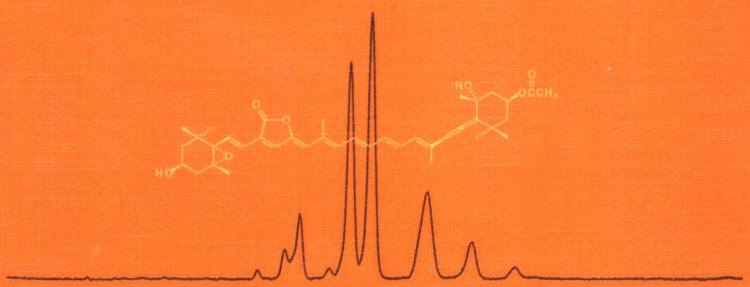
Carotenoids

Volume 1A: Isolation and Analysis

Edited by G. Britton
S. Liaaen-Jensen
H. Pfander



Birkhäuser

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Birkhäuser Verlag
Basel · Boston · Berlin

OTTO ISLER

4 August 1910 - 28 September 1992

All those associated with the preparation of this series of books on carotenoids were deeply saddened by the death on 28th September 1992 of Otto Isler, editor of the last major text on the subject, published in 1971.

Isler is, of course, best known for his outstanding work on the synthesis of vitamin A and the carotenoids. He was the pioneer in their manufacture and commercial exploitation. His numerous collaborators have good cause to remember his inspiring leadership, and many workers in other laboratories in various countries benefited greatly from his encouragement and help.

Otto Isler was born in Schaffhausen, near Lake Constance. In due course he studied chemistry at the ETH in Zürich, where he completed his doctorate under the supervision of Nobel laureate Leopold Ruzicka. There followed a most rewarding year with S.A. Jacobs at the Rockefeller Institute for Medical Research in New York.

In 1936 Isler joined what was then a small department in Hoffmann-La Roche in Basle and started to realize his ambition to work on vitamins. A year later he synthesized vitamin K_1. In 1938, in collaboration with Paul Karrer, he achieved the first synthesis of a biologically active vitamin E preparation. Later, he set himself the daunting task not only of synthesizing vitamin A, when many previous attempts had failed, but of doing so on an industrial scale. His team produced the first crystals of synthetic vitamin A in 1947, and the route used is still the major source of this biologically important substance.

In 1950 Isler's team turned their attention to the synthesis of β-carotene. For the first commercially viable route they modified an approach developed by H.H. Inhoffen. The product is widely used as a colorant in margarine and as a provitamin A. Later they improved the Karrer route from β-carotene to canthaxanthin, and developed a synthesis of ethyl 8'-apo-β-caroten-8'-oate. Both pigments are produced commercially for use in chicken feed.

Isler was a man with a vision. He clearly saw the advantages of using carotenoids, which have always been part of a normal human diet, to colour food and pharmaceuticals, rather than using unnatural alien pigments. This led him to exploratory studies on the synthesis and use of lycopene, crocetin, bixin, zeaxanthin, and many other carotenoids, work which has been actively and successfully pursued by his former collaborators after his retirement in 1975.

There can be no doubt that Otto Isler earned himself an honoured place in any roll of major contributors to studies on carotenoids and related compounds. The present volume is dedicated to him in recognition of his unique achievements.

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Foreword by the late Otto Isler

Carotenoids are a most important group of natural pigments in the vegetable and animal kingdoms. Historically, it may be considered that research on carotenoids began in 1906, when Tswett invented column chromatography in order to separate leaf pigments. This allowed Willstätter, shortly afterwards, to determine the correct molecular formula of several carotenoids and, subsequently, Zechmeister to advance the concept that carotenes have polyene structures.

In 1928, both Paul Karrer and Richard Kuhn began working on carotenoids. Their research schools were highly competitive and produced very important results which are still relevant to modern carotenoid chemistry. This, in fact, is a very good example of how competition can act as a spur to success and thus to the benefit of science. On the one hand, Karrer recognized the symmetrical nature of lycopene, β-carotene, zeaxanthin and crocetin. On the other, Kuhn provided additional proof of the structure of lycopene and β-carotene by a stepwise chromic acid oxidation. Karrer introduced the provitamin A concept by showing the constitution of vitamin A to be half the structure of β-carotene with an added molecule of water in the aliphatic end group. This concept, which was entirely new at the time, was later to become of great scientific and economic importance. The structures of the provitamins, cryptoxanthin, echinenone and γ-carotene, as well as that of astaxanthin, were elucidated by Kuhn's school.

In 1948, in their famous carotenoid book, Karrer and Jucker were able to describe 28 carotenoids of known structure, and 37 of partly known or unknown structure. In 1947, Isler *et al.* synthesized crystalline vitamin A; this opened up the field of carotene and carotenoid syntheses. Crystalline β-carotene was first synthesized in 1950 by Karrer and Eugster who used a Grignard reaction and followed the scheme:

$$C_{16} + C_8 + C_{16} = C_{40}$$

In a series of papers, Karrer and Eugster described the syntheses of several C_{40} carotenoids. In 1954, the large-scale industrial synthesis of β-carotene was established at Hoffmann-La Roche by following Inhoffen's Grignard reaction:

$$\beta\text{-}C_{19} + C_2 + \beta\text{-}C_{19} = C_{40}$$

The β-C_{19}-aldehyde was synthesized from the β-C_{14}-aldehyde by enol-ether reactions to lengthen the chain by two and then three C-atoms. This procedure is still in use. Other current industrial procedures are based on Wittig reactions and Julia's sulphone syntheses.

In the next twenty years, the number of known carotenoids increased to about 300 and the carotenoid field developed enormously with regard to isolation, spectroscopy, biosynthesis and metabolism. In 1971, I edited a multi-author book which was dedicated to the late Professor Karrer who, before his death, had followed the planning of the book with great interest.

Now, after more than twenty years, that book is out of print and partly obsolete, and the number of known naturally occurring carotenoids has increased to about 600. The IUPAC-Symposia on Carotenoids, which are held every three years, show how much the field has broadened. Major developments have come from the work of George Britton in Liverpool, Synnøve Liaaen-Jensen in Trondheim and Hanspeter Pfander in Bern. These scientists are now editing this new series of books, which will cover all aspects of the carotenoid field and which, like their two predecessors, are printed in Basel by Birkhäuser. A group of extremely good and renowned scientists, both academic and industrial, has been assembled as advisers and contributors. This complete revision and update of the carotenoid field is not directed simply towards carotenoid specialists. It should also be of particular importance to workers in other fields, *e.g.* those interested in the medical potentialities of β-carotene, which promises to be a protective agent against some cancers and cardiovascular disease. In addition, and this was always a matter of concern to me, non-toxic carotenoids should play a more important role as colorants in food processing, and in the near future they should replace the yellow and red azo-dyes which have come under suspicion as possible carcinogens.

The Nobel laureate, Paul Karrer, wrote in his memoirs: "The carotenoids are the most interesting class of compounds with which I have worked, and they have given me a great deal of pleasure." I can endorse that view and I hope that all readers and users of this new series of books will experience the same enjoyment and pleasure in working in the carotenoid field.

Preface to the Series

With this volume begins the third generation of books published by Birkhäuser under the title '*Carotenoids*'. The first one, by P. Karrer and E. Jucker, published in German in 1948 and in English translation by E. A. Braude (Elsevier, Amsterdam) two years later, and the second, edited by O. Isler and published in 1971, became classics which are still widely used as valuable sources of information. To emulate the high scientific quality and value of these books is a difficult challenge.

The field of carotenoid research has expanded enormously over the years and has spread into many areas of science. These changes are reflected in the changing style, content and philosophy of the '*Carotenoids*' books. In 1948, the elucidation of carotenoid structures was an exciting challenge of natural product chemistry, and rapid progress was being made. The book of Karrer and Jucker collected the main findings of that work as a survey which described the structures and properties of each carotenoid that was then known, and the properties of several others whose constitution had not, at that time, been established; some 70 to 80 carotenoids were covered. By the time of Isler's book, the number of known carotenoids had increased to around 260, and great advances had been made in spectroscopic methods and chemical synthesis, and also in many aspects of the biochemistry and biology of carotenoids. The commercial production and applications of β,β-carotene and some other carotenoids were well established. The need for a monograph that gave a broader coverage of the carotenoid field was amply fulfilled by the Isler book which brought together comprehensive surveys of different aspects of carotenoid chemistry and biochemistry, presented as a series of authoritative chapters written by eminent carotenoid researchers from universities and industry.

These are not the only books on carotenoids. A monograph by L. Zechmeister on 'Cis-trans *Isomeric Carotenoids, Vitamins A and Arylpolyenes*' (Springer, Vienna, 1962) still stands as the basic reference work on this specialized branch of carotenoid and polyene chemistry. The list of naturally occurring carotenoids that was included as a Chapter in the Isler book, later appeared (Birkhäuser, 1976) in an expanded form as a '*Key to Carotenoids*', edited by O. Straub. The rate of discovery and characterization of new carotenoids since then has been rapid, and a second edition of the *Key to Carotenoids*, edited by H. Pfander and listing 563 carotenoids, was published by Birkhäuser in 1987. Complementary to these are several books which deal with other aspects of carotenoids, especially their biochemistry, functions and applications. In 1952, T. W. Goodwin published his '*Comparative Biochemistry of the Carotenoids*' (Chapman and Hall London), which contains exhaustive tabulated data on the carotenoid compositions of many hundreds of species of plants, animals and microorganisms. The second edition, published in two volumes in 1980 (plants and microorganisms) and 1984 (animals), brought the coverage up to date and is now the primary reference work for information on the occurrence and distribution of carotenoids in all living organisms.

Interest in carotenoids is not purely academic. They are also of considerable commercial importance, especially in the area of food and nutrition. The technological aspects of carotenoids, especially their uses and applications, formed the subject of another extensive

monograph '*Carotenoids as Colorants and Vitamin A Precursors*' edited by J. C. Bauernfeind (Academic Press, New York, 1981).

In 1966 an International Symposium on Carotenoids was held in Trondheim, Norway, under the sponsorship of the International Union of Pure and Applied Chemistry (IUPAC) and the text of the main lectures were published in the official IUPAC journal *Pure and Applied Chemistry* (*Pure Appl. Chem.*, **14**, 215-278, 1967). This proved to be the first of a prestigious series of meetings, held at 3-yearly intervals. The published proceedings of these Symposia, held in Las Cruces, USA, 1969 (*Pure Appl. Chem.*, **20**, 365-553, 1969), Cluj-Napoca, Romania, 1972 (*Pure Appl. Chem.*, **35**, 1-130, 1973), Bern, Switzerland, 1975 (*Pure Appl. Chem.*, **47**, 97-243, 1976), Madison, USA, 1978 (*Pure Appl. Chem.*, **51**, 435-675, 857-886, 1979), Liverpool, UK, 1981 (*Carotenoid Chemistry and Biochemistry*, ed. G. Britton and T. W. Goodwin, IUPAC-Pergamon, Oxford, 1982), Munich, Germany, 1984 (*Pure Appl. Chem.*, **57**, 639-821, 1985), Boston, USA, 1987 (*Carotenoids: Chemistry and Biology*, ed. N. I. Krinsky, M. M. Mathews-Roth and R. F. Taylor, Plenum Press, New York, 1990), Kyoto, Japan, 1990 (*Pure Appl. Chem.*, **63**, 1-176, 1991) and again in Trondheim in 1993 (*Pure Appl. Chem.*, **66**, 931-1076, 1994) provide regular progress reports on all aspects of carotenoid science.

More than twenty years after Isler's book, there is clearly a need for a new version of '*Carotenoids*'. Discussions began in 1987 during the 8th International Symposium on Carotenoids in Boston. An International Editorial Advisory Board was constituted, consisting of leading academic and industrial authorities on different aspects of carotenoid science, from several different countries. A trio of Editors was assigned the task of producing the new version, and generous sponsorship was granted by F. Hoffmann-La Roche and BASF to make the venture possible. It is pleasing to note that the new edition, like the previous ones, is published by Birkhäuser, and that tradition is maintained through the use, once again, of the simple title '*Carotenoids*'. But this is where tradition stops. The new '*Carotenoids*' is different, perhaps radically different, in concept, philosophy and presentation, in order to meet what are seen as the different needs of today. The most immediately obvious difference is that, because of the greatly increased amount and diversity of information now available, the new '*Carotenoids*' is published not as a single volume, but as a series of volumes. Collectively, these will cover the entire carotenoid field, and there will be extensive cross-referencing, but each volume will also stand as a self-contained work on its own particular topic.

A most important innovation lies in the philosophy behind the series, and the new objectives that the editors seek to achieve. This is not to be simply a revised or updated 'new Isler'. The Karrer and Jucker and the Isler monographs presented information and remain essential reference books for anyone in the carotenoid field. The new '*Carotenoids*' series will also present information, in particular information and ideas that are new since 1971. The main objective, however, is for the older generation of carotenoid workers to pass on experience and expertise in a way that will be of value not only to established carotenoid researchers, but especially to workers coming new to carotenoids, either those who are beginning a scientific career in carotenoids or those whose work in, for example, the fields of

food science, medicine, or photosynthesis leads them, perhaps temporarily, into the carotenoid field.

It is a commonly held belief that working with carotenoids is difficult. For the newcomer to the field it can be, and even experienced workers should never become complacent or forget how easy it is to destroy carotenoids, to produce artifacts that modern analytical methods can detect, and to generate misleading results. In their own carotenoid research, the authors and editors have made many mistakes, but have learned a great deal from them. This new series gives newcomers an opportunity to learn from our experience and to avoid repeating the mistakes that we have made.

The books in the new *Carotenoids* series are intended to be workbooks that have a place in the laboratory, not simply on the reference shelf. This is especially true of the coverage of general methods of carotenoid isolation and analysis which forms the subject of the two parts of Volume 1. The emphasis is on practical guidance, *i.e.* guidance on what can and cannot be done, advice on the choice of the most appropriate methods and equipment, and instruction on how best to carry out the recommended procedures. A novel but particularly important feature is the inclusion of Worked Examples in which the most important and effective procedures are described in detail, almost as an instruction manual or recipe that any researcher can follow.

The Worked Examples in Vol. 1A provide access to a range of authentic reference compounds by isolation from well documented natural sources. Some of these Examples could be used as student practical exercises.

Each volume in the series will be a planned and coordinated multi-author book consisting of approximately 350 pages and will be given the title '*Carotenoids*' followed by an informative subtitle. The Editors and Editorial Advisory Board will formulate the overall plan for the volume and will select the most suitable authors for the different topics that are to be covered. Initially, six volumes are planned, but the series is open-ended to allow for the addition of further topics, as new developments dictate.

This first volume, published in two parts, provides an introduction to carotenoids and covers the general methods of isolation and analysis and the application of spectroscopic methods to analysis and structure elucidation.

Acknowledgements. Although we are privileged to be the editors of these books, their production and publication would not have been possible without the help of a great many other people. We are grateful for the advice and support of the members of the International Editorial Advisory Board. We greatly appreciate the dedicated work of the authors, their patience, their prompt attention to requests and questions, and their gracious acceptance of the drastic editing that was often necessary to avoid duplication and to meet the stringent limitations of space. We owe a great debt of gratitude to our co-workers, whose careful reading of manuscripts and proofs has been so valuable, and to those people who have undertaken the arduous task of preparing, in Liverpool, uniform camera-ready manuscripts and figures with great skill and patience. Finally, we gratefully acknowledge Hoffmann-La Roche and BASF, without whose sponsorship this project could not have been realized, and Cito Habicht, Elizabeth Beckett and their colleagues at Birkhäuser for their guidance

throughout the preparation work, their tolerance in the face of numerous delays and their efforts in achieving publication so soon after receipt of the final manuscripts.

George Britton
Synnøve Liaaen-Jensen
Hanspeter Pfander

Editors' Notes on the Use of these Books

The *Carotenoids* books are planned to be used with the *Key to Carotenoids,* Second Edition. Throughout the series, whenever a known, natural carotenoid is mentioned, its number in the *Key to Carotenoids* or the *Appendix* (Vol. 1A, Chapter 8) is given in bold print. Other compounds, including purely synthetic carotenoids, that do not appear in the *Key* are numbered separately, in italics, in sequence for each Chapter, and their formulae are shown. Note that the *Key* numbers given are those in the Second Edition of the *Key to Carotenoids* (1987). The numbering of compounds in the original *Key to Carotenoids* (1976) and the list in the 1971 Isler *Carotenoids* book is different.

There is substantial cross-referencing between Volume 1A and 1B, which constitute two parts of a single volume and are intended to be used together.

Trivial names

The IUPAC semi-systematic names for all known naturally occurring carotenoids are given in the *Key to Carotenoids*, 2nd Edition, and in the Appendix (Vol. 1A, Chapter 8). Trivial names for many carotenoids are, however, well-established and convenient, so the practice of using these trivial names rather than the often cumbersome IUPAC semi-systematic names will, in general, be maintained in this series. An important exception is the naming of those compounds which bear the trivial name '(prefix)-carotene'. To avoid confusion caused by the use of Greek-letter prefixes that do not correspond to the series of prefixes used to designate end groups in the IUPAC system (*e.g.* 'γ-carotene' does not contain γ end groups) the old names α-carotene, β-carotene, γ-carotene, δ-carotene and ε-carotene are abandoned according to the IUPAC recommendation, and replaced by β,ε-carotene, β,β-carotene, β,ψ-carotene, ε,ψ-carotene and ε,ε-carotene, respectively.

References

Selective key references for major papers and review articles are given, rather than comprehensive lists of original literature sources.

Indexing

The indexes have been prepared primarily to aid the use of these books as general information and practical guides. No author index or index of biological sources of carotenoids is given. For information on individual carotenoids the *Key to Carotenoids*, which gives the trivial and semi-systematic names of each carotenoid, and references to its occurrence, isolation, spectroscopic properties, synthesis, *etc.* should be used. Chapters 1 and 2 of Vol. 1A are accounts of the historical development of the carotenoid field and the challenges that carotenoid research now offers, and they thus provide a background context for the whole series. They do not contain any direct practical guidance and therefore have not been covered in the Index.

The Index should be used together with the List of Contents to direct the reader to particular concepts, definitions, phenomena, techniques and procedures during the practical use of the books.

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