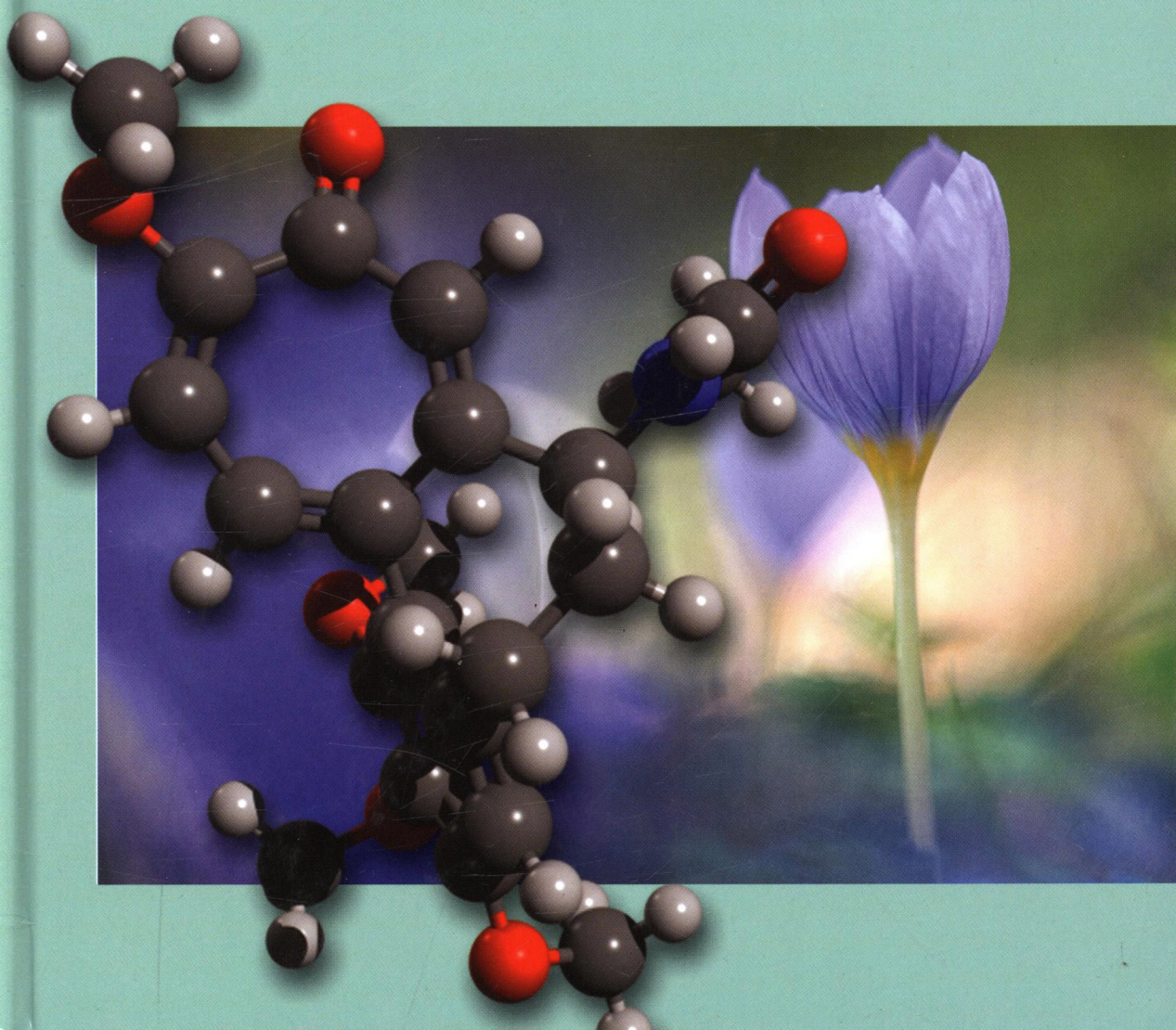


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Reinhard W. Hoffmann

# Classical Methods in Structure Elucidation of Natural Products





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**T**he structures of many natural products are given in standard textbooks on organic chemistry as 'established facts'. Yet for those natural products whose structures were determined between 1860 and 1960 by classical chemical methods, the lines of evidence are frequently buried under any number of investigations that led to dead ends and to later revised structure assignments. Since very little knowledge still prevails about the structure clarification of these products at present, this volume serves to shed light once again on the achievements of previous generations of chemists, who worked with minimal experimental and analytical tools.

The selection of the 25 representative examples is subjective and arbitrary, dictated by the author's pleasure in recovering fundamental milestones in organic chemistry, with each chapter devoted to one organic compound. The time period covered, however, is more precisely defined: 1860 represents the

advent of structure theory, prior to which there was no conceptual framework to address the 'structure' of a compound. One hundred years later, 1960 approximately marks the change from classical structure elucidation to the era in which structure elucidation is mainly based on spectroscopic evidence and X-ray crystallography.

Rather than simply provide a history of structure elucidation of particular natural products, the author combines results from historic experiments to trace a line of evidence for those structures that are nowadays accepted as established. This line of evidence may follow the path put forward by the original contributors, yet in some cases the experimental facts have been combined to form another, hopefully shorter, line of evidence. As a result, readers are able to ascertain for themselves the 'facts behind the established structure assignments' of a number of important natural products.



Hoffmann

Classical Methods in Structure  
Elucidation of Natural Products

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# Classical Methods in Structure Elucidation of Natural Products

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**Classical Methods in Structure Elucidation of Natural Products**



## 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

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## 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

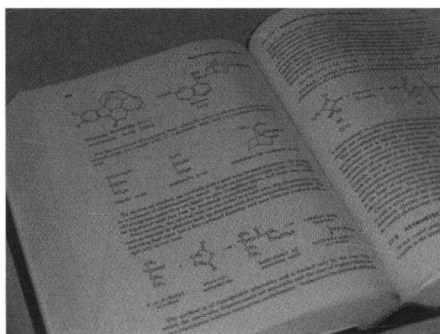


Figure 1

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:

Kekulé (1857)

Cooper (1858)

Butlerov (1861)

Stereochemistry:

Le Bel (1874)

van't Hoff (1875)

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

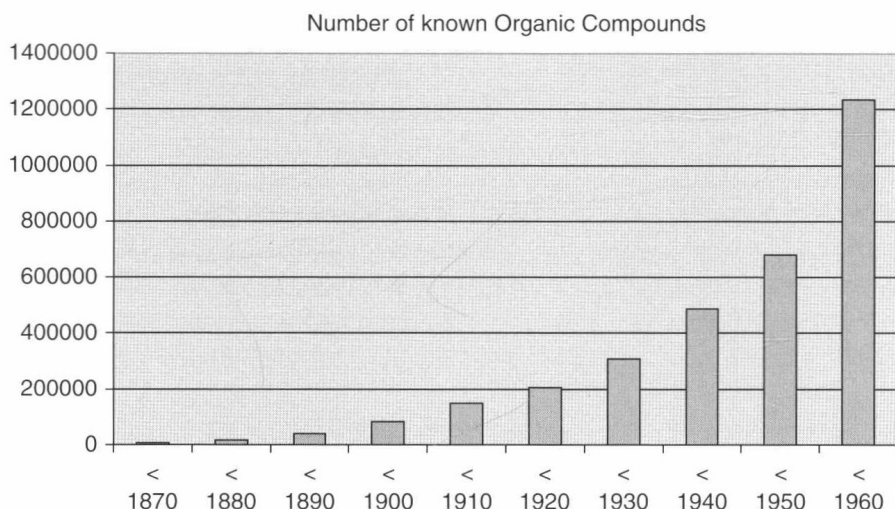
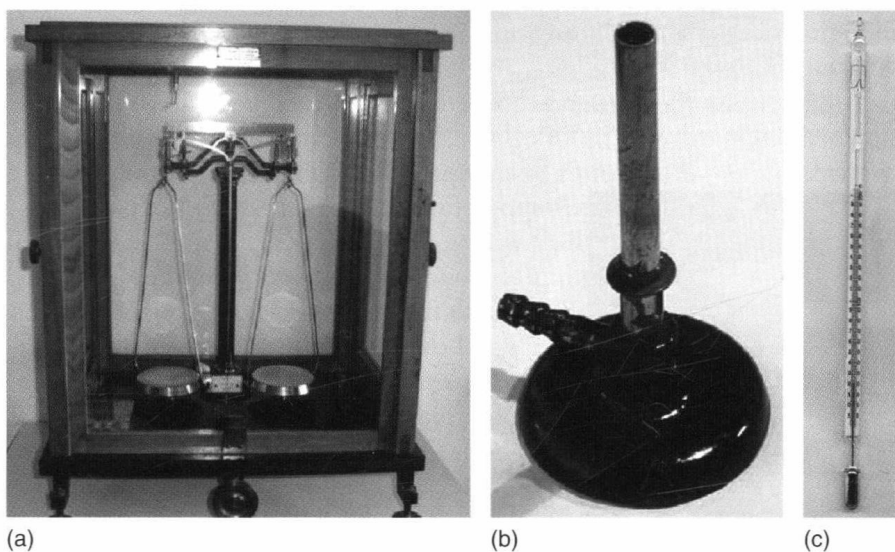


Figure 2



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

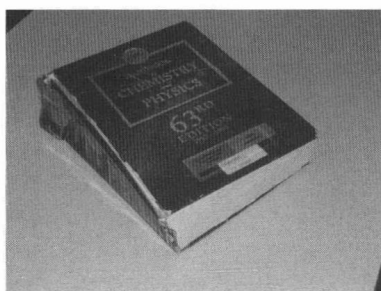
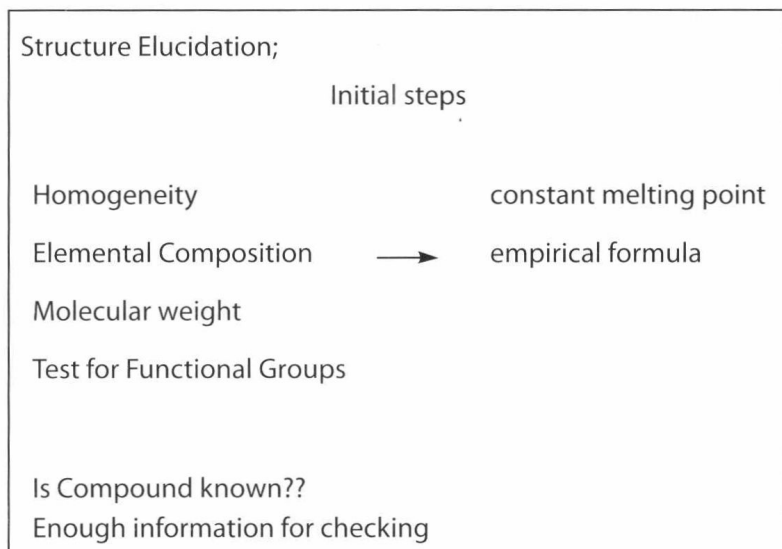
The next step in structure elucidation concerned the kind of functional groups present. The nature of the elements present in the compound provided a hint, as to which qualitative tests [8] for functional groups should be conducted.

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)



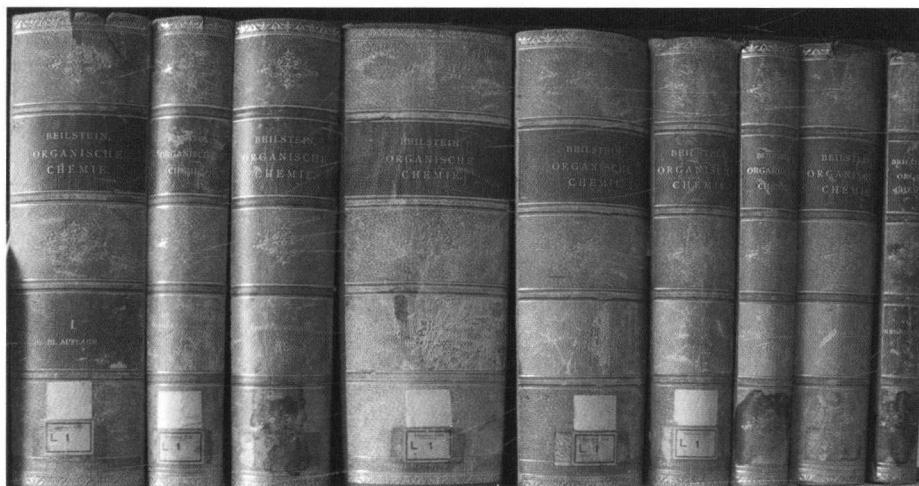
known compounds, listed according to the melting point, and searching for a hit with the same characteristics.

### Information Box 2



**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)**  $C_9H_{16}O = H_2C < \begin{smallmatrix} CO \cdot CH_2 \\ CH_2 \cdot CH_2 \end{smallmatrix} > CH \cdot CH(CH_3)_2$  (E I 23).

B. Bei der Hydrierung von Isopropylldihydroresorcin in Alkohol oder Aceton in Gegenwart von Palladium-Kohle (SIEFERT, D.R.P. 389815; C. 1924 II, 889; *Frdl.* 14, 1457). —  $K_{p15}$ : 94° (S.). Löst sich in wäßr. Natriumsalicylat-Lösung (BAYER & Co., D.R.P. 386486; C. 1924 I, 2633).

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

8. **1-Isopropyl-cyclohexanon-(4)**  $C_9H_{16}O = OC < \begin{smallmatrix} CH_2 \cdot CH_2 \\ CH_2 \cdot CH_2 \end{smallmatrix} > CH \cdot CH(CH_3)_2$  (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). —  $K_{p10}$ : 85–86°.  $D^{25}_D$ : 0,915.  $n^{25}_D$ : 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  $C_9H_{17}ON = (CH_3)_2CH \cdot C_6H_9 \cdot N \cdot OH$ . F: 33–35°;  $K_{p12}$ : 129–130° (VAVON, CALLIER, *Bl.* [4] 41, 679).

Semicarbazon  $C_{10}H_{19}ON_3 = (CH_3)_2CH \cdot C_6H_9 \cdot N \cdot NH \cdot CO \cdot NH_2$  (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  $\text{HNO}_3$ , alkaline  $\text{KMnO}_4$ , or  $\text{CrO}_3$  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 $\text{HNO}_3$
	alkaline $\text{KMnO}_4$
	$\text{CrO}_3$ in acetic acid
	refluxing aq. $\text{Ba(OH)}_2$

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 Proceß in seinen einzelnen Phasen zu studiren, weil die Menge der Zwischenproducte (Cinchoninsäure und Cinchomeronsäure) oft verschwindend klein werden kann.