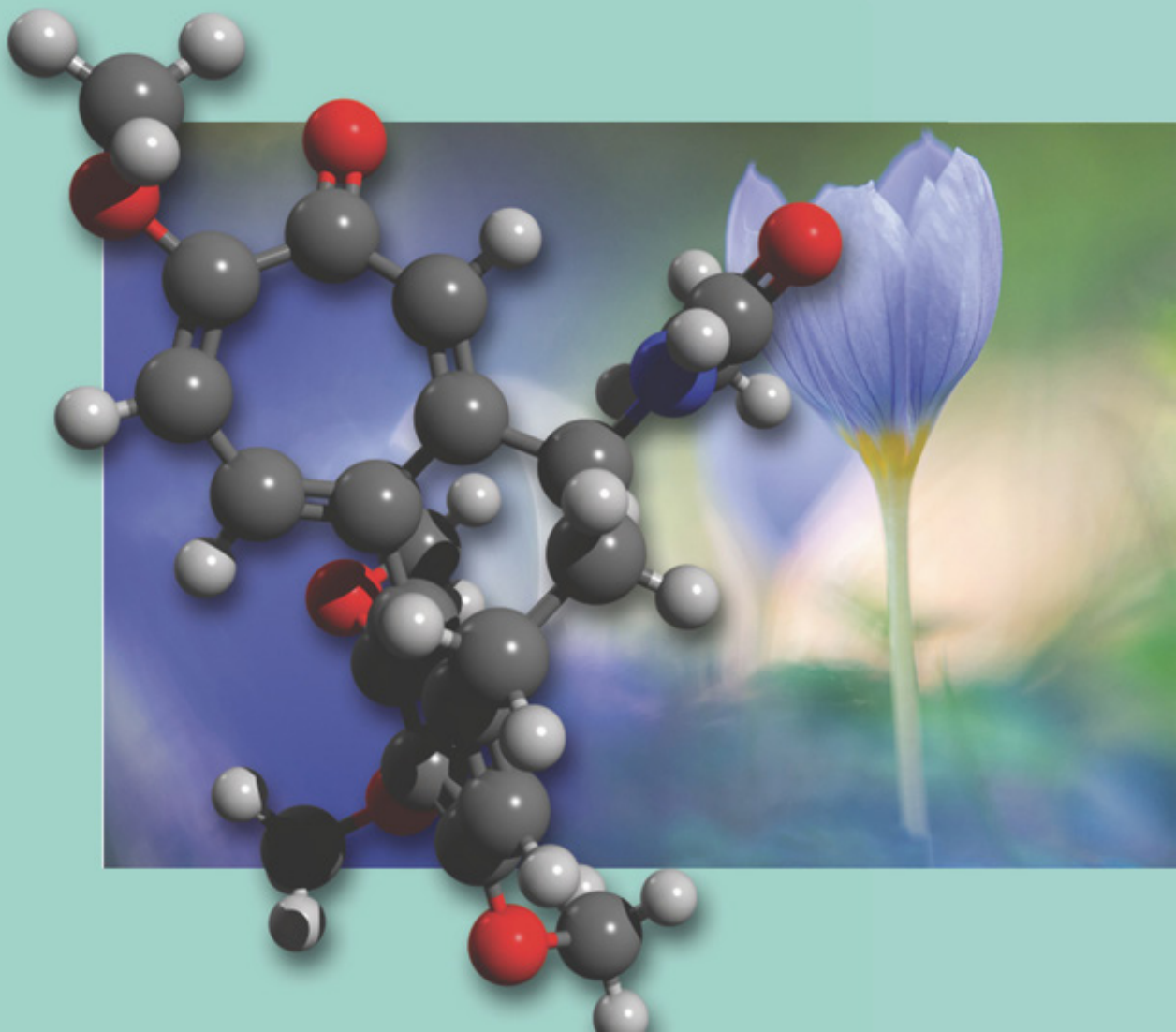
WILEY-VHCA

Reinhard W. Hoffmann

Classical Methods in Structure Elucidation of Natural Products



Classical Methods in Structure Elucidation of Natural Products

Classical Methods in Structure Elucidation of Natural Products

R. W. Hoffmann

WILEY-VHCA

Author

Prof R. W. Hoffmann
University of Marburg
Department of Chemistry
Hans-Meerwein-Straße 4
35032 Marburg
Germany

Cover
Getty #: 539191220 / Jill Ferry Photography

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Bibliographic information published by the Deutsche Nationalbibliothek
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at <http://dnb.d-nb.de>.

Print ISBN: 978-3-906390-73-4
ePDF ISBN: 978-3-906390-79-6
ePub ISBN: 978-3-906390-78-9
Mobi ISBN: 978-3-906390-80-2
obook ISBN: 978-3-906390-81-9

Typesetting SPi Global Private Limited, Chennai, India
Printing and Binding

Printed on acid-free paper

Contents

Preface

It is a well-accepted standard in the education of scientists to advise the novices that they should not hesitate to question the validity of "established" facts. Facts and "dogmas" in science acquire the status of being "established" by no longer being questioned. Doubting established facts in an experimental science requires checking whether the underlying experimental data and deductions are rigorous and unambiguous.

The structures of many natural products are depicted in standard textbooks of Organic Chemistry as "established facts." But how solid are the experimental data that predicate a particular structure given for a natural product? In the case of natural products, the structures of which were elucidated in the period 1860–1960 by classical chemical methods, the lines of evidence are frequently buried under a plethora of degradation studies, that is, investigations that repeatedly led into culs-de-sac and to revised structure assignments.

It is the aim of this treatise to bring those lines of evidence to light for a number of representative natural products. The choice of the examples is subjective and arbitrary, dictated by the pleasure to recover fundamental achievements of Organic Chemistry. In doing so, the author gained further profound respect for the intellectual achievements of past generations of chemists, who carried out these structure elucidations with minimal experimental tools.

This work pays tribute to the great scientists of the past generation, whose contributions are no longer appreciated by the present generation of young chemists. Those presently being educated, for example, in the United States or Japan, frequently lack a linguistic background to read and appreciate the contributions of German, French, or Italian chemists of the nineteenth and twentieth centuries published in their native tongues. It is all the more important to highlight their accomplishments at least by presenting some examples.

Finally, I had the feeling to be prepared for such a task, as my chemistry education comprised just the methods and reasoning of classical chemistry that form the content of this treatise. To prevent any misunderstanding, this treatise does not give the history of structure elucidation of particular natural products. Rather, the results from historic experiments are combined to derive a line of evidence for the structures that are accepted as "established" today. The line of evidence may follow the path put forward by the original contributors. In some

instances, however, the experimental facts have been combined to another, hopefully shorter, line of evidence. Eventually, it is the aim to put the reader into a position to trace the "facts behind the established structure assignments" of some important natural products.

July 24, 2014 Reinhard W. Hoffmann

Hundred Years of Structure Elucidation

Those natural products, the structures of which appear in the textbooks, are the basic representatives that form the core of organic chemistry. Most of these structures have been elucidated in the period that present-day chemists consider the "Stone Age" of organic chemistry. All the more, chemists should be willing to question the validity of these structure assignments. How solid are the facts that support the structure assignment? How cogent are the connections of these facts to the final conclusion? Surprisingly, very little knowledge on the structure elucidation of those natural products prevails at present; reason enough to bring these achievements of the previous generations of chemists to light again.

Hence, this treatise deals with exemplary structures elucidated in the hundred years from 1860 to 1960. While the facts presented are historic, this is not a history of the structure elucidations. This would be much too detailed, as the structure elucidations of most of the products covered here were highly ramified with many culs-de-sac.

Rather, one should justify the limits 1860 and 1960. The year 1960 approximately marks the change from classical structure elucidation by degradation to the era in which structure elucidation is mainly based on spectroscopic evidences and X-ray crystallography. Since it is the emphasis of this treatise to address classical structure elucidation, efforts made after 1960 are only considered in exceptional cases.

The other limit, 1860, has to do with the notion of structure. Prior to the advent of structural theory [1], there was no conceptual framework to address

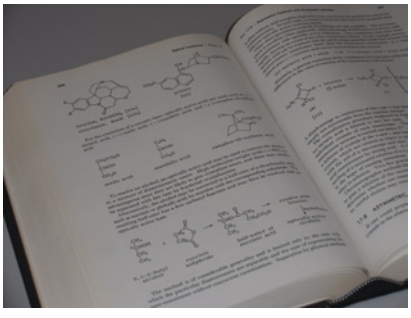


Figure 1

Classical Methods in Structure Elucidation of Natural Products. First Edition, R. W. Hoffmann.

the "structure" of a compound. This framework was provided by Kekulé (1857) [2], Couper (1858) [3], and Butlerov (1859) [4, 5]. The insight that the C-atom has four valencies and that C—C bonds form the backbone of organic compounds constituted the basis of the structural theory; it is the distinct connectivity between the atoms that defines the structure of an organic compound [5]. This theory made it for the first time comprehensible why (and how many) isomeric substances could exist for a given elemental composition of a compound.

Information Box 1

Structure Elucidation;

What is STRUCTURE ??

Structure Theory:	Stereochemistry:
Kekulé (1857)	Le Bel (1874)
Cooper (1858)	van't Hoff (1875)
Butlerov (1861)	

Of similar importance was the foundation of stereochemistry by LeBel [6] (1874) and van't Hoff [7] (1875), making it comprehensible why (and how many) "stereoisomeric" substances could exist for a given constitution of a compound.

Subsequently, the determination of such connectivities, that is, the structure of any compound of interest, became the predominant task of organic chemistry. Yet, this task constituted a challenge of unprecedented dimensions for the chemists at the end of the nineteenth century, because the structure of a new compound had to be related to that of structurally known compounds in, what one could call, a self-consistent network of information. Obviously, at the beginning, there were only a rather small number of structurally characterized compounds, which fortunately expanded rapidly with every decade of dedicated work. Obviously, in the course of time, it was increasingly easier to reach a known compound upon degradation of an unknown compound.

The situation was aggravated by the fact that the tools to elucidate structures of a given compound, that is, to establish the relation to other known compounds, were deplorably limited. Indeed, all that chemists had at their disposal were next to simple glassware, a balance, a *Bunsen* burner, and a few thermometers. All the more, one has to admire and respect the achievements of the chemists of that period.

The concept of structure could be attributed only to a "uniform" compound, that is, the sample to be studied had to be homogeneous. In the absence of any chromatographic or spectroscopic means to establish *homogeneity,* there was only the criterion of the invariance of the melting point. This means that the melting point of the sample did not change upon repeated crystallization, preferably from different solvents.

As a next step in structure elucidation, the elemental composition was to be established, both qualitatively and quantitatively, to arrive at the molecular formula. Quantitative combustion analysis provided the ratio of the elements in

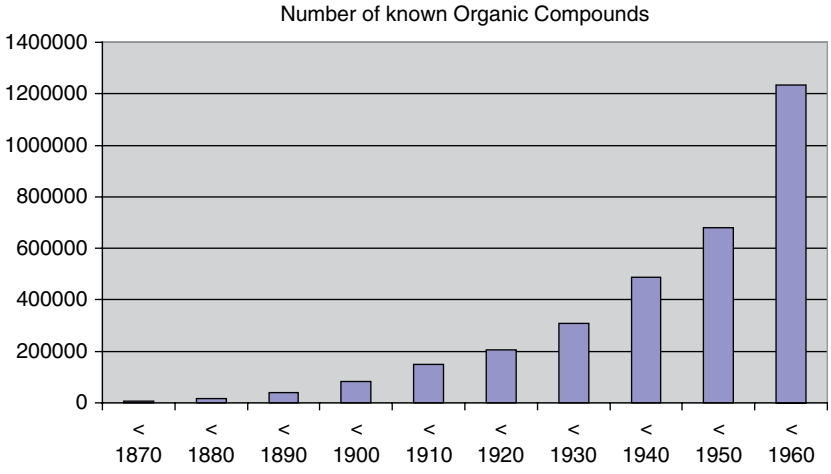


Figure 2

Figure 3 *Source:* (a) ref. [16] (b) With kind permission of Dr. Timo Mappes, www.musoptin.com; (c) ref. [17].

the compound, that is, $(C_xH_yN_zO_w)_n$. In those days, the methods to arrive at the molecular formula, that is, to determine n by vapor-density measurement, cryoscopy, or ebullioscopy, were known. Nevertheless, in most cases, n was assumed to be 1, and molecular weights were determined only when in doubt.

The next step in structure elucidation concerned the kind of functional groups present. The nature of the elements present in the compound provided a hint, as to which qualitative tests [8] for functional groups should be conducted.

The information reached at this level (melting point, molecular formula, and functional groups present) was sufficient to decide whether one deals with a known or a new compound, by consulting a compendium [9] of (common)

known compounds, listed according to the melting point, and searching for a hit with the same characteristics.

Information Box 2

Structure Elucidation;

Initial steps

Homogeneity		constant melting point
Elemental Composition	→	empirical formula
Molecular weight		
Test for Functional Groups		

Is Compound known??
Enough information for checking

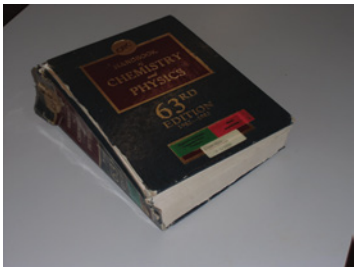


Figure 4 CRC Handbook of Chemistry and Physics.

When the compound at hand was not listed in the standard compendia, one would consult *Beilsteins Handbuch der Organischen Chemie*. There, compounds are listed in a systematic manner, according to which, for each compound with a given molecular formula and distinct functional groups, there is a unique place, where the compound should be listed. One could in this way check whether a compound with the same melting point was listed or which isomeric compounds of the proper composition were known, leaving the remaining isomers as possible candidates. For such a search, there was a limitation due to the closing date of a particular volume. To acquire information after such a closing date, one would have to search the formula registers of the annual volumes of the *Chemisches Zentralblatt* and later of *Chemical Abstracts* and, from these, the abstracts, and then the original papers to find out whether a compound with the same characteristics as that of the one at hand has already been described. A major hurdle in

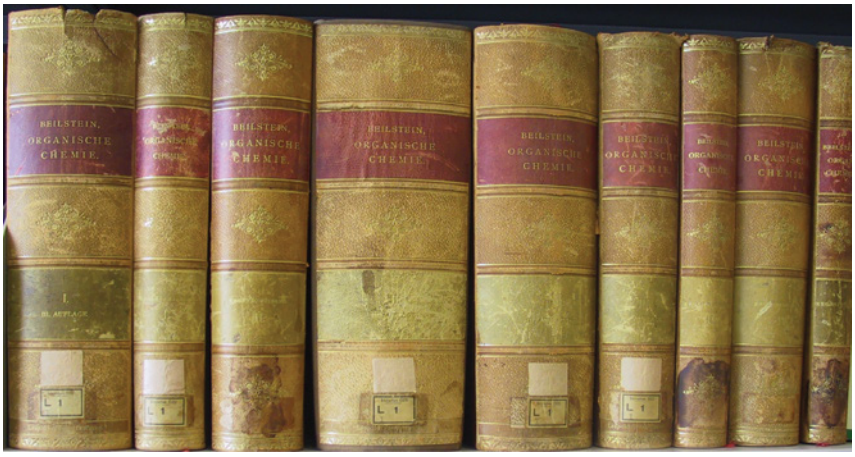


Figure 5 Beilsteins Handbuch der Organischen Chemie.
Source: With kind permission of Engelbert Zass, Zürich.

doing this was the fact that the nomenclature used for individual compounds has changed several times over the years.

One aspect became immediately evident in doing such searches: the range of melting points, commonly between 20 and 320 °C, is with about 150 data points not sufficient to distinguish ten thousands of compounds. Hence, there was the requirement of preparing (crystalline) derivatives of the compound at hand and to compare their melting points as well with the published data. Actually, the compendia and *Beilstein* list the derivatives and their melting points right alongside the data of the parent compound. However, a single derivative may not be enough to differentiate between two known compounds, as seen in the case of the 3- and 4-isopropyl-cyclohexanones. Hence, it became customary to prepare at least two derivatives of a parent compound for definitive identification. When the melting points matched, identification was considered as accomplished.

Information Box 3 Beilsteins Handbuch der Organischen Chemie, 2. Supplementary work, 1948, 7, p.31.

7. ***1-Isopropyl-cyclohexanon-(3)*** $C_9H_{16}O = H_2C<\begin{matrix}CO\cdot CH_2\\CH_2\cdot CH_2\end{matrix}>CH\cdot CH(CH_3)_2$ (E I 23). *B.* Bei der Hydrierung von Isopropyldihydroresorcin in Alkohol oder Aceton in Gegenwart von Palladium-Kohle (SIEFERT, D.R.P. 389815; *C.* **1924** II, 889; *Frdl.* **14**, 1457). — Kp_{15}: 94° (S.). Löst sich in wäßr. Natriumsalicylat-Lösung (BAYER & Co., D.R.P. 386486; *C.* **1924** I, 2633).

Semicarbazon $C_{10}H_{19}ON_3 = (CH_3)_2CH\cdot C_6H_9{:}N\cdot NH\cdot CO\cdot NH_2$ (E I 23). F: 187° (SIEFERT, D.R.P. 389815; *C.* **1924** II, 889; *Frdl.* **14**, 1457).

8. ***1-Isopropyl-cyclohexanon-(4)*** $C_9H_{16}O = OC<\begin{matrix}CH_2\cdot CH_2\\CH_2\cdot CH_2\end{matrix}>CH\cdot CH(CH_3)_2$ (H 28; E I 23). *B.* Durch Oxydation des bei der katalytischen Hydrierung von 4-Isopropyl-phenol erhaltenen Gemisches von cis- und trans-1-Isopropyl-cyclohexanol-(4) mit Chromschwefelsäure (VAVON, CALLIER, *Bl.* [4] **41**, 678; vgl. V., C., *Bl.* [4] **41**, 360). — Kp_{10}: 85–86°. D^{23}: 0,915. n_D^{23}: 1,4563. — Bei der Hydrierung in Gegenwart von Platinschwarz in Äther oder Eisessig entsteht ein Gemisch von cis- und trans-1-Isopropyl-cyclohexanol-(4).

Oxim $C_9H_{17}ON = (CH_3)_2CH\cdot C_6H_9{:}N\cdot OH$. F: 33–35°; Kp_{12}: 129–130° (VAVON, CALLIER, *Bl.* [4] **41**, 679).

Semicarbazon $C_{10}H_{19}ON_3 = (CH_3)_2CH\cdot C_6H_9{:}N\cdot NH\cdot CO\cdot NH_2$ (H 28). F: 187–188° (VAVON, CALLIER, *Bl.* [4] **41**, 679).

At this point, the conclusion could as well be that the compound at hand is not known and that the structure had to be determined by chemical means. This endeavor would start with a degradation of the compound to smaller (hopefully known) compounds. Degradation relied on oxidative cleavage at or near the functional groups present in the molecule, such as ozonolysis of C=C bonds, oxidative cleavage at C=O groups effected with refluxing HNO_3, alkaline $KMnO_4$, or CrO_3 in acetic acid. Admittedly, this approach is crude, very similar to the attempts to learn something about a Chinese porcelain figurine in a dark room by knocking it to pieces, collecting them, and to examine them later by light. But this approach was the only one chemists could apply at the end of the nineteenth century. Accordingly, Williams recommended [10]:

> ". . . it is desirable to split the molecule with the mildest possible reagent in order that splitting will occur only at the weakest point. This avoids the production of many confusing fragments all at once. . . As the splitting process continues one obtains simpler and simpler substances and ultimately all the fragments will be found to be substances already known and described. . ."

Information Box 4

if it is not a known compound

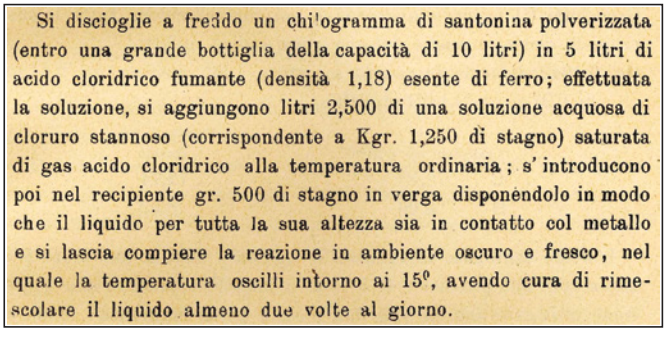
start **degradation**:

by **cleavage** at the functional groups

typically
- refluxing HNO_3
- alkaline $KMnO_4$
- CrO_3 in acetic acid
- refluxing aq. $Ba(OH)_2$

Such degradation studies required large amounts of the material to be studied in order to obtain in the end not only something that crystallized but also the product in sufficient amounts to be characterized and to be – when necessary – degraded further. Experimental sections typically read as follows [11, 12]:

> Jede Operation wurde mit wenigstens einem halben Kilo ausgeführt; bei Anwendung kleinerer Mengen ist es kaum möglich den Procefs in seinen einzelnen Phasen zu studiren, weil die Menge der Zwischenproducte (Cinchoninsäure und Cinchomeronsäure) oft verschwindend klein werden kann.



Si discioglie a freddo un chilogramma di santonina polverizzata (entro una grande bottiglia della capacità di 10 litri) in 5 litri di acido cloridrico fumante (densità 1,18) esente di ferro; effettuata la soluzione, si aggiungono litri 2,500 di una soluzione acquosa di cloruro stannoso (corrispondente a Kgr. 1,250 di stagno) saturata di gas acido cloridrico alla temperatura ordinaria; s' introducono poi nel recipiente gr. 500 di stagno in verga disponendolo in modo che il liquido per tutta la sua altezza sia in contatto col metallo e si lascia compiere la reazione in ambiente oscuro e fresco, nel quale la temperatura oscilli intorno ai 15°, avendo cura di rimescolare il liquido almeno due volte al giorno.

It is obvious that these experimental aspects restricted structure elucidation to those compounds that could be obtained – from nature or otherwise – in large quantities.

Degradation thus furnished a small number of fragments that reflect the position(s) of the functional groups in the parent molecule. Nevertheless, conclusions regarding the structure of the original molecule remained difficult.

Fortunately, the kind of transformation effected by a particular degradation reaction, for example, the ozonolysis of a C=C bond, may serve as a guideline in reconstructing the original structure.

Information Box 5

Stepwise degradation until known compounds are reached

generates an incomplete set of pieces of a structure puzzle

? how to put them together ?

need information on the **backbone** by (reductive) defunctionalization.

distillation from Zn-dust at 400°C

Even then, there would usually remain manifold possibilities to arrange the fragments in order to arrive at a potential structure of the original compound. Hence, there was the necessity to gain information on the *nature of the backbone* of the parent compound. The task is to rid the backbone of the attached heteroatoms, that is, the functional groups. Baeyer was the one that introduced the Zn-dust distillation for this purpose [13]. This treatment soon became the standard technique to unveil the backbone of aromatic compounds. Much later, this technique was complemented for alicyclic compounds by the Se-dehydrogenation [14], a method by which alicyclic compounds were converted to aromatic compounds with a related backbone.

Once the backbone of a compound was recognized with the aid of one of these methods, it was usually possible on the basis of the fragment compounds from the degradation studies to allocate the position(s) of the functional groups at the backbone, leading to a rational proposal for the structure of the compound at hand.

Such a structural proposal was, however, nothing more than a working hypothesis. Confirmation of such a hypothesis had then to be reached by *synthesis.* Obviously, the planning of such a synthesis benefited from all the information on the peculiarities of the compound and its degradation products acquired by the degradation studies. Nevertheless, the synthesis had to fulfill the requirements of providing proof for the proposed structure, implying that it could enlist only such reactions, which were well established and reliable in their course. Moreover, the synthesis had to proceed only in short straightforward steps, the results of which could be individually checked by going one step backward. But such restrictions were really seminal in developing the art of synthesis in the late nineteenth century.

The practical aspects of structure elucidation relied almost exclusively on melting points for the characterization of compounds and mixed melting points for the identification of compounds. Hence, crystallization of a compound from the crude products of a reaction – chromatography had not been invented yet – became the essential capability of the chemists at that time. In turn, compounds that did not crystallize had only a small chance to be investigated in detail. Nevertheless, even with all these limitations, chemists were remarkably successful to establish the structures of almost all abundant natural products.

Very little progress though, if at all, was made in determining the stereostructure of the compounds of interest. The relative configuration of substituents at a C=C bond ((*E*) or (*Z*)), or a ring (cis or trans), could be addressed by attempting to link these substituents forming a new cycle, such as a five-membered cyclic anhydride or lactam. This would succeed only when the substituents were in a cis-disposition.

Otherwise, an attempt could be made in a top-down approach to excise from the molecule by degradation the substructure containing the stereogenic center(s) and to try to correlate this fragment compound obtained with a compound of known configuration. In turn, there was the option of a bottom-up approach in synthesizing the various stereoisomers and to see which one matched the compound of interest. But this option was generally foiled by the inability of effecting stereoselective synthesis with a foreseeable stereochemical outcome. Rather, reactions that generated stereogenic centers were in most cases stereounselective, leading to mixtures of (dia)stereoisomers, which had to be separated by fractional crystallization in a laborious manner. Even when separated, the compounds would not reveal their configuration by themselves.

Therefore, in the first 100 years after structural theory had been conceived, neither the analytical methods nor those of stereoselective synthesis were apt to readily address the stereostructure of natural products. This situation changed dramatically with the advent of spectroscopic methods for structure elucidation, which occurred in the time span of 1930–1960. From then on, structure elucidation became the task of spectroscopy, rendering chemical means of structure elucidation more and more obsolete.

There was also an important corollary regarding synthesis: confirmation of a hypothetical structure was no longer solely the task of synthesis. Natural-product synthesis was freed from the chains to be structure-proof and could develop to a creative field of chemistry in its own right [15].

References

1 Staab, H.A. (1958) *Angew. Chem.*, 70, 37–41.
2 (a) Kekulé, A. (1857) *Ann. Chem. Pharm.*, 104, 129–150; (b) Kekulé, A. (1858) *Ann. Chem. Pharm.*, 106, 129–159.
3 (a) Couper, M.A. (1858) *C.R. Hebd. Seances Acad. Sci.*, 46, 1157–1160; (b) Couper, A.S. (1859) *Ann. Chem. Pharm.*, 110, 46–51.
4 Butlerow, A. (1859) *Ann. Chem. Pharm.*, 110, 51–66.
5 Konovalov, A.I. (2011) *The Butlerov Theory* in 'Special print for the International Congress on Organic Chemistry', Kazan.
6 LeBel, J.-A. (1874) *Bull. Soc. Chim. Paris*, 22, 337–347.
7 van't Hoff, J.-H. (1875) *Ann. Chem. Pharm.*, 23, 295–301.
8 Shriner, R.L., Fuson, R.C., Curtin, D.Y., and Morrill, T.C. (1980) The Systematic Identification of Organic Compounds, 6th edn, John Wiley & Sons, Inc., New York.
9 (a) Utermark, W. and Schicke, W. (1963) Schmelzpunkttabellen Organischer Verbindungen, 2nd edn, Akademie Verlag, Berlin; (b) Weast, R.C. and Astle, M.J. (eds) (1982) CRC Handbook of Chemistry and Physics, 63rd edn, CRC Press, Boca Raton, FL.
10 Baldwin, R.S. (1975) *J. Nutr.*, 105, 1–14.
11 Weidel, H. (1874) *Liebigs Ann. Chem.*, 173, 76–116.
12 Andreocci, A. (1895) *Gazz. Chim. Ital.*, 25(Pt. 1), 452–568.
13 Baeyer, A. (1866) *Liebigs Ann. Chem.*, 140, 295–296.
14 Plattner, P.A. (1942) *Angew. Chem.*, 55, 131–137; 154-158.
15 Eschenmoser, A. (1974) *Naturwissenschaften*, 61, 513–525.
16 Dr. Guenther, http://de.wikipedia.org/wiki/Datei:Analysenwaage2.jpg
17 G.M. Hofman, https://commons.wikimedia.org/wiki/File:Maxima-Thermometer.jpg. Licensed under CC-BY-SA 3.0

Part I

Compounds with only Oxygen Functionalities

1

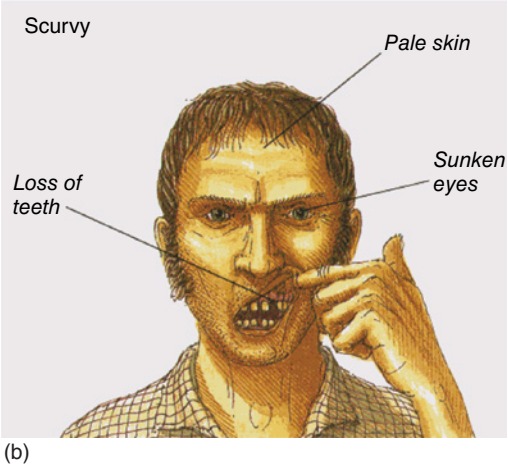
Ascorbic Acid

Figure 1.1 Albert Szent-Györgyi (b. 16.9.1893–d. 22.10.1986), in 1911, started to study medicine at the University of Budapest. This was followed by his cosmopolitan years in Prague, Berlin, Hamburg, Leiden, Groningen, Cambridge, and Rochester, before accepting a chair at the University of Szeged in 1930. In 1947, he moved to the Marine Biology Laboratories in Woods Hole (MA) in the United States. In 1937, Albert Szent-Györgyi was awarded the *Nobel* Prize for Medicine for his research on vitamin C. *Source:* J.W. McGuire, https://commons.wikimedia.org/wiki/File:Albert_Szent-Gy%C3%B6rgyi_cropped.jpg. CC-public domain.

A strongly reducing substance, $C_6H_8O_6$, was isolated from the adrenal glands in 1928 by Szent-Györgyi [1]. This substance was later identified as vitamin C, the essential food constituent, the lack of which leads to scurvy (in French, "scorbut"). Hence, this substance was given the name **ascorbic acid** [2].

Classical Methods in Structure Elucidation of Natural Products. First Edition, R. W. Hoffmann.

(a)



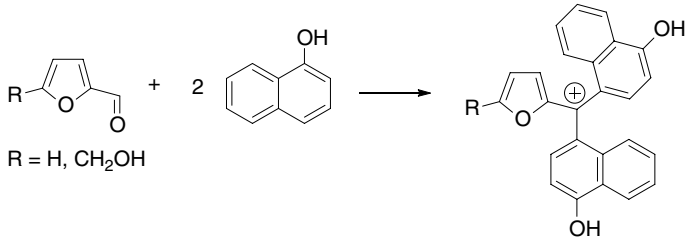
(b)

Figure 1.2 (a) Fruits containing vitamin C. (b) Signs of scurvy. *Source:* (a) Serg64/Shutterstock, www.gettyimages.com (b) Dorling Kindersley Ltd, Gütersloh, Germany. With kind permission of Dorling Kindersley Ltd, Gütersloh, Germany.

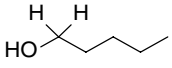
The highly oxygenated nature of this substance indicated a relationship with carbohydrates, and, indeed, ascorbic acid showed a positive *Molisch* test. The molecular formula suggests ascorbic acid to be a dehydrogenated (–4H) hexose.

Information Box 1 Molisch Test for Pentoses and Hexoses [3, 4].

Pentoses and hexoses, therefore carbohydrates in general are dehydrated by sulfuric acid to generate furfural or hydroxymethyl-furfural. When this is effected in the presence of a phenol, such as α-naphtol, condensation to give a red or violet colored triphenyl-methane dye is initiated.



1.1 ***Preliminary Findings.*** Upon treatment with chloro(triphenyl)methane, ascorbic acid readily formed a trityl ether [5]. Hence, ascorbic acid contains a primary alcohol function. Upon treatment with HCl, ascorbic acid formed furfural quantitatively [6]. Accordingly, ascorbic acid contains at least five C-atoms in a linear chain.



Ascorbic acid readily formed an acetonide [7]. It should, therefore, be a 1,2- or 1,3-diol. Finally, it was established with a *Zerewitinoff* test that ascorbic acid contains four H-atoms active toward *Grignard* reagent CH_3MgI [8].

Information Box 2 Zerewitinoff Test for Active Hydrogen [9].

Methyl-Grignard reagents such as CH_3MgI react with all compounds containing a –OH, $–NH_2$, –NH, –SH, –C≡CH group under evolution of methane. The methane evolved can be quantified volumetrically to determine the number of -XH groups in the compound investigated.

$$CH_3MgI + R\text{-}XH \longrightarrow CH_4 + R\text{-}X\text{-}MgI$$

As the name implies, ascorbic acid is acidic, with a pK_A value of 4.1 [5]. Ascorbic acid thus readily yielded a sodium salt $C_6H_7O_6Na$ [8]. Upon reaction of ascorbic acid with CH_2N_2, two (acidic) OH groups were methylated to give a dimethoxy compound **1.1** $C_8H_{12}O_6$ [8, 10].

Ascorbic acid as well as its sodium salt gave a strong positive result for the Fe^{3+} color test for enols [8], whereas that of the dimethoxy compound was negative. Therefore, at least one of the acidic H-atoms in ascorbic acid belongs to an enol, and the other one may belong to a second enol or to a carboxylic acid.

1.2 Cleavage of ascorbic acid into smaller fragments was accomplished by oxidation: upon oxidation with NaOI, 1 equiv. of oxalic acid was obtained [6]. Oxidation of ascorbic acid by $KMnO_4$ furnished oxalic acid and a 2,3,4-trihydroxybutanoic acid **1.2**; Scheme 1.1 [6, 11].

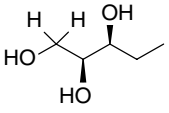
$$C_6H_8O_6 \xrightarrow{KMnO_4} HOCH_2\text{–}CHOH\text{–}CHOH\text{–}COOH + HOOC\text{–}COOH$$

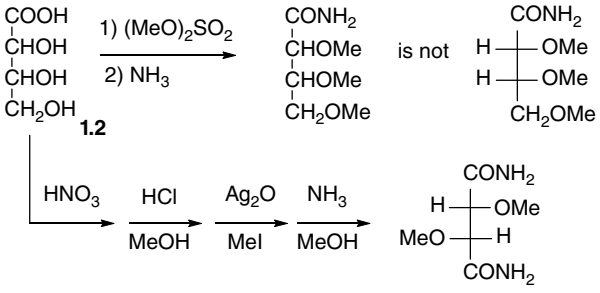
1.2

Scheme 1.1

1.3 Since there are two diastereomeric forms of 2,3,4-trihydroxybutanoic acid, the relative configuration of **1.2** was addressed at this point. To this end, compound **1.2** was permethylated with $(MeO)_2SO_2$/KOH, and the methyl ester obtained was converted with NH_3 to a crystalline amide (Scheme 1.2).

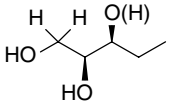
This amide turned out to be different from the known amide of *erythro*-2,3,4-trimethoxy-butanoic acid. Hence, compound **1.2** ought to be the *threo*-diastereomer thereof [11].





Scheme 1.2

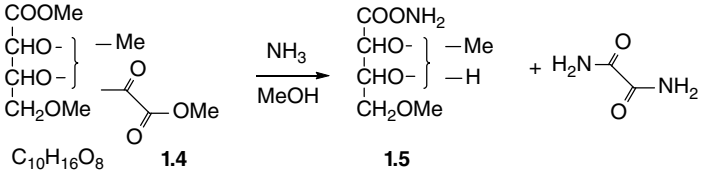
The *threo*-configuration was established by oxidizing compound **1.2** with HNO_3 (Scheme 1.2). This led to a tartaric acid, which was identified after esterification, O-methylation, and amide formation as being the (*R*,*R*)-tartaric acid. These findings established the following partial structure of ascorbic acid:



1.4 The as-yet-unidentified right-hand part of ascorbic acid contains one more C-atom and three O-atoms including the enol function, identified under Section 1.1. Further evidence was sought by methylating (blocking) all OH-functions followed by cleavage of the C=C bond, starting with the dimethoxy compound **1.1** $C_8H_{12}O_6$ described under Section 1.1, which still contained two OH groups. Thus, compound **1.1** was methylated with MeI/Ag_2O to give a tetramethoxy compound $C_{10}H_{16}O_6$ (**1.3**) [11].

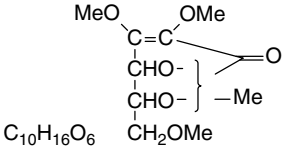
The latter should still contain the enolic C=C bond, which could be cleaved with O_3 to give a neutral compound $C_{10}H_{16}O_8$ (**1.4**) (Scheme 1.3). Compound **1.4** retained all 10 C-atoms of its precursor **1.3.** Therefore, the enolic C=C bond in the tetramethoxy compound $C_{10}H_{16}O_6$ must have been part of a ring! Ozonolysis of an enol ether gives rise to an ester. To cleave the ester moiety, compound **1.4** was treated with NH_3, resulting in oxamide and the amide **1.5** of a hydroxy-dimethoxy-butanoic acid [11].

In this transformation, three CO—NH_2 moieties have been generated. Therefore, **1.4** must have contained three ester functions. Yet, bookkeeping of the atoms



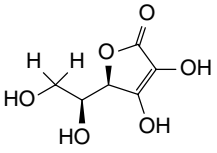
Scheme 1.3

allows for only two methyl esters in compound **1.4.** Hence, the third ester function should be a lactone unit. Accordingly, the precursor tetramethoxy compound $C_{10}H_{16}O_6$, the permethylated ascorbic acid, **1.3,** must have been a lactone and a dimethyl ether of an ene-diol.



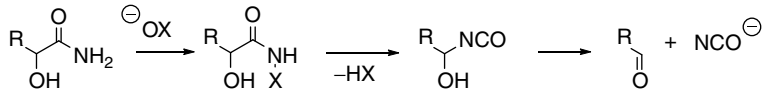
1.3

1.5 All that remained at this point was to determine the ring size of the lactone. This could be determined by locating the position of the free OH group in the hydroxy-dimethoxy-butanamide **1.5.** To this end, compound **1.5** was subjected to *Weerman* degradation by the action of NaOCl [12]. The liberation of cyanate in this process evidenced the presence of an α-*hydroxy*-carboxamide in **1.5.** Hence, the lactone ring must have been five-membered, and already present in ascorbic acid, the structure of which was thus established as the enol form of 3-keto-L-gulonolactone:



Information Box 3 Weerman Degradation [12].

In the Hofmann degradation a carboxamide R-$CONH_2$ is cleaved to R-NCO and CO_2. Weerman found that when the residue R contains an α-hydroxy-function, the resulting isocyanate undergoes hydrolysis to cyanate and an aldehyde. This reaction has been applied to the stepwise degradation of carbohydrates.



References

1 Szent-Györgyi, A. (1928) *Biochem. J.*, **22**, 1387–1409.
2 Szent-Györgyi, A. and Haworth, W.N. (1933) *Nature*, **131**, 24.
3 Molisch, H. (1886) *Monatsh. Chem.*, **7**, 198–209.
4 Bredereck, H. (1931) *Ber. Dtsch. Chem. Ges.*, **64**, 2856–2859.
5 Karrer, P., Schwarzenbach, G., and Schöpp, K. (1933) *Helv. Chim. Acta*, **16**, 302–306.
6 Cox, E.G., Hirst, E.L., and Reynolds, R.J.W. (1932) *Nature*, **130**, 888.

7 von Vargha, L. (1932) *Nature*, **130**, 847.
8 Karrer, P., Salomon, H., Schöpp, K., and Morf, R. (1933) *Helv. Chim. Acta*, **16**, 181–183.
9 Zerewitinoff, T. (1907) *Ber. Dtsch. Chem. Ges.*, **40**, 2023–2031.
10 Micheel, F. and Kraft, K. (1933) *Z. Physiol. Chem.*, **215**, 215–224.
11 Herbert, R.W., Hirst, E.L., Percival, E.G.V., Reynolds, R.J.W., and Smith, F. (1933) *J. Chem. Soc. (London)*, 1270–1290.
12 Weerman, R.A. (1917) *Recl. Trav. Chim. Pays-Bas*, **37**, 16–51.