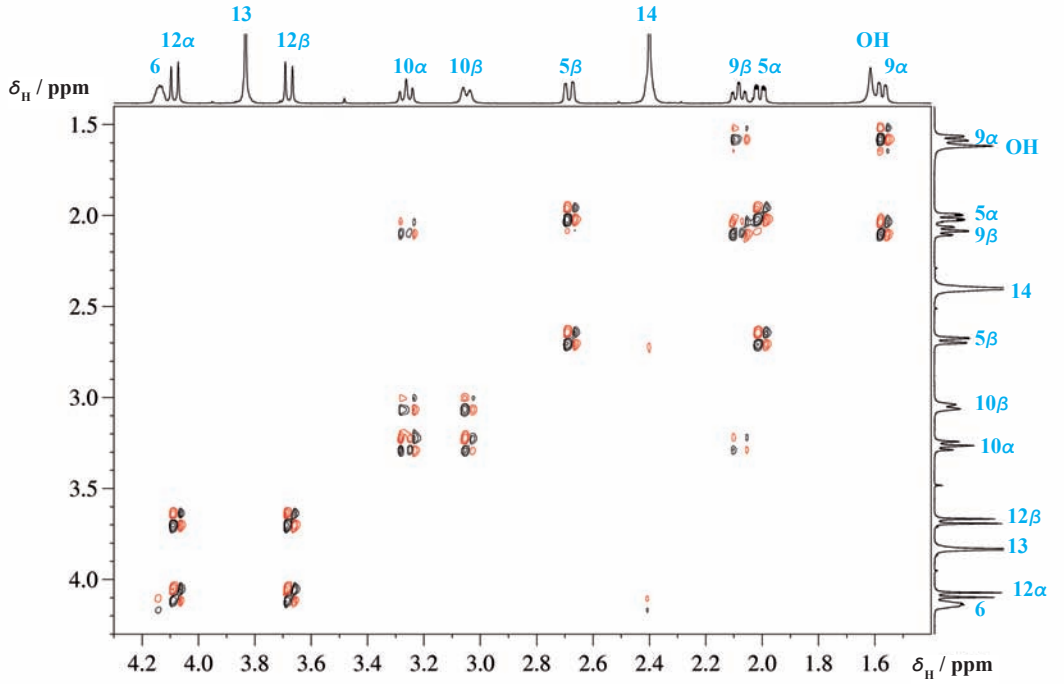


Fig. 1.6-18 Expansion of the DQF-COSY spectrum in the aliphatic region

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

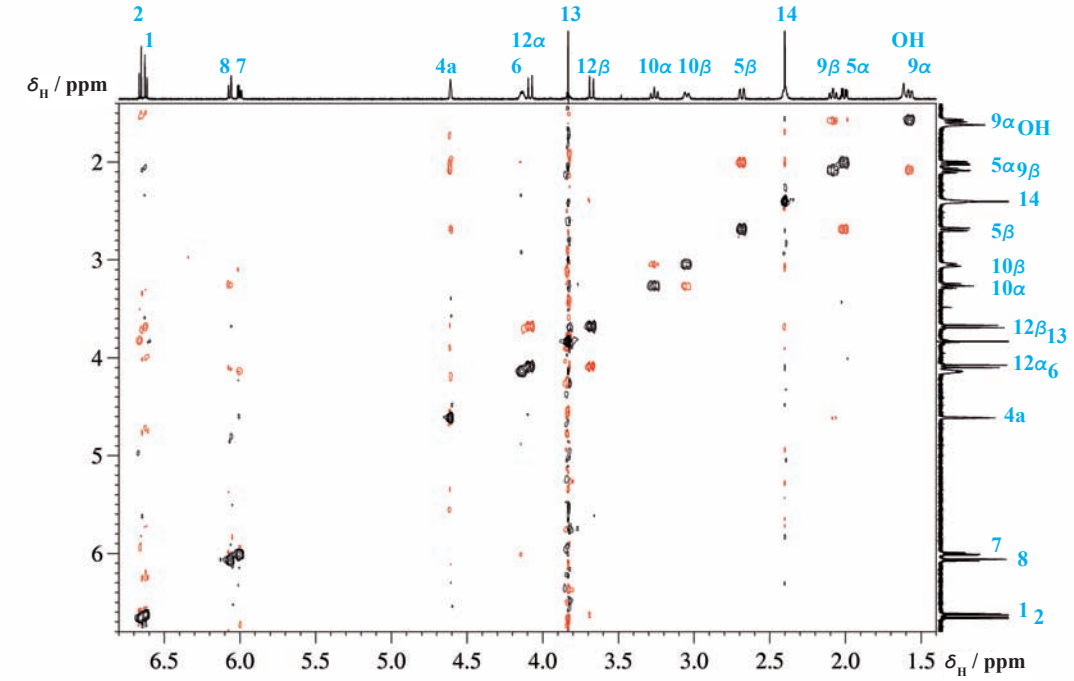
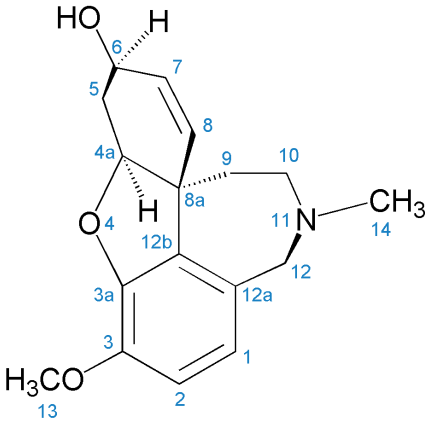


Fig. 1.6-20 NOESY spectrum

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

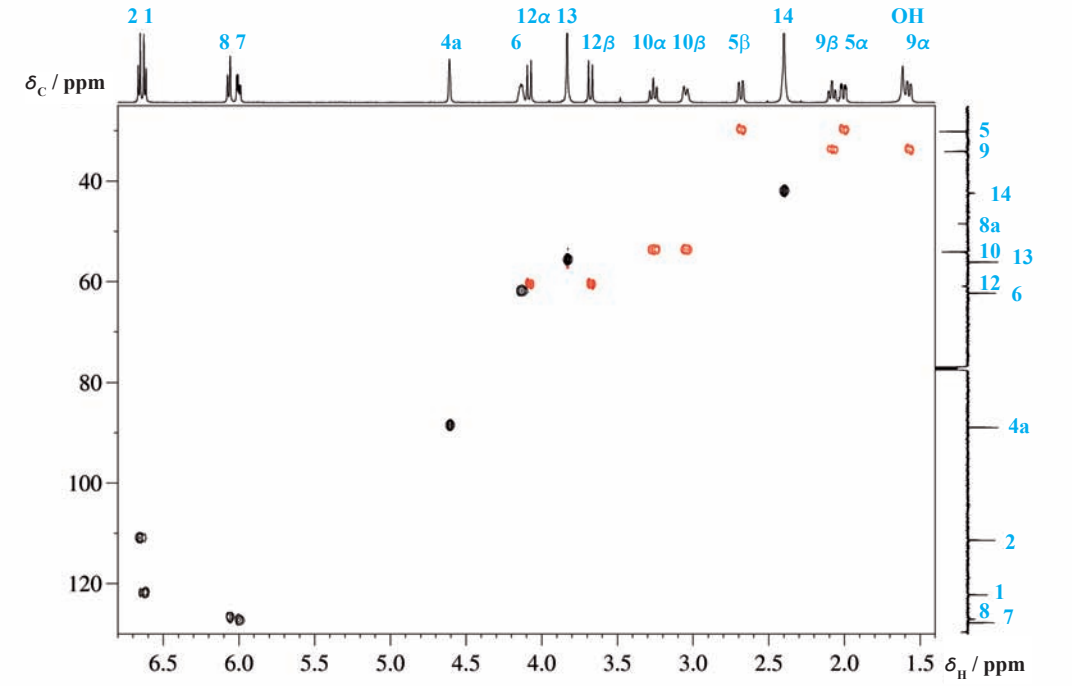


Fig. 1.6-21 HSQC spectrum

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.

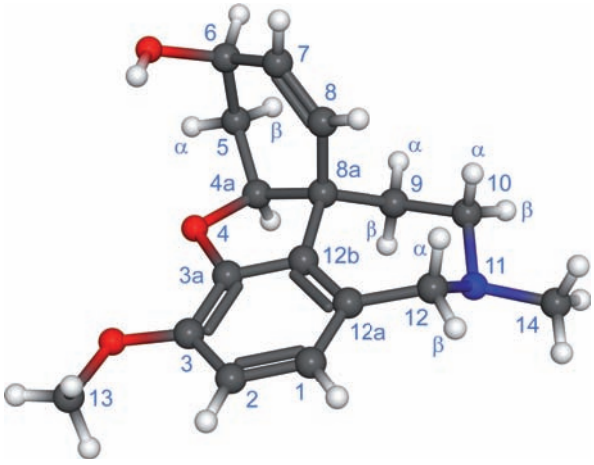


Fig. 1.6-22 Molecular model of galanthamine

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

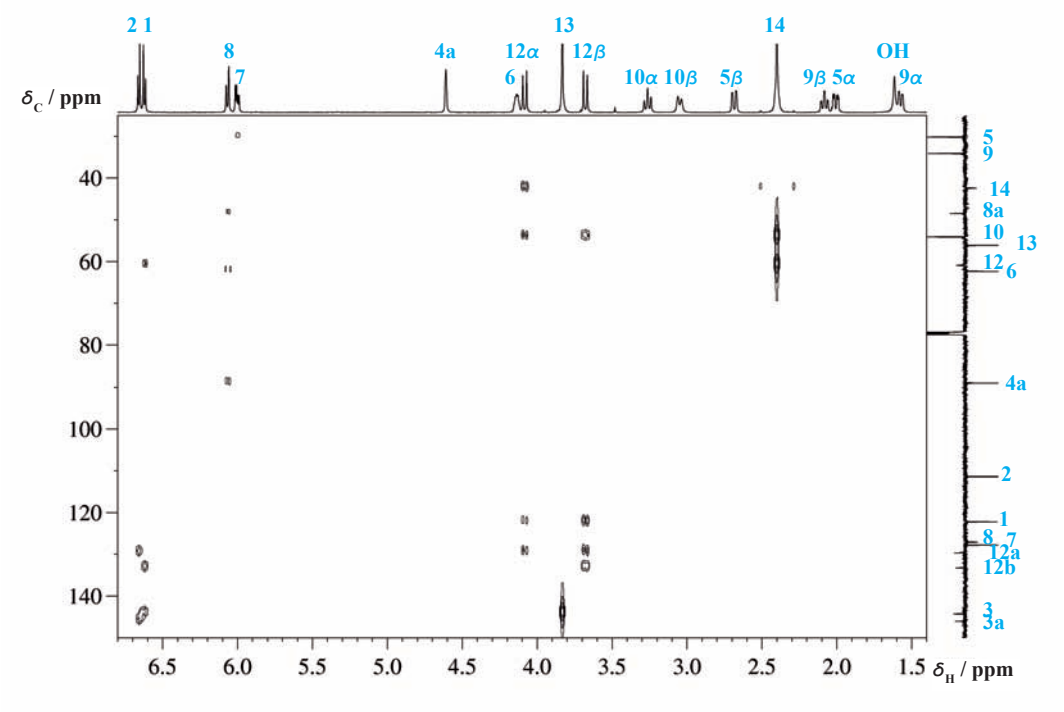


Fig. 1.6-23 HMBC spectrum

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

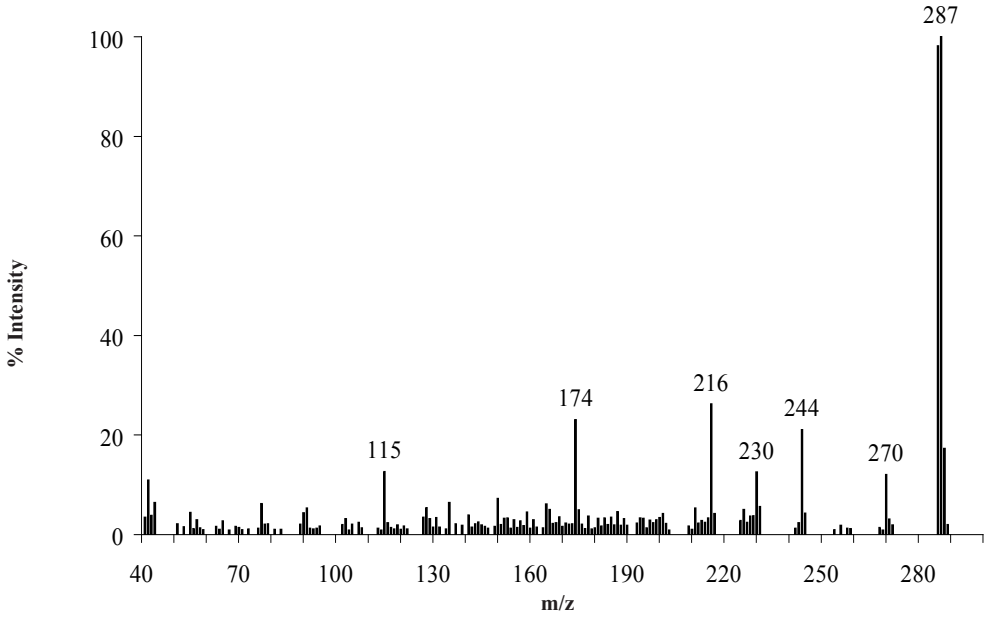
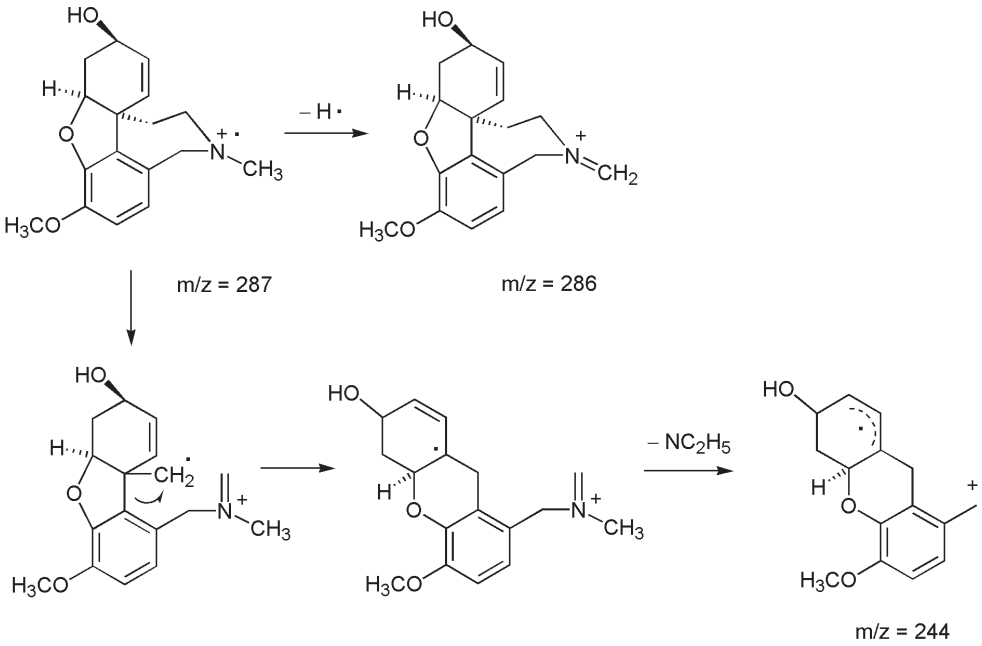
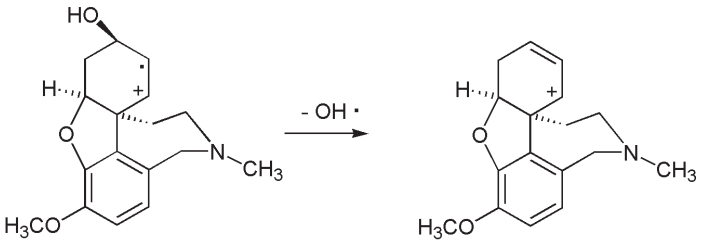


Fig. 1.6-24 Mass spectrum (EI)

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

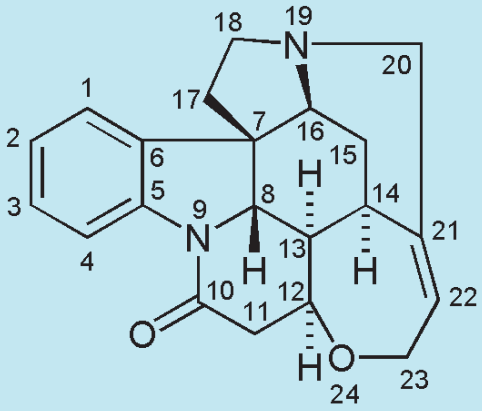
CAS RN 57-24-9, BRN 52979

$[\alpha]_D^{21}$ –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.

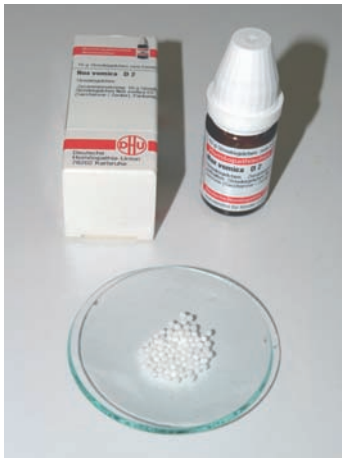


Fig. 1.7-2 Homeopathic strychnine pills

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

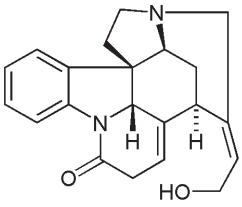
scientist, was beheaded for his leading position in the pre-revolutionary government as administrator of the *Ferme Générale* − a hated private tax collection company. Therefore, Pelletier's father had to look for a new possibility to get his son professionally educated. He sent him as an associate to 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*).

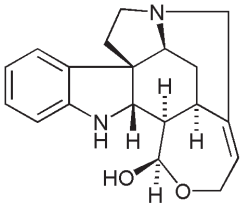
No wonder that strychnine, as soon as its structure was known, became a synthesis target of the best chemists of the world. The first to complete the total synthesis was Woodward, who received the Nobel Prize in Chemistry in 1965 for this and other outstanding achievements. He reported a synthesis of 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.

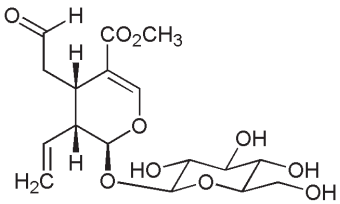
David Livingstone (1813−1873)
A Popular Account of Dr. Livingstone's Expedition to the Zambesi and its Tributaries and the Discovery of Lakes Shirwa and Nyassa



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

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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.

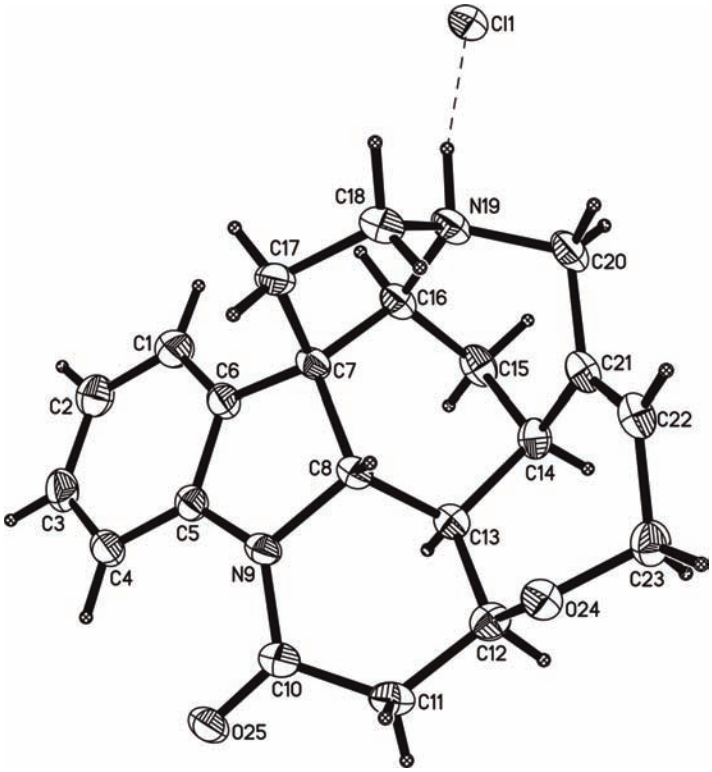
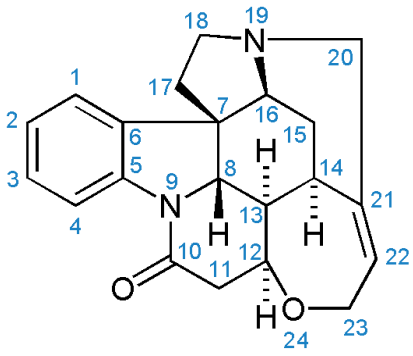


Fig. 1.7-3 X-ray structure
Details of the crystallographic data can be obtained from the Cambridge Crystallographic Data Centre, No. 697488



Scheme 1.7-2

4. Spectra and Comments

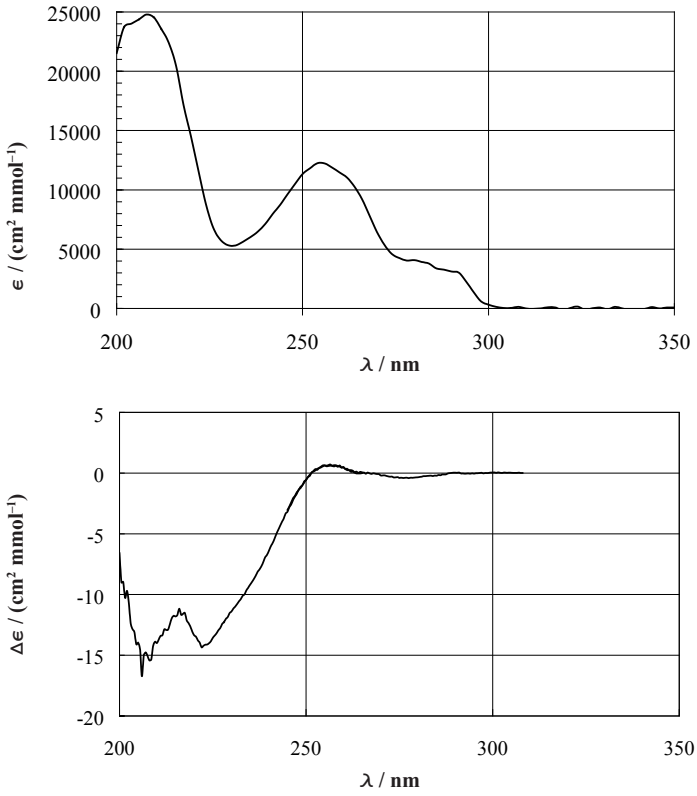


Fig. 1.7-4 UV and CD spectra in ethanol

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

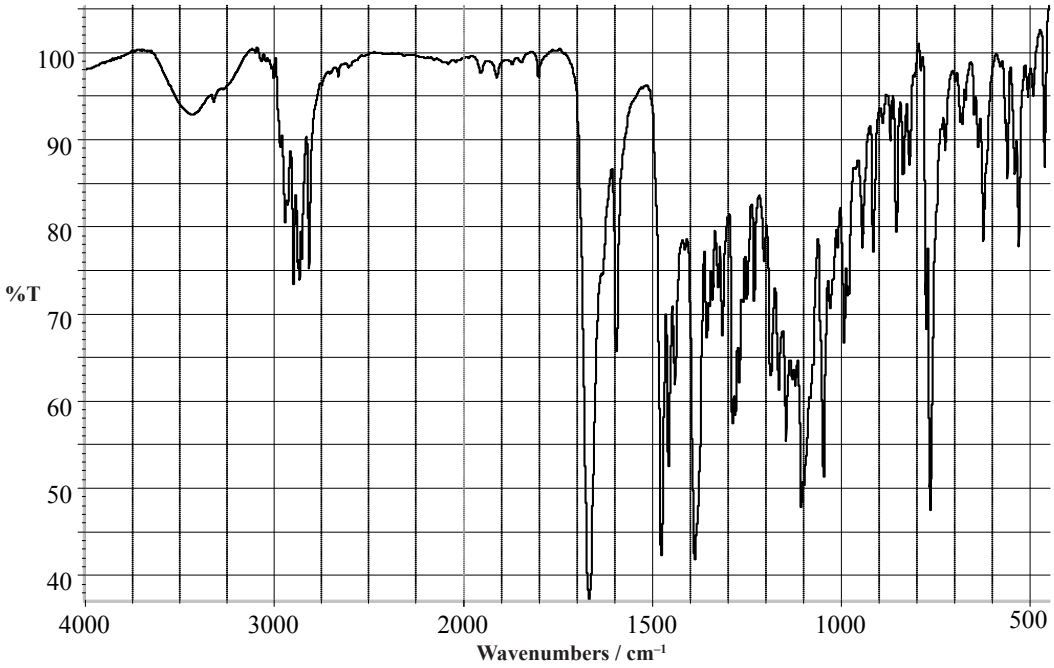


Fig. 1.7-6 IR spectrum in KBr

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}.

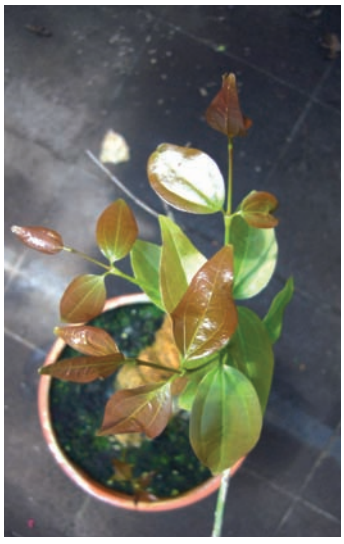
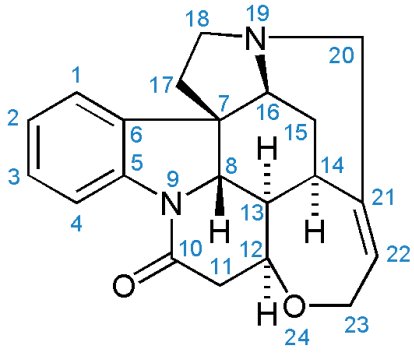


Fig. 1.7-7 A young plant of *Strychnos nux-vomica*



Scheme 1.7-3

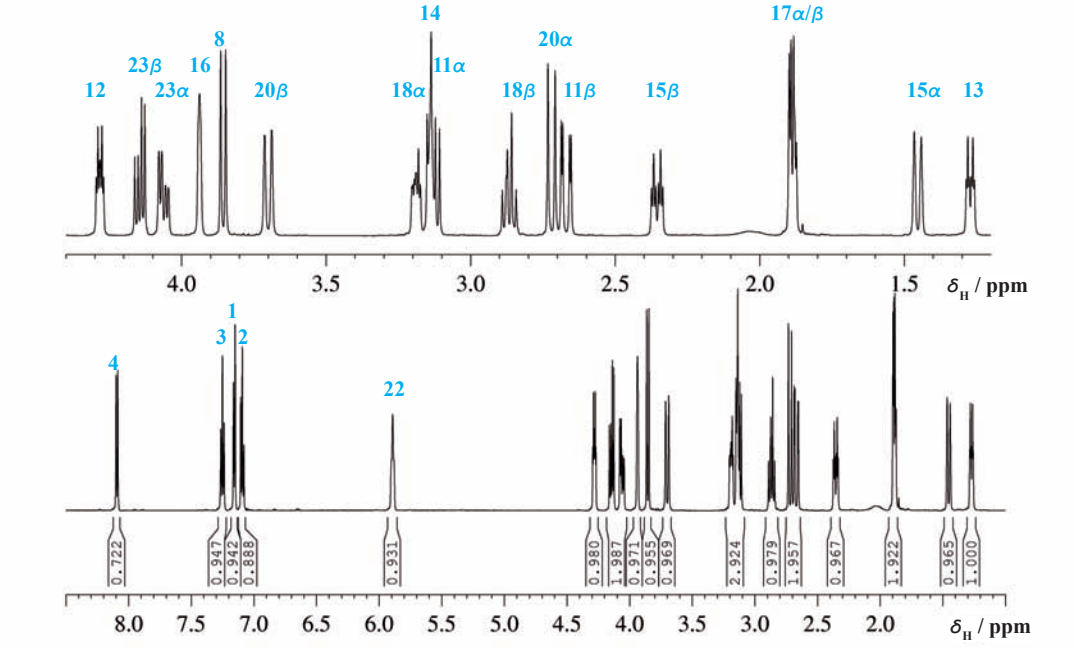


Fig. 1.7-8 ^{1}H NMR spectrum at 600 MHz in $CDCl_3$

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.

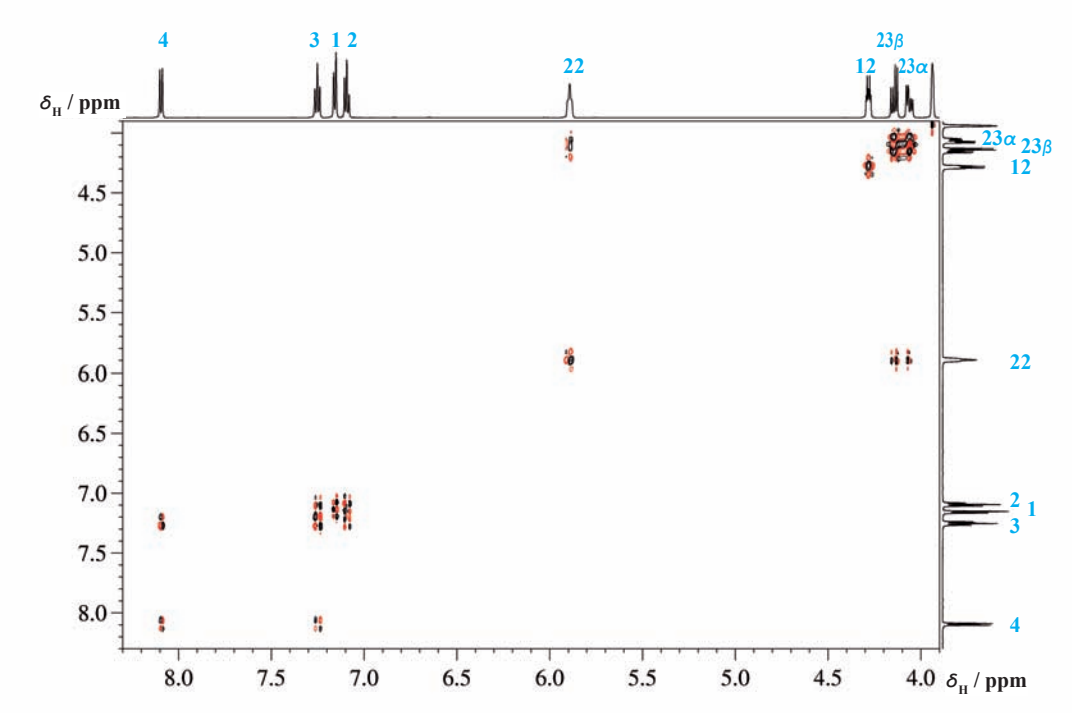


Fig. 1.7-9 Expansion of the DQF-COSY spectrum in the olefinic aromatic region

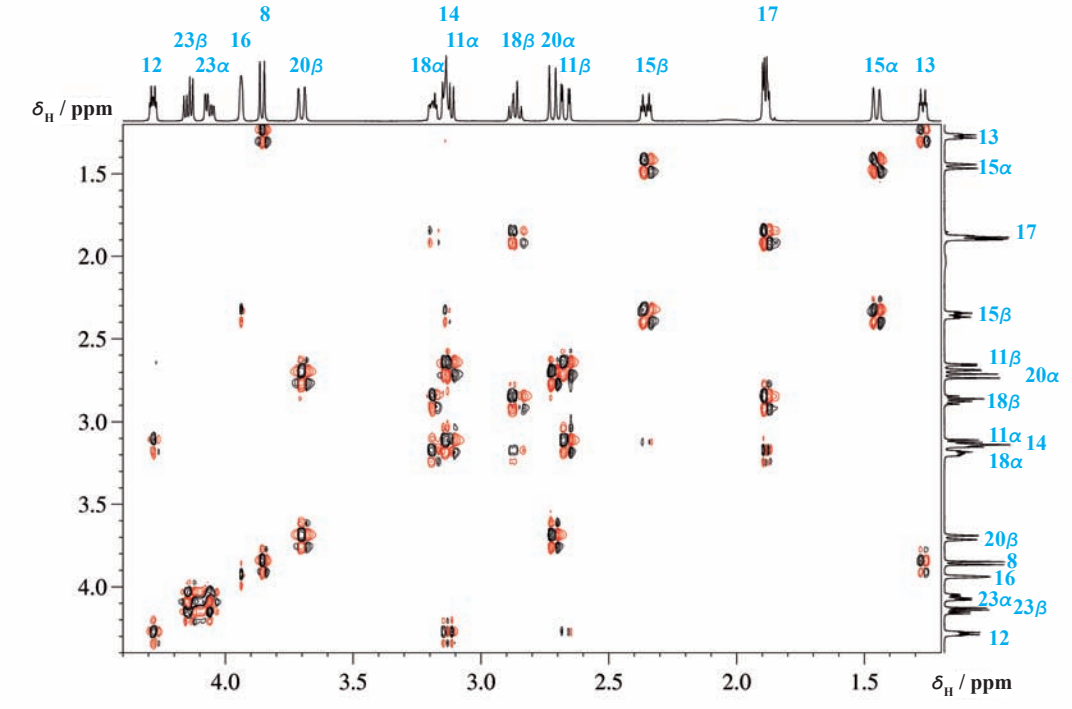
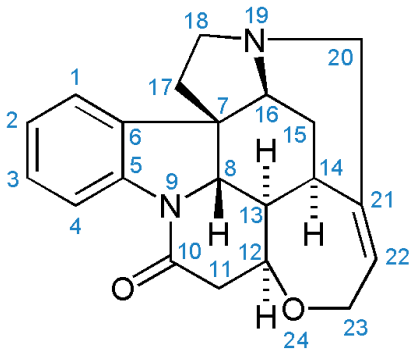


Fig. 1.7-10 Expansion of the DQF-COSY spectrum in the aliphatic region



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*

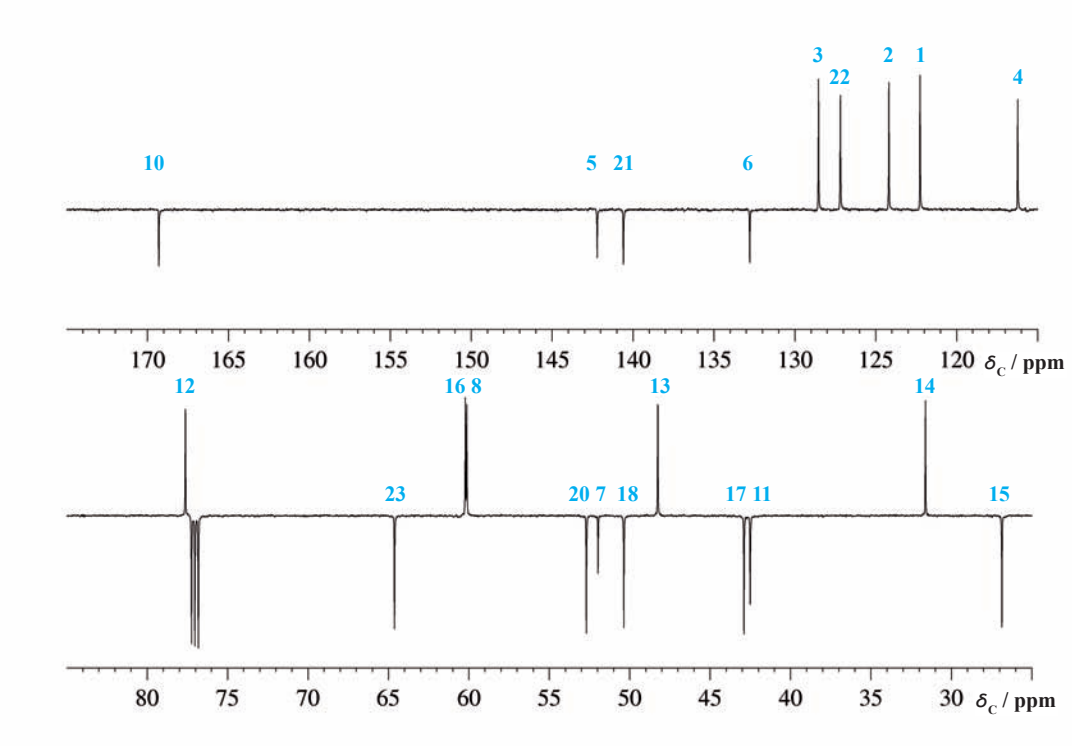
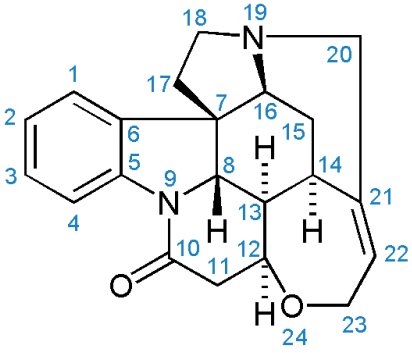


Fig. 1.7-12 APT ^{13}C NMR spectrum at 150 MHz in $CDCl_3$

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*

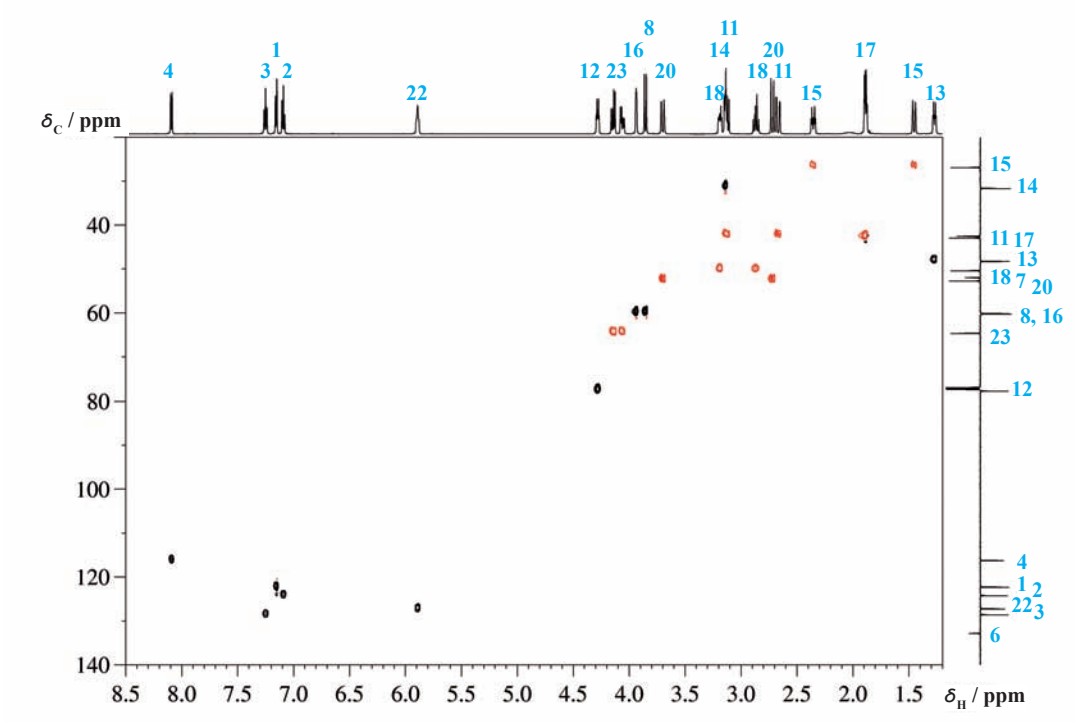


Fig. 1.7-14 HSQC spectrum

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.

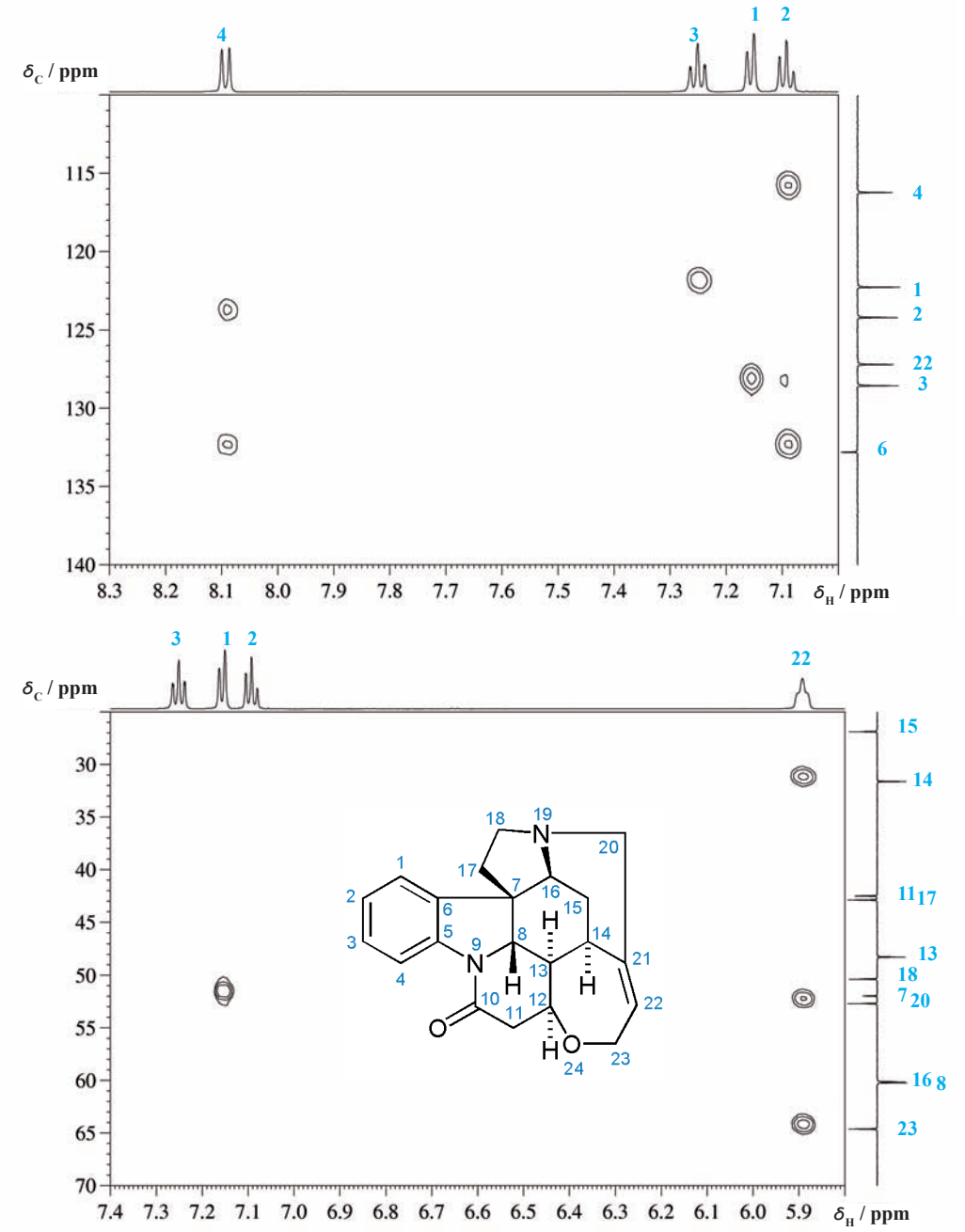


Fig. 1.7-15 Expansions of the HMBC spectra

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.

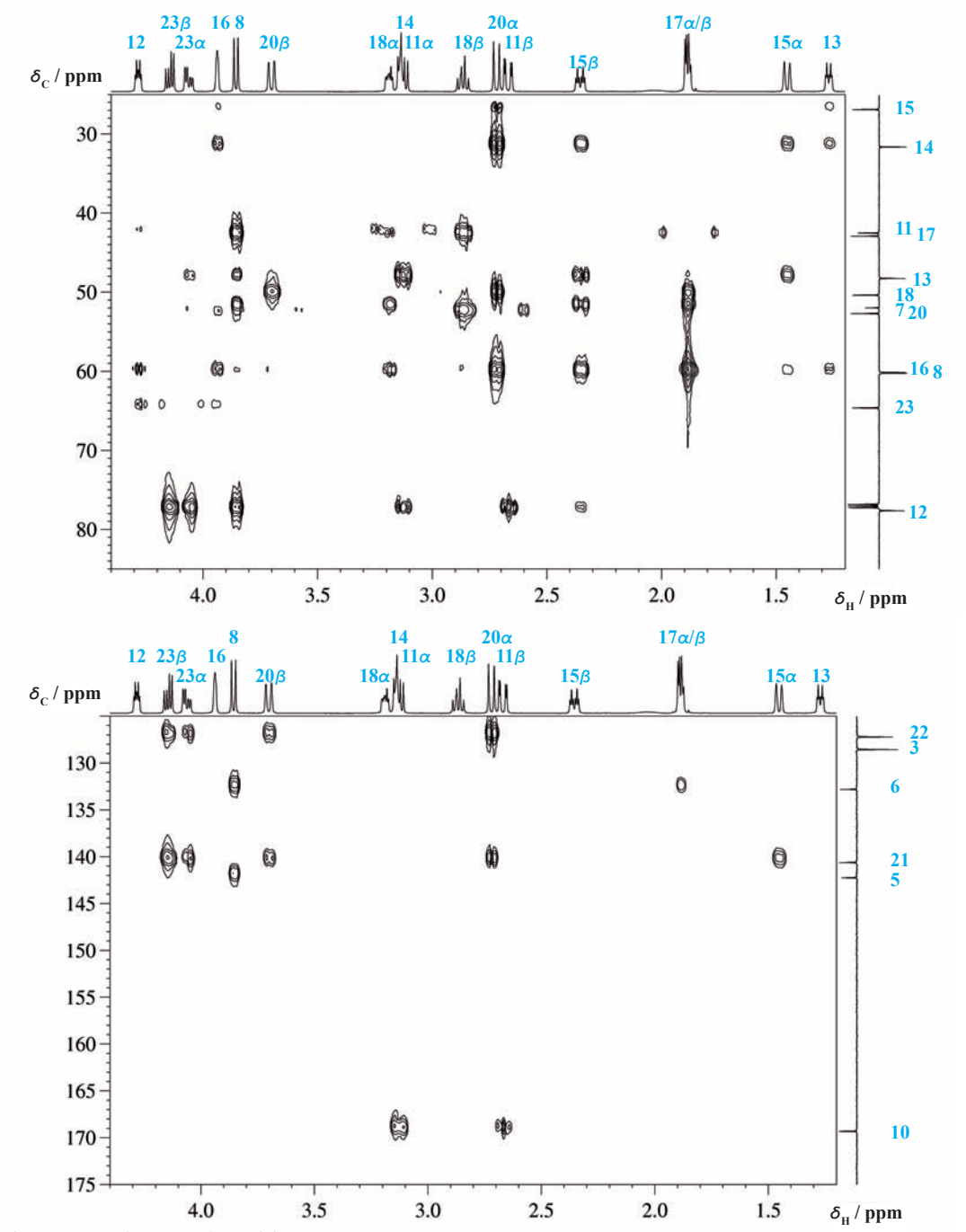


Fig. 1.7-16 Further expansions of the HMBC spectra

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.

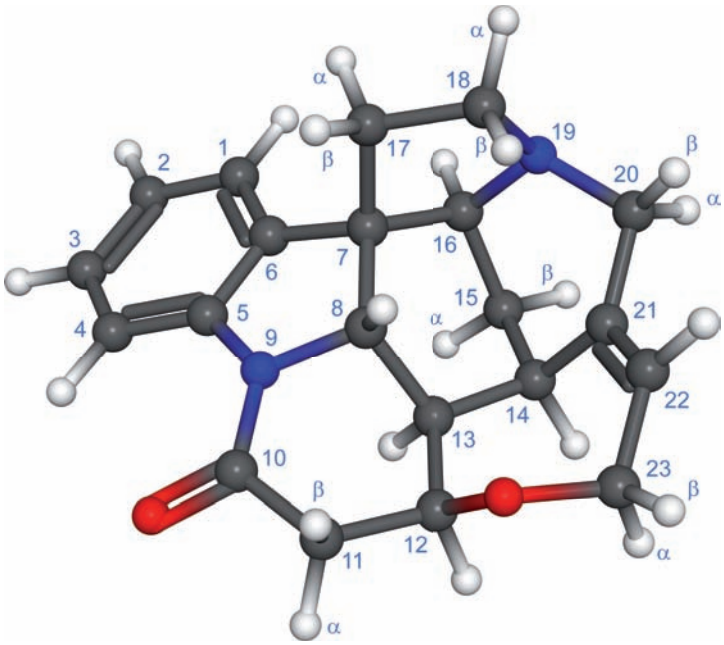


Fig. 1.7-17 Molecular model of strychnine

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.

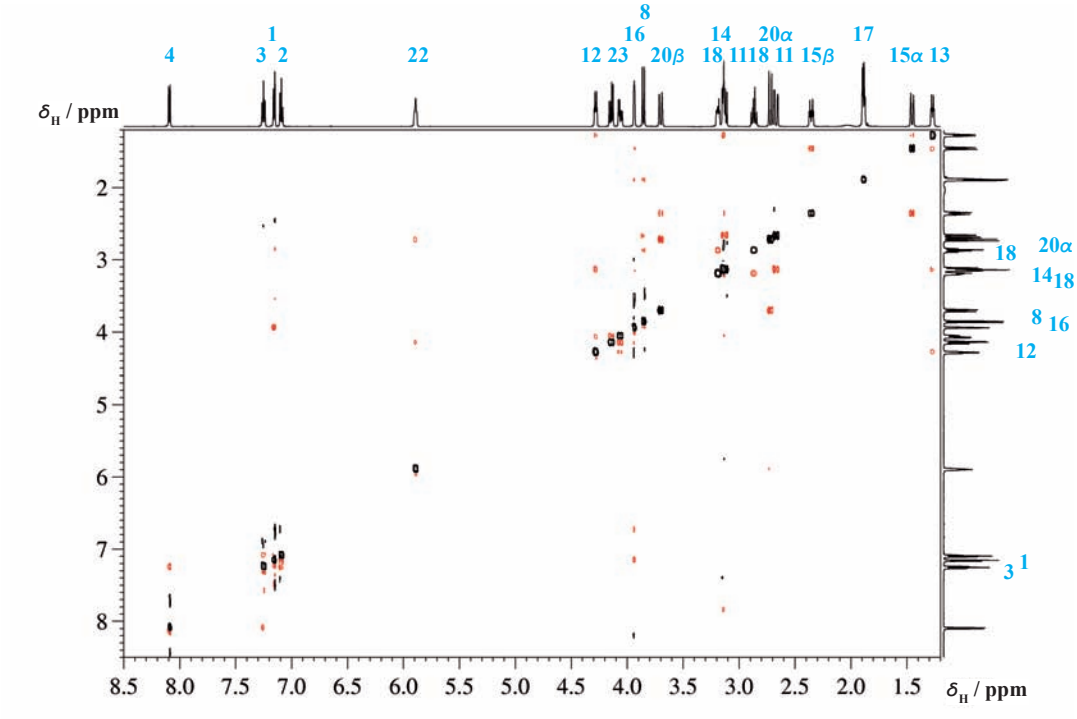


Fig. 1.7-18 NOESY spectrum

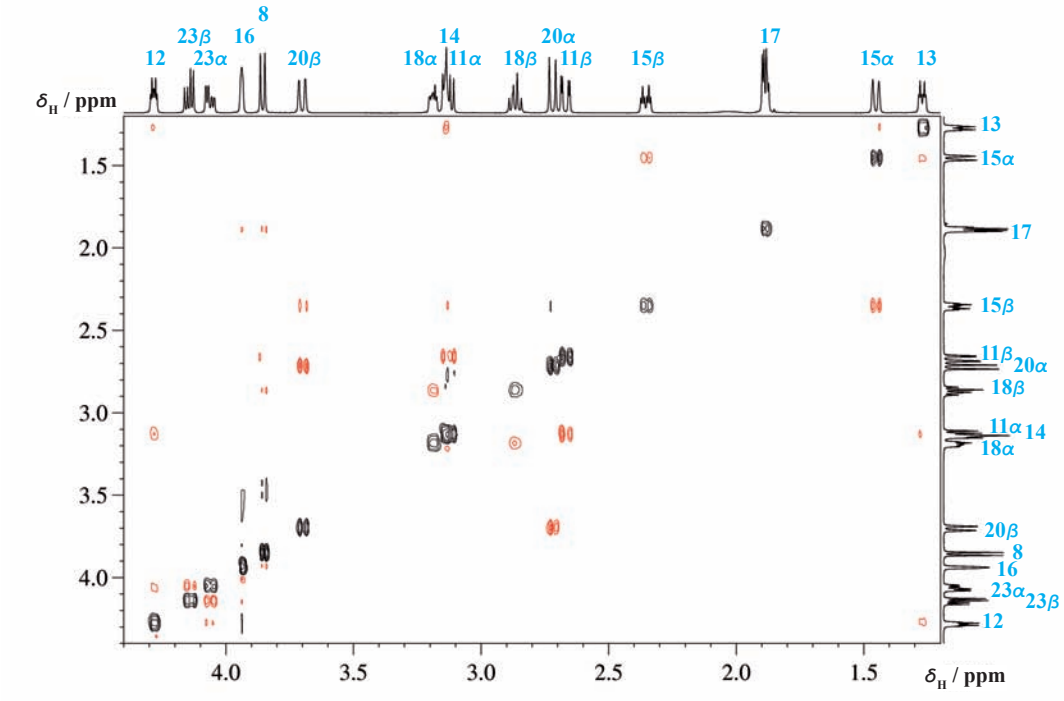


Fig. 1.7-19 Aliphatic expansion

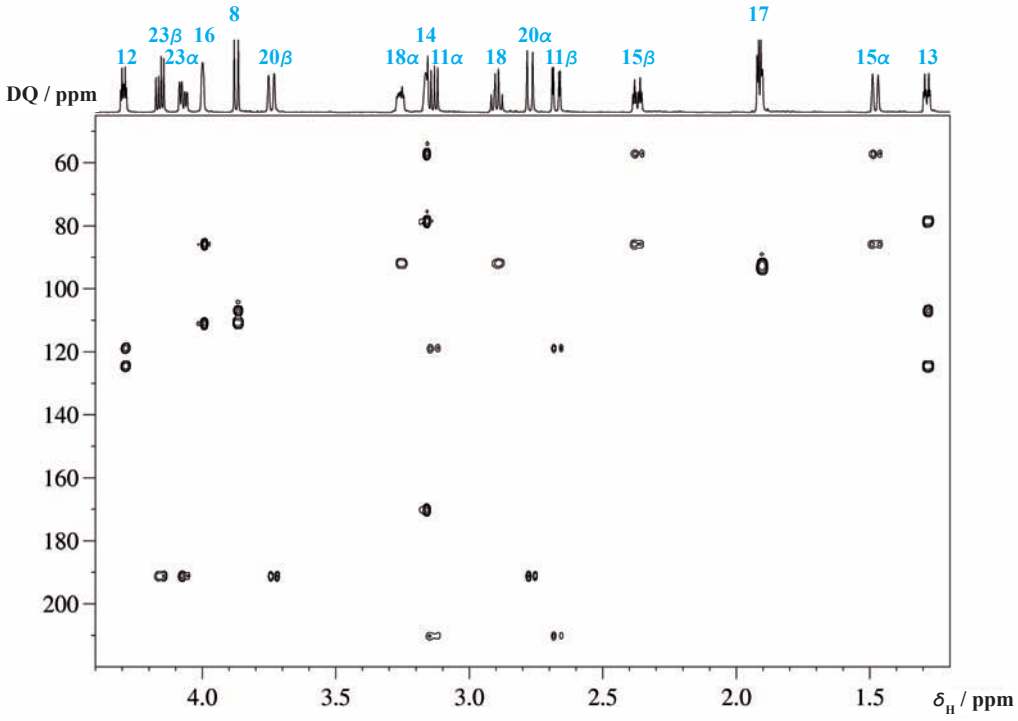
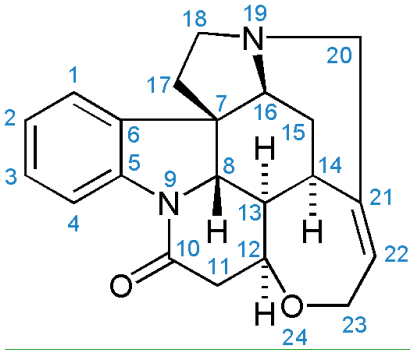


Fig. 1.7-20 ADEQUATE spectrum at 175 MHz

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 $\delta_H = 4.28$; two correlation signals at the double-quantum frequencies $\delta_C = 119.3$ 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 ($\delta_H = 4.0$ 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 $\delta_H = 1.27$. C-13 at $\delta_C = 48.22$ 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

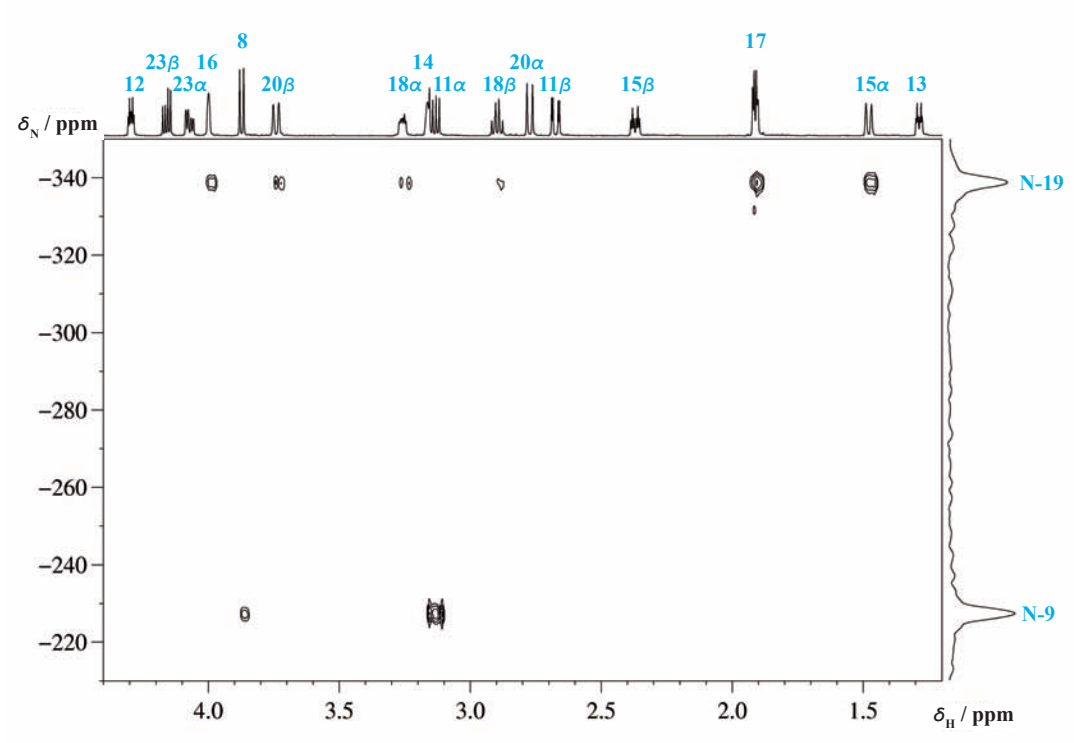


Fig. 1.7-23 ^{1}H ^{15}N HMQC spectrum

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.

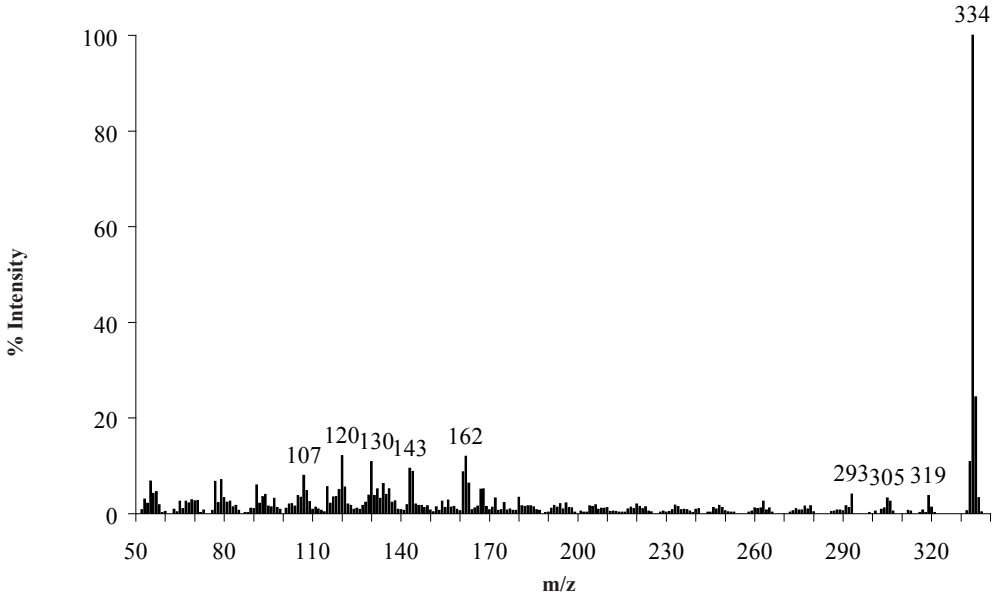
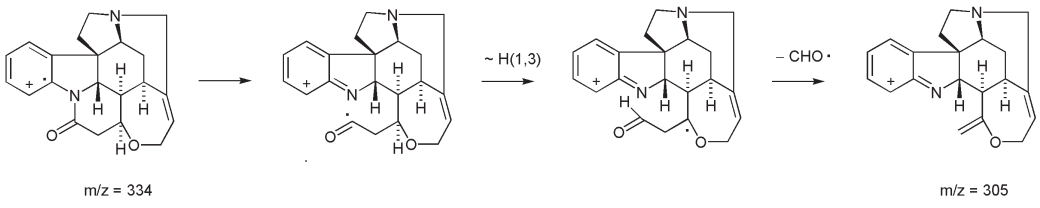


Fig. 1.7-23 Mass spectrum (EI)

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