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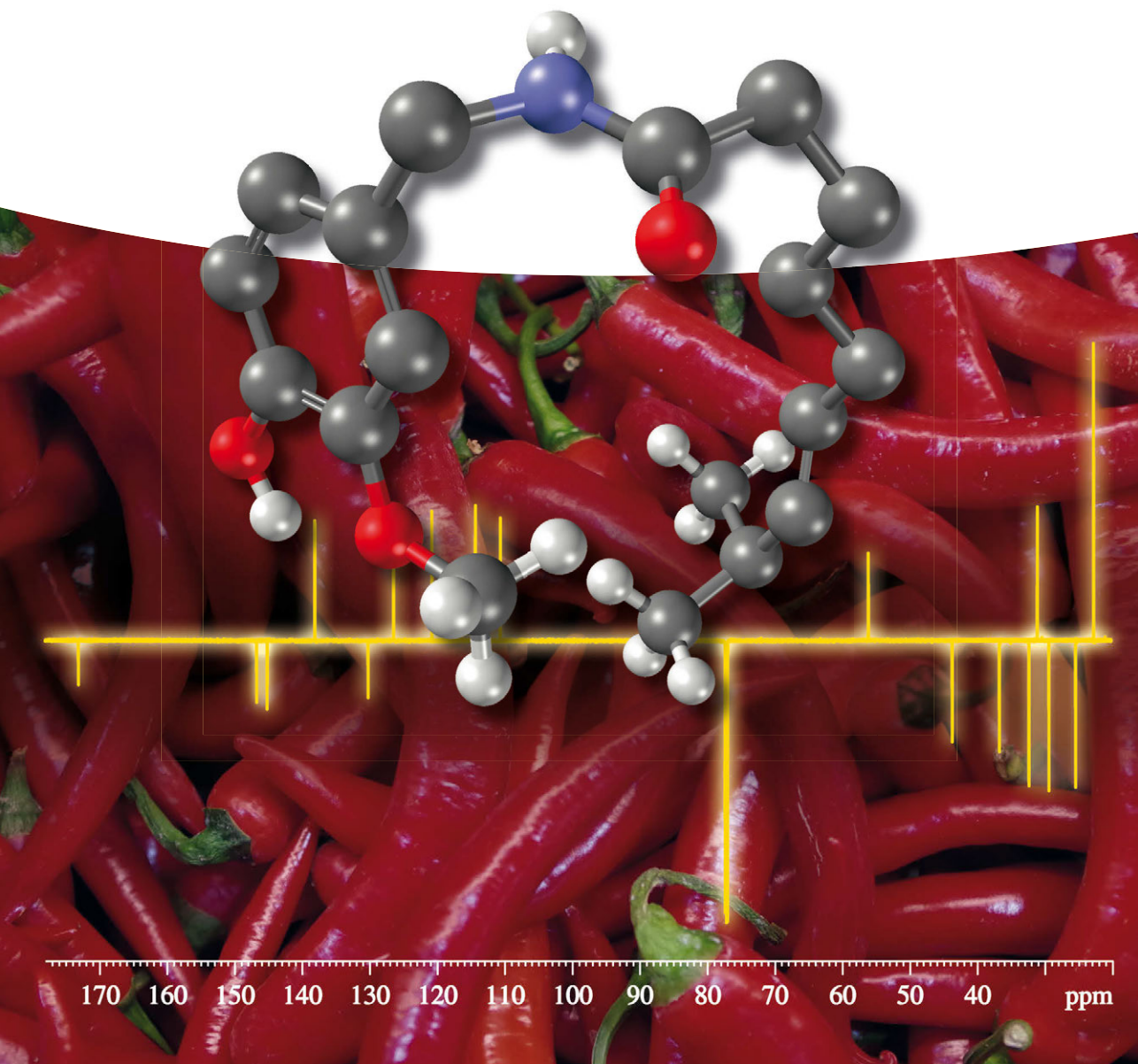
Isolation, Structure Elucidation, History



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Cover
The structure of capsaicin obtained through quantum chemical calculations and its ^{13}C NMR spectrum. The background picture depicts pepperoncini fruits containing capsaicin.

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Preface

Natural products – Isolation, Structure Elucidation, History

In 2009 we published a first book on this subject entitled "Classics in Spectroscopy − Isolation and Structural Elucidation of Natural Products". This volume originated from practical courses at our university in both organic and analytical chemistry, in which the authors were in charge of teaching students the appropriate techniques in their respective disciplines. It contained the description of 30 natural products based on bachelor dissertations and indepth studies from many of our students and was met with considerable interest by the chemical community. It even won the prize for the best chemical literature in 2009 from the German Fonds of the Chemical Industry.

After a time for reflection, we started an article series in the German educational journal "Chemie in unserer Zeit" in 2013, which again comprises 30 new natural products and which will run until the end of 2018. Since this journal only appears in German, we thought it advisable to provide a second book in English for an international readership and agreed with the publisher on a selection of 20 articles for the present volume.

For this series of articles and the new book, the team of authors has been enlarged. Prof. K.-P. Zeller considerably intensifies the interpretation of the mass spectra and Prof. H.-U. Siehl is responsible for the quantum chemical calculations of the structures and of the ^{13}C chemical shifts.

The structural analysis of natural products develops parallel to the increasing complexity of the research topics in this field. The arsenal of methods becomes broader and more efficient. However, the basis remains an understanding of the spectroscopic techniques that are applied to the compounds that have been isolated in pure form.

Structural diversity is again the aim of this book. Twenty natural products have been arranged in five sections, describing three alkaloids, five coloured compounds, three carbohydrates and glycosides, seven terpenoids, and two aromatic compounds. An important selection criterion was that every dedicated reader should have access to the source of the natural product. Each chapter consists of the following paragraphs:

1. Background provides the reader in a journalistic style with a certain amount of cultural history of the specific compound and its natural raw material. Often, astonishing links between different fields of life are manifested. Sometimes, personal experience of the authors has been added. Usually, we lead you from the discovery of the compound to its daily use. As in the first book, we regard these background stories as our particular feature that combines "chemistry" with "culture" in an enriching and inspiring way.

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

3. Spectra and Comments gives you, in as much detail as 20 pages per compound allows, the spectral result, always starting with the UV/vis-spectrum and, if appropriate the CD-spectrum. This is sometimes followed by the IR-spectrum. The main part consists of many different NMR-spectra that are discussed in considerable detail, followed by the quantum chemical calculation of the structure and the ^{13}C chemical shift. All NMR spectra can be inspected or downloaded from http://www.nmrshiftdb.org/collections/ChiuZ|Classics+in+Spectroscopy. Finally, the mass spectra come with extensive comments. The layout is arranged such that a numbered formula is always present close to the spectra and the pertinent text. Therefore, pages do not have to be turned and it is easy to follow the, in part, ambitious discussion. Owing to the space limit, we have decided to offer supplementary information in which additional material is discussed. This is arranged in a cloud file and can be reached by the reader via: https://www.zenodo.org/ or obtained via email from the authors.

4. Questions are sometimes rather intricate and will certainly demand a fair amount of deliberation by the reader. All these questions are answered in detail in the supplementary information.

5. Literature can of course not be an extensive or complete survey, since the literature on the compounds described in this book is enormous; sometimes more than 10.000 references exist. We have, where possible, cited the early, significant papers on the first isolation and structural elucidation of the compound, and then included some reviews on its importance. Finally, we cite some specialized and recent spectroscopic papers.

Red Margins. As in our first book we include some quotations and many, mainly our own, pictures in the margin to enhance the special feature mentioned above. These quotations are intentionally not from chemistry, but from poetry and fiction. Actually, these quotations really document the global importance of the selected compounds. Authors from all ages, continents and cultural backgrounds contribute to this.

Producing such a book is not only a scientific task. Firstly, the German text of the articles had to be translated. We are deeply grateful to Dr. Colin Liddiard for this enormous task. To create a stimulating text in a convincing layout a person was needed who was fully committed to this project and this person is our secretary Mrs. Uta Zeller. We are extremely grateful for her many valuable contributions.

The polarimetric values were recorded by the first author. All NMR spectra were personally recorded and processed by the last author, and thus he is the only one to blame for eventual insufficiencies. Mrs. Katrin Steinke recorded all the UV/vis and IR spectra, some CD spectra and was an invaluable help for many HPLC runs. Mrs. Heike Petzold provided essential and skilful assistance in the lab.

The 20 compounds described were prepared by the dedicated work of the following students:

Johannes Appun, Sebastian Blanke, Philipp Drosky, Katrin Eckhardt, Ying Ying Gao, Georg Heß, Elena Jose, Anne Klaproth, Markus Leutzsch, Peter Mettke, Marija Najdanova, Kalaiselvi Natarajan, Huang Pei, Thi Thuy Duong Pham, Agneta Prasse, Franziska Reuß, Alexander Roth, Anna Rudo, Madlen Sander, Franziska Schulze, Anne Sehl, Raina Seupel, Sandro Spiller, Juliane Titus, Markus Winkler and Peter Wonneberger

Their individual contributions are often shown in the last reference in each section.

We further thank Dr. Christiane Albrecht, Prof. Günther Gauglitz, Dr. Peter Haiss, Dr. Lothar Hennig, Dr. Bettina Jee, Prof. Mirjana Minceva, Prof. Kazuhide Nakata, Ramona Oehme, Dr. Harry Pearson, Prof. Joachim Sieler and Dr. Dorothee Wistuba for various help during this project.

The authors look forward to any comment or criticism.

March 2018

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Principio genus herbarum viridemque nitorem
terra dedit circum collis camposque per omnis,
florida fulserunt viridanti prata colore,
arboribusque datumst variis exinde per auras
crescendi magnum inmissis certamen habenis.

Titus Lucretius Carus (99–54) De rerum natura, Liber V, 783-787.

In the beginning, earth gave forth, around
The hills and over all the length of plains,
The race of grasses and the shining green;
The flowery meadows sparkled all aglow
With greening colour, and thereafter, lo,
Unto the divers kinds of trees was given
An emulous impulse mightily to shoot,
With a free rein, aloft into the air.

Translated from Latin by William Ellery Leonard and E. P. Dutton, 1916.

Contents

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

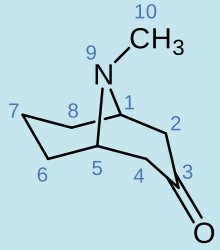


Fig. 1.1-1 Structure of pseudopelletierine

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.

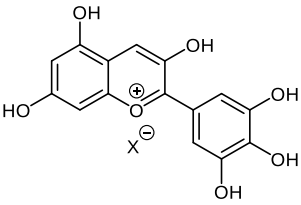
The Song of Solomon (Chapter 7, 10-13, Chapter 8, 1-2) about 500 B.C.

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.

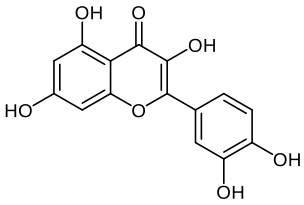
All parts of the fruit can be used. This is even valid for the skin with its high content of the tanning agents gallotannins and ellagitannins. Infusions of the skin are administered in traditional medicine for dysentery and diarrhoea. The sweet tasting seeds are used in the oriental and in the meantime 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.

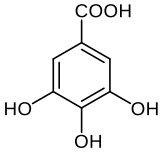
Derived from the French word for the pomegranate, grenadier, is grenadine, a syrup obtained from pomegranate juice, which no well stocked bar should be without. Grenadine lends for example Tequila Sunset its red colour and fruity taste.



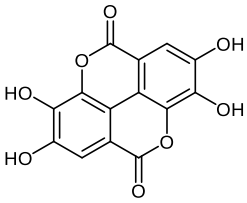
Delphinidin



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).

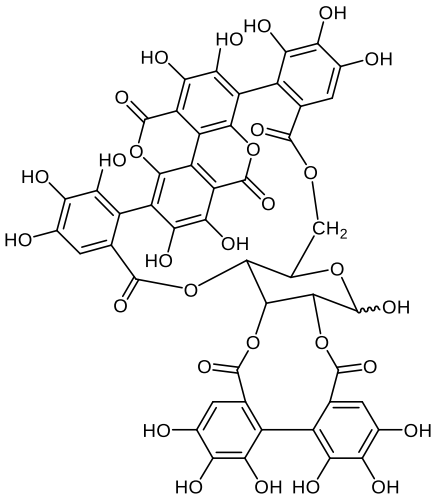


Fig. 1.1-5 Punicalagin

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

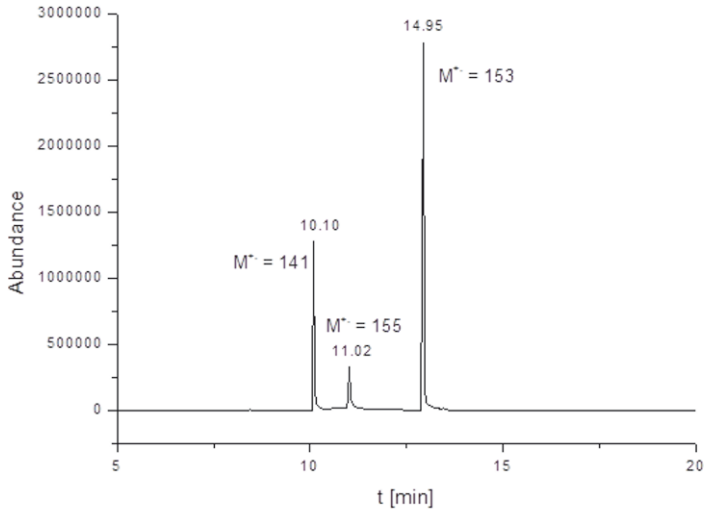


Fig. 1.1-11 GC-MS Investigation of an extract of the alkaloids of the root-bark of the pomegranate tree (for extraction, GC conditions and mass spectra see *supporting information*)

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*).

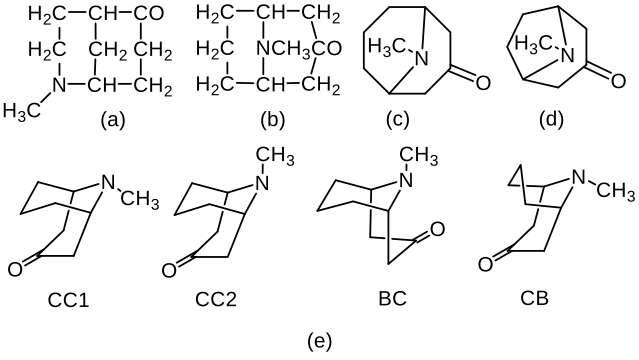


Fig. 1.1-13. First suggestion for the structure of pseudopelletierine (a), corrected and today still valid planar structural formula (b), planar representation of pseudopelletierine (c) and tropinone (d), various chair (C) and boat (B) conformations of pseudopelletierine (e)

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 20th 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).

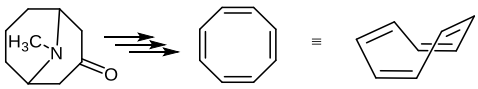


Fig. 1.1-14 From pseudopelletierine to cyclooctatetraene

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*.

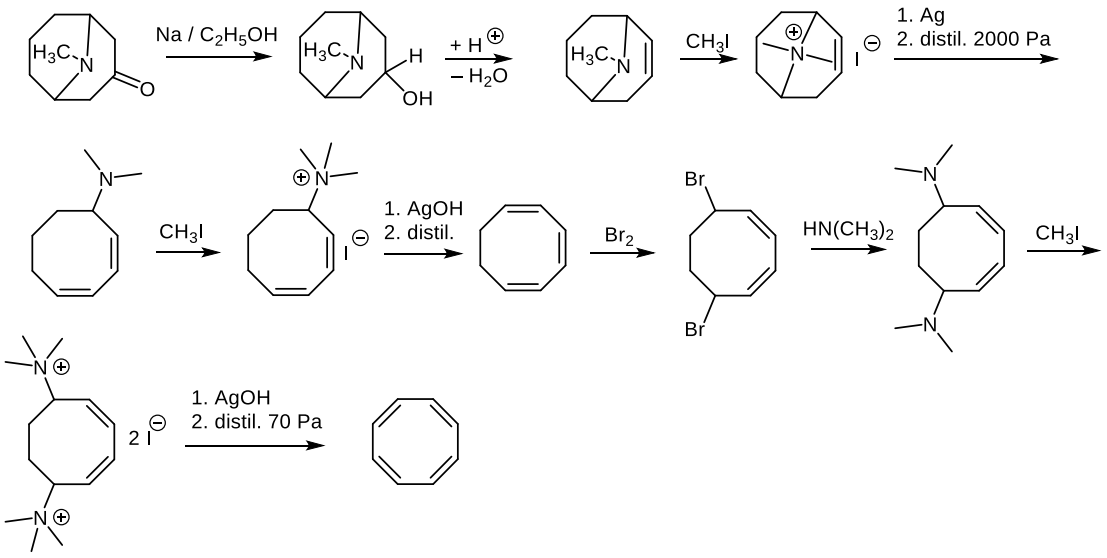


Fig. 1.1.16 Willstätter's synthesis of cyclooctatetraene from pseudopelletierine

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

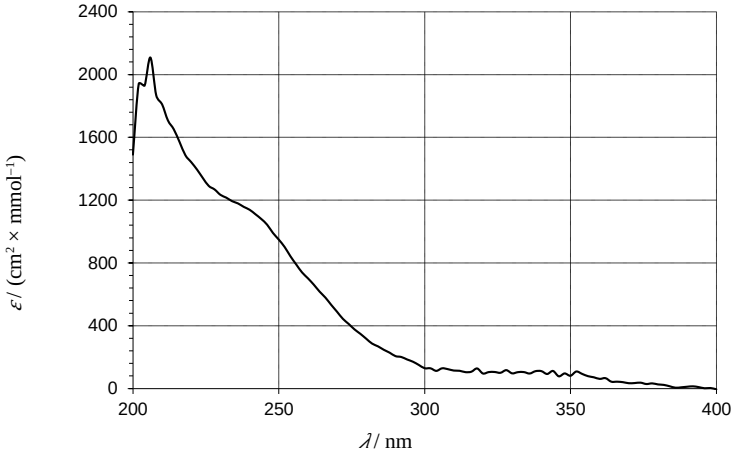


Fig. 1.1-17 UV spectrum of pseudopelletierine

IR Spectrum in KBr

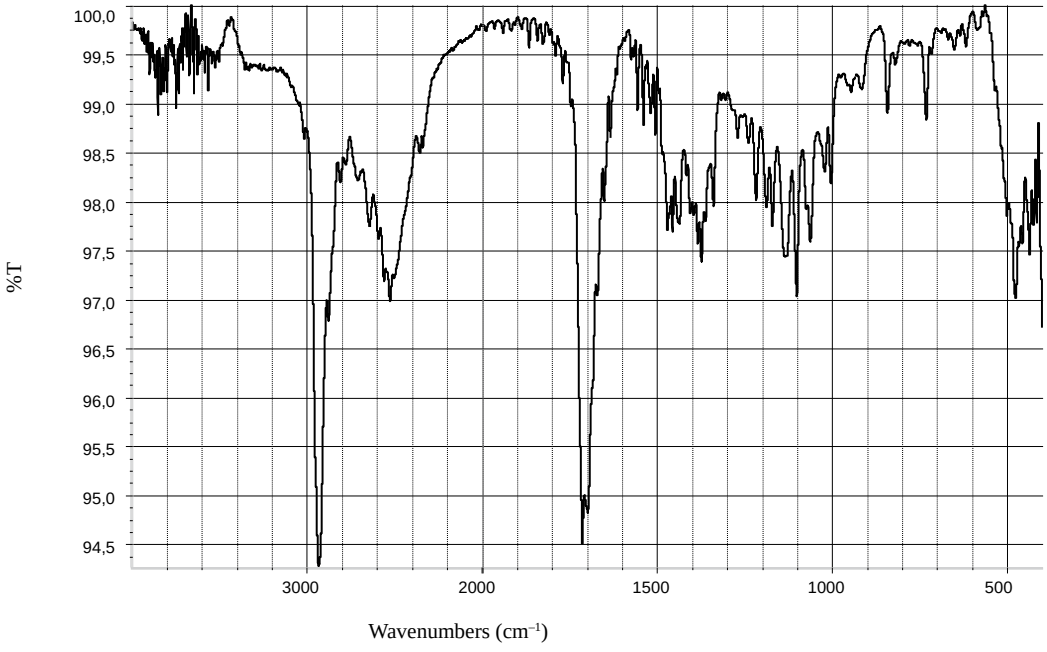


Fig. 1.1-18 IR spectrum of pseudopelletierine

700 MHz NMR Spectra in $CDCl_3$

^{1}H NMR Spectrum

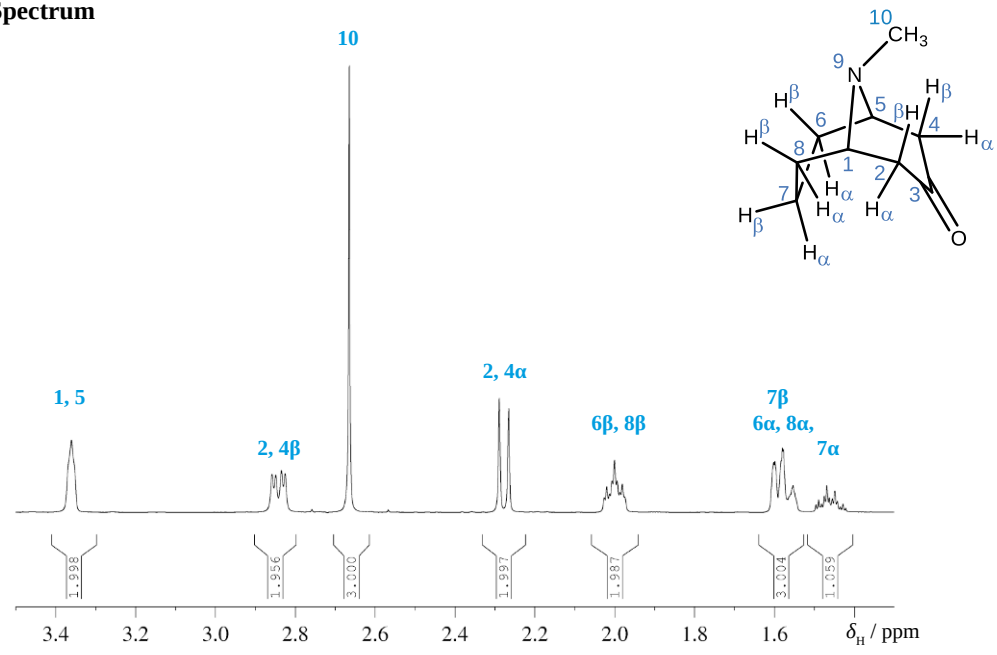


Fig. 1.1-19 ^{1}H NMR spectrum of pseudopelletierine

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 δ_H = 2.67, which can easily be assigned to the *N*-methyl group, is obvious.
The most strongly deshielded protons at δ_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 δ_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 δ_H = 2.00 and the two protons of the group of signals at δ_H = 1.59 belong to the methylene groups H-6 and H-8. The two remaining proton signals at δ_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

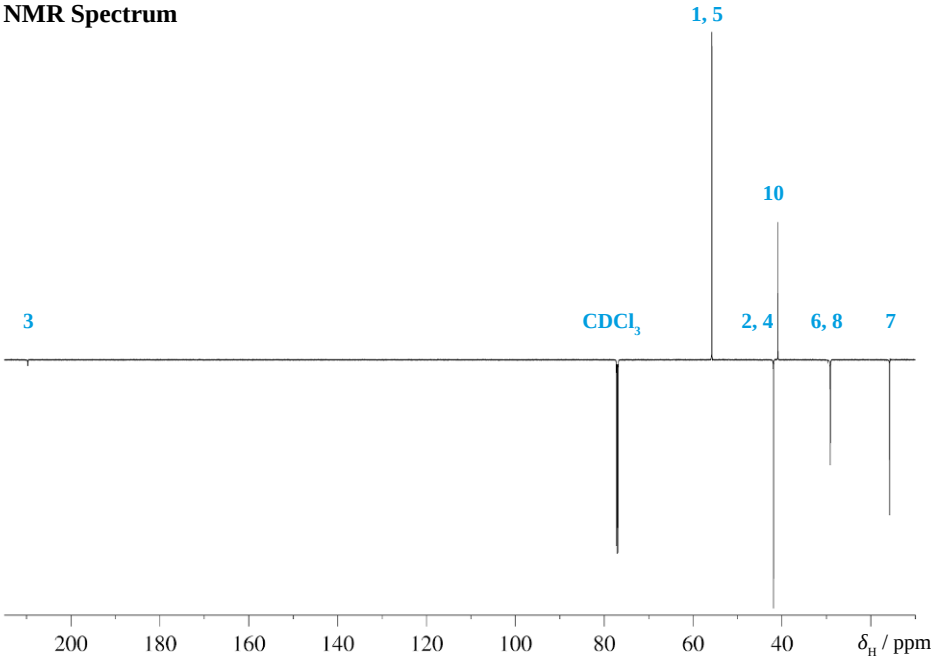


Fig. 1.1-20 APT ^{13}C NMR spectrum of pseudopelletierine

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

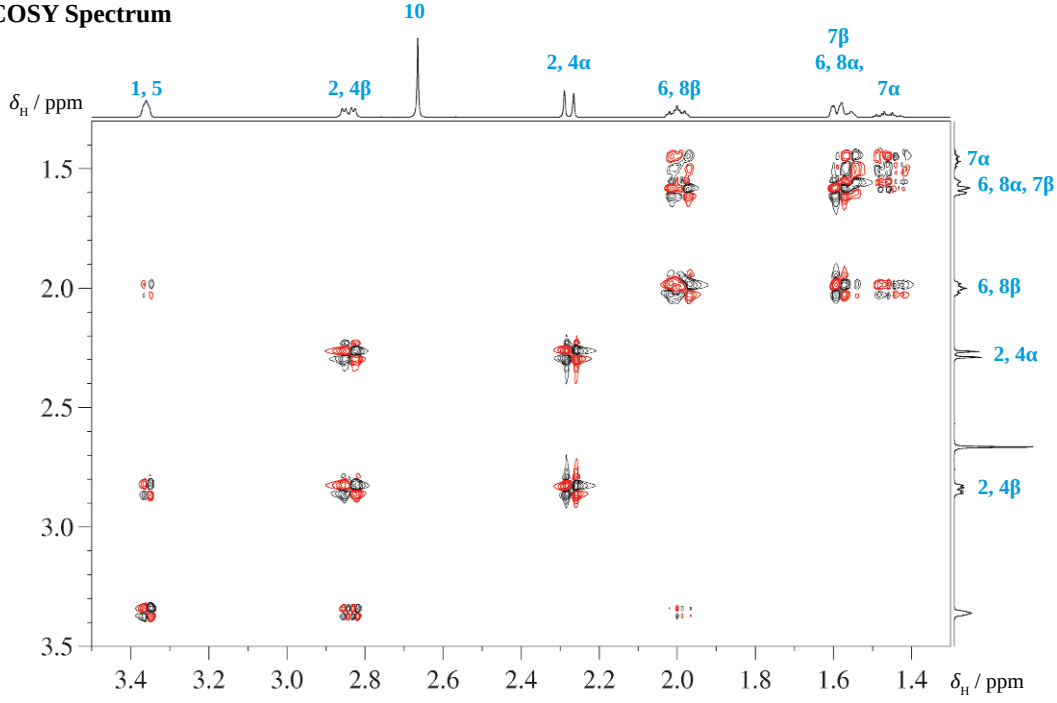


Fig. 1.1-21 COSY spectrum of pseudopelletierine

NOESY Spectrum

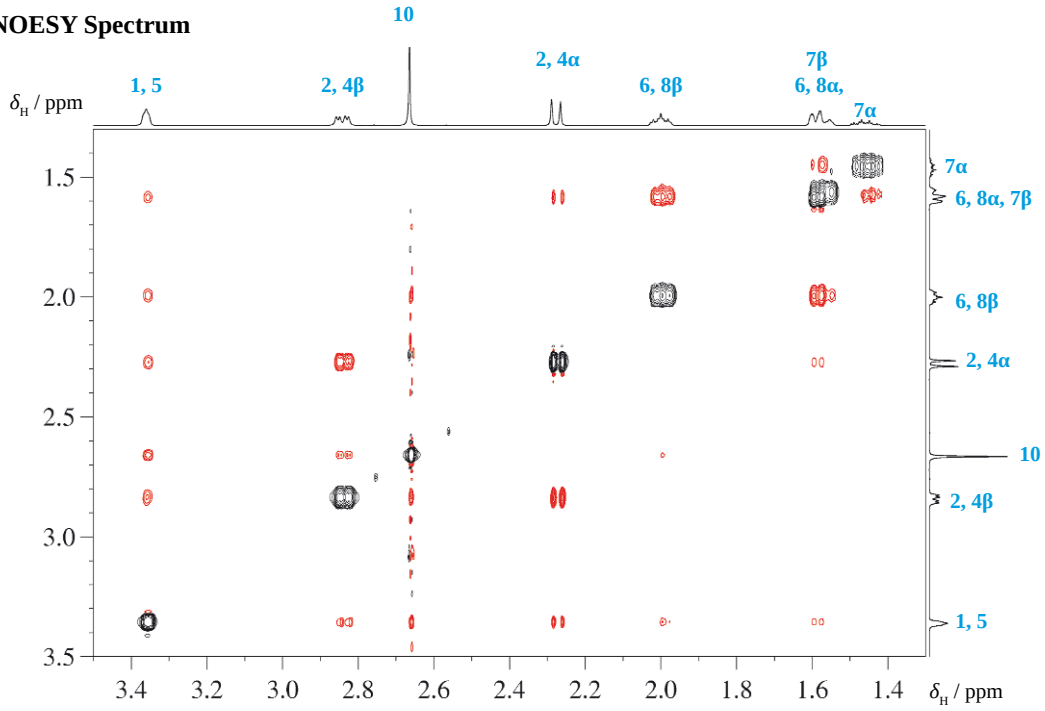


Fig. 1.1-22 NOESY spectrum of pseudopelletierine

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 δ_H = 2.84 but not to H-2/4 at 2.27. This means, that the protons at δ_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 δ_H = 2.27 and the signal from H-6/8 at δ_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 δ_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 δ_H = 2.27 and the signal of H-7 at δ_H = 1.46 shows, that the latter must also be in the α-position.

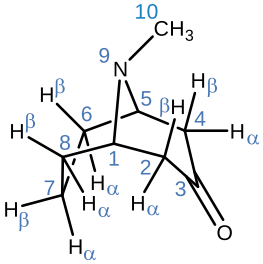


Fig. 1.1-23 Fruit bearing pomegranate tree on Crete in autumn

HSQC Spectrum

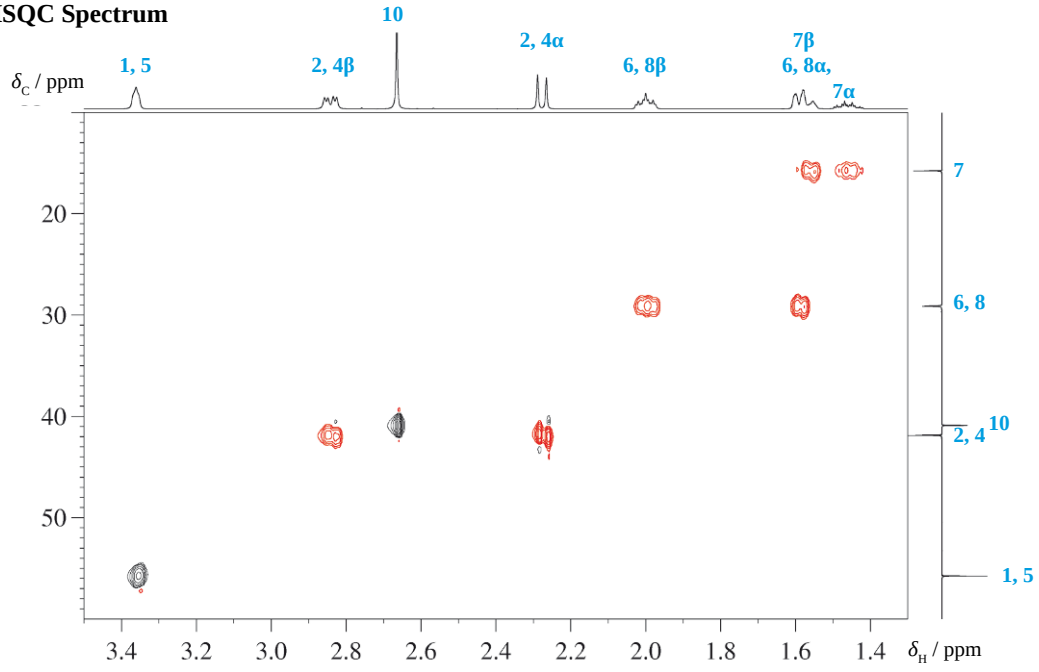


Fig. 1.1-24 HSQC spectrum of pseudopelletierine

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

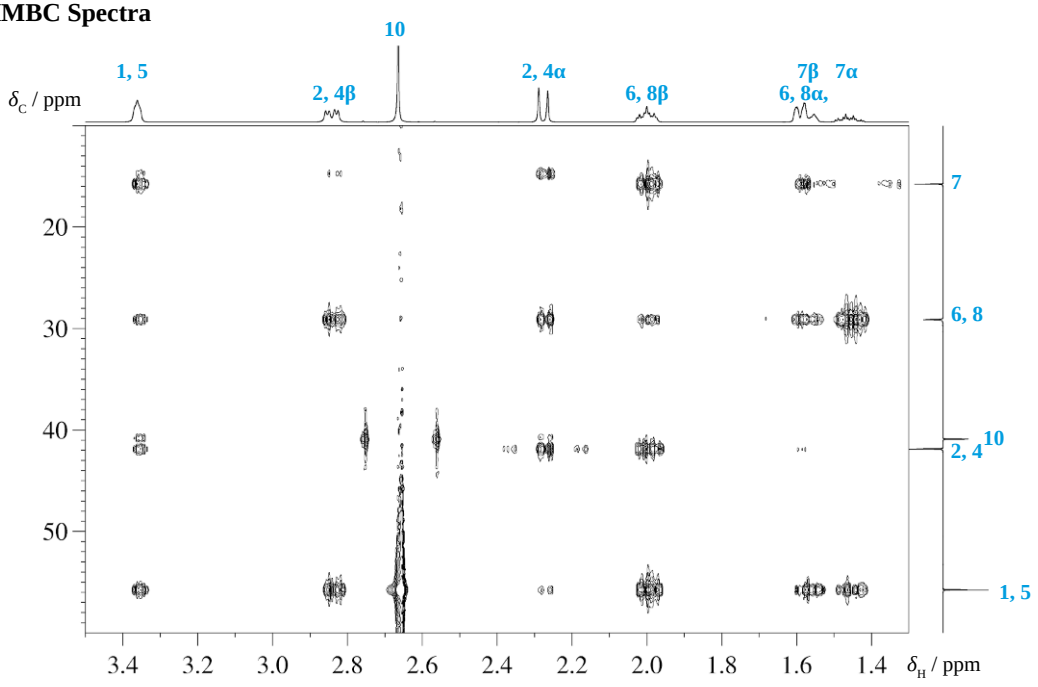


Fig. 1.1-25 Excerpt 1 of the HMBC spectrum of pseudopelletierine

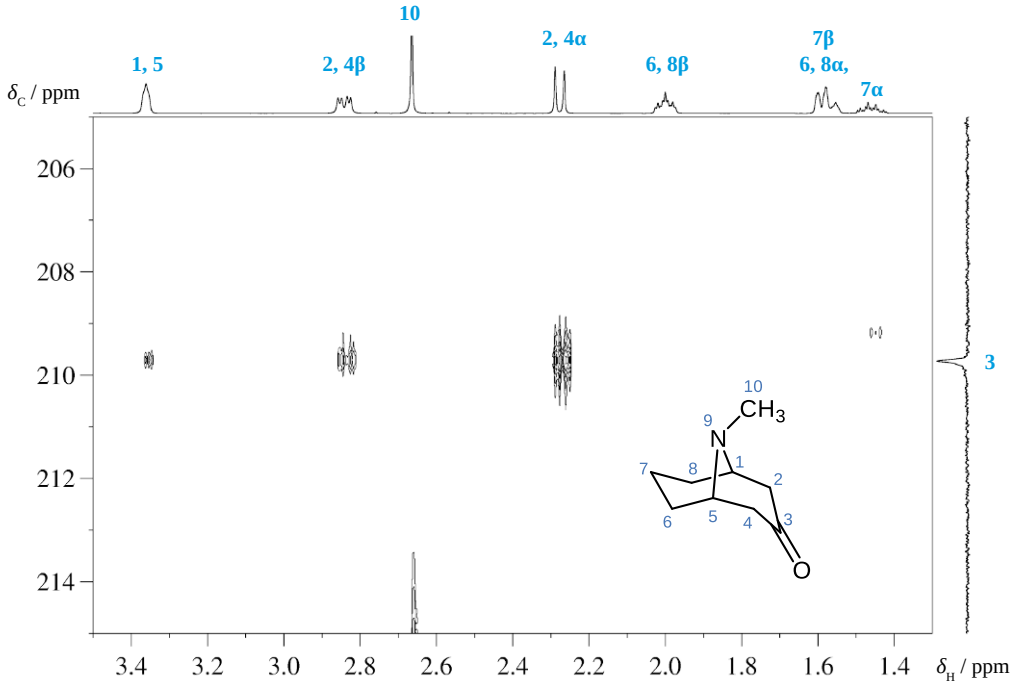


Fig. 1.1-26 Excerpt 2 of the HMBC spectrum of pseudopelletierine in the carbonyl region

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)

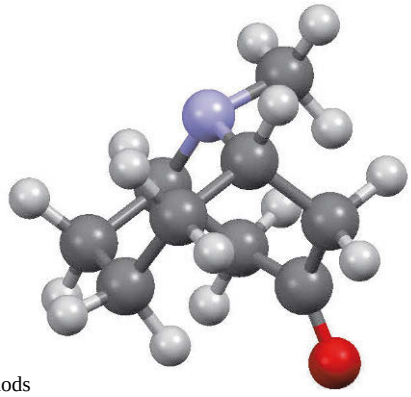


Fig. 1.1-27 3D structure of pseudopelletierine calculated with *ab initio* methods

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).

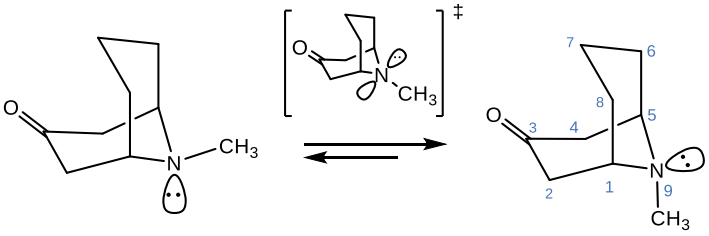


Fig. 1.1-28 Inversion at the N-atom

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 δ [ppm]	Type of C-atom	Assignment	^{1}H-NMR signal δ [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 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 α: 1.59	1/5, 2/4, 7	H-10 H-2/4e	22.9	36.5	25.9
15.8	CH_2	C-7	β: 1.55 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

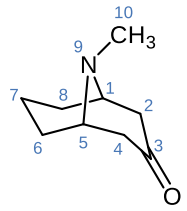
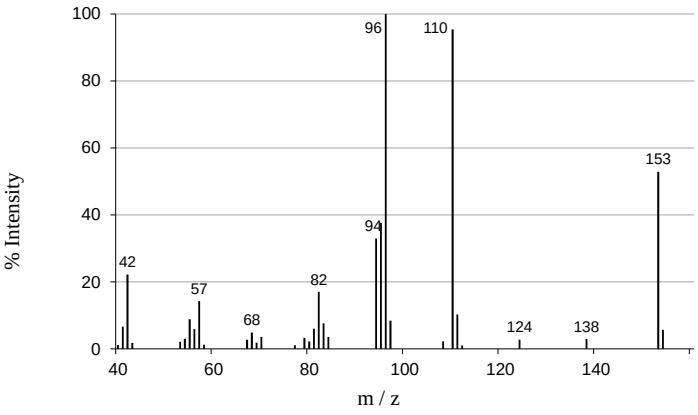


Fig. 1.1-29 EI mass spectrum of pseudopelletierine

EI Mass Spectrum



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

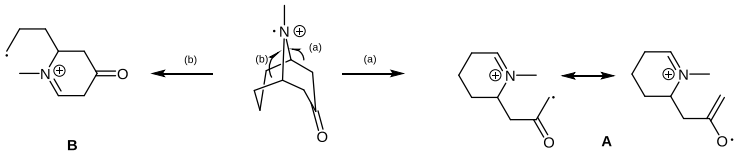


Fig. 1.1-30 Two possible α-cleavages in the mass spectrum of pseudopelletierine

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).

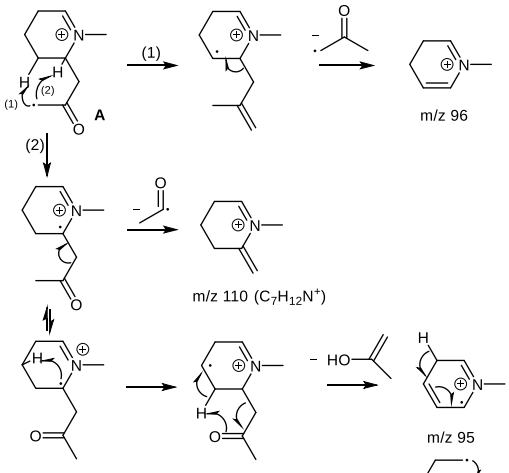


Fig. 1.1-31 Formation of fragment ions from the α-cleavage product **A**

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).

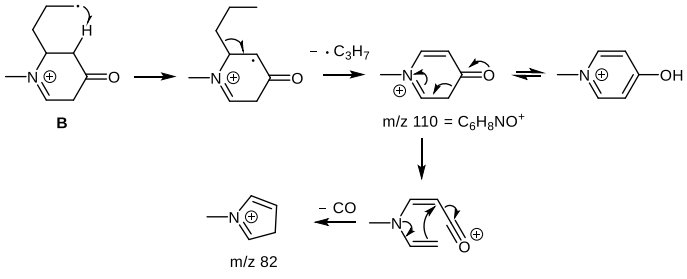


Fig. 1.1-32 Formation of fragment ions from the α-cleavage product **B**

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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