# Chapter 1 Alkaloids

## 1.1 Pseudopelletierine

**From the pomegranate tree to cyclooctatetraene**

9-Methyl-9-azabicyclo[3.3.1]nonan-3-one

Synonyms: pseudopunicine, granatonine, granatan-3-one, ψ-pelletierine

**From the root-bark of the pomegranate tree** *Cortex punica granatum* L. (Lythraceae)

$C_9H_{15}NO$, MW 153.22 g×mol$^{-1}$

CAS RN 552-70-5

Colourless crystals, mp 54°C


Fig. 1.1-1 Structure of pseudopelletierine

Fig. 1.1-2 Pomegranate trees in Andalusia

# 1. Background

My first acquaintance with a pomegranate (*punica granatum*) is now more than twenty years ago and took place in Jordan. On the way back from an excursion to Jerash, in antiquity Gerasa, to which a Jordanian colleague had invited me, we stopped by a trader on the highway and bought several of these special fruits. Back in Amman we ate these delicacies, which because of the complicated internal structure was not so easy. The lasting impression was, that there could be no better refreshment after a hot, dusty day.

The origin of the pomegranate tree (Fig. 1.1-2) is in Asia (Indian Subcontinent, Persia, China). In biblical times, it was cultivated in the Near East and spread throughout the entire Mediterranean region. The name of the genus *punica* indicates, that the Phoenicians, whom the Romans called *punici*, introduced it into the Roman Empire. The Spaniards cultivated the pomegranate in their American colonies. Today pomegranate plantations can be found in all subtropical regions, which has allowed this fruit that was once reserved for monarchs to find its way onto the shelves of supermarkets.

I am my beloved's, and his desire is toward me.
Come, my beloved, let us go forth into the field; let us lodge in the villages.
Let us get up early to the vineyards; let us see if the vine flourish, whether the tender grape appear, and the pomegranates bud forth: there will I give thee my loves.
The mandrakes give a smell, and at our gates are all manner of pleasant fruits, new and old, which I have laid up for thee, O my beloved.
O that thou wert as my brother, that sucked the breasts of my mother! when I should find thee without, I would kiss thee; yea, I should not be despised.
I would lead thee, and bring thee into my mother's house, who would instruct me: I would cause thee to drink of spiced wine of the juice of my pomegranate.

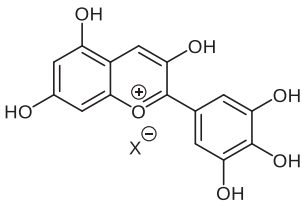
The Song of Solomon (Chapter 7, 10-13, Chapter 8, 1-2) about 500 B.C.

The pomegranate is regarded today as belonging to the loosestrife family (*Lythraceae*), but is placed by some sources together with other species of punica in its own family (*Punicaceae*). The deciduous plant reaches a maximum height of 5 m and is often shrub-like. It has about 10 cm long, shiny, lanceolate, leathery leaves. In spring and summer, it forms large bell shaped flowers on the end of its twigs, which are coloured yellow to orange-red and contain numerous stamina. The fruits which are apple shaped and mottled red and orange, have a leathery, shiny skin, upon which the sepals sit like a small crown. Cut open, the pomegranate displays a rich interior, made up of chambers, separated by a membrane, which are filled with many seeds. The Latin *granatum* means rich in grains. A glassy, juicy, deep red coloured seed coat (sarcotesta) surrounds each seed.

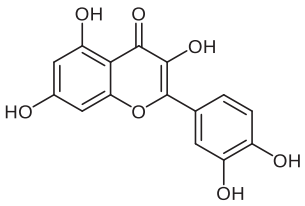
All parts of the fruit can be used. This is even valid for the skin with its high content of the tanning agents gallotannins and ellagitannins. Infusions of the skin are administered in traditional medicine for dysentery and diarrhoea. The sweet tasting seeds are used in the oriental and in the meantime European cuisine for the embellishment of food. The oil that is obtained from the seeds is rich in γ-linolenic acid and is therefore used in anti-aging products.

The red colour of the seed coat and its juice comes from flavonoids (delphinidin-3,5-diglucoside and quercetin). Although the mineral and vitamin content are only average, the pomegranate is particularly rich in phenolic acids (ellagic acid and gallic acid, Fig. 1.1-4) or punicalagin (Fig. 1.1-5). The phenolic acids are considered to be the main cause of the excellent antioxidative effect, which even exceeds that of green tea. In more than 250 studies pomegranate juice has been attributed a positive effect for cardiovascular disease, cancer and arthritis [1]. In most cases these investigations were carried out on cell cultures, so it seems premature, to awaken too much hope. However, the sweet-sour pomegranate juice is not only a delicious refreshment; it is also good for health.

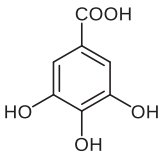
Derived from the French word for the pomegranate, grenadier, is grenadine, a syrup obtained from pomegranate juice, which no well stocked bar should be without. Grenadine lends for example Tequila Sunset its red colour and fruity taste.



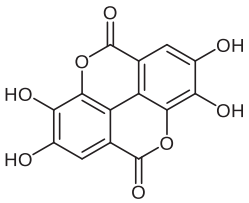

Delphinidin




Quercetin




Gallic acid




Ellagic acid

Fig. 1.1-4 Polyphenolic components of the pomegranate

Fig. 1.1-3 Albrecht Dürer (1471 – 1528): Maximilian I Emperor of the Holy Roman Empire of the German Nation "The last Knight" (1459 – 1519) Kunsthistorisches Museum, Vienna

Along with olives, dates, figs, grapes, almonds and locust beans the pomegranate belongs to the symbolic fruits of the bible. In many old cultures and scripture religions, it has a particular significance. The pomegranate is involved in many myths and stories that have found their place also in European poetry and art [2]. Its symbolism stands for life, fertility, earthly and heavenly love, the blood of Christian martyrs a well as for wealth, power and abundance. Paris is reputed to have settled the dispute between Hera, Athena and Aphrodite, about who was the most beautiful, by handing Aphrodite a pomegranate. No wonder, that this fruit above all others is associated with beauty and eternal youth and has been discovered by the modern cosmetic industry as an ingredient for its products. A women's journal "Burda Style" (9/2014) identified no less than 16 beauty products with ingredients from the pomegranate. In addition, there is a pomegranate-based series of care products that are sold exclusively in the pharmacy (Fig. 1.1-7).

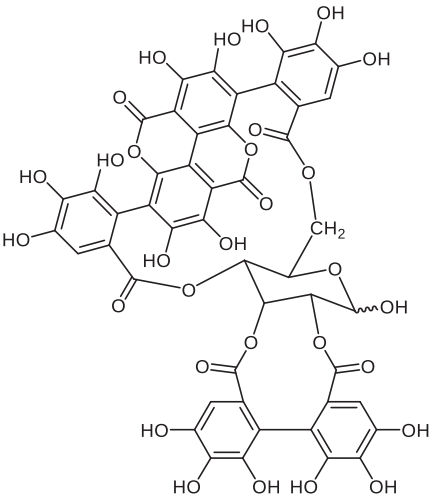



Fig. 1.1-5 Punicalagin

Fig. 1.1-6 Ripe pomegranates


Fig. 1.1-7 Beautifying shower gel with *Punica granatum*

Fig. 1.1-8 Root-bark of the pomegranate tree, from which pseudopelletierine was isolated.

The deep red gem stone garnet (German: Granat), an orthosilicate with the formula $Ca_3Al_2(SiO_4)_3$, gets its name from the pomegranate. We can agree to that but not with the misuse of its name for a weapon of war, the grenade.

Contrary to the allegorically deified fruit, the root-bark has received no mention in poetry. The root-bark (Fig. 1.1-8) is poisonous [3]. However, from antiquity until into the 20th century it has had a use, about which people concerned with it unwillingly speak. In the indigenous region of the pomegranate, medicine knew from long ago about the anthelmintic effect (tapeworm ejecting effect) of an infusion made from the root-bark. This knowledge first came to Europe in 1807, when the Scottish doctor Buchanan, who was stationed at a British dependency in India, reported it. The German edition of a French medical encyclopaedia published in 1838 states, that an infusion of the root-bark was (at that time) by far the most effective and almost always successful anthelmintic. Still after World War I this was the predominant opinion as shown by the quotation from "Merck's Warenlexikon" 1920, at the lower part of the margin. The cover of volume 5 of "Universallexikon der praktischen Medicin und Chirurgie" is reproduced in the *supporting information*. The quotation on the left margin can be found there on p. 281ff.

Today one sees it more critically. The adverse effects include hypertension, sight disorders, vomiting, collapse etc. up to death by respiratory paralysis, so that in Germany the drug is regarded to be an obsolete anthelmintic, the use of which is emphatically discouraged [3]. Which components of the bark lend it its anthelmintic properties?

An infusion of this medication is most often used. The usual dose is 2 oz. of the root-bark of the pomegranate tree in 2 pints of water, which is evaporated to 1 pint and imbibed in one day….
…sometimes adverse effects such as nausea, vomiting, colic and even dizziness may occur, however, these disappear again in a short time.

Universallexikon der
praktischen Medicin und Chirurgie, 1838

The prize winning French pharmacist and chemist Charles Tanret (1847–1917) (Fig. 1.1-9) extracted four basic compounds from the root-bark that he characterized as salts and in honour of the pioneer of botanical chemistry, Pierre J. Pelletier, (1788–1842), (Fig. 1.1-10) called them pelletierine ($C_8H_{15}NO$), isopelletierine ($C_8H_{15}NO$), methylpelletierine ($C_9H_{17}NO$) and pseudopelletierine ($C_9H_{15}NO$) [4].

The gas chromatogram of the alkaloids extracted from the root-bark shows essentially three intensive peaks (Fig. 1.1-11) that according to MS in the order of their intensity can be attributed to pseudopelletierine ($C_9H_{15}NO$, $M^{+\bullet} = 153$), pelletierine or isopelletierine ($C_8H_{15}NO$, $M^{+\bullet} = 141$) and finally methylpelletierine ($C_9H_{17}NO$, $M^{+\bullet} = 155$). But where is the fourth alkaloid that Tanret described?

The root-bark in contrast to the ineffective root-wood is a known anthelmintic and contains apart from considerable amounts of tannic acids 0.5 – 1% alkaloids, namely pelletierine and some of its derivatives. On chewing it tastes bitter and colours the saliva yellow, provided it is not too old and has thereby become ineffective……. To obtain real root-bark one must resort to a reliable source and obtain it mainly from Italy and Greece.

From "Merck's Warenlexikon für Handel, Industrie und Gewerbe", 7. edition. Publ. by Adolf Beythien and Ernst Dressler. Gloeckner, Leipzig 1920

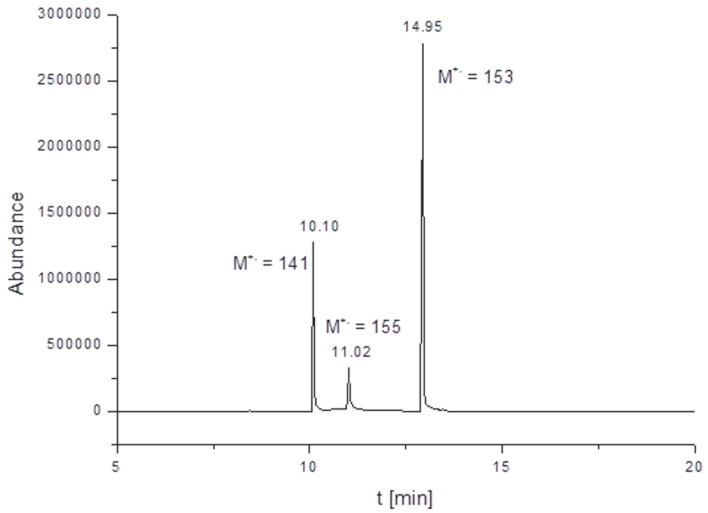



Fig. 1.1-11 GC-MS Investigation of an extract of the alkaloids of the root-bark of the pomegranate tree (for extraction, GC conditions and mass spectra see *supporting information*)

JULIET Wilt thou be gone? it is not yet near day:
It was the nightingale, and not the lark,
That pierced the fearful hollow of thine ear;
Nightly she sings on yon pomegranate-tree:
Believe me, love, it was the nightingale.

William Shakespeare (1564–1616)
Romeo and Juliet, III,V

This story is very complicated. Tanret described pelletierine as optically active, in contrast to isopelletierine. A sample of the pelletierine sulphate that Tanret produced in 1880 is preserved in the Muséum d'Histoire Naturelle in Paris. Over 80 years later, this sample was reanalysed [5]: mp 135-138°C (decomp.), $[\alpha]_D^{25}$ −29.5° (c 10.5 mg/mL $H_2O$). Later workers isolated only the optically inactive isopelletierine but no pelletierine. The answer to this puzzle is, that the optically active pelletierine from the biosynthesis racemizes totally or in part and after salt formation with acids crystallizes in the form of racemic compounds, which have different melting points than the corresponding salts of (−)-pelletierine.

Fig. 1.1-9 Charles Joseph Tanret (1847 – 1917)

The racemization of (−)-pelletierine, as was later shown, is a base catalysed process. Clearly, it depends on the influence of bases during the isolation process, to what degree (−)-pelletierine is racemized to isopelletierine = (±)-pelletierine. In the *supporting information*, GC experiments with chiral phases are shown that illustrate this fact.

Before the structures of pelletierine and isopelletierine could be elucidated, a discussion broke out that today one can hardly understand and which was first ended by J. Meisenheimer in Tübingen [6] by the successful total synthesis of (±)-pelletierine and (±)-methylpelletierine. We return to this subject and to further aspects of the "Pelletierine Story" in the *supporting information* and restrict ourselves at this point to showing the structural formulae of the "pelletierines" in Fig. 1.1-12, which identify them as piperidine derivatives.

Fig.1.1-10 Pierre-Joseph Pelletier (1788 – 1842)
French Pharmacists and Chemists

Piperidine alkaloids are often found in the plant world. The best known is perhaps coniine found in poison hemlock (*Conium maculatum*) [7]. Note that the different attribution of pelletierine and coniine to *R* and *S* results from the priority rules of the CIP-system.

The structure elucidation of pseudopelletierine took less than 20 years, a remarkably short time in the pre-spectroscopic era. This was primarily

R = H (2*R*)-(–)-Pelletierine

R = $CH_3$ (2*R*)-(+)-N-Methylpelletierine

(2*S*)-(+)-2-Propylpiperidine = (2*S*)-(+)-Coniine

Fig.1.1-12 Piperidine alkaloids

Another mythical exposition of our view of sexual pleasure as the assertion of the will to live beyond the individual life, as an attainment to life which is brought about for the first time by this means, or as it were a renewed assignment of life, is the Greek myth of Proserpine, who might return from the lower world so long as she had not tasted its fruit, but who became subject to it altogether through eating the pomegranate. This meaning appears very clearly in Goethe's incomparable presentation of this myth, especially when, as soon as she has tasted the pomegranate, the invisible chorus of the Fates—
"Thou art ours!
Fasting shouldest thou return:
And the bite of the apple makes thee ours!"

The World As Will And Idea by Arthur Schopenhauer
Translated from German by R. B. Haldane, M.A. and J. Kemp, M.A.
Vol. I.

due to Ciamician and Silber, who in sunny Bologna not only founded organic photochemistry but also, amongst other things, investigated the structure of pseudopelletierine. In a series of experiments [8] they confirmed the molecular formula $C_9H_{15}NO$, proved the existence of a tertiary amine and a ketone and with the help of numerous transformations recognized pseudopelletierine to be an homologue of tropinone without, however, suggesting an exact structure.

*"At the present time, we do not consider it to be propitious, to speculate about the probable structure of pseudopelletierine, although the facts that we have observed allow us to speculate and recognize a great deal."*

Apart from this, they found the name given by Tanret to be:

*"… too long, complicated and thoroughly unsuitable, to accurately describe the derivative that we have obtained."*

The new suggestion for the name, granatinone, was derived from *punica granatum* and was supposed to underline the analogy to tropinone (a derivative of atropine from *Atropa belladonna*). Tanret, who was not involved in the structure elucidation, defended in a, at that time usual, note of protest [9] his right as discoverer to determine the name and was successful. If molecules were endowed with the emotions of humans, then the main alkaloid of the pomegranate tree would surely be unhappy about the prefix "pseudo". Who would want to be termed a pseudo-artist in the midst of artists?

We return to the structure elucidation. After Ciamician and Silber had obtained numerous products from the transformation of pseudopelletierine that were comparable to the transformation products of tropinone, obtained in an analogous way, it was clear, that pseudopelletierine is a homologue of tropinone. However, since the structure of tropinone was still in dispute, also the first suggestion for the structure of pseudopelletierine could not be correct (Fig. 1.1-13 (a)) [10]. In 1899 A. Piccinini, a co-worker of Ciamician, found the correct linking of the atoms of pseudopelletierine, as he succeeded in oxidatively cleaving the backbone and by further degradation steps arrived at suberic acid (octanedioc acid) [11]. (Details see *supporting information*).

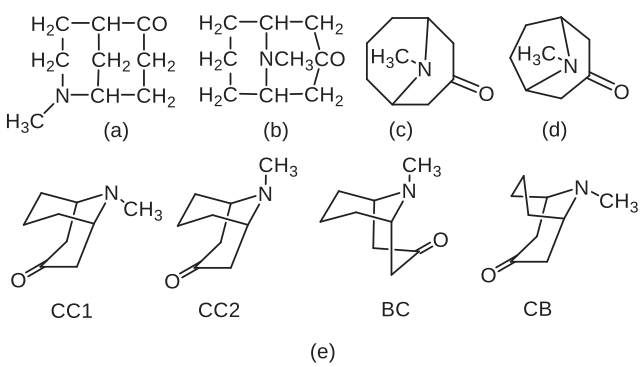



Fig. 1.1-13. First suggestion for the structure of pseudopelletierine (a), corrected and today still valid planar structural formula (b), planar representation of pseudopelletierine (c) and tropinone (d), various chair (C) and boat (B) conformations of pseudopelletierine (e)

It was therefore clear, that an unbranched chain of 8 C-atoms in a closed ring form is present as a substructure in pseudopelletierine. The correct linking of the atoms that was derived from this is shown in Fig. 1.1-13 (b) and the present version in Fig. 1.1-13 (c).

From the different conformations (Fig. 1.1-13 (e)) that are in principle possible, mainly the chair-chair structures CC1 and CC2 are important. These are interconvertible by inversion at the N-atom (see Quantum Chemical Calculation).

The research groups of Ciamician and Willstätter, Willstätters fundamental work will be addressed below, needed considerable amounts of precious pseudopelletierine for their investigations. Calculated back, the result is an amount of root-bark of the pomegranate tree in the order of hundreds of kilograms. Where could this be obtained? At that time, the firm Merck in Darmstadt extracted and isolated the pomegranate alkaloids on an industrial scale, to supply the world market with the indispensable cure against tapeworms. The acknowledgements of the publications show, that Ciamician and Willstätter obtained fractions enriched in pseudopelletierine from this production.

If today anyone requires several grams of pseudopelletierine, he is well advised not to start with root-bark but to use the perfectly devised Robinson-Schöpf reaction. Modified for pseudopelletierine, glutaraldehyde (1,5-pentanedial), methylamine and acetonedicarboxylic acid react together in a one-pot reaction directly to pseudopelletierine [12]. A procedure described in Organic Synthesis [13] reports yields of up to 70%. It comprises a double *Mannich reaction*, which take place under mild, so-called physiological conditions. The reaction scheme and the biosynthesis [14] of the alkaloids of *Punica granatum*, which has some similarities with the laboratory synthesis, are shown in the *supporting information*.

Pseudopelletierine proved to be a stroke of luck for the up-and-coming organic chemistry of the 20$^{th}$ century. Basically, we are dealing with an aza-bridged cyclooctane. Willstätter recognized its potential as a wonderful starting material for carbocyclic eight-membered rings and by skilfully chosen degradation sequences made the way to cyclooctane and olefinic $C_8$-rings accessible. The climax of a whole series of investigations was the synthesis of cyclooctatetraene (COT) published in 1911 [15, 16] (Fig. 1.1-16).


Fig. 1.1-14 From pseudopelletierine to cyclooctatetraene

The discovery, that cyclooctatetraene is a yellow coloured compound with a high degree of unsaturation, disappointed the expectations, that COT, if it could be synthesized, would demonstrate a vinylogous relationship to benzene. The disappointment, that COT proved to be a polyolefine, aroused doubt on Willstätters degradation of pseudopelletierine, particularly as some attempts, to reproduce this unusual synthesis sequence, were unsuccessful. In the 1930s the predominant opinion was, that the product had been wrongly interpreted.

First, the tetramerisation of acetylene with a nickel catalyst by the BASF-chemist Reppe [17], which made COT available in unlimited amounts and the reproduction of the degradation of pseudopelletierine to COT by Cope [18] confirmed Willstätter's historic achievement. In the following decades, it inspired many scientists to investigate the "secret" of aromaticity. In contrast to benzene, COT is not planar but in its most stable form exists in a tub-shaped conformation. Willstätter’s work is described in more detail in the *supporting information*.

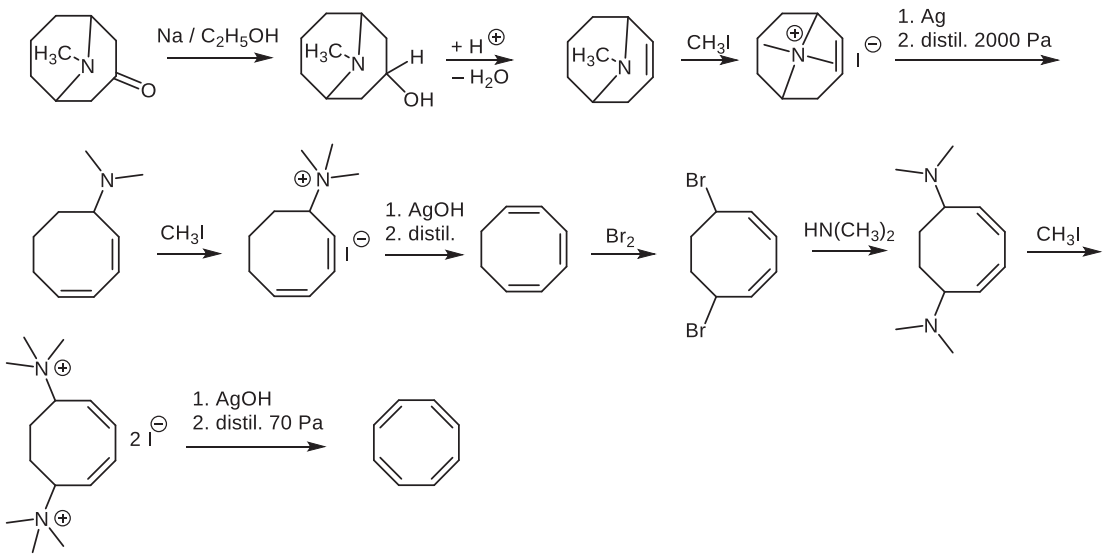



Fig. 1.1.16 Willstätter's synthesis of cyclooctatetraene from pseudopelletierine

Fig. 1.1-15 Richard Martin Willstätter, born 13.8.1872 in Karlsruhe, Germany, died 3.8.1942 in Muralto, Switzerland. Studied at the LMU in Munich. Lecturer and professor in Munich, Zurich, Berlin and Munich. 1915 Nobel Prize for research into the pigments of plants, particularly chlorophyll. 1924 resigned as professor in protest against increasing antisemitism. 1938 flew from the Gestapo into Switzerland with the help of his student A. Stoll. Willstätter was awarded the Iron Cross in World War I for the development of the first effective gas mask for the absorption of chlorine and phosgene.

# 2. Isolation

## 2.1 Principle

Basic alkaloids occur in plants most often with a protonated amino function, i.e. in cationic form. Frequently the salt has an organic anion. By treatment in strongly basic medium, the organic ammonium salt can be deprotonated, so that the solubility of the alkaloid in water is reduced and simultaneously the solubility in non-polar solvents increased. The alkaloid can then be extracted into an organic phase. However, all other lipophilic substances also go into the organic phase. The aim of the extraction is, to separate the alkaloid as selectively as possible. The basicity of the alkaloid is used to differentiate it from the other lipophilic, organic substances. On extracting an organic phase that contains alkaloids with a strongly acid aqueous phase, the amino group is again protonated to an ammonium salt. Being highly hydrophilic it is selectively re-extracted into the acidic aqueous phase. After a further deprotonation the alkaloid transfers to another organic phase. Pseudopelletierine (and its companions) can be isolated from the bark extract using this procedure.

This work was inspired by the work of Tanret [4] and a newer article about quinine from cinchona bark that describes the process used by the firm Buchler [19].

## 2.2 Method

NB. The root-bark of the pomegranate tree is a speciality and a natural product that is not always commercially available. If you want to duplicate our procedure, you should in good time search in the Internet for a source that can deliver this raw material.

The shredded root-bark of the pomegranate tree (46.4 g) is pulverized to a coarse powder in a kitchen mill (*La Moulinette* from the firm Tefal). Calcium oxide (20.0 g), sodium hydroxide (1.0 g) and water (145 mL) are mixed to a suspension of low viscosity that is then mixed with the root-bark. The paste-like mixture that results is ochre to red-brown in colour. The mixture is stirred overnight in an ice-bath, whereby the mixture becomes more homogenous in its consistency and less viscous. The suspension is diluted with water (285 mL) and the solids removed by filtration under suction. The filtration is repeated five times to obtain a clear filtrate (425 mL). The filtrate is extracted four times with chloroform (4×200 mL). The united, colourless organic phases are dried over $MgSO_4$ and filtered. The solvent is removed to dryness under reduced pressure, to obtain a yellowish oil (103.1 mg).

The oil is dissolved in chloroform (10 mL) and extracted twice with 20% $H_2SO_4$ (2×5 mL). The united sulphuric acid phases are cooled in an ice-bath and aq. NaOH (12 mL, 4.6 M) added in small drops to attain a pH of 11. A precipitate of $Na_2SO_4$ forms that is removed by filtration under suction and the aqueous solution is extracted three times with diethyl ether (3×20 mL). The united ether phases are dried over $MgSO_4$ and filtered. The solvent is removed to dryness under reduced pressure. A yellowish oil (45.8 mg) remains that according to TLC contains pseudopelletierine.

## 2.3 Purification

Thin Layer Chromatography (TLC) of the "Pomegranate Alkaloids"

As eluant for TLC a mixture of dichloromethane and methanol in the ratio $CH_2Cl_2/CH_3OH$ 4:1 (v/v), as given in the literature [20], was used. To increase the selectivity 2% (v/v) concentrated aq. $NH_3$ was added to the eluent. For this purpose the addition of triethylamine was also tried, however, it proved to be unsuitable, because this tertiary amine reacted with the Dragendorff reagent, which was used for detection (see below for composition of Dragendorff reagent).

Before the purification by column chromatography, the investigation at each step of the isolation by TLC on silica gel coated aluminium plates always showed the same three spots for alkaloids, which were detected with the Dragendorff reagent. They had the $R_f$ values 0.25, 0.43 and 0.72. From the intensity of the spots, it was assumed, that the spot with the highest $R_f$-value came from pseudopelletierine. This was later confirmed by the NMR spectrum of the corresponding fraction from the column chromatography.

The spots with the $R_f$-values 0.25 and 0.43, which unlike the spot from pseudopelletierine showed a strong tailing, originate presumably from the other main alkaloids of the root-bark of the pomegranate tree, namely pelletierine and *N*-Methylpelletierine. However, an exact assignment was not possible, because the amounts obtained were too small.

The Dragendorff reagent for alkaloids

The detection is based on the brown colouration of the alkaloid spot, caused by the formation of a sparingly soluble salt of the tetraiodobismuthate anion and the alkaloid ammonium cation. According to procedures described in the literature, the reagent can be prepared by mixing basic bismuth nitrate ($BiO(NO_3)\times H_2O$, 0.85 g) and L-(+)-tartaric acid (10.11 g) in water (25 mL), whereby a white precipitate forms. A solution of potassium iodide (8.2 g) in water (20 mL) is added. The now reddish suspension is stirred for an hour, then filtered and the red-brown solution stored in a brown glass bottle in the refrigerator.

For the application a freshly prepared solution of sodium nitrite (1 g) in water (10 mL) is required.

For the detection of the alkaloid spots on the TLC plate a suitable quantity of the Dragendorff stock-solution is diluted with water in the ratio 1:3 and sprayed onto the developed and dried TLC plate, followed immediately by spraying with the $NaNO_2$ solution. Because of the aerosol produced by spraying and the nitrous fumes from the reaction, the procedure should be conducted in a fume cupboard.

On drying the plate, light to dark brown spots form, where an alkaloid is present. The formation of the spots can take several hours, although generally the spots appear immediately.

The pseudopelletierine spot shows a specific and helpful effect: on spraying with the Dragendorff reagent the spot acquires an intense violet colour that disappears entirely on spraying with the $NaNO_2$ solution. The otherwise colourless spot is then surrounded with a dark brown corona and after some time becomes completely brown.

**Purification of the raw pseudopelletierine by column chromatography**

Column Size: length: 450 mm, diameter 25 mm
Stationary Phase: Merck Silica Gel 60 (35 – 79 µm)
Eluent: $CH_2Cl_2$/MeOH (4:1 v/v) with addition of 2% v/v aq. ammonia solution (25%)
Size of fraction: 10 mL for fractions 1-4, 4 mL for all following fractions

For the chromatography, the product obtained from the isolation (45.8 mg) was mixed with product obtained from a previous test isolation (11.6 mg).

The product from the isolation (57.4 mg) is dissolved in the eluent (3 mL) and added to the column. After a pre-elution (4×10 mL) fractions of 4 mL are collected. The fractions are investigated by TLC (detection with Dragendorff reagent), whereby the fractions 16 – 26 are shown to contain pseudopelletierine. These fractions are united and the solvent completely removed under reduced pressure. A colourless, crystalline solid (19.9 mg) that from the melting point and spectra is identified as pseudopelletierine is obtained.

Proportionally 15.9 mg were obtained from 46.4 g of root-bark, equivalent to a yield of 0.03%.

Melting point: 56 – 61°C
Lit. 64 – 65°C (ligroin) A. C. Cope, *J. Amer. Chem. Soc*. **1951**, *73*, 3416–3418.

# 3. Spectra and Comments

## UV Spectrum in Ethanol

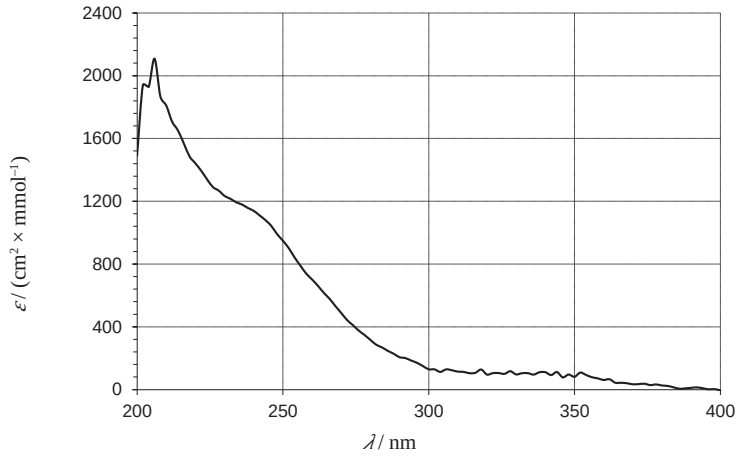



Fig. 1.1-17 UV spectrum of pseudopelletierine

## IR Spectrum in KBr

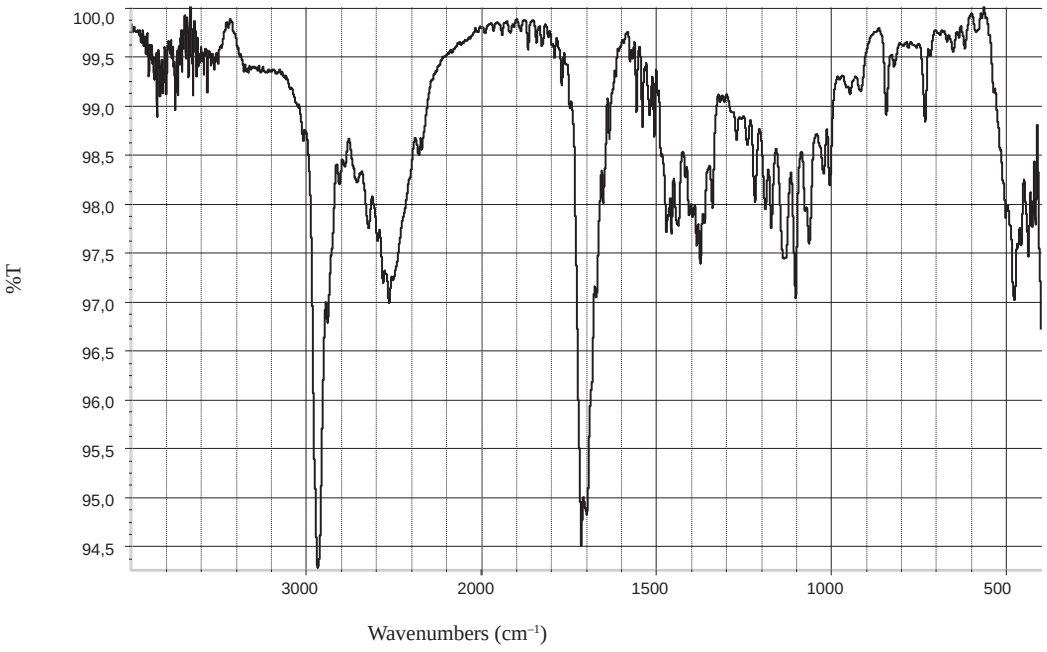



Fig. 1.1-18 IR spectrum of pseudopelletierine

## 700 MHz NMR Spectra in $CDCl_3$

### $^1$H NMR Spectrum

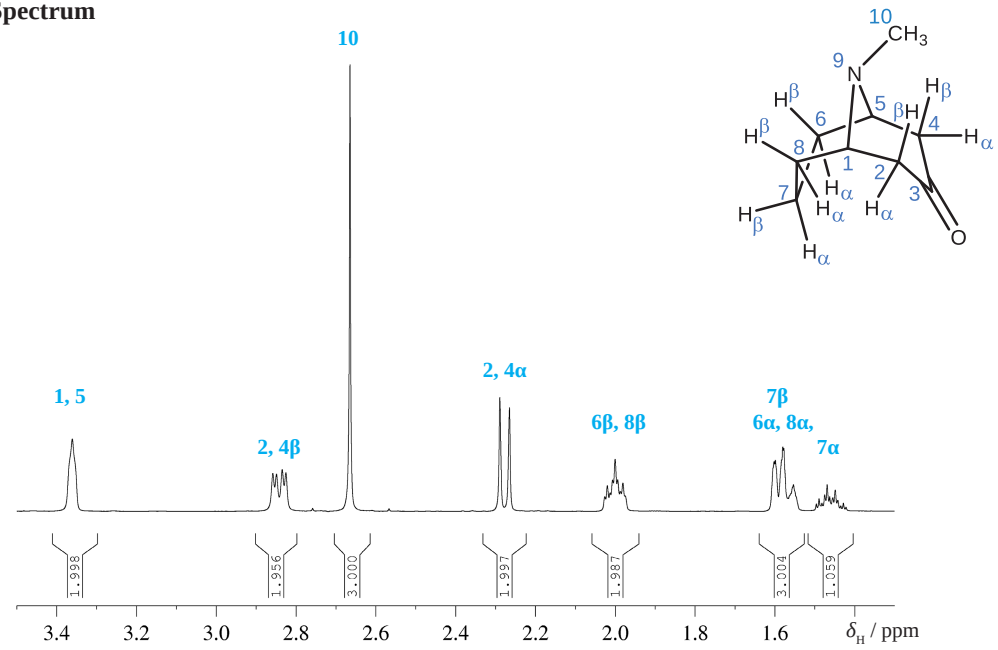



Fig. 1.1-19 $^1$H NMR spectrum of pseudopelletierine

Pseudopelletierine has a mirror plane and thus $C_s$-symmetry. Although with C-1 and C-5 two stereogenic centres exist, because of the reflectional symmetry the entire molecule is achiral. As a result of the mirror plane the α- and β-protons and the $^{13}$C-atoms at the positions 1,2 and 8 are isochronic with those at the positions 5, 4 and 6 respectively. In the $^1$H NMR spectrum (Fig. 1.1-19) the singlet at $\delta_H$ = 2.67, which can easily be assigned to the *N*-methyl group, is obvious.
The most strongly deshielded protons at $\delta_H$ = 3.36 belong to the bridgehead protons H-1 and H-5, their shift is determined by their proximity to the nitrogen atom. For the methylene protons the α/β-nomenclature is used, whereby α stands for protons below the mean molecular plane. The two signals at $\delta_H$ = 2.84 and 2.27 couple with each other and must be assigned to the α/β protons 2 and 4 next to the carbonyl group. The signal at $\delta_H$ = 2.00 and the two protons of the group of signals at $\delta_H$ = 1.59 belong to the methylene groups H-6 and H-8. The two remaining proton signals at $\delta_H$ = 1.55 and 1.46 are attributed to H-7.

There sits Death at the table and invites me (to eat)
And many pages with fine thin hands
And shoes of black velvet, which glide silently,
Carry wonderful dishes out:
Whole peacocks and fish with silver scales
And purple fins, in the small teeth
(Which are gilded) stick laurel branches
And grapes with gold-red rust and open
Pomegranates, which glow on soft cushions
Of fresh violets, and Death
Wears a coat made of white velvet
And seats me next to himself
And is very polite….

Hugo von Hofmannsthal (1874-1929) The Maiden and Death

**APT $^{13}$C NMR Spectrum**

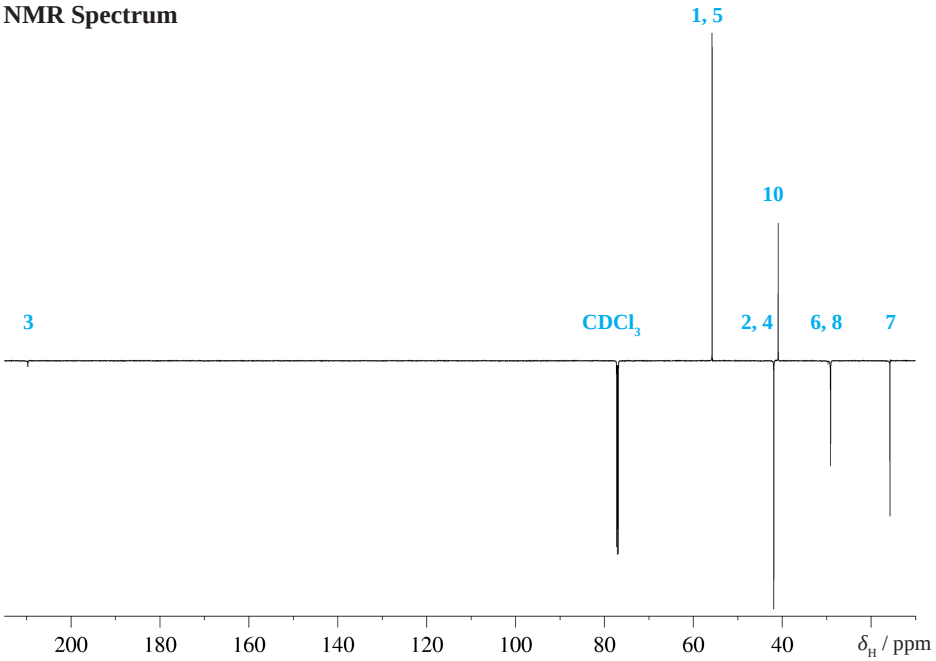



Fig. 1.1-20 APT $^{13}$C NMR spectrum of pseudopelletierine

The very simple $^{13}$C NMR spectrum (Fig. 1.1-20) shows as expected two positive and four negative signals. These are all well separated from one another, so that with the known rules for $^{13}$C chemical shifts the assignment presents no problems.

**COSY Spectrum**

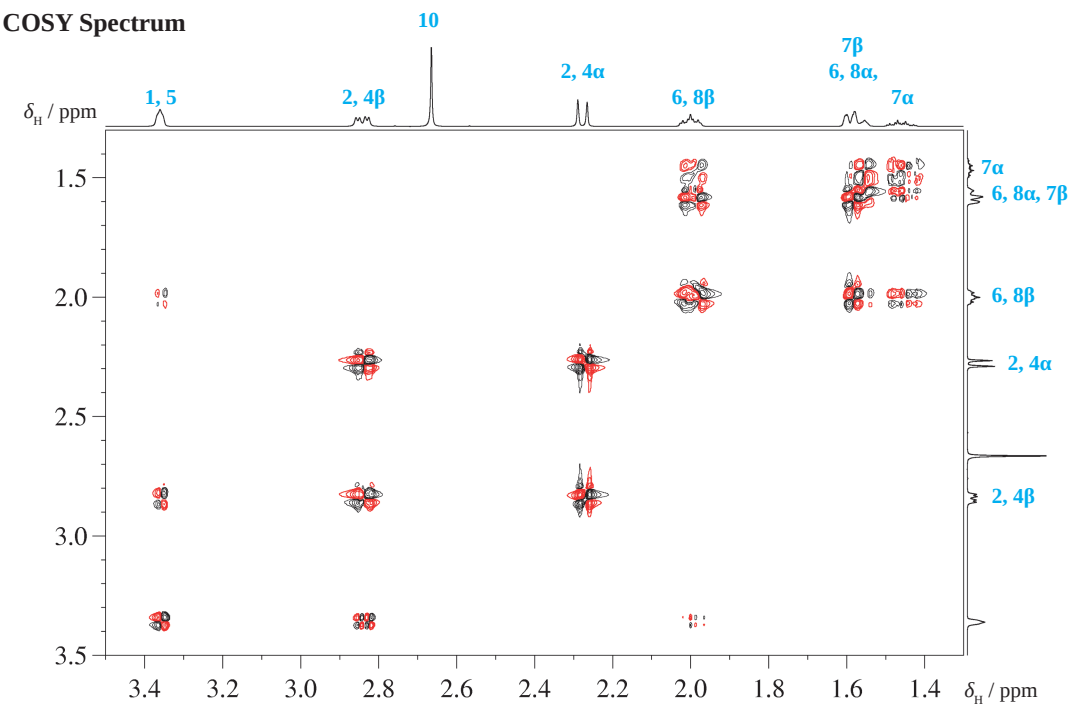



Fig. 1.1-21 COSY spectrum of pseudopelletierine

**NOESY Spectrum**

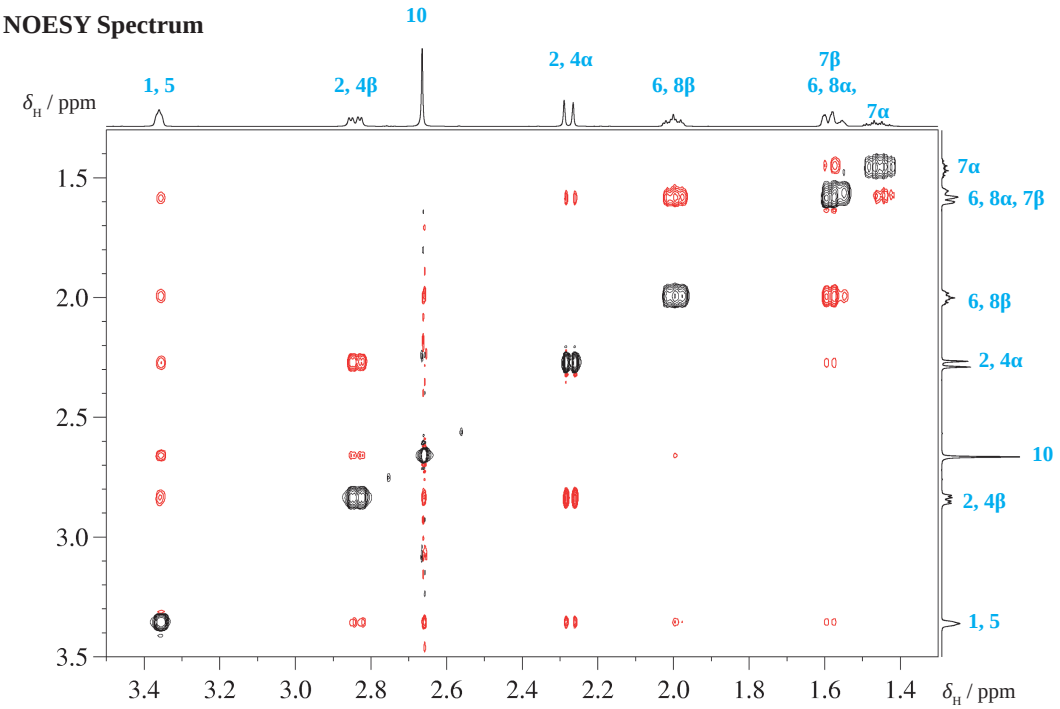



Fig. 1.1-22 NOESY spectrum of pseudopelletierine

For the proton signals the allocation of the α/β-positions for the methylene groups H-2/4, H-6/8 and H-7 can be achieved with help of the NOESY spectrum (Fig. 1.1-22). The signal of the methyl group shows a cross relaxation peak to H-2/4 at $\delta_H$ = 2.84 but not to H-2/4 at 2.27. This means, that the protons at $\delta_H$ = 2.84 must be on the same side of the molecule as the methyl group, i.e. in the β-position for the expected CC2 conformation. Similarly, there is an NOE cross relaxation between the α-protons of H-2/4 at $\delta_H$ = 2.27 and the signal from H-6/8 at $\delta_H$ = 1.59. This means that these protons must also be in the α-position. In addition, this is confirmed by a distinct cross relaxation peak between the *N*-methyl group and the signals of the H-6/8 in the β-position at $\delta_H$ = 2.00. This NOESY signal proves, that the inversion at the pyramidal N-atom under the conditions of the measurement at room temperature is fast on the NMR time-scale.
The missing NOESY signal between the H-atoms of N-$CH_3$ and the β-H-atom of C-7 indicates, that under the conditions of the measurement the chair-boat conformations CB1 and CB2 make no appreciable contribution to the conformational equilibrium of pseudopelletierine. Otherwise, a NOESY signal, especially for the CB1-conformation, would be expected. Finally a weak NOE signal (not visible in Fig. 1.1-22) between the α-protons of H-2/4 at $\delta_H$ = 2.27 and the signal of H-7 at $\delta_H$ = 1.46 shows, that the latter must also be in the α-position.

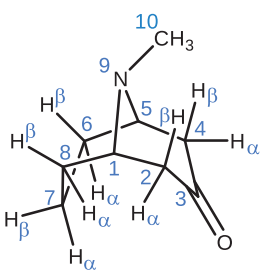



Fig. 1.1-23 Fruit bearing pomegranate tree on Crete in autumn

**HSQC Spectrum**

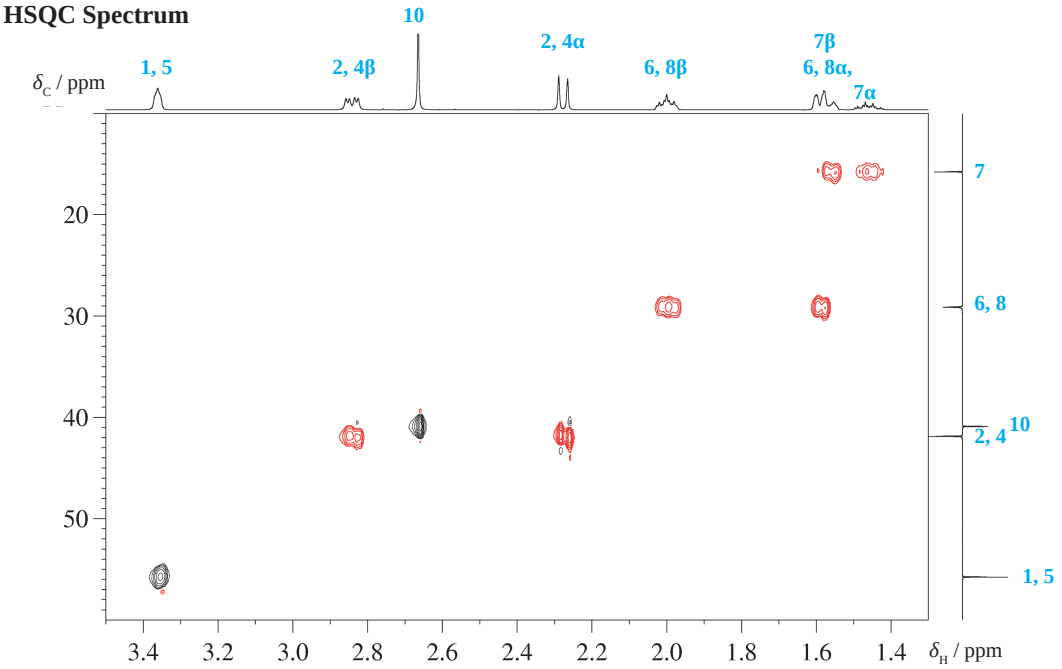



Fig. 1.1-24 HSQC spectrum of pseudopelletierine

The $^{13}C$ assignments are obvious from the HSQC spectrum (Fig. 1.1-24) because of the secured assignments of the protons [21,22].

**HMBC Spectra**

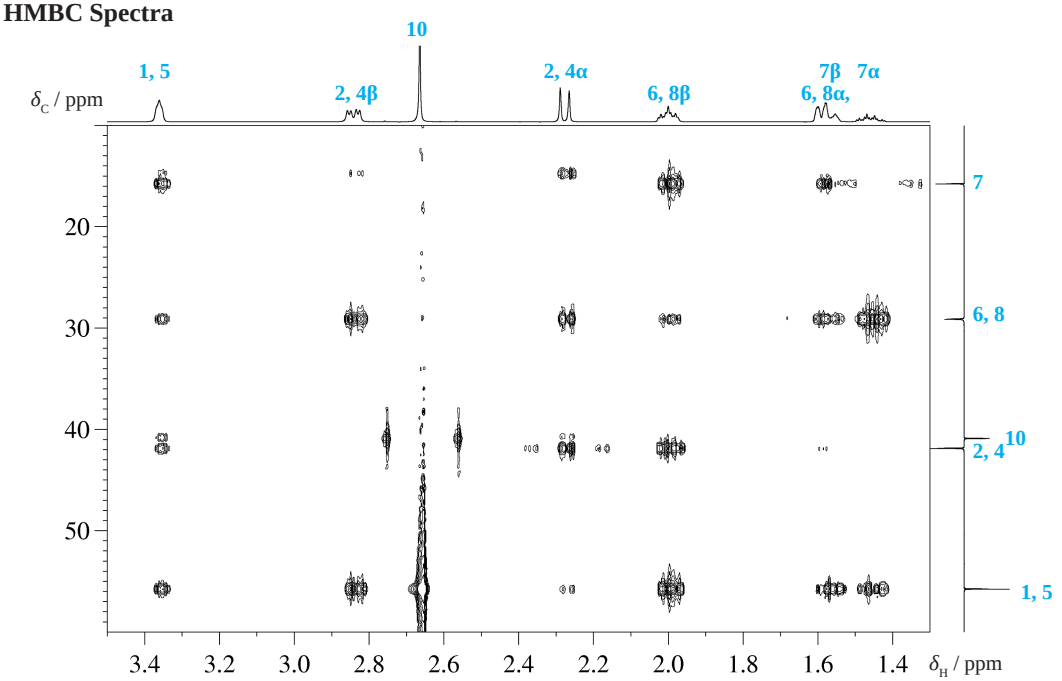



Fig. 1.1-25 Excerpt 1 of the HMBC spectrum of pseudopelletierine

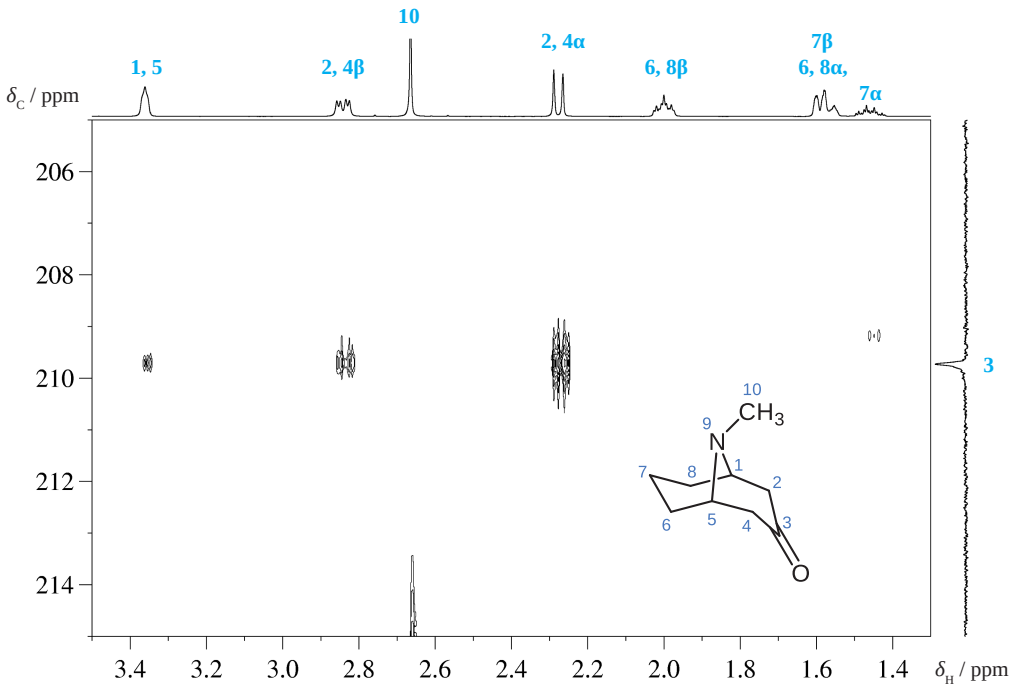



Fig. 1.1-26 Excerpt 2 of the HMBC spectrum of pseudopelletierine in the carbonyl region

## Quantum Chemical Calculation

**Memories from Greece**

Pomegranates offers and vines
Reconciling every year
And today life is sweet
As it was for the ancestors

Emanuel Geibel (1815–1884)

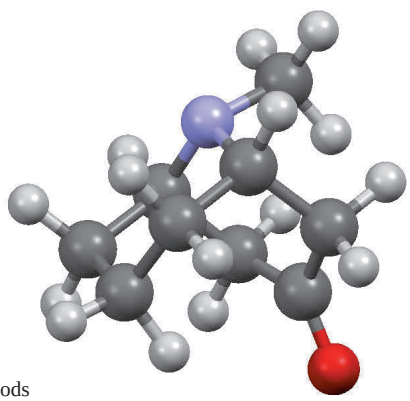


Fig. 1.1-27 3D structure of pseudopelletierine calculated with *ab initio* methods

The $^{13}C$ NMR chemical shifts for pseudopelletierine predicted by the program ChemBioDraw® for the bridgehead atoms C-1 and C-5 (Δδ = +17 ppm) and the methylene groups C-2/4 (Δδ = +7.5 ppm), C-6/8 (Δδ = -6.2 ppm) and C-7 (Δδ = +5 ppm), show considerable deviations from the measured values. This could be caused by lack of data for the azabicyclo[3.3.1]nonanes, or by the dynamic stereochemistry of these bicyclic compounds. For 9-methyl-9-azabicyclo[3.3.1]nonan-3-one (pseudopelletierine) chair-boat-chair ring inversion of both rings and the pyramidal inversion at the N-atom can lead to an averaging of the NMR signals of various positions. The NOESY spectra give no indication for a contribution of chair-boat conformations (CB1, CB2) under the conditions of the measurement. Both energetically favoured conformations CC1 and CC2, in which the rings are in the chair-chair conformation, are stereoisomers with a different arrangement of the methyl group at the $sp^3$-hybridized nitrogen. They are interconvertible via a transition state with a planar arrangement of the $sp^2$-hybridized N-atom (Fig. 1.1-28).

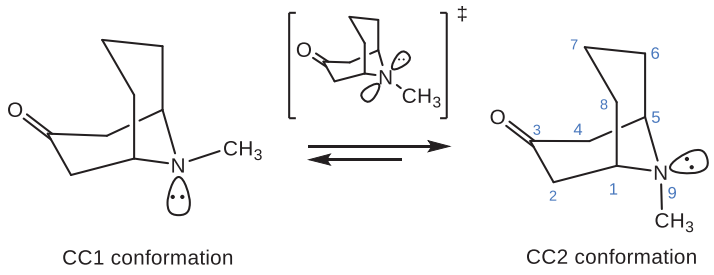



Fig. 1.1-28 Inversion at the N-atom

Our quantum chemical model calculations for the gas-phase show in agreement with recent experimental and theoretical investigation in solution [23, 24a, b] a slight energetic advantage (1.7 kJ×mol$^{-1}$; MP2/Def2TZV) for the conformation CC2 with an axial *N*-$CH_3$ group in the piperidone ring (Fig. 1.1-28).

This is explained by the reduced steric hindrance of an axial substituent in the flattened piperidone ring. From the energy difference a Boltzmann distribution at 298.15 K of ~0.5 (CC1:CC2 ~ 34:66) is calculated. $\Delta G^{\ddagger}$ for the inversion of the pyramidal N-atom via the planar transition state is ~ 30.6 kJ×mol$^{-1}$ (MP2/Def2TZV). Under the conditions used for the measurement, the inversion equilibrium is fast on the NMR time scale. Experimentally, this is confirmed by the two NOESY cross peaks from the H-atoms of the *N*-$CH_3$ group to the β-oriented H-atoms on C-2/4 and C-6/7. At room temperature a population weighted averaging of the chemical shifts of the C-atoms of the main conformations CC2 and CC1 determines the $^{13}$C NMR signals of pseudopelletierine.

The structure of both conformations CC1 and CC2 of pseudopelletierine were calculated with a DFT-hybrid method, a triple-ζ-type basis set and consideration of dispersion for $C_s$-symmetry (B3LYP/Def2TZVP EmpiricalDispersion=GD3BJ). The $^{13}$C chemical shifts for the isolated molecule calculated with wave functional methods (GIAO MP2/cc-pVTZ) are shown in the assignment table.

As to be expected for the gauche interaction of the axial arrangement of the *N*-$CH_3$ group in CC1 and CC2, the greatest differences in shift are calculated for the C-2/4 methylene groups in the piperidone ring and for the C-6/8 methylene groups in the piperidine ring. The differences in shift between CC1 and CC2 is small for all other positions.

A good agreement between the calculation and the experimental NMR spectrum is dependent upon many factors. Apart from the choice of the method of calculation, the solvent, the pH and the temperature have an influence on the conformational equilibrium and the rate of inversion of the pyramidal N-atom. Therefore, a population weighted averaging of the calculated shifts of various conformers was not carried out.

## Assignment Table

| $^{13}$C-NMR signal $\delta$ [ppm] | Type of C-atom | Assignment | $^{1}$H-NMR signal $\delta$ [ppm], $J$ [Hz] | Proof (HMBC coupling from proton to C-atom) | Proof (NOE from proton to proton | $^{13}$C-NMR signal predicted by ChemBio-Draw® | CC2 conformation *) | CC1 conformation *) |
|---|---|---|---|---|---|---|---|---|
| 209.7 | $C_q$ | C-3 | | 1/5, 2/4 | | 207.3 | 201.6 | 202.0 |
| 55.8 | CH | C-1/5 | 3.36 | 2/4, 6/8, 7, 10 | | 73.1 | 62.5 | 60.5 |
| 41.9 | $CH_2$ | C-2/4 | β: 2.84<br>α: 2.27 | 1/5, 6/8 | H-10 | 47.4 | 42.6 | 51.3 |
| 40.9 | $CH_3$ | C-10 | 2.67 | | | 39.5 | 44.3 | 43.7 |
| 29.1 | $CH_2$ | C-6/8 | β: 2.00<br>α: 1.59 | 1/5, 2/4, 7 | H-10<br>H-2/4e | 22.9 | 36.5 | 25.9 |
| 15.8 | $CH_2$ | C-7 | β: 1.55<br>α: 1.46 | 1/5, 6/8 | H-2/4e | 20.8 | 19.6 | 20.8 |

*) Calculation of structure: B3LYP/Def2TZVP EmpiricalDispersion=GD3BJ, NMR Calculation: GIAO MP2/cc-pVTZ

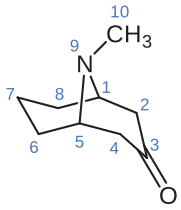
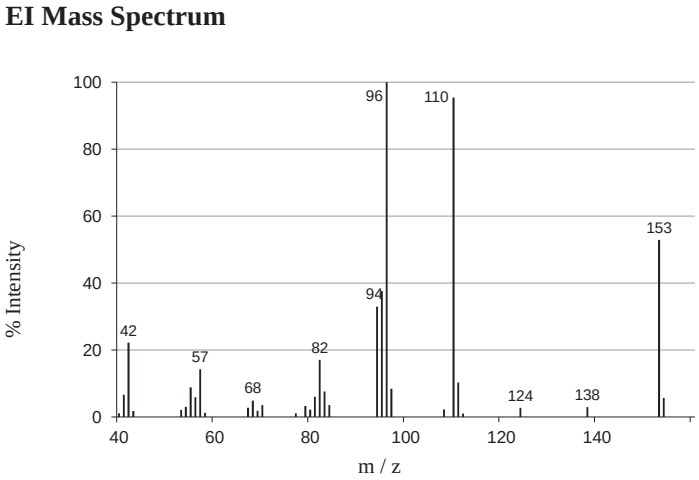



Fig. 1.1-29 EI mass spectrum of pseudopelletierine




In principle, two α-cleavages are possible that could break the bicyclic structure of ionized pseudopelletierine (Fig 1.1-30).

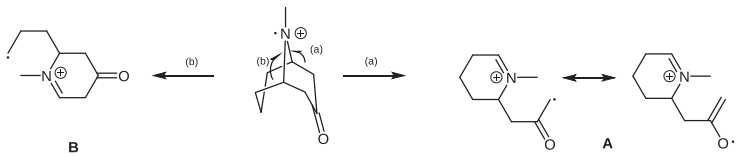



Fig. 1.1-30 Two possible α-cleavages in the mass spectrum of pseudopelletierine

Starting from **A** the base peak at m/z 96 can be explained by a radical induced H-abstraction via a six-membered transition state (Path (1)) and subsequent α-cleavage (Fig. 1.1-30). H-abstraction via a five-membered transition state (Path (2)) and subsequent α-cleavage leads to the fragment $C_7H_{12}N^+$ at m/z 110. Since m/z 96 is an ion with an even number of electrons, it cannot be the precursor of m/z 95, i.e. an independent route must exist for the formation of this ion, such as shown in Fig. 1.1-31. H-elimination from m/z 95 finally gives the *N*-methylpyridinium ion (m/z 94).

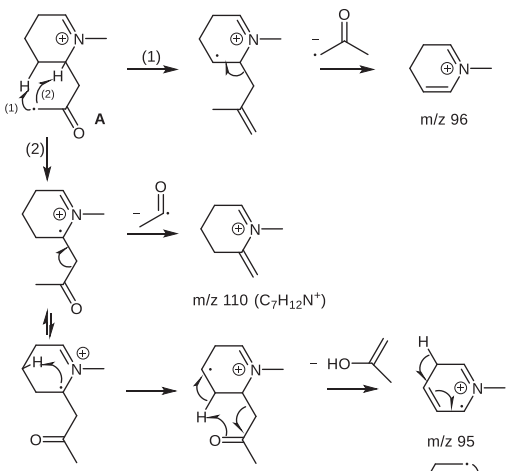



Fig. 1.1-31 Formation of fragment ions from the α-cleavage product **A**

A high-resolution spectrum indicates, that the $C_7H_{12}N^+$-ion (calc. 110.0985, measured 110.0977) formulated in Fig. 1.1-30 contributes only about 43% to the peak with m/z 110.
The greater part has the composition $C_6H_8NO^+$ (calc. 110.0616, measured 110.0613). For the formation of the isobaric ion the product of α-cleavage **B** is involved, which after an intramolecular H-shift can eliminate a propyl radical (Fig. 1.1-32).

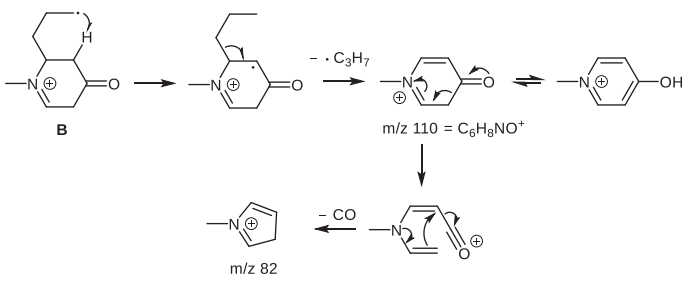



Fig. 1.1-32 Formation of fragment ions from the α-cleavage product **B**

## 4. Questions

A. Eating a pomegranate is not so easy and often ends with coloured stains on the table cloth, shirt or blouse. Suggest how these can be removed.

B. What is to be understood by the term "alkaloid"? Do all alkaloids have a common structural element?

C. Which alkaloid was the first to be isolated and from what? Give examples for basic and non-basic alkaloids.

D. Which alkaloid was first isolated on an industrial scale? From what was it isolated and for what was it used?

E. Which plant gained strategic importance in World War 2, because of the alkaloids it contains?

F. Explain the yellow colour of cyclooctatetraene.

G. The sharp doublet from H-2/4α is strongly reminiscent of a similarly situated proton of a bicyclic compound discussed in this book. Which one?

## 5. Literature

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Fig. 1.1-33 Pomegranate wine and juice at a market in Xian, China

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Fig. 1.1-34 Pomegranates among tropical fruits on a market in Kathmandu, Nepal

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[25] This article was first published by G. Heß, P. Haiss, D. Wistuba, H.-U. Siehl, S. Berger, D. Sicker, K.-P. Zeller "Pseudopelletierin – Vom Granatapfelbaum zum Cyclooctatetraen" *Chem. Unserer Zeit*, **2016**, *50*, 34–43.

# 1.2 Colchicine

**Poisonous, dangerous, useful and unique**

*N*-[(7*S*)-1,2,3,10-Tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide

**From the seeds of the autumn crocus**
*Colchicum autumnale* L. (Colchicaceae)

$C_{22}H_{25}HO_6$, MW 399.43 g×mol$^{-1}$

CAS RN 64-86-8

Pale yellow, amorphous powder,
mp 150-157°C

$[\alpha]_D^{26}$ – 64.4° (c 45 mg×mL$^{-1}$, $CHCl_3$)

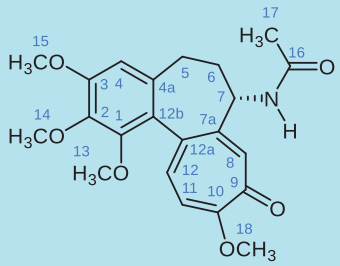



Fig. 1.2-1 Structure of colchicine

Fig. 1.2-2 Autumn crocuses on the site of an opencast mine at Nochten in Saxony

# 1. Background

My first contact with colchicine, the poison from the autumn crocus, was long ago. It was dramatic, "Ow, ow, my big toe hurts again, it's agony – quick bring me my drops!" my father-in-law implored me.


Fig. 1.2-3 Colchysat (Colchicina) drops

What he wanted were colchicine drops from the autumn crocus (Fig. 1.2-3). What made him limp was the gout. The swollen, hot and red big toe was a classical symptom. Deposits of uric acid crystals, which form, when the level of uric acid in blood is too high, cause the inflammation of the metatarsophalangeal joint of the big toe. This is the result of a defective purine metabolism of the kidney. It would go too far, to discuss in detail the possible causes here, to which diabetes mellitus or an excessive consumption of meat and alcohol can contribute.

The illness, also known as podagra, is as old as mankind. Often it affected those, who were particularly well situated when it came to eating and drinking. Therefore, it was formerly regarded to be the illness of monarchs. Famous sufferers from gout include Frederick the Great, Charles V, Louis XIV, Goethe, Voltaire and Bismarck.

Even so, gout is not the price for dissolute living but should be seen to be the result of a congenital, metabolic illness that causes a deficient excretion of uric acid. Inadvisable eating and drinking habits can be the trigger but not the root cause. Beneficial is only an unspectacular low-purine diet, such as bread, milk and potatoes.

What surprised me then, was the speed, with which the drops helped against the pain but naturally not against the gout itself. It was already known in antiquity, that an extract from the autumn crocus brings relief. Today we know why. Colchicine hinders the work of the leucocytes in blood. Leucocytes are the scavenger cells of the immune system that are engaged in absorbing and destroying the uric acid crystals. In so doing they emit cytokine as a messenger substance, which causes a painful inflammation. By hindering the work of the leucocytes, colchicine suppresses for the moment the occurrence of pain.

The dosage of colchicine is, however, not simple. This toxic alkaloid belongs to the poisons with a high effectivity but a narrow therapeutic window. A single dose of 2 mg and up to a maximum of three doses per day is tolerable. However, it is not a case of the more the better; 20 mg are already potentially lethal.

What sort of plant is it that produces such a highly active natural product? The autumn crocus (Poisonous Plant of the Year 2010) grows in moist, fertile meadows, sometimes in masses.

It is not a crocus (Iridaceae) even if there are extraneous similarities. The yearly rhythm of the plant is mysteriously strange and is worthy of note. It first flowers in early autumn but already produces its seeds in early summer. Isn't that the wrong way round? This tuber plant is a geophyte, i.e. some parts that should survive the winter remain underground. That functions as follows: in the summer, the corm of the herbaceous plant produces a side shoot. In September, it brings forth between one and three pink to violet flowers. Leaves do not grow in autumn. Insects pollinate the flowers. The information "fertilized" remains under the earth. During the winter, the

On the grave of two children, who had eaten poison (verses 1 – 3 of 11)

As in the beauteous blossom time
Two children went out
And in innocent joy
Picked the prettiest flowers,
There in the meadow they found
Seeds of the autumn crocus,
Pleased by their find,
They bathed in delight.

They opened the seed-vessel
And found some grains within
As white as snow, and what
Delight they felt!
That must be sugar crystals
They said, and believed,
Deceived by the appearance,
The seeds to be sugar.

What misfortune, nobody was there,
Who could tell them:
These seeds are poisonous
As are many fine berries;
And to go to school
They were too young and not compelled
Or else they could have learned from
A book about poisons.

Michael von Jung (1781 – 1858) Songs of Graves

mother corm wastes away. The side shoot now becomes the new mother corm. In the next spring leaves grow that are capable of photosynthesis and can produce all that is needed for seed formation and the new growth cycle. After the development of the seeds in an egg shaped capsule in May/June, these are elevated above the earth, as were the leaves. The capsule is brown and swollen (Fig. 1.2-4).

Fig. 1.2-4 Autumn crocus in summer

The small brown-black seeds are sticky and can be transported by ants. In September, the new corm has developed so far, that the next flowers can be produced.

The approximately 30 cm long, narrow leaves of the autumn crocus like those of the lily of the valley can easily be mistaken for those of ramsons, which in either case could possibly lead to a deadly poisoning. The circumstance, that with this plant flowers and leaves are never seen together, increases the risk of poisoning, when with the best of intentions "wholesome, wild herbs" are collected. Great care is required! It would be equally fatal to confuse the corm with an edible onion, but even that has occurred.

The name Colchicum for the plant family refers to the region of origin of the plant, Colchis, a region on the Black Sea in present-day Georgia. There, in the Land of the Golden Fleece the mythological figure Medea, a sorceress and mixer of poisons, was supposedly active. This illustrates the poisonousness of the plant, which contains colchicine in all its parts. Already in 78 A.D. the famous Greek pharmacologist Pedanius Dioscorides warned in his main work "De materia medica" against imbibing preparations of colchicum (Fig. 1.2-5). The alkaloid seems to have been notorious as a poison suitable for murders, as can well be imagined.

The attribute autumnale is the Latinised reference to the period, in which the plant flowers. The vulgar names of a not particularly poetic nature are autumn crocus, meadow saffron and naked lady (Fig. 1.2-6).

Colchicine is a highly effective cytotoxic agent. The maliciousness of a poisoning with colchicine lies in the period of latency of between 2 and 6 hours between its intake and its effect. As little as 20 mg is a fatal dose for an adult (about 60 g of fresh leaves) and as little as 5 mg for a child (equivalent to

The bulb has a central partition at which it sends out the flower. It grows abundantly in Messenia and at Colchos. Eaten, it kills by choking, similar to mushrooms. We have described it so that it may not lie hidden and be eaten instead of bulbus, for it is strangely alluring to the inexperienced for its pleasantness. To help those who eat these, give them whatever helps those who eat mushrooms [above], and cow's milk (taken as a drink) so that when this is at hand they need no other help.

Pedanius Dioscorides (40 – 90) De materia medica. Book 4-84 English translation by T. A. Osbaldeston First Edition, 2000. Published by IBIDIS Press, Johannesburg, South Africa.


Fig. 1.2-5 Pedanius Dioscorides, Codex Aniciae Julianae picturis illustratus, German National Library, Leipzig


Fig. 1.2-6 Naked Lady (autumn crocus) in May

1.5 g of seeds). Difficulties to swallow, burning in the mouth, vomiting and bloody diarrhoea are the symptoms. Respiratory paralysis and circulatory collapse can lead to death. Children are particularly endangered.

Animals such as horses and pigs are also greatly at danger from the wrong feed, this is particularly true for horses put out to graze. Cows, pigs, sheep, goats, hares, dogs and cats can also poison themselves. The milk of sheep or goats that have eaten autumn crocuses has proved to be poisonous.

If a case of colchicine poisoning is suspected the emergency poison centre or an emergency doctor should be consulted immediately and the circumstances explained emphatically.

However, the toxicity is just one aspect of colchicine. The alkaloid is used against pericarditis. A lifelong (!) medication with colchicine helps inhabitants of the east Mediterranean region, who suffer from familial Mediterranean fever. This hereditary disease is manifested by periodic bouts of fever accompanied by amyloidosis, an accumulation of protein fibres caused by a deficient protein folding. Since colchicine inhibits the division of cells, by binding to the protein tubulin and preventing the formation of the spindle apparatus [1], an application as an anticancer agent is conceivable. However, the already mentioned, narrow therapeutic window has prevented an approval for this indication. In human genetics colchicine is used as an adjuvant to obtain a karyogram. This is an ordered overview of all the chromosomes in a cell, which can be evaluated using an optical microscope, allowing for example the recognition of hereditary illnesses.

Because of its antimitotic effect, the alkaloid has been a much valued tool in botany for the cultivation of plants. Here it leads to diploid or even polyploid plants, which produce a higher yield. What is so particular about colchicine from the perspective of organic chemistry?

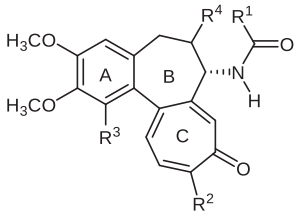
At first sight, the structure of colchicine appears to be unique. There is no association to another natural product. At the same time, it does not appear to be so complicated. However, what is unusual is immediately apparent; it is the two seven-membered rings in the 6-7-7-ring system. Using colchicine as the subject many things can be exemplified, to which belong:

1) the development of the concept of structure and the historical stages that organic and inorganic chemistry have gone through, to arrive at the present-day level of structure elucidation,
2) the clarification of the biogenesis, a biochemical task,
3) the development of ingenious total syntheses.

Colchicine total syntheses have been reviewed in detail [1].

Colchicine was first isolated [2] in 1820 by the first two grand masters of al-

kaloid chemistry, Pelletier and Caventou, who also isolated strychnine from the poison nut [3] and obtained the antimalarial drug quinine from cinchona bark on an industrial scale in a factory. Mistakenly both regarded what they had isolated to be the already known veratrine, which is known to be a mixture of other alkaloids. The pharmacists Geiger and Hesse established in 1833, that it was a new compound [4]. The name colchicine comes from them. However, not even the molecular formula was known not to mention the structure. Following the erroneous results of others, the chemist Simon Zeisel (Fig. 1.2-7) from Moravia found the correct molecular formula in 1883: $C_{22}H_{25}NO_6$ [5]. In 1856 the most important co-alkaloid colchiceine was discovered, the molecular formula of which $C_{21}H_{23}NO_6$ was also established by Zeisel [5].




| $R^1$ | $R^2$ | $R^3$ | $R^4$ | Name |
|---|---|---|---|---|
| $COCH_3$ | $OCH_3$ | $OCH_3$ | H | Colchicine |
| $COCH_3$ | OH | $OCH_3$ | H | Colchiceine |
| $CH_3$ | $OCH_3$ | $OCH_3$ | H | Demecolcine |
| $COCH_3$ | $OCH_3$ | OGlc | H | Colchicoside |
| $COCH_3$ | $SCH_3$ | $OCH_3$ | H | Thiocolchicine |
| $COCH_3$ | $OCH_3$ | $OCH_3$ | OH | Colchicilline |

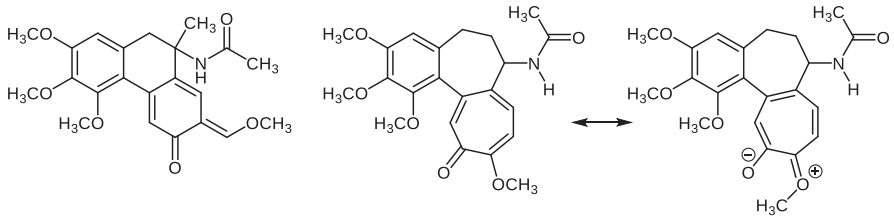
Fig. 1.2-8 Structural analogues of colchicine

At that time structural elucidation was performed with the aid of degradation reactions, by which one hoped to find a known degradation product that by clever interpretation could be related to its unknown precursor. For the tetramethoxy-compound colchicine Zeisel himself developed the classical method that is named after him (Zeisel method) to determine the number of alkoxy-groups by cleavage of the ether bond with boiling hydriodic acid followed by the gravimetric determination of the iodine in the alkyl iodide formed after reaction with silver nitrate to silver iodide. What effort, what skill, what precision was necessary! However, it was worth it, since very many natural products are methoxy-substituted. It was also Zeisel, who recognized the character of the alkaloidal nitrogen contained in a non-basic acetamide group. In 1924, over 100 years after the first isolation, Windaus [6] achieved an important step in the structural elucidation. He recognized, that colchicine contains three annulated rings. However, he speculated, that it was a derivative of phenanthrene, which is a 6-6-6-ring system. Three annulated rings was thinking big, but not “big” enough, if compared with the reality of the 6-7-7-ring system.

Fig. 1.2-7 Professor Simon Zeisel (1854 – 1933) College for Agriculture, Vienna

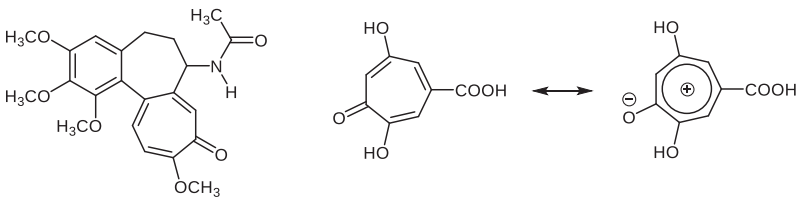
The British theoretical chemist M. J. S. Dewar had the first success concerning the structure of the ring system in a paper in 1945 [7]. Known for his creative ideas and new structural suggestions, he had earlier elucidated the structure of stipitatic acid, which contained something new, namely 2-hydroxycyclohepta-2,4,6-trienone, that is a specially substituted seven-membered ring with a cyclic, conjugated 6π-electron system. Dewar called it α-tropolone. Dewar then recognized colchicine to be the first akaloid that contains this substructure. Only the position of the keto group was not correctly determined. The search continued. X-ray crystallography, at that time a new but successful technique, helped further. It measures the diffraction of monochromatic X-rays by a crystal and correlates these to the atomic arrangement. A difficulty was, that colchicine itself is not crystalline but amorphous.

However, King *et al*. discovered, that it crystallizes together with diiodomethane and analysed these crystals [8]. This delivered the first correct structural formula for colchicine in 1952. One question was still unanswered, that of the absolute configuration on C-7. Here a classical degradation experiment helped. On ozonolysis one of the degradation products found was a nitrogen containing, optically active carboxylic acid $C_7H_{11}NO_5$ that could be unambiguously identified by Corrodi *et al*. as N-acetyl-L-glutamic acid [9]. It is paradoxical, that the authors related this determination of the configuration to the structural proposal of Dewar. However, in toto after 132 years the structure of a physiologically highly active natural product was clarified – a time interval that one might anticipate would not be very different for many other natural products with an unusual structure.



A. Windaus, 1924

M.J.S. Dewar, 1945, Resonance structures of colchicine



M.V. King, 1952

M.J.S. Dewar, 1945, Resonance structures of stipitatic acid

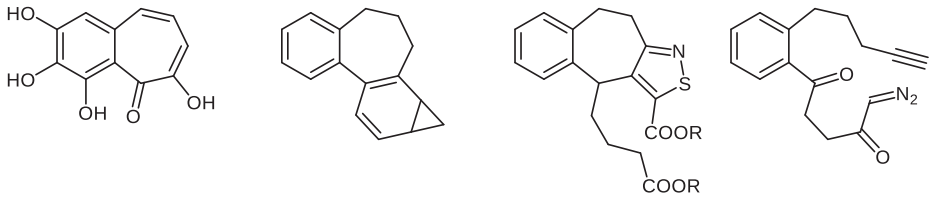
Fig. 1.2-9 Suggestions for the structure found on the path to the correct structural formula

The unusual ring system does not reveal at the first glance, what the original biochemical building blocks are. It required decades of work, to clarify the individual steps of the biosynthesis. As is normal in this field of research, unanswered questions were clarified by skillful isotopic labelling and incorporation experiments. The credit for this belongs to Battersby and Leete et al. [10, 11].

It has been shown, that the naturally occurring amino acids tyrosin and phenylalanine are the precursors. A biogenetic scheme is given in [1] and shown in the *supporting information*.

Colchicine continues to motivate the further development of organic synthesis [1]. Why is this so? The compound was already synthesized in 1959. Natural products are of great value because of the abundance of unconventional and highly diverse, physiologically active structures that they contain. Many active pharmaceutical ingredients are either themselves natural products or further developments based on the structures of natural products. The aim of the latter being an increased effectivity with the least possible side-effects. Exactly that is the target of present-day colchicine synthesis. Similar to the concept of automobile production, a platform, similar to the structure of colchicine, should exist, based on which different models can be constructed. Chemically these would be structurally diversified deriva-

tives or analogues with pharmaceutically usable properties. The laboratories that, 50 years after the structural elucidation, are engaged in this work are involved with "The Taming of the Structural Shrew".




Van Tamelen *et al.* 1959 Eschenmoser *et al.* 1959 Woodward 1963 Schmalz *et al.* 2004

Fig. 1.2-10 Four different starting materials from four different retrosynthetic concepts for colchicine

# 2. Isolation

## 2.1 Principle

The autumn crocus is not a protected plant. However, it is not easy, to obtain material for the extraction of colchicine. The corm of the plant is not available from businesses that sell the bulbs of crocuses, daffodils, tulips etc. This is apparently due to the poisonousness of the plant, although it is found in the flowerbeds of public parks. As has already been described, its biological rhythm is relatively complicated. However, pharmaceutical preparations that contain colchicine are produced commercially. Therefore, it must be possible for the manufacturers of such products to find a reliable source of material for the extraction of colchicine. After some searching, we found a farmer in Thuringia, who planted autumn crocuses and sold us a sufficient quantity of seeds, to carry out the isolation described here. The seeds supposedly contain about 0.5% colchicine.

The first problem that is encountered, if colchicine is to be isolated from seeds, which are dark brown and about the size of mustard seeds, is of a mechanical nature. The seeds are extremely hard. Using the milling equipment of kitchen machines (e.g. a Moulinette) it is practically impossible to grind them. They can be ground under liquid nitrogen in a steel or porcelain mortar, whereby the low temperature produces a brittleness that can be exploited. However, not a powder but at best a course grit is obtained. A ball mill, if available, produces a much better result.

Colchicine is an alkaloid but it is not basic. This means, that the usual procedure used for a basic alkaloid is not applicable. Colchicine is an organic compound of medium polarity, which dissolves for example in ethanol or chloroform but not in petroleum ether or hexane. 45 g dissolve in 1 L of water at room temperature. That is a surprisingly large quantity and at first glance at the structural formula may not be expected. We found, that an aqueous extraction is the best method to remove colchicine relatively selectively from the seeds. Hydrophobic components that are possibly present in the seeds are removed prior to the aqueous extraction by a Soxhlet extraction with *n*-hexane. The aqueous extract is then extracted with chloroform, whereby most of the colchicine goes into the organic phase. The raw colchicine obtained from the chloroform extract is first purified by a normal flash chromatography. Preparative HPLC removes the last impurities.

Colchicine has only been described as an amorphous and not as a crystalline substance, which leads to widely varying values for the melting point. We

consider values that lie between 150 and 160°C to be reliable. The published data for the specific rotation is extremely confusing. All possible values between +119° and –152° can be found. Amongst authoritative sources, there seems to be agreement, that colchicine is laevorotatory. We are convinced, that the value of – 64.4° measured by us using a state of the art instrument (automatic polarimeter POLARTRONIC MHZ-8 from Schmidt & Haensch) is reliable.

## 2.2 Method

Ground seeds of the autumn crocus (110 g) are extracted in a Soxhlet extractor on a water bath for 6 h with *n*-hexane (700 mL). The resulting colourless extract, which according to an analysis by TLC (silica gel aluminium plate UV254, eluent chloroform-methanol 9:1 v/v) contains no colchicine (i.e. no spots showing fluorescent extinction that are typical for colchicine or related compounds are seen), is discarded. The seeds are dried in air.

The dried seeds (50 g) are then extracted six times with water (each time 500 mL for 30 minutes) at 40°C in a single necked flask (1 L) with vigorous stirring using a large magnetic stirring bar. A turbid suspension forms and the water takes on a brown colour. The extracts are united and filtered under suction through a Büchner filter funnel. Using the TLC conditions given above a TLC analysis on this extract shows a spot for colchicine ($R_f$ = 0.46) and a further spot with $R_f$ = 0.35, which are visible by fluorescence extinction. The volume of water is reduced to a half on a rotary evaporator under reduced pressure. The concentrated aqueous extract is extracted with chloroform (5×150 mL). The chloroform phases are united and dried over $MgSO_4$. The chloroform is removed to dryness under vacuum. An ochre yellow, paste-like residue remains that on drying under oil pump vacuum turns into a brittle foam. A yield of 186 mg (0.37% of the mass of seeds) is obtained.

## 2.3 Purification

The purification is carried out by flash chromatography.
Column: length of separation zone 250 mm, diameter 30 mm
Stationary phase: silica gel 60 (0.063 – 0.200 mm)
Eluent: chloroform – methanol (20:1 v/v)
Volume of fraction: 7 mL

The raw material (115 mg) is dissolved in the eluent (4 mL) and with the addition of further eluent (6 mL) transferred to the column and eluted. A dark brown start zone is formed. After an initial elution (200 mL) fractions are collected. During the course of the elution the entire column acquires a pale yellow colour but no differentiable colour zones are observed. In total 35 fractions are collected. The fractions are characterized by TLC using the conditions described above. Fractions 1 – 5 show no spots exhibiting fluorescence extinction and are discarded. The fraction 6,7 and 8 after dipping the TLC plate in Seebach's phosphomolybdic acid reagent and heating show a spot at $R_f$ = 0.54 and are discarded. "Clean" spots from colchicine at $R_f$ = 0.46 are found in the fractions 12 – 22. In the fractions 23 – 35 a further spot below that of colchicine at $R_f$ = 0.35 is visible by fluorescence extinction. The fractions 12 to 22 are united as are the fractions 23 -35 and each of the united fractions is evaporated to dryness in vacuum. From the fractions 12 - 22 colchicine (72.7 mg) is obtained as a pale yellow, amorphous solid and the fractions 23 – 35 yield a colourless, amorphous solid (23.7 mg) that

The final hay was harvested weeks ago. When I stride over the meadow, the one I mean, I am struck by – round about wafts a fine, damp cold, milky white mist – a magically stemmed, pale lilac coloured word: colchicum.
Alone or together in long, dense patches, hauntingly the strange, pale, lonely flower, the only ones, the very last; still over the expanse of the bare meadow, in their haunting, hectic beauty; and still bringing memories of the jolly fresh crocus of early spring.
Timeless, timeless* – why are they thus called? It seems to have a special meaning. They, on the threshold, on which all sprouting, thriving life contracts and withdraws within itself as if in its last, immeasurable, with the senses encompassed entity; within, where without space and time only its innermost, unlosable centre of power [exists].
But that is a treacherous region. And it is known, that they are poisonous.

*The German name for the autumn crocus is Herbstzeitlose, literally autumn timeless
Johannes Schlaf 1862 – 1941 Neue Erzählungen aus Dingsda (Further Stories from Whatsit)

contains colchicine as the main component. The melting point of the first solid with the highest content of colchicine is between 150 – 157°C. The $^1$H NMR spectrum is recorded for each sample in $CDCl_3$, the spectra show, that the first sample is of sufficient purity. The specific rotation of this sample is $[\alpha]_D^{26}$ – 64.4° (c 45 mg×mL$^{-1}$, $CHCl_3$).

Further Purification by Preparative HPLC

Colchicine (20 mg) from the fractions 12 – 22 is purified by preparative HPLC (column: Knauer and Merck, Eurospher 100 C18 5µm, length 250 mm, diameter 25 mm). The sample is dissolved in methanol (5 mL) and purified in five runs, in each of which 1 mL is injected. The eluent is methanol (flow rate: 1 mL×min$^{-1}$). For detection a wavelength of 290 nm is used. The retention time is 33 – 37 minutes. Pure colchicine (8 mg) is obtained.

Colchicine purified in the above manner was used to record the 600 MHz NMR spectrum shown here.

## 3. Spectra and Comments

### UV and CD Spectra in Ethanol

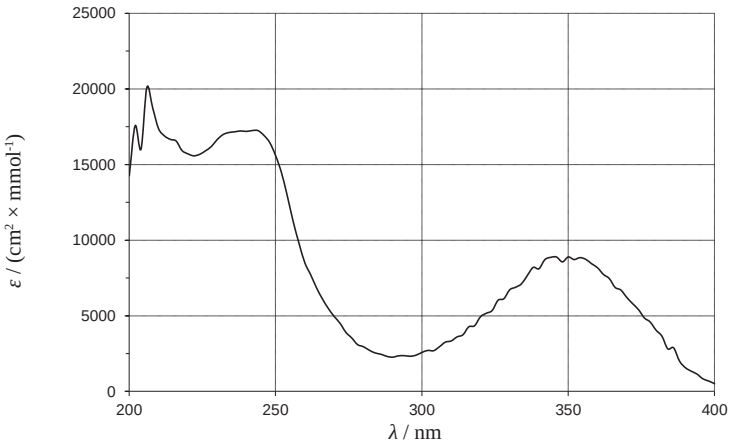



Fig. 1.2-11 UV spectrum of colchicine

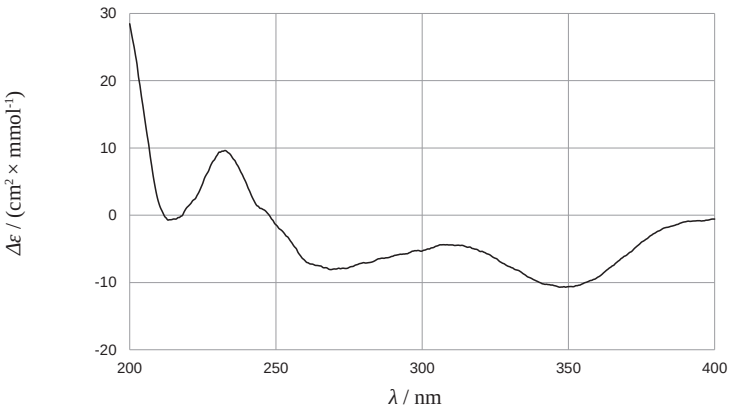



Fig. 1.2-12 CD spectrum of colchicine

Colchicine has three chromophoric groups, an aromatic ring with three auxochromic methoxy groups, the tropolone system and finally the amide group. All three contribute to the distinctive UV spectrum (Fig. 1.2-11) with maxima at 230 and 350 nm having values of ε of around 10,000 cm$^2$ × mmol$^{-1}$. In the CD spectrum of the chiral compound (Fig. 1.2-12) the two bands have opposite signs. The luminescence of the compound and the corresponding Jablonski diagram are comprehensively discussed by H. Roigt and R. M. Leblanc [13].

## 600 MHz NMR Spectra in $CDCl_3$

### $^1$H NMR Spectrum

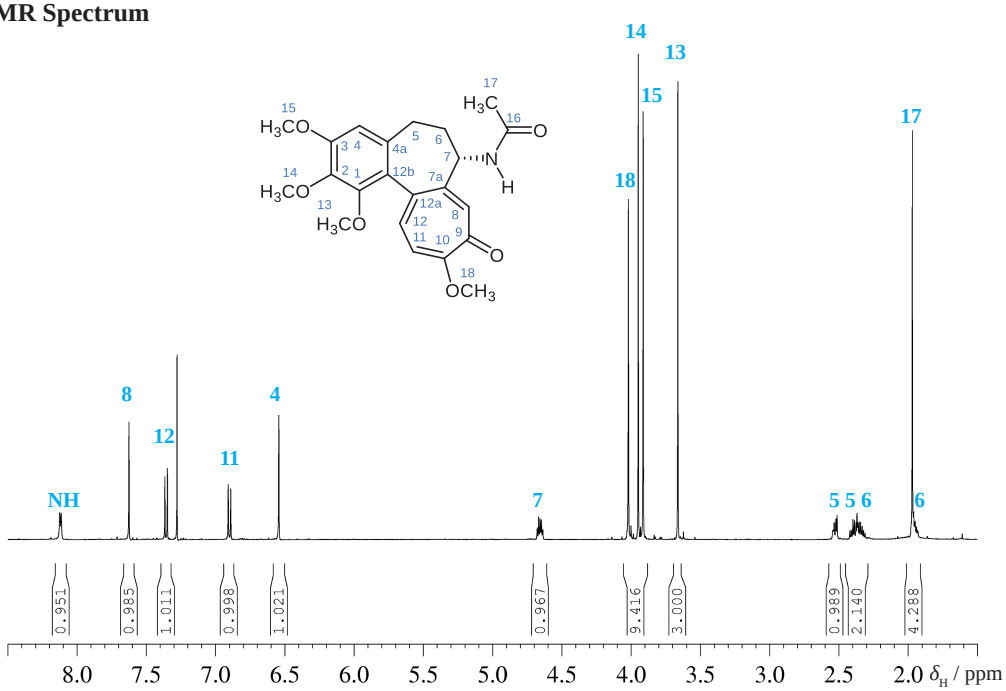



Fig. 1.2-13 $^1$H NMR spectrum of colchicine

In the first $^{13}$C NMR paper [15] because the field strength and resolution were too weak many errors in the assignments occurred, however, in the correction [16] two errors still exist. The assignments given here are in agreement with those given in more recent publications [17, 18]. As is often the case for the $^1$H NMR spectra of alkaloids, the resonances are distributed across the entire spectrum and are therefore relatively easy to assign (Fig. 1.2-13). The NH-proton at $\delta_H$ = 8.12 appears as a doublet and its coupling partner H-7 can be found at $\delta_H$ = 4.66. The two singlets in the aromatic region belong to the isolated protons H-8 and H-4, whereby H-4 because of the +M-effect of the neighbouring methoxy group is more strongly shielded.

The AX spin system that can be seen in the same region belongs to H-12 and H-11, again because of the +M-effect of the methoxy group H-11 is more strongly shielded and appears at $\delta_H$ = 6.90. The spin coupling of 11 Hz is considerably larger than the corresponding coupling in benzene derivatives. The individual assignment of the methoxy groups as well as the methylene protons 5 and 6 require the aid of the 2D COSY, NOESY and HMBC spectra.

The meadow is poisonous but pretty in the autumn
The cows that graze there are slowly poisoned
Meadow-saffron the colour of lilac and of shadows
Under the eyes grows there your eyes are like those flowers
Mauve as their shadows and mauve as this autumn
And for your eyes' sake my life is slowly poisoned

Children from school come with their commotion
Dressed in smocks and playing the mouth-organ
Picking autumn crocuses which are like their mothers
Daughters of their daughters and the colour of your eyelids
Which flutter like flowers in the mad breeze blown
The cowherd sings softly to himself all alone
While slow moving lowing the cows leave behind them
Forever this great meadow ill flowered by autumn

Guillaume Apollinaire (1880 – 1918) Les colchiques)
Translation Oliver Bernard
Source of the translation: http://www.artofeurope.com/apollinaire/apo4.htm

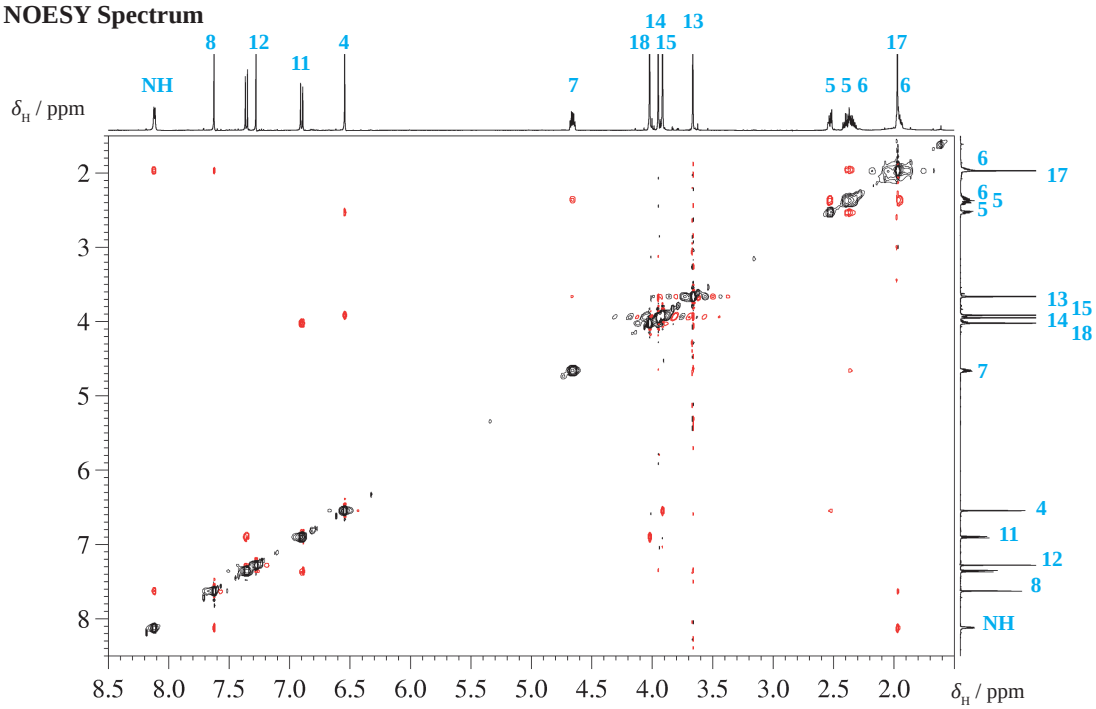



Fig. 1.2-14 NOESY spectrum of colchicine

Starting from the NH-proton the expected NOE cross signal to H-8 and the methyl group H-17 can be found (Fig. 1.2-14). In addition H-8 shows an NOE cross peak to the methyl group H-17, which proves, that the acetamido group can rotate freely. Two methoxy group signals, H-15 and H-18, can be assigned directly with the aid of the NOESY-spectrum, since they interact with their neighbouring protons H-4 and H-11. Moreover H-4 shows a cross peak to one of the methylene protons 5 at $\delta_H$ = 2.56, which can be designated as H-5'. This must be in a pseudo-equatorial position, consistent with a relative deshielding by the aromatic ring current.
The acetamido group can be assumed to be in a pseudo-equatorial position and therefore, H-7 is arranged pseudo-axially. H-7 shows an NOE-cross peak to an H-6 at $\delta_H$ = 2.35, the latter must stand pseudo-equatorially, as predicted by the calculated stereostructure, and is designated H-6'. Two further cross signals belong to H-6', one is to its geminal, pseudo-axial partner H-6 at $\delta_H$ = 1.95 and the other to H-5'.

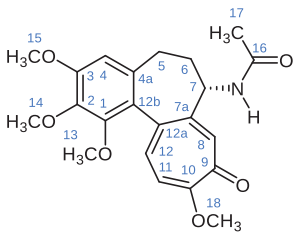
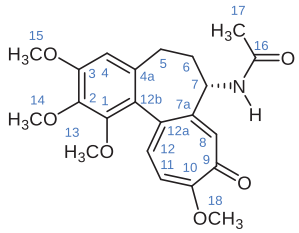



Fig. 1.2-15 Seeds of the autumn crocus




**HSQC Spectrum**

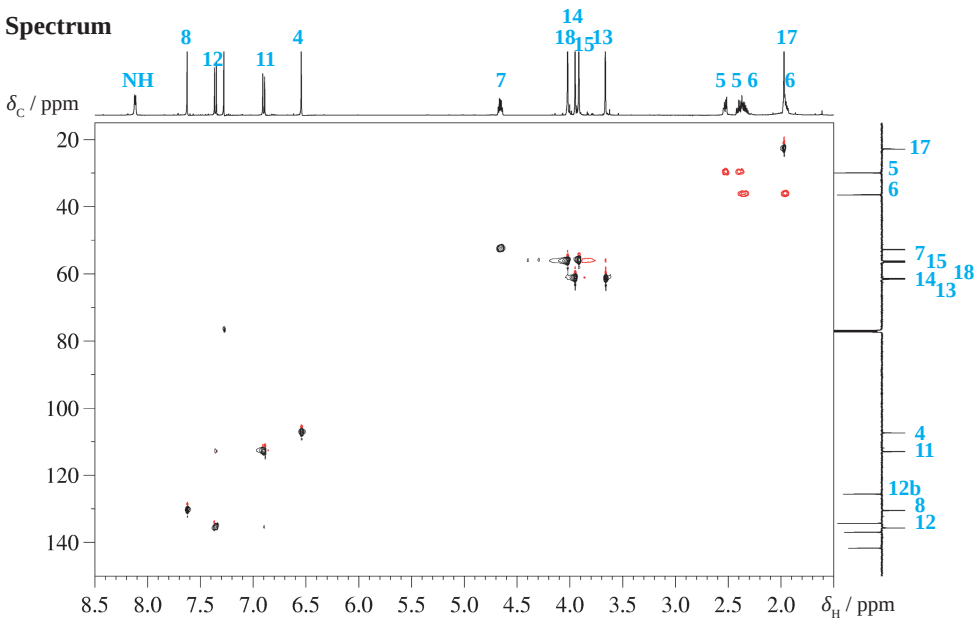



Fig. 1.2-16 HSQC spectrum of colchicine

Since the aromatic and olefinic proton signals have already been assigned, the allocation of the $^{13}C$ chemical shifts in this region is simple. The same applies to C-7 and both methoxy groups C-15 and C-18. The red coloured signals of the C-atoms 5 and 6 clearly shows the diastereotopism of the proton signals of the methylene groups (Fig. 1.2-16).

## HMBC Spectrum

**a) $\delta_C$ 15-70 ppm**

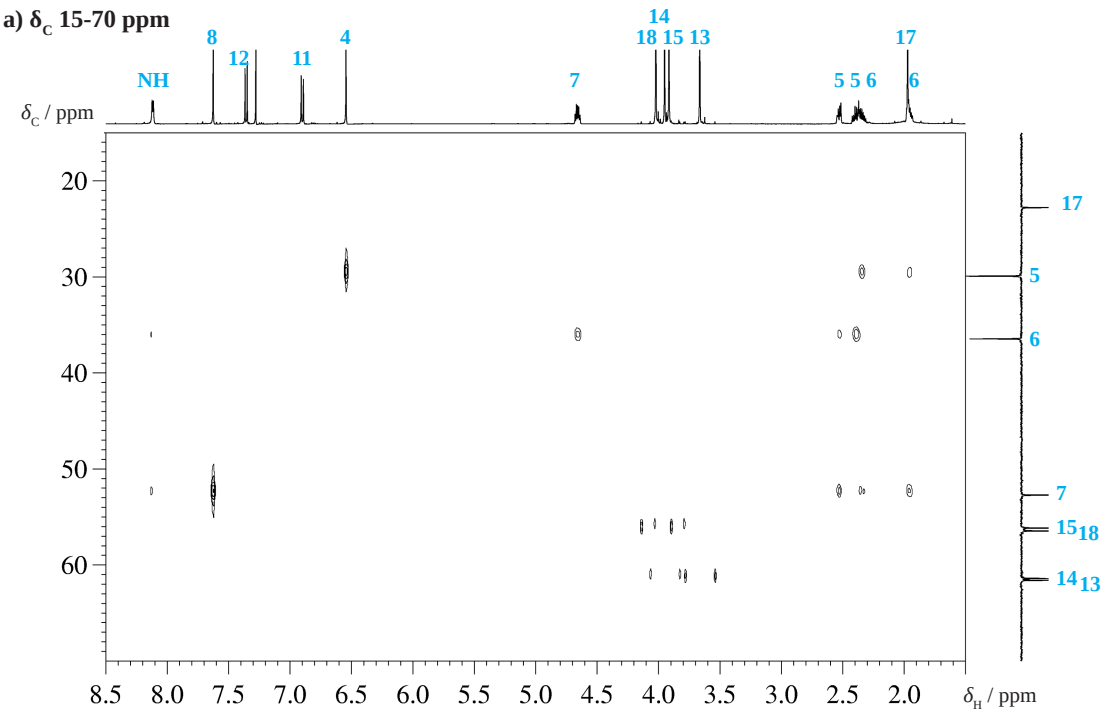



Fig. 1.2-17 HMBC spectrum of colchicine for the aliphatic carbon atoms

**b) $\delta_C$ 100-180 ppm**

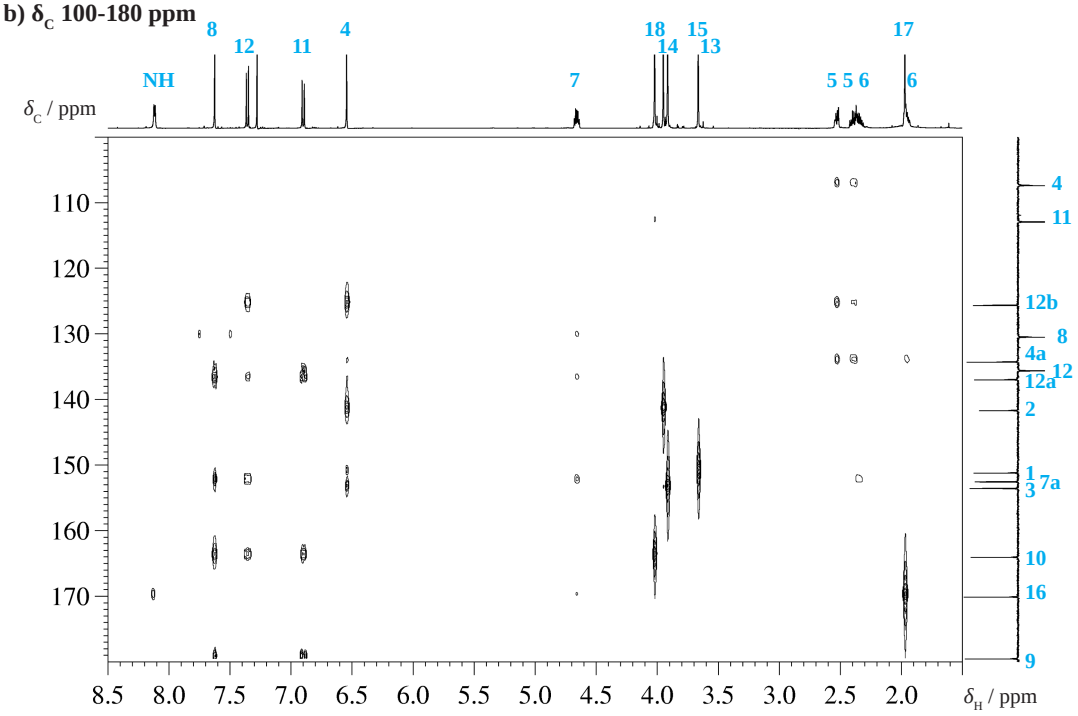
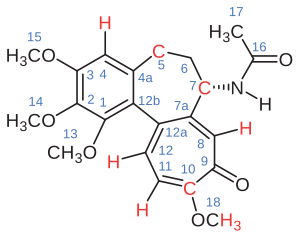
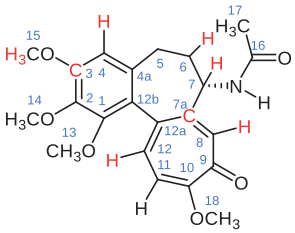



Fig. 1.2-18 HMBC spectrum of colchicine for the $sp^2$ hybridized carbon atoms

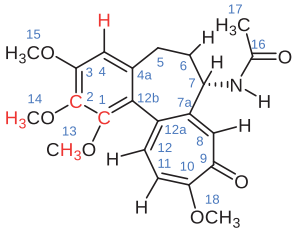
The HMBC spectra (Fig. 1.2-17 and 18) clarifies any remaining questions regarding the assignment. In the top expansion a) two strong cross signals can be seen that join H-8 with C-7 and H-4 with C-5 and thus confirm the assignments made for the proton spectrum. In the bottom expansion b) it can be seen, that the four proton signals of H-8, H-11, H-12 and H-18 are connected to the $^{13}$C-signal at $\delta_C$ = 164.1. Therefore, this must be attributed to C-10.



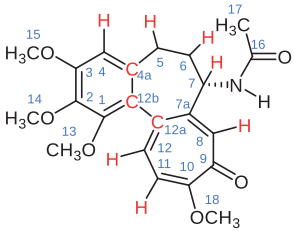
The proton signals of H-4 and H-15 correlate with the $^{13}$C-signal at $\delta_C$ = 153.7 that therefore belongs to C-3. Correspondingly, the proton signals of H-8, H-12, H-7 and H-6' correlate with the $^{13}$C-signal at $\delta_C$ = 152.6, so that it is established to be C-7a.



The third of the closely situated $^{13}$C-signals of the quaternary C-atoms at $\delta_C$ = 151.2 is connected by a very weak cross peak (over four bonds) to the signal from H-4 and that of a methoxy group at $\delta_H$ = 3.66. It can therefore be assigned to C-1 and the proton signal at $\delta_H$ = 3.66 to the methoxy group 13. The next quaternary carbon signal at $\delta_C$ = 141.7 belongs to C-2, because it shows a strong correlation peak to H-4 and the methoxy group H-14 at $\delta_H$ = 3.94.



The protons H-12, H-11, H-8 and H-7 show a strong correlation to the $^{13}$C signal at $\delta_C$ = 137.0 that can only belong to C-12a. The signal from C-4a can be identified by the correlation with H-4, H-5 and H-6. The signal of the last remaining quaternary C-atom 12b at $\delta_C$ = 125.6 is confirmed by the link to H-12, H-4 and H-5.



## Quantum Chemical Calculation

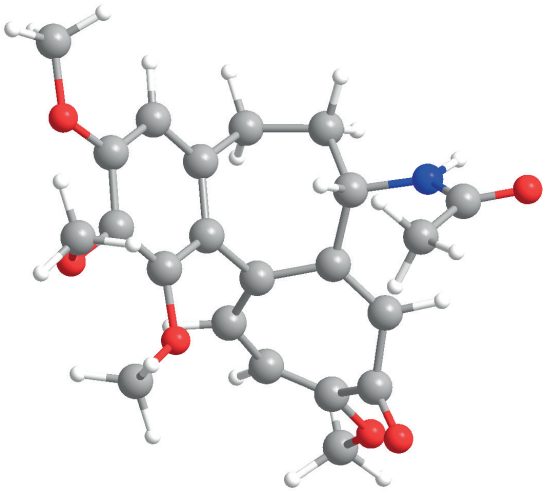


Fig. 1.2-19 3D structure of colchicine calculated with *ab initio* methods

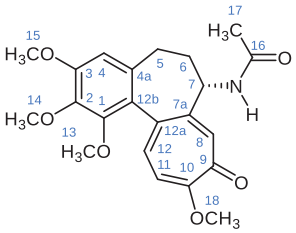
The signals of the $^{13}C$ spectrum of colchicine measured in $CDCl_3$ are estimated using ChemBioDraw® software with a satisfactory accuracy of –2 to +7 ppm. However, some signals have a larger deviation of up to +12 ppm.
The $^{13}C$ chemical shifts are calculated quantum chemically with a DFT method for **one** quantum chemically calculated minimum structure (Fig. 1.2-19) of colchicine with **one** specific conformational arrangement of the mobile substituents, the four methoxy groups and the acetamide group. Conformational equilibria and solvent effects are not considered. Calculated chemical shifts show in part a good agreement of within –1 to +4 ppm, however, some larger deviations up to a maximum of +9 ppm exist.

For us, for whom the doorposts, on which our childhood years were marked inch by inch, have been burned. In our garden, we planted no tree, in the growing shadow of which we could place our chair.
We, who sit down on the hillside, as if we were shepherds of the cloud-sheep that wander over the blue meadow above the elms.
For us, who are always travelling – a life-long journey, as if between two planets – searching for a new beginning.
For us the autumn crocuses stand up on the brown meadow of the summer, and the woods become filled with brambles and rose hips --
That we look into the mirror and learn to read our face, in which the future slowly unravels.

Hilde Domin (1909 – 2006) Autumn crocuses

## Assignment Table

| $^{13}$C-NMR signal $\delta$ [ppm] | Type of C-atom | Assignment | $^{1}$H-NMR signal $\delta$ [ppm], $J$ [Hz] | Proof (HMBC coupling from proton to C-atom) | Proof (NOE from proton to proton) | $^{13}$C-NMR signal predicted by ChemBio-Draw® | $^{13}$C-NMR chemical shifts calculated with B3LY-P/6-31G(d)// HCTH407/ TZVP |
|---|---|---|---|---|---|---|---|
| 179.5 | $C_q$ | C-9 | | H-8, H-11 | | 179.4 | 174.5 |
| 170.1 | $C_q$ | C-16 | | NH, H-17 | | 170.7 | 166.1 |
| 164.1 | $C_q$ | C-10 | | H-8, H-11, H-12, H-18 | | 164.9 | 169.5 |
| 153.6 | $C_q$ | C-3 | | H-4, H-15 | | 152.2 | 157.2 |
| 152.6 | $C_q$ | C-7a | | H-7, H-8, H-12 | | 151.1 | 149.3 |
| 151.2 | $C_q$ | C-1 | | H-4, H-13 | | 150.9 | 154.7 |
| 141.7 | $C_q$ | C-2 | | H-4, H-14 | | 140.6 | 146.6 |
| 137.0 | $C_q$ | C-12a | | H-7, H-8, H-11, H-12 | | 139.2 | 138.6 |
| 135.6 | CH | C-12 | 7.356, $J$ = 10.9 | | | 135.5 | 135.8 |
| 134.3 | $C_q$ | C-4a | | H-4, H-5, H-6 | | 134.4 | 137.9 |
| 130.5 | CH | C-8 | 7.63 | | | 130.8 | 135.9 |
| 125.6 | $C_q$ | C-12b | | H-4, H-5, H-12 | | 126.6 | 129.6 |
| 112.9 | CH | C-11 | 6.90, $J$ = 10.9 | | | 112.4 | 108.1 |
| 107.4 | CH | C-4 | 6.55 | H-5 | | 108.3 | 104.1 |
| 61.6 | $CH_3$ | C-13 | 3.66 | | H-7 | 61.7 | 62.3 |
| 61.4 | $CH_3$ | C-14 | 3.94 | | | 60.8 | 60.9 |
| 56.5 | $CH_3$ | C-18 | 4.01 | | H-11 | 58.4 | 56.0 |
| 56.2 | $CH_3$ | C-15 | 3.91 | | H-4 | 56.1 | 56.1 |
| 52.7 | CH | C-7 | 4.66 | | | 58.4 | 60.9 |
| 36.5 | $CH_2$ | C-6 | 6: 1.956<br>6': 2.35 | H-5, H-7 | H-7 | 36.1 | 45.9 |
| 29.9 | $CH_2$ | C-5 | 5: 2.39<br>5': 2.52 | H-6 | H-4 | 29.1 | 35.9 |
| 22.8 | $CH_3$ | C-17 | 1.97 | | | 23.7 | 20.6 |



## EI Mass Spectrum

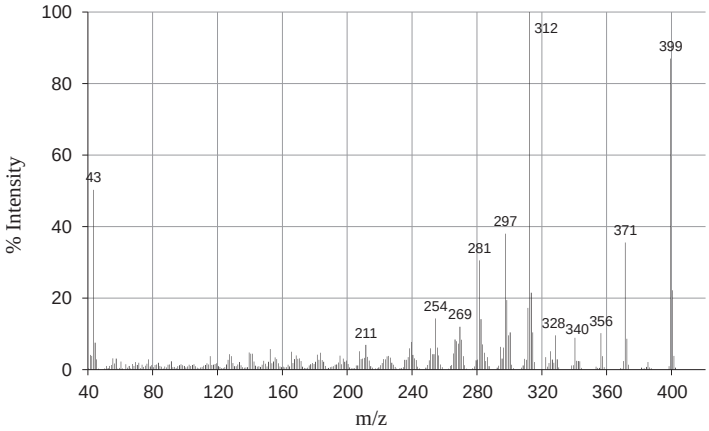



Fig. 1.2-20 EI mass spectrum of colchicine

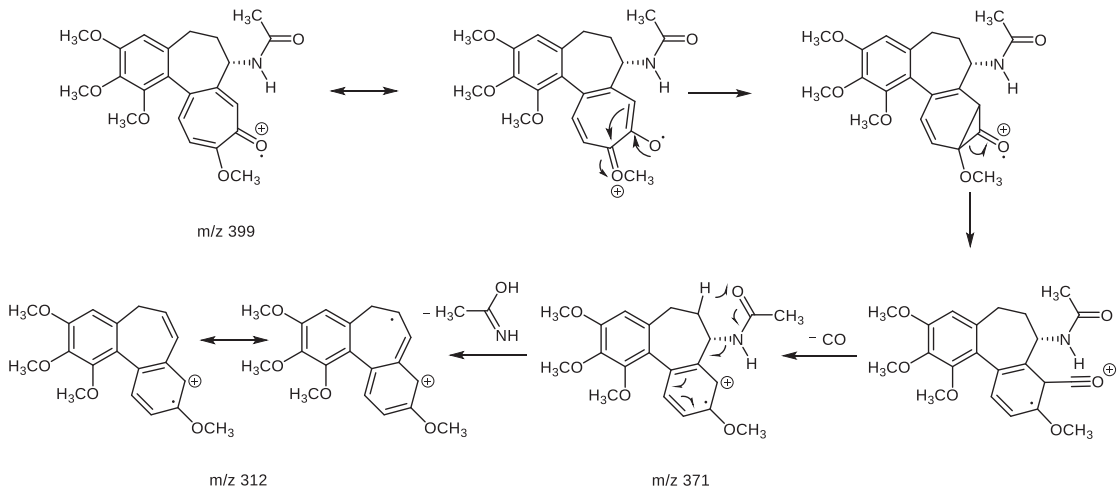



Fig. 1.2-21 Formation of the most intensive fragment ion (m/z 312) by sequential elimination of CO and McLafferty rearrangement.

The marked tendency of the molecular ion of tropone and tropolone derivatives to eliminate CO and to form a benzenoid ring [19] is also found in colchicine [20]. This degradation produces the ion with m/z 371 (Fig. 1.2-21). Because of the partial amide structure a further fragmentation of the molecular ion by a McLafferty rearrangement to an ion with m/z 340 is to be expected. The most intensive fragment in the mass spectrum of colchicine is the ion with m/z 312, its formation is possible by a McLafferty rearrangement following the elimination of CO ($M^{+\bullet} \rightarrow$ m/z 371 $\rightarrow$ m/z 312) (Fig. 1.2-21) or by the reverse sequence of the two steps ($M^{+\bullet} \rightarrow$ m/z 340 $\rightarrow$ m/z 312) (Fig. 1-2-22).

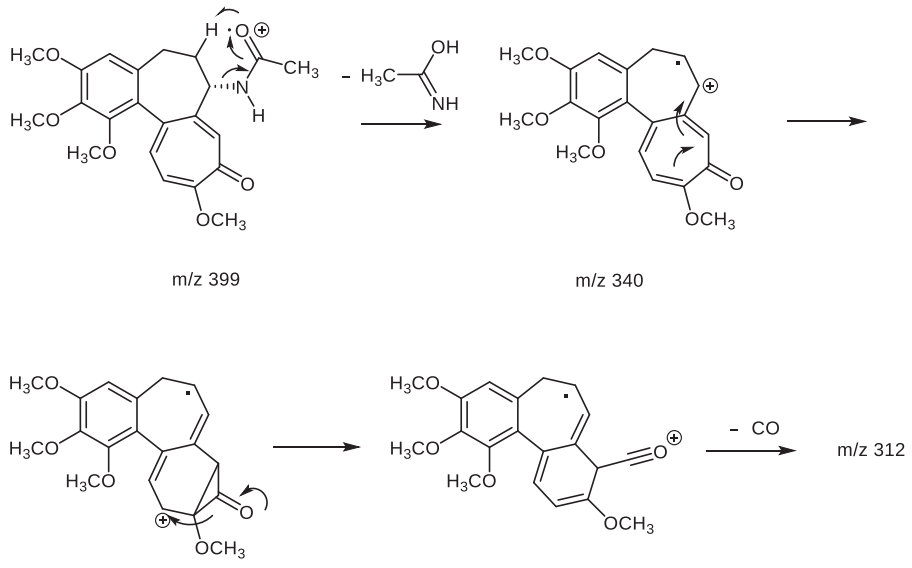



Fig. 1.2-22 Alternative route to m/z 312 by the reversed order of the fragmentation steps

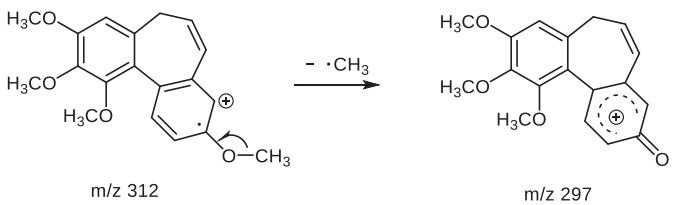



Fig. 1.2-23 Methyl elimination from the m/z 312 ion by α-cleavage

The cleavage of the tautomeric acetamide from the $[M\text{-}CO]^+$ ion shown in Fig. 1.2-21 deserves some comment. It is general opinion, that a McLafferty rearrangement requires the carbonyl O-atom to have radical character, so that a radical attack on a γ-H-atom is possible. Correspondingly, Fig. 1.2-22 implicitly assumes, that colchicine is partially ionised at the amide function, so that a McLafferty rearrangement can be formulated.
In the $[M\text{-}CO]^+$ ion (m/z 371) the radical and charge are delocalised in the aromatic π-system and cannot reach the amide function because the conjugation is interrupted by an $sp^3$-hybridised C-atom. Therefore, a radical attack on the γ-C-atom cannot initiate the acetamide cleavage. In this case the fragmentation is started instead by a radical attack on the C(7)-N-bond.
As a radical cation the main fragment with m/z 312 is converted by the loss of a methyl radical to an ion with an even number of electrons (m/z 297). Further possibilities, for the loss of the radical character, can be found in the spectrum by the elimination of an H-atom (m/z 311) or a methoxy radical (m/z 281) (see Question F).

## 4. Questions

A. Why is colchicine not basic?

B. What is the quintessence of the isolation procedure of basic alkaloids such as nicotine, cytisine or galanthamine?

C. How can the relatively high solubility of colchicine in water be explained, when it contains no functional groups such as OH or COOH?

D. What do such structures as benzene, the cyclopentadienyl anion and the tropylium cation have in common?

E. What effect causes the increase in the vicinal coupling constant of unsaturated seven-membered rings compared with aromatic six-membered rings?

F. Apart from the cleavage of a methyl radical, explained in Fig. 1.2-23, the radical cation with m/z 312 makes use of the elimination of $H^{\cdot}$ (m/z 311) and $CH_3O^{\cdot}$ (m/z 281) to lose its radical character. Suggest how this fragmentation can occur.

G. Discuss the formation of the ions with m/z 356, m/z 328 and m/z 43 (second most intensive fragment).

Fig. 1.2-24 Blossoming autumn crocus

## 5. Literature

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Fig. 1.2-25 An autumn crocus plant in mid-May.

# 1.3 Capsaicin

**Hot, Hotter, Capsaicin!**

(6*E*)-*N*-[(4-Hydroxy-3-methoxyphenyl)methyl]-8-methylnon-6-enamide

Synonyms: (*E*)-Capsaicin, Capsicine, Capsicin

**From sun-dried, Kenyan "African Bird's Eye Chilies"** *Capsicum frutescens* L. (Solanaceae) without stalk

$C_{18}H_{27}NO_3$, MW 305.41 g×mol$^{-1}$

CAS RN 404-86-4

Colourless crystals, mp 68-69°C (tetrahydrate)

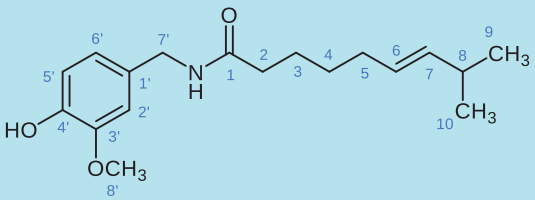



Fig. 1.3-1 Structure of capsaicin

Fig. 1.3-2 Peperoncini

## 1. Background

I have literally "hot" memories of peppers and chilli (Fig. 1.3-2). In 1976 I was camping with my girl-friend on the Lipno Lake in Bohemia, when during our evening meal, because of some innocent looking Hungarian wax peppers (green and pointed), a drama began: "Oooh, that's hot! What can I do? Tell me, what I can do!" she cried in agony. I knew, that drinking tea or eating bread would not help, although drinking milk might but we did not have any. I recalled a "hot dare" during military service. On a Saturday evening in the barracks, we ate a jar of pickled chillies. They were really incredibly hot but the rules of the game were to keep a straight face and demand stoically "Give me another!" If a player gave up, he was allowed to drink a vodka, which had been smuggled in, as a chaser. So, in the Bohemian Forest I crawled into the tent and brought out the bottle of Becherovka and this alcoholic beverage helped my girl-friend by removing the pungent capsaicin from her tongue. We shall see later, what the scientific explanation for this trick is.

Our colleague Klaus Roth in 2010 in his ebullient article "Some like it hot" in *Chem. Unserer Zeit* has already highlighted from all angles the members of the capsicum family such as peppers and chilli [1]. This includes:

- An overview of the botanical diversity and the cultivation of plants of the genus paprika (*Capsicum*) from the family of nightshade plants (*Solanaceae*), to which chilli also belong, in Middle and South America.
- A culinary overview of the carotenoid dyes that excite our senses. We have already described the isolation of one of the two "paprika ketones", i.e. capsanthin (Fig. 1.3-3), from noble sweet paprika [2a].

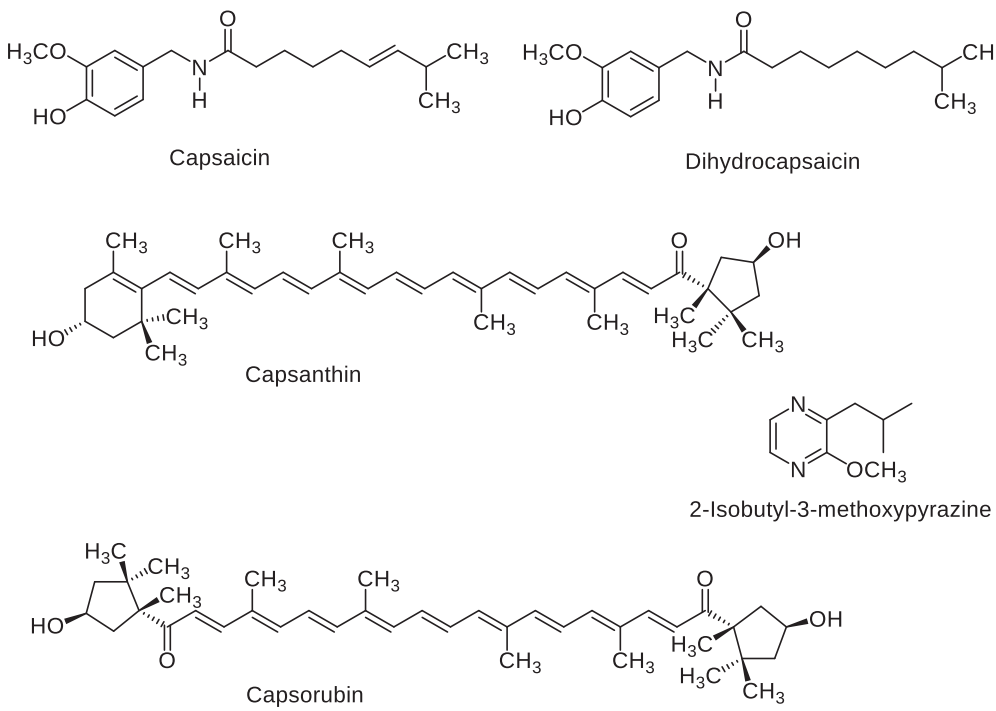



Fig. 1.3-3 Some important components of paprika

- A detailed view of the biosynthesis of both paprika ketones and therefore also that of capsorubin (Fig. 1.3-3).
- An eulogy to 2-isobutyl-3-methoxypyrazine, the "paprika pyrazine" (Fig. 1.3-3), the origin of the pleasant aroma, which fortunately can be detected by our noses in extreme dilution.
- As the main theme, the structure of the four pungent substances from paprika including capsaicin and dihydrocapsaicin (Fig. 1.3-3) and their biosynthesis from the amino acids L-phenylalanine and L-leucine and where exactly their pungency is located in the fruit.
- Naturally also observations on the Scoville scale, which is legendary to lovers of hot, hotter and hottest food. It was developed in 1912 by the American chemist W. L. Scoville as an organoleptic test of pungency. It has immortalized his name in a way that is normally only reserved for the discoverers of a named reaction in the *Scoville Heat Unit* (SHU), which can extend to a value of 16 million.
- The surprising but in detail proven statement, that we do not taste pungency in the way, in which we taste sweet, sour, salty or bitter but we experience it by a different type of receptor, namely the TRPV1 ion canal for *temperature*. This sensation is transported by a different nerve, the trigeminal nerve – that you hopefully do not know from neuralgia. Heat and pungency activate the same receptor. Maybe this is the reason, why in English spicy food is referred to as being "hot".
- Knowledge about the structure-pungency relationship, for which the vanillyl residue and the medium sized carbon chain (8 to 11 carbon atoms) are essential, whereby the double bond of capsaicin is not necessary for its pungency.
- Reports on how the components of paprika can be used other than for culinary purposes. For example:

  - as the red food colourant E160c

  - as a component of the so-called *pepper spray*, which if taken literally it is not, because not the pungent alkaloid piperine [2b] from black pepper but the capsaicinoid from chilli is sprayed.

  - the oleoresin from capsicum is used in therapeutic hot plasters to increase the blood circulation in the skin, to treat sprains or rheumatism. Unfortunately it is also misused, to dope horses in equestrian sport, by making the front legs of a show jumper more sensitive to pain, so that when jumping the horse anxiously lifts its legs as high as possible.
- Our intention is to supplement the above article with the practical aspect, how the most pungent substance known, capsaicin, can be isolated in a pure state and how its structure can be confirmed, using analytical methods.


Fig. 1.3-4 Particularly hot: Red habaneros

The "pungent essence" of paprika was first isolated in an impure form in 1816 by Buchholz [3]. He gave his substance the name capsicin after the genus *Capsicum*. The name used today, capsaicin, was given 60 years later by Tresh, who isolated it in an almost pure state [4a, 4b]. Micko obtained the first pure capsaicin in 1898 [5a, 5b]. In 1919, Nelson elucidated the chemical structure [6]. The first of many total syntheses was published in

1930 [7]. For this natural product also, more than a century lies between the first isolation and the still classic, not spectroscopic structure elucidation.

How can capsaicin be isolated from chillies (Fig. 1.3-5)? Firstly, using acetone an oleoresin is extracted. From this in a partition chromatographic process with 90% ethanol and *n*-hexane the capsaicinoid fraction is transferred to the ethanol fraction. The skilful part is now to separate the very similar compounds capsaicin and dihydrocapsaicin. To do this, the only structural difference, the double bond in capsaicin, is utilized. With this capsaicin can form a Ag-π-complex but dihydrocapsaicin cannot. On a silica gel column that is massively impregnated with $AgNO_3$, capsaicin is eluted after dihydrocapsaicin, which is not "retarded" by $Ag^+$.

Now our knowledge about the lipophilic properties of capsaicin can be of everyday use. Since capsaicin is only very slightly polar, it is not possible to rinse the taste of "hot" food out of the mouth with water, lemonade or tea. Partially lipophilic drinks like an emulsion such as milk can help but it must be a fat containing whole milk or even better cream. A spoonful of olive oil is effective, if you can stand it, or hard liquor, since alcohol dissolves capsaicin, which is used in a step in the isolation.

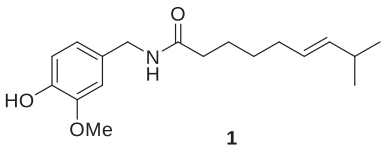
In the beginning was the spice. Since the Romans in the course of their travels and wars were the first to enjoy the burning or numbing, the biting or exhilarating ingredients of the orient, the occident can and will not miss the "especeria", the Indian spices, in the kitchen and cellar. Then until well into the Middle Ages, Nordic food remained unbelievably insipid and dreary. It will take a long time, until the most common crops used today, such as potatoes, maize and tomatoes, are regarded as domestic products in Europe. Still, the lemon is hardly used to acidify or sugar to sweeten, still the fine stimulants of coffee and tea not discovered. Even the nobility and the distinguished conceal the spiritless monotony of meals by stupefying gluttony. But oh wonder: simply a single grain of Indian spice, a few specks of pepper, a dried blossom of nutmeg, a pinch of ginger or cinnamon mixed into even the most coarse of dishes and already the favoured palate senses the foreign, delicious exciting appeal. Between the blatant major and minor of sour and sweet, of pungent and flat come the vibrations of exquisite culinary overtones and intermediary tones. Very soon, the barbaric sense of taste of the Middle Ages cannot get enough of these new excitations. A dish is first regarded as being perfect, when it is totally over-peppered and crassly seasoned. Ginger is even thrown into beer and wine is heated with powdered spices, until each mouthful burns like gunpowder in the throat.

Stefan Zweig (1881–1942) Magellan, the Man and his Deed, Navigare necesse est

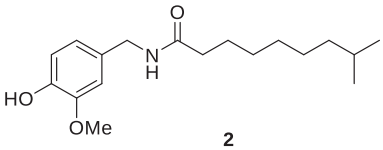

Fig. 1.3-5 "African Bird's Eye Chilies" used for the extraction

# 2. Isolation

## 2.1 Principle




**1**




**2**

Fig. 1.3-6 The isolated capsaicinoids: capsaicin (1) and dihydrocapsaicin (2).

The isolation starts from dried chillies and has several steps. Firstly, the hydrophobic substances such as dyes and compounds of medium polarity, to which the capsaicinoids belong, are extracted with acetone. Saccharides and glycosides do not dissolve. After the removal of the acetone a red oleoresin remains, from which the strongly hydrophobic substances are removed with *n*-hexane by partition with 90% ethanol and *n*-hexane in a separating funnel. The orange, resinous residue that remains after the removal of ethanol is subjected to column chromatography over silica gel using diethyl ether as the eluent, to obtain the capsaicinoid fraction, which contains **1** and **2**. This can be crystallised on a multiple gram scale.

The skilful part follows. This involves separating the two substances, which are exceedingly similar in their properties, by column chromatography over silica gel that has been richly impregnated with silver nitrate. This artifice makes use of the only difference available, i.e. the double bond. Capsaicin can form complexes but dihydrocapsaicin cannot. The principle of the separation is based on the formation of a π-complex between the silver ions and the double bond of the unsaturated fatty acid residue of capsaicin **1**, whereby the retention time is increased in comparison to dihydrocapsaicin **2**, thus facilitating chromatographic separation. The procedure is taken from the separation of olefinic compounds, such as unsaturated fatty acids.

Using trichloromethane as the eluent the separation is very slow and the DC-$R_f$-values are small ($R_f$(**1**) = 0.025; $R_f$(**2**) = 0.065) but this is advantageous for the separation. Silver nitrate is also practically insoluble in this solvent and is not eluted. Acetone for example would lead to a faster elution but would mobilize the silver nitrate, which is not desired. In this way both **1** and **2** can be isolated and obtained in a pure form suitable for spectroscopy after crystallisation. An X-ray crystallographic analysis was also conducted on capsaicin **1**. The inspiration for this work came mainly from a patent: S. Kato, S. Murasugi, H. Segi, S. Yamada, Alps Pharmaceutical Ind. Co. Ltd.: EP0891966 B1, **1997**.

The dried chillies used are commercially available. Sundried "African Bird's Eye Chilies" (*C. frutescens*) without stalk from Kenyan production, traded by the firm *Equator Kenya Ltd*. and imported by *Oriental Merchant*, are used.

Cayenne, the capital of French Guiana (with 900 houses and 1500 inhabitants). The island is situated near South America, is in part mountainous, where habitable it is swampy and therefore unhealthy and was used earlier as a penal colony. Cayenne pepper, an extremely fiery spice, made from the husk or seeds of the so-called Spanish pepper, gets its name from it. The seeds are removed from the pod of Capsicum baccatum (which amongst other members of the genus are cultivated in our garden and grow wild in Hungary) and ground to a fine powder, as are the husks after drying. The powder is kneaded with wheat flour and yeast and baked. The cake is powdered again and stored in well-sealed glass jars protected from sunlight and humidity, as both reduce its strength. Cayenne pepper is used in large amounts in America. For our taste, it is a so pungent spice, that a grain of it placed on the tongue causes a day long, initially almost unbearable, burning. Hungarians eat the entire pod with vinegar, oil and pepper, just as we eat cucumber salad.

From: The Lady's Conversation Lexicon, Volume 2, Leipzig 1834

## 2.2 Method

Safety Instruction:

All capsaicinoids are strong irritants. Therefore, gloves should always be worn when working with these compounds, to avoid contact with the skin. Injured or already damaged skin, mucous membrane and the eyes are particularly sensitive. Furthermore, the inhalation of dust or aerosols must be

avoided, because a strong irritation of the respiratory tract is to be expected, leading to coughing and sneezing. All procedures should be carried out in a fume cupboard.

Dried chilli fruits (1 kg; *Capsicum frutescens, "African Bird's Eye Chilies"*) are shredded and then extracted three times with acetone (2 L for each extraction) for 15 minutes at 50°C with continuous rotation of the flask. The extracts are united and the solvent removed under reduced pressure. A red-orange oleoresin (69.5 g) is obtained. This is dissolved in a mixture of 90% ethanol (300 mL) and *n*-hexane (300 mL) and intensively shaken in a separating funnel. The hexane phase is separated and discarded. The ethanol phase is extracted again with *n*-hexane (300 mL) and the hexane phase again discarded. The solvent is removed under reduced pressure from the ethanol phase, whereby an orange, oily-resinous residue (17.4 g) remains.

The residue is dissolved in diethyl ether (50 mL) and subjected to column chromatography over silica gel. Diethyl ether is used as the eluent. Each fraction is investigated by TLC. The $R_f$-value with diethyl ether as eluent is 0,5.

A total of 27 fractions (each 20 mL) are united. The diethyl ether is removed under reduced pressure to yield a yellow-orange oil (7.7 g). This is dissolved in diethyl ether (200 mL) and powdered activated carbon is added stepwise, the solution being filtered under gravity intermittently, to determine the degree of discolouration. The addition of activated carbon (7.5 g) is continued until the solution is almost completely colourless. After removal of the diethyl ether under reduced pressure, the capsaicinoid mixture (6.70 g) remains as a resinous, yellow oil, which according to the $^1$H NMR spectrum contains capsaicin and dihydrocapsaicin in the ratio 60:40.

To initiate crystallisation, the resinous, yellow oil is dissolved at room temperature in 2-propanol/*n*-hexane (100 mL; 15:85 v/v). In the deep-freeze overnight the capsaicinoids crystallize out (3.14 g) with a ratio of **1:2** of 70:30 according to the $^1$H NMR spectrum and a mp of 65-66°C. After the removal of solvent, a second crystal fraction (880 mg) is obtained with a ratio of **1:2** of 50:50.

Total yield: 3.92 g

## 2.3 Purification

Analytical thin layer chromatography (TLC) is carried out on Merck DC silica gel 60 $F_{254}$ plates. For detection a UV-lamp with wavelength 254 nm is used or the capsaicin spot is coloured by spraying with a solution of 2,6-dichloroquinone-4-chloroimide (0.5 g 2,6-dichloroquinone-4-chloroimide in 100 mL ethanol) and developed in ammonia vapour in a glass chamber, whereby the capsaicin spot is coloured an intense blue.

As a structurally related reference substance commercially available Nonivamide (pseudocapsaicin, Boehringer Ingelheim) is used.

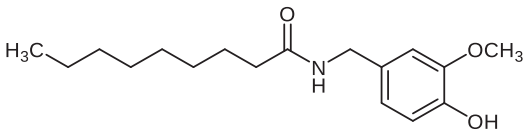



Fig. 1.3-7 Structure of nonivamide (pseudocapsaicin)

For the $AgNO_3$ assisted column chromatography flash-silica gel of the type Geduran (40–63 µm) from the firm Merck is used. To impregnate the silica gel for the separation of the capsaicinoids, silver nitrate (21.70 g) is dissolved in deionised water (250 mL) and mixed with flash-silica gel (123 g). Most of the water is removed under reduced pressure at 60°C. The pre-dried silica gel is dried further overnight under vacuum (oil pump) and then used in the usual way for chromatography.

Capsaicin (**1**)

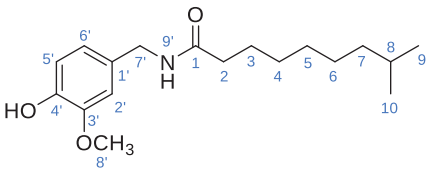
A slurry of the prepared gel (vide supra) in trichloromethane is prepared and filled into the chromatography column (400 mm×35 mm). A portion of the first yield of capsaicinoid crystals (1.004 g) is dissolved in trichloromethane (5 mL) and applied to the column. After an initial elution of trichloromethane (3 L) the collection of a total of 100 fractions containing capsaicinoids (each 20 mL) begins. The investigation of each fraction with TLC is carried out on silica gel plates impregnated with silver nitrate. These are prepared from commercially obtainable silica gel plates for TLC by dosing the plates uniformly with a 50% aqueous solution of silver nitrate from a pipette. The plates are dried at 80°C in an oven. The $R_f$ value for capsaicin **1** is 0.025 and for dihydrocapsaicin **2** 0.065 with trichloromethane as the eluent. According to TLC the fractions 60-100 contain capsaicin. These fractions are united and the solvent is removed under reduced pressure. A pale yellow oil (702 mg) remains. To initiate crystallisation this is dissolved at room temperature in 2-propanol/*n*-hexane (15 mL; 15:85 v/v) and placed in the deep-freeze in a closed container. Pale yellow crystals (403 mg) with a mp of 68-69°C are obtained. The product contains traces of 2-propanol and an olefinic impurity at 6.16 ppm ($^1$H NMR).

To obtain entirely colourless crystals, capsaicin is recrystallized. Capsaicin (200 mg) is dissolved in methyl *tert*-butyl ether (1.5 mL) and *n*-pentane (0.7 mL) is carefully added. For crystallisation the solution is placed in a closed container in the deep-freeze. After filtration pure capsaicin **1** (176 mg) is obtained.

Mp: 68-69°C, Literature value 64-65°C in P. M. Gannett, D. L. Nagel, P. J. Reilly, T. Lawson, J. Sharpe, B. Toth, *J. Org. Chem.* **1988**, *53*, 1064–1071.

Dihydrocapsaicin (**2**)

The fractions 36 to 46 are particularly rich in dihydrocapsaicin. These fractions are united and the solvent removed under reduced pressure to yield a pale yellow oil (147 mg).




**2**

Fig. 1.3-8 Numbered structure of dihydrocapsaicin (**2**)

To initiate crystallisation the oil (120 mg) is dissolved at room temperature in 2-propanol/*n*-hexane (5 mL; 15:85 v/v) and placed in the deep-freeze in a closed container. Pale yellow crystals (76 mg) with a mp of 59-60°C (literature value 63-64°C ibid) are obtained.

The NMR spectra of dihydrocapsaicin are given in the *supporting information.*

## 3. Spectra and Comments

### UV Spectrum in Ethanol

Paprika. – As a spice, only the ground whole fruit is used. However, recently a powder, which is recommended as an additive for minced meat and is made after removal of the placenta, has appeared. It seems to be tasteless and can therefore be regarded to be a colourant, which is forbidden by the directive on meat inspection. In powdered form, P. is likely to be subject to adulteration. The addition of flour, bran, sandalwood, powdered brick, ochre and other minerals and artificial colourants have been observed, even samples that have been totally extracted with alcohol are traded.

From "Merck's Warenlexikon für Handel, Industrie und Gewerbe", 7. edition. Publ. by Adolf Beythien and Ernst Dressler. Gloeckner, Leipzig 1920

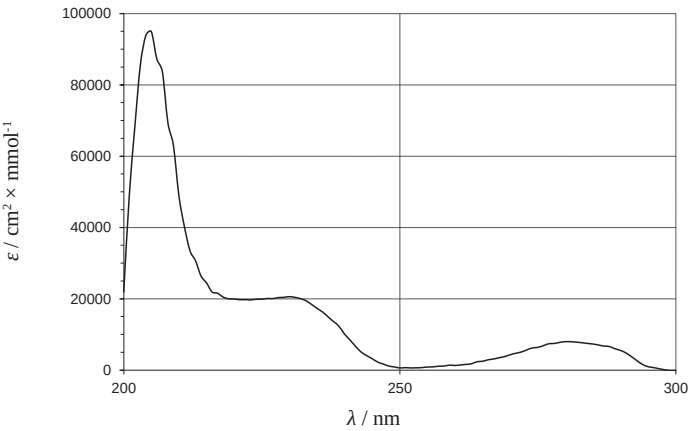



Fig. 1.3-9 UV spectrum of capsaicin

Capsaicin contains three not conjugated chromophores, the amide group, the double bond between C-6 and C-7 and the aromatic ring substituted with two auxochromes. For the aromatic residue a $\pi \rightarrow \pi^*$-transition that is quite typical for this structure element (α-band of the phenyl ring [8, 9]) appears at 280 nm with a value for $\varepsilon$ of 8000 $cm^2 \times mmol^{-1}$. The other absorptions are difficult to assign individually but are unusually intense (Fig. 1.3-9)

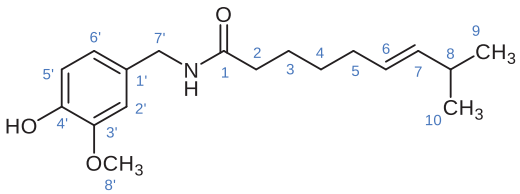




Fig. 1.3-10 Products from chilli: Diverse hot sauces, repellent spray, Finalgon® heat ointment, Habanero sauce "Reudnitz Scorpion"

## IR Spectrum in KBr

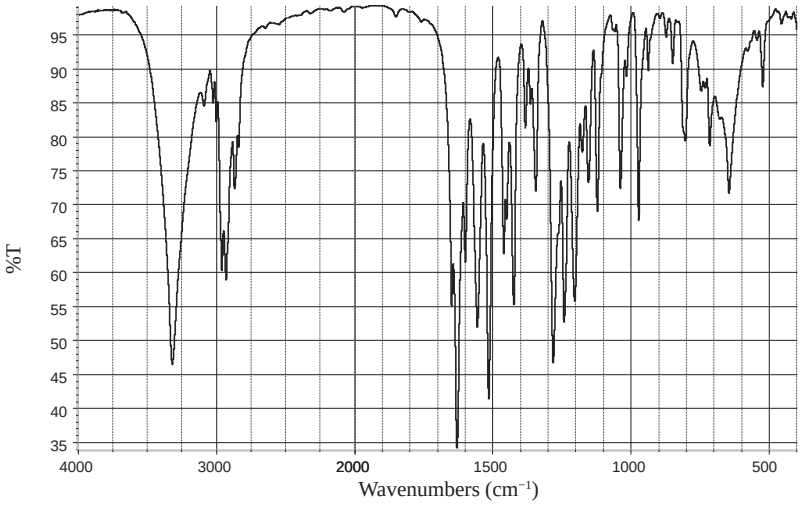



Fig. 1.3-11 IR spectrum of capsaicin

## 700 MHz NMR Spectra in $CDCl_3$

### $^1$H NMR Spectrum

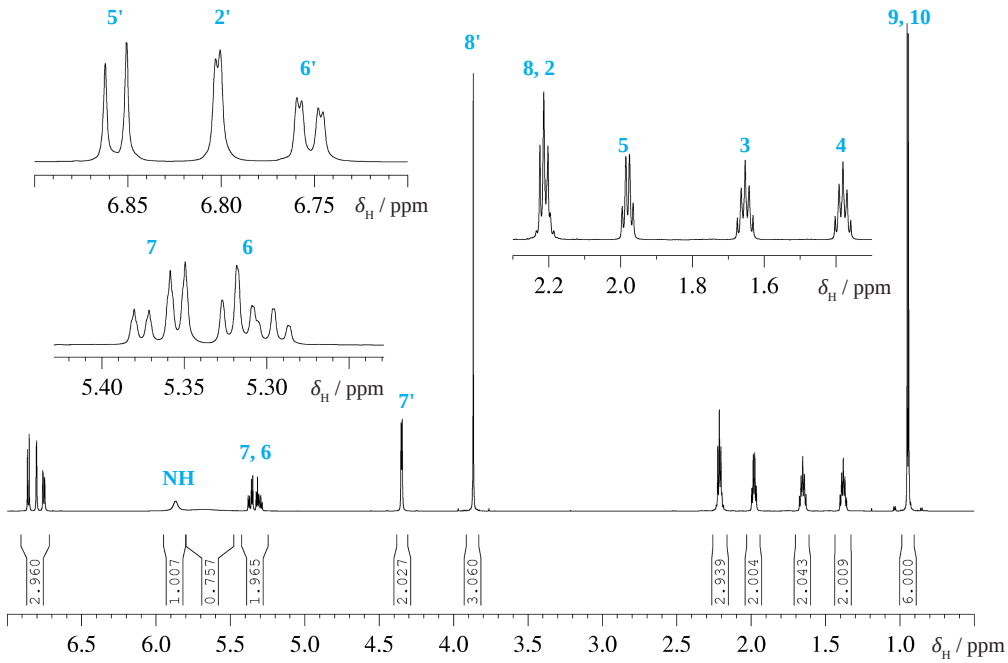



Fig. 1.3-12 $^1$H NMR spectrum of capsaicin

The $^1$H NMR spectrum of capsaicin can easily be interpreted (Fig. 1.3-12). Firstly, at $\delta_H$ = 6.8 the typical pattern of a 1,2,4-substituted aromatic compound is found [10, 11]. The exchange broadened NH-proton appears at $\delta_H$ = 5.9 followed by the resonances of the H-atoms 6 and 7, the individual assignment of which is immediately apparent from the coupling pattern, since H-6 couples with the two H-5 protons but H-7 couples with only one methine proton H-8. The signal of this methine proton at $\delta_H$ = 2.2 overlaps with the signal of the methylene group 2. The order of the remaining aliphatic resonances can be taken from the COSY spectrum (Fig. 1.3-15).


Fig. 1.3-13 Mediterranean verve: Viagra peperoncini on the Gulf of Naples

## APT $^{13}$C NMR Spectrum

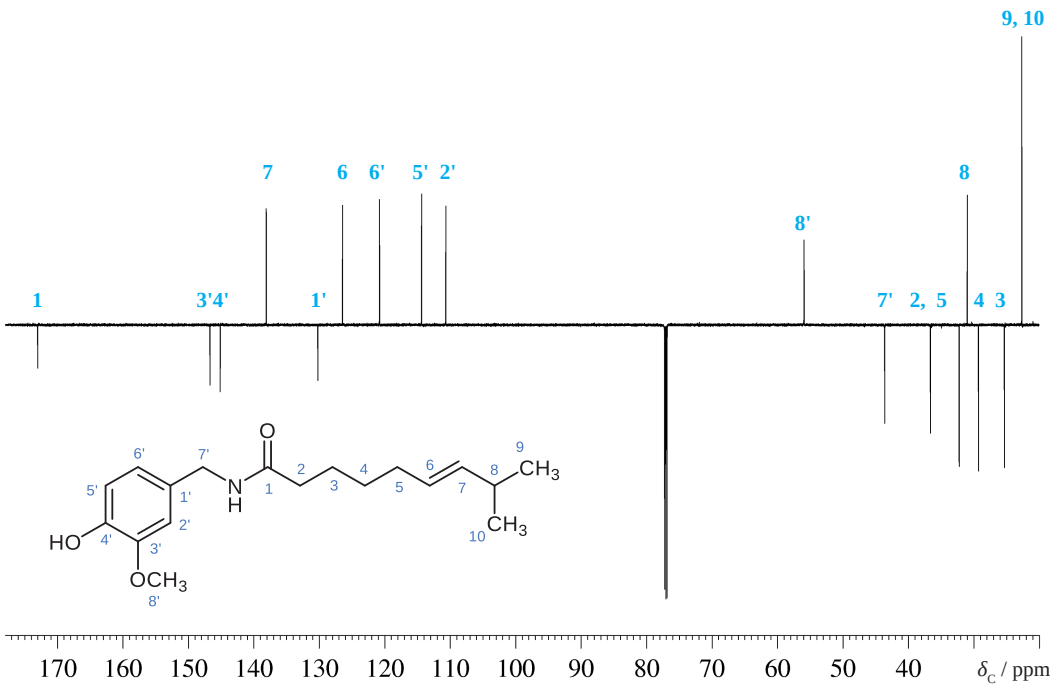



Fig. 1.3-14 APT $^{13}$C NMR spectrum of capsaicin

**COSY Spectrum**

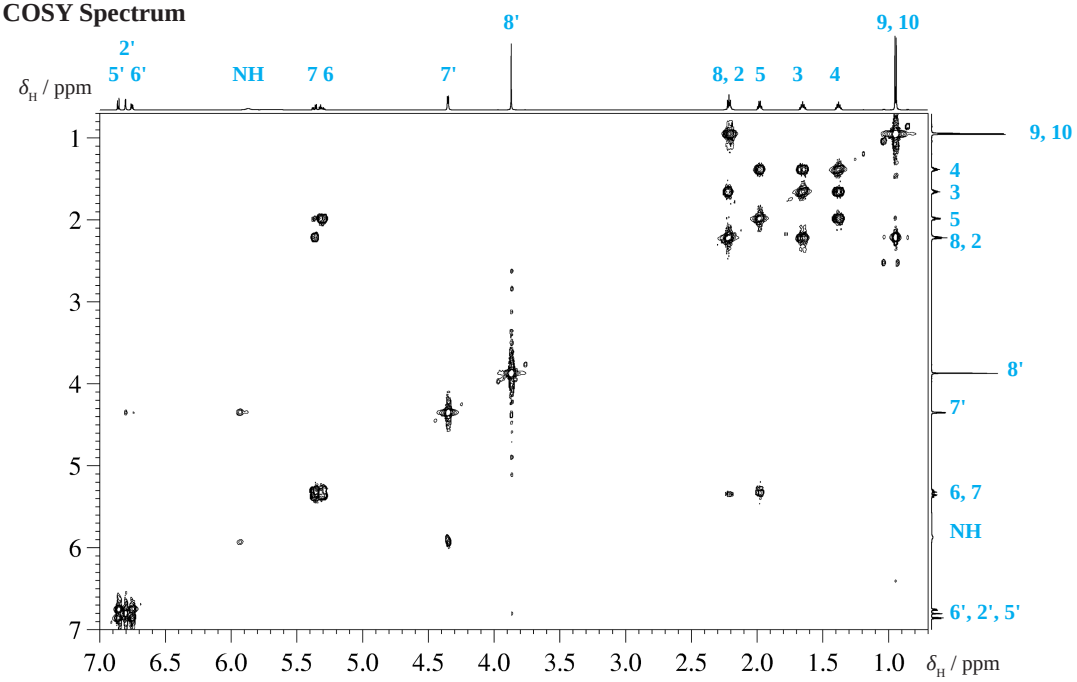



Fig. 1.3-15 COSY spectrum of capsaicin

**NOESY Spectrum**

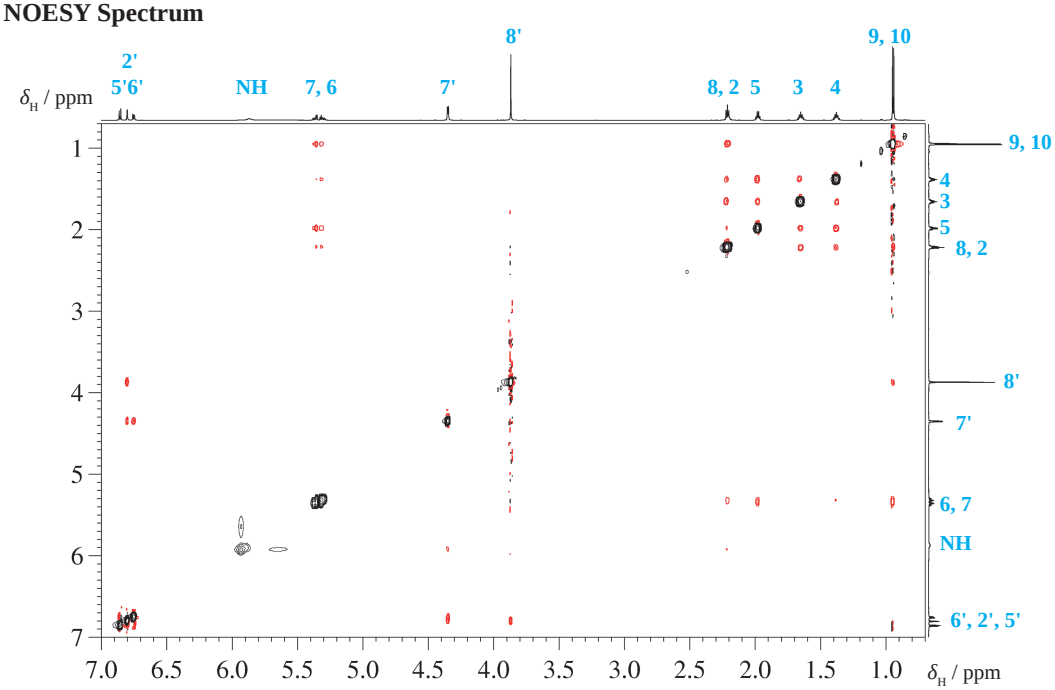



Fig. 1.3-16 Excerpt of the NOESY spectrum

The NOESY spectrum demonstrates very clearly the exchange of the NH-proton with the strongly broadened signal of the OH-group on C-4'. A cross peak between H-8' and H9/10 indicates the spacial nearness of these protons, as is seen in the analysis of the crystal structure and the quantum chemical calculation.

**HSQC Spectrum**

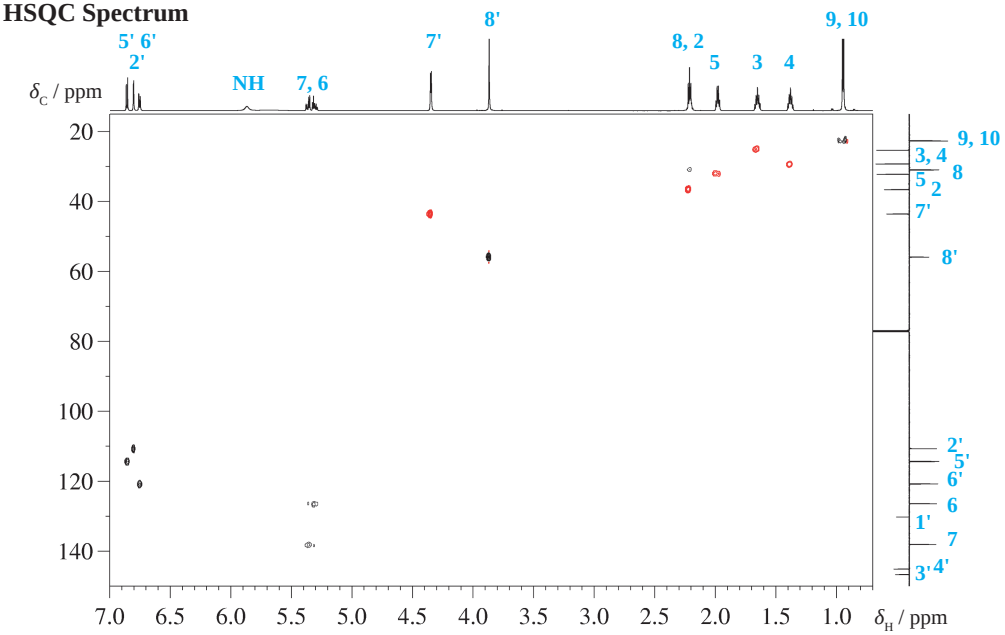



Fig. 1.3-17 HSQC spectrum of capsaicin

Since with the help of the COSY spectrum (Fig. 1.3-15) the $^1$H NMR spectrum of capsaicin could be completely assigned, the allocation of all proton-bearing C-signals is easily accomplished with the HSQC spectrum (Fig. 1.3-17). Capsaicin has only 4 quaternary C-atoms. The assignment of these is confirmed as usual by the HMBC spectrum.

**HMBC Spectrum**

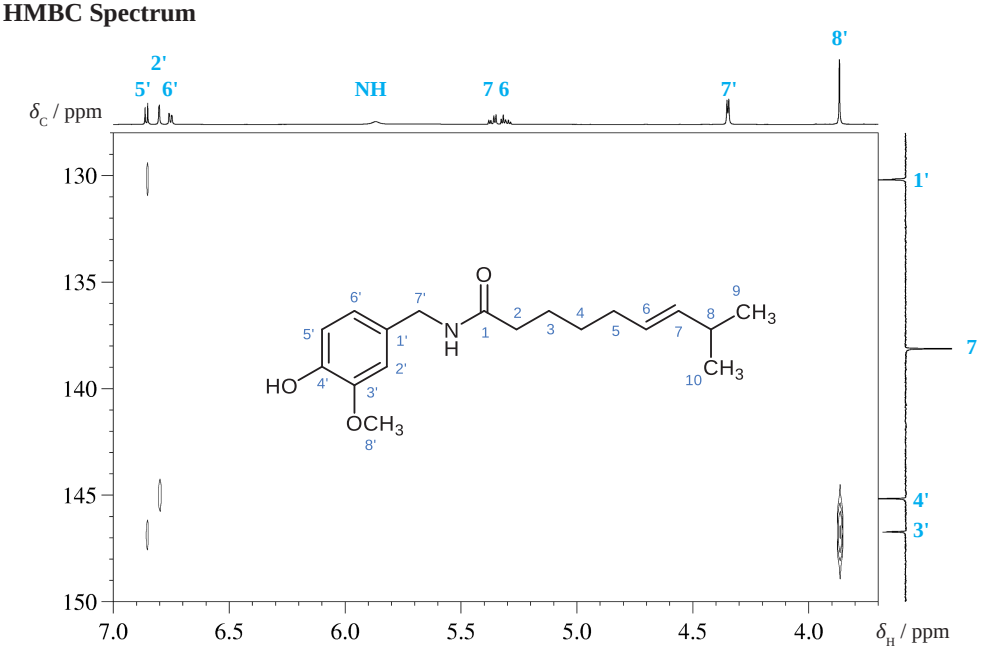



Fig. 1.3-18 Excerpt 1 from the HMBC spectrum of capsaicin

While the assignment of C-1 at $\delta_C$ = 173.1 requires no further confirmation, it can be seen in Fig.1.3-18 that the methoxy group H-8' couples with the signal at $\delta_C$ = 146.7. This is therefore allocated to C-3'. The other aromatic C-atom connected to an oxygen containing functional group at $\delta_C$ = 145.2 couples over three bonds to H-2' and can therefore be assigned to C-4'. The signal at $\delta_C$ = 130.2 remains for the assignment to C-1' and this signal is seen over three bonds by H-5'.

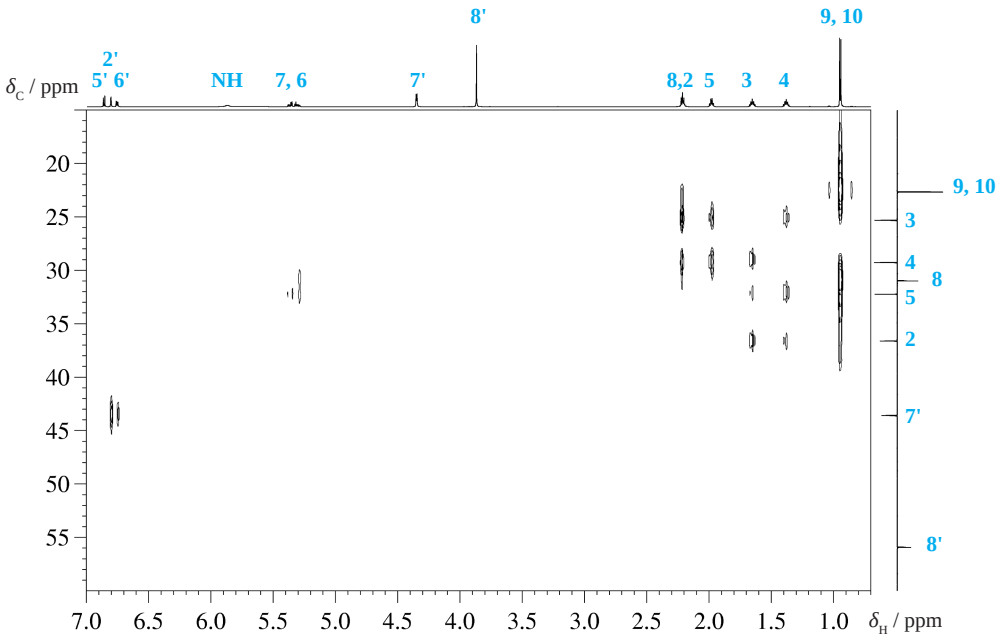



Fig. 1.3-19 Excerpt 2 of the HMBC spectrum of capsaicin

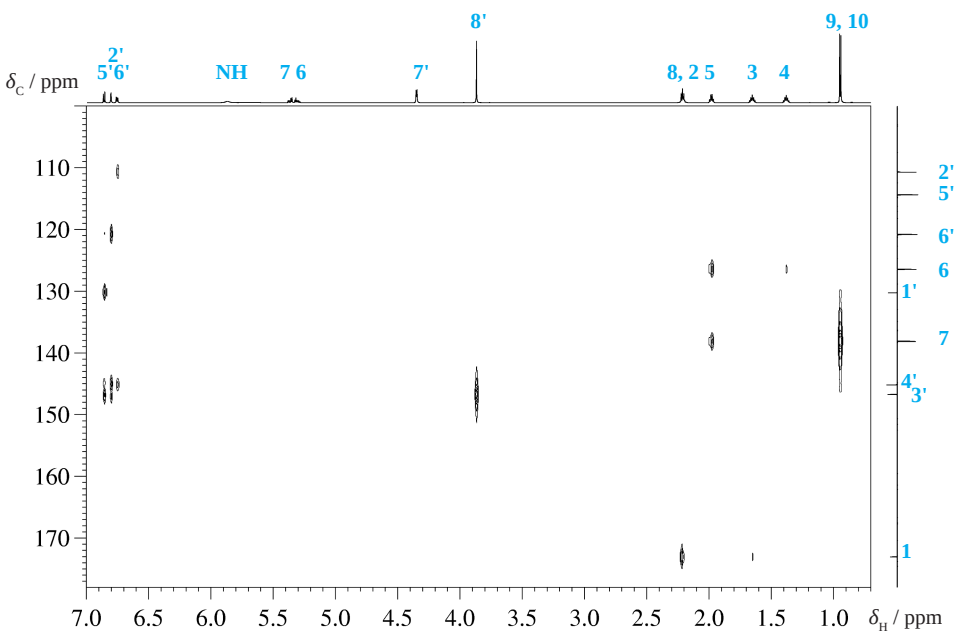



Fig. 1.3-20 Excerpt 3 of the HMBC spectrum of capsaicin

**Hot-Cold Chocolate**

100 g plain chocolate, 30 mL sunflower oil, ½ tsp chilli powder
Melt the chocolate at 45°C, mix it with sunflower oil that is as neutral to taste as possible and very finely ground chilli powder. Allow it to solidify in small portions and cool them to -18°C in the deep-freeze. On savouring the ice-cold chocolate, the chilli powder produces of very pleasant cold-warm/hot feeling in the mouth.

From T. A. Vierich, T. A. Vilges "Aroma, the Art of Seasoning", Stiftung Warentest Berlin 2013

Fig. 1.3-21 Nepalese cuisine – vegetarian but fiery

## X-Ray Analysis

Capsaicin crystallizes as monoclinical crystals with the space group $P2_1/c$ [12]. The nine-carbon chain of the nonenoic acid clearly shows an (*E*)-configuration. This is also shown in Fig. 1.3-22. The torsional angle between the atoms C6-C7-C8-C9 is 3.3°. The bond length of C6-C7 of 1.319 Å confirms the double bond character. An *intra*molecular hydrogen bond exists between the atoms O2-H2o-O3 (Fig. 1.3-23). The bond parameters are O3-H2o 0.89 Å; O2-H2o 2.21 Å; O2-O3 2.677 Å; O2-H2o-O3 112.2°. In the amide group of the molecule the C1-O1-bond with 1.240 Å has double bond character. This also applies to the bond length C1-N1 of 1.329 Å, this value is near the ideal C-N double bond of 1.29 Å (C-N single bond 1.49 Å).

For the crystal structure, two further *inter*molecular hydrogen bonds are important [8]. The amide hydrogen N1-H1n hydrogen bonds to the O1-atom with the parameters N1-H1n 0.85 Å; N1-O1 2.13 Å; N1-O1 2.980 Å; N1-H1N-O1 176.°, additionally the oxygen atom O2 participates in a second hydrogen bond: O2-H2o 0.89 Å; O1-H2o 1.93 Å; O2-O1 2.760 Å; O2-H2o-O1 156°.

The first hydrogen bond is realized through the c-glide plane of the space group $P2_1/c$, the second hydrogen bond through the screw axis $2_1$. The cell parameters agree with literature values [13].

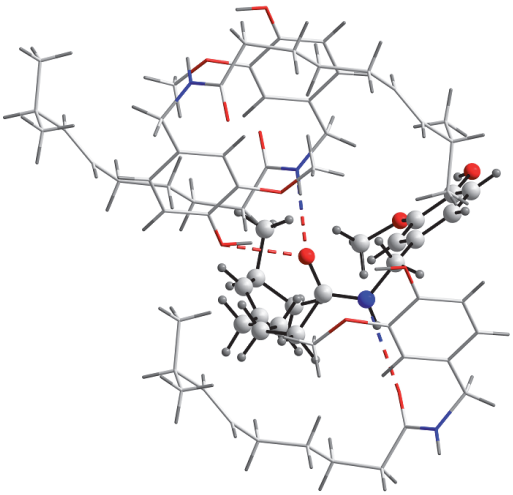


Fig. 1.3-22 Intermolecular hydrogen bonding of capsaicin (red: oxygen, blue: nitrogen shown by the xray crystallographic structure of capsaicin)

## Quantum Chemical Calculation

The isolated structure of capsaicin calculated with the hybrid DFT functional B3LYP and a triple ζ-basis set (TZVP) shows overall good agreement with the data for the crystal structure of capsaicin obtained experimentally (Fig. 1.3-23).

The average deviation of the bond length is 0.076 Å. The bond angle shows an average deviation of 1.7°. The torsional angle between the *trans*-configured C-atoms of the double bond is 1.8°, the bond length of the double bond is 1.33 Å. The calculated parameters of the bonds of the phenolic group and the methoxy group (O-H 0.98 Å; H- - -O-$CH_3$ 2.09 Å; O-O 2.65 Å; O-H-O 114.4°) confirm the hydrogen bonding found experimentally for the crystal. The resonance of the amide function with the C=O double bond (1.22 Å), the short C-N bond (1.36 Å) and the planar geometry (torsional angle O-C-N-C 1.6°) is also confirmed by calculation. The torsional angles calculated for an isolated capsaicin structure deviate on average by 7.6° from the values obtained experimentally for the crystal. The maximum deviation is -54° for the dihedral angle H(C7', syn to C=O)-C7'-C1'-C6'. Rotations around C-C single bonds have low energy barriers ($C^2$-$C^3$-bond in *n*-butane ~ 20 KJ×$mol^{-1}$). Different conformations in the isolated structure and the crystal can be caused by packing effects in the solid state and intramolecular interactions such as intramolecular hydrogen bonding.

The NMR chemical shifts were calculated with the DFT functional HCTH407 using the cc-pVTZ basis set and simulation of methanol as solvent for the B3LYP/TZVP-optimized structure of capsaicin. Different conformers were not considered and the calculated $^{13}C$ NMR chemical shifts were not scaled. Deviations of the order of 3-4 ppm, for individual positions even up to 9 ppm, in comparison to the experimental data are to be expected, since in solution an equilibrium between conformers exists that leads to an energy weighted average of the chemical shifts of all relevant conformations.

The empirically estimated shifts which are based on data measured experimentally in solution, therefore show smaller deviations from the experimental shifts than the quantum chemically calculated NMR shifts for a single conformation of the capsaicin molecule.

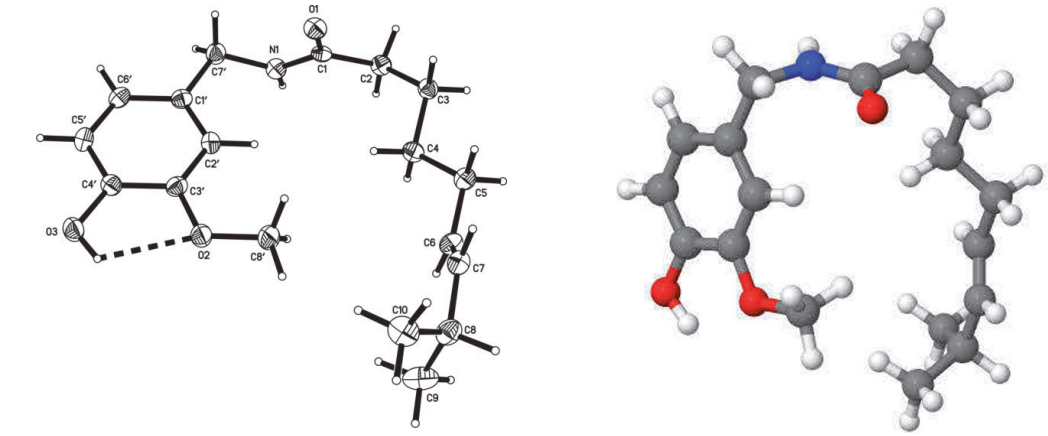
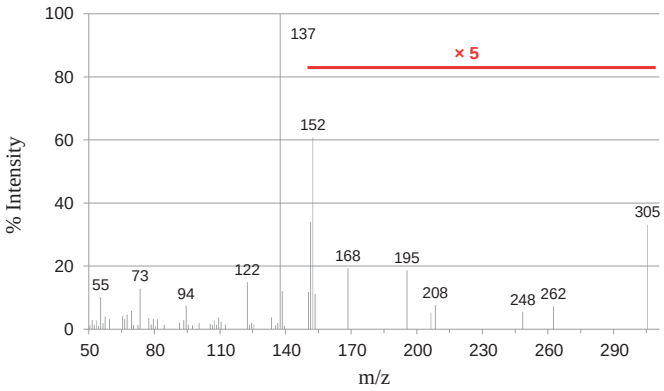



Fig. 1.3-23 Comparison of the crystal structure and the 3D structure of capsaicin calculated with *ab initio* methods

## Assignment Table

| $^{13}$C-NMR signal $\delta$ [ppm] | Type of C-atom | Assign-ment | $^{1}$H-NMR signal $\delta$ [ppm], $J$ [Hz] | Proof (HMBC coupling from proton to C-atom) | Proof (NOE from proton to proton) | $^{13}$C-NMR signal pre-dicted by ChemBio-Draw® | $^{13}$C-NMR chemi-cal shifts calculated with B3LYP/TZVP// HCTH407/cc-pVTZ (pcm, solvent $CH_3OH$)) |
|---|---|---|---|---|---|---|---|
| 173.1 | $C_q$ | C-1 | | H-7', H-2, H-3 | | 172.9 | 169.5 |
| 146.7 | $C_q$ | C-3' | | H-5', H-2', H-8' | | 147.3 | 147.5 |
| 145.2 | $C_q$ | C-4' | | H-5', H-2', H-6' | | 146.7 | 146.5 |
| 138.1 | CH | C-7 | 5.37 $J_{76}$ = 15.3, $J_{78}$ = 6.27 | H-5, H-8, H-9 | H-9,10, H-5 | 139.6 | 138.5 |
| 130.2 | $C_q$ | C-1' | | H-5', H-7' | | 130.9 | 134.1 |
| 126.5 | CH | C-6 | 5.31 $J_{67}$ = 15.3, $J_{65}$ = 6.46 | H-5, H-8, H-4 | H-9,10, H-5 | 129.3 | 129.7 |
| 120.8 | CH | C-6' | 6.75 $J_{6'5'}$ = 8.03 | H-5', H-2', H-7' | H-7' | 123.2 | 120.0 |
| 114.4 | CH | C-5' | 6.86 $J_{5'6'}$ = 8.03 | | | 115.4 | 110.3 |
| 110.7 | CH | C-2' | 6.80 $J_{2'6'}$ = 1.71 | H-2', H-7' | H-7', H-8' | 109.6 | 110.0 |
| 56.0 | $CH_3$ | C-8' | 3.87 | | H-2' | 56.1 | 56.9 |
| 43.6 | $CH_2$ | C-7' | 4.35 $J_{7'NH}$ = 5.20 | H-2', H-6' | H-2', NH | 43.9 | 47.7 |
| 36.6 | $CH_2$ | C-2 | 2.22 | H-4, H-3 | H-4, H-3 | 36.6 | 39.9 |
| 32.2 | $CH_2$ | C-5 | 1.98 | H-6, H-7 | H-4, H-3 | 33.3 | 41.4 |
| 31.0 | CH | C-8 | 2.22 | H-9,10 | H-9,10 | 31.7 | 37.6 |
| 29.3 | $CH_2$ | C-4 | 1.38 | H-5, H-2, H-3 | H-5, H-2, H-3 | 28.9 | 36.2 |
| 25.3 | $CH_2$ | C-3 | 1.65 | H-5, H-2, H-4 | H-5, H-2, H-4 | 27.7 | 32.0 |
| 22.7 | $CH_3$ | C-9,10 | 0.95 $J_{98}$ = 6.83 | H-9,10 | H-8 | 22.6 | 22.4 |
| | NH | | 5.87 | | | | |
| | OH | | 5.68 | | | | |

## EI Mass Spectrum



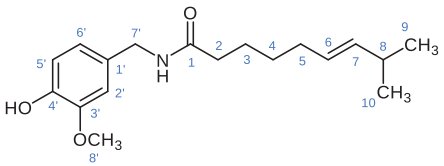



Fig. 1.3-24 EI mass spectrum of capsaicin, amplified by a factor of 5 starting from m/z 150

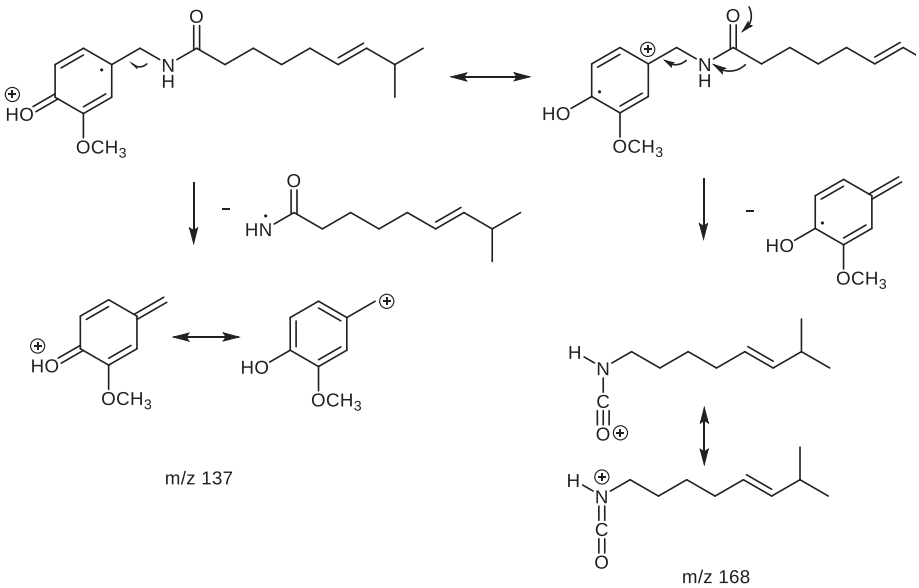



Fig. 1.3-25 Benzyl cleavage in the molecular ion of capsaicin

The EI mass spectrum of capsaicin (Fig. 1.3-24) is dominated by the cleavage of the benzylic bond to give the ion with m/z 137 (Fig. 1.3-25). The breaking of the bond can in part occur with the migration of the charge to the amide fragment. We exclude the possibility, that the formation of the ion with m/z 168 can be explained by an acyl nitrenium ion. This energetically unfavourable species with a sextet on the N-atom can be avoided by a migration of the alkyl residue to the N-atom synchronous to the benzylic cleavage.

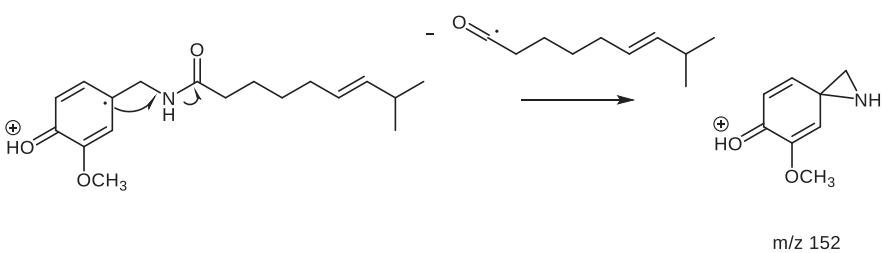



Fig. 1.3-26 Breaking of the amide bond in the molecular ion of capsaicin

Apart from the benzylic bond, the neighbouring amide bond can also be broken. Here it can also be assumed that the ion formed at m/z 152 is not a nitrenium ion, but that with the participation of the neighbouring aryl residue a cation that is stabilized by mesomerism is formed (Fig. 1.3-26).

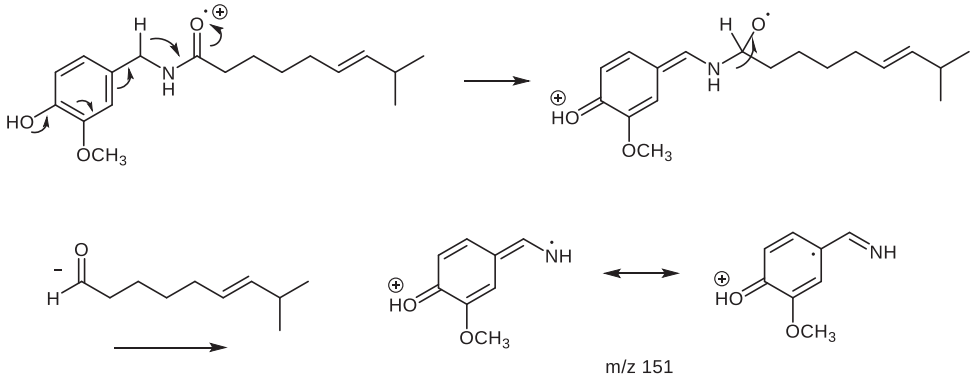



Fig. 1.3-27 Formation of the ion with m/z 151

The ion at m/z 151 cannot be formed by H-elimination from m/z 152 (even electron rule). For its formation, an intramolecular redox reaction that occurs before cleavage of the C-N-bond is suggested (Fig. 1.3-27). This presumes, that molecular ions exist that are ionized on the carbonyl group. The radical cation formed corresponds to an ionized aldimine.

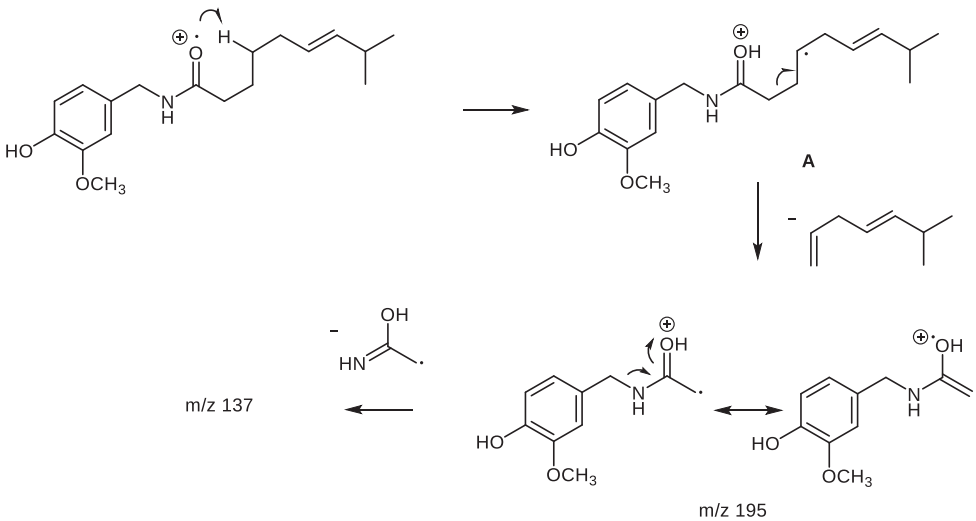



Fig. 1.3-28 McLafferty rearrangement in the molecular ion of capsaicin

The existence of an ion from a McLafferty rearrangement at m/z 195 is an indication for the presence of molecular ions that are ionized on the carbonyl group (Fig. 1.3-28). In principle, the ion from the McLafferty rearrangement (m/z 195) could contribute to the formation of the base peak at m/z 137. Further ions appearing in the EI mass spectrum are explained in the *supporting information*.

## 4. Questions

A. Why are the capsaicinoids not basic alkaloids?

B. Apart from capsicum species, such as chilli, pepper and ginger are regarded as further pungent spices. The question arises if there is possibly a common structural element in those components that are found to be pungent. This can actually be recognized, if the structures of capsaicin, piperine (from pepper), gingerol (from ginger) and shogaol (a product of dehydration of gingerol) are compared. Find the structures of the first three named substances in the literature and name the common feature.

C. What is to be understood by the term oleoresin? From what and how are oleoresins obtained and for what are they used?

D. The NOESY spectrum shows the diagonal signals in black and the NOE cross signals in red, to demonstrate the different phases. The exchange signals between the NH-group and the OH-group have the same phase as the diagonal signals. Why?

E. Of which named reaction in organic chemistry does the suggested explanation for the formation of the ion with m/z 137 in Fig. 1.3-25 remind you?

F. An ion with m/z 122 that can only be explained by the elimination of $CH_3^{\cdot}$ from m/z 137 can be found 15 a. m. u. below the signal with m/z 137. What is the problem?

## 5. Literature


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Fig. 1.3-29 Harissa – a chili paste from Tunisia

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Fig. 1.3-30 Making of a hot chili dish in a wok in Chongqing/ China