EXPERIENTIA

Vol. 26 – Fasc. 7 Pag. 697–816 15. 7. 1970

Developments in the Carotenoid Field*

By SYNNØVE LIAAEN-JENSEN

Organic Chemistry Laboratories, Norway Institute of Technology, University of Trondheim, Trondheim (Norway)

1. *Introduction*

It was more than 40 years ago that PAUL KARRER first started his investigations in the carotenoid field, an interest he maintained throughout his scientific career. There can be no doubt that no single scientist has contributed more to research in this field.

The total number of KARRER's publications on carotenoids exceeds 200, distributed over 40 years production. The symmetrical formula of lycopene advanced in 1931, the post-war elucidation of the structures of the epoxidic and furanoid carotenoids, arriving in time to be included in KARRER and JUCKER's standard book *Carotinoide*[1], the first total synthesis of β-carotene published in 1950 and later the synthesis on which the technical production of canthaxanthin is based, may be regarded as highlights of his carotenoid research.

Any major research contribution will also indirectly influence the further development of the field. In the following the more recent general progress in the carotenoid domain will be reviewed and some examples of the more indirect influence of KARRER's contribution to the development of this field will be given.

2. *Biosynthesis and function*

The empirical isoprene rule, used in chemical studies on isoprenoid compounds, has been experimentally proved also for carotenoids (Figure 1). It is today recognized that the biosynthesis of carotenoids proceeds via mevalonic acid and isopentenyl pyrophosphate to geranylgeranyl pyrophosphate, 2 moles of which are condensed tail-to-tail to give the C_{40}-skeleton. The first C_{40}-compound appears to be phytoene. By stepwise dehydrogenation the coloured carotenoids are formed from the colourless C_{40}-precursor by the so-called Porter-Lincoln series (Figure 2). Oxygen functions are generally introduced at a later biosynthetic stage. The cyclization step is not fully understood, although cyclization is suspected to occur at the stage of neurosporene. The pre-phytoene steps are based on enzymatic studies with cell-free preparations. Post-phytoene reactions were originally based on studies of mutants and endogenous synthesis, recently supported by transformations in cell-free systems[2].

KARRER took great interest in the biological vitamin A activity of carotenoids[1,3]. Today other functions are established, like indirect participation in photosynthesis by absorption and transfer of light energy to chlorophyll, role in phototaxis, in oxygen transport, and as protectors against photodynamic destruction[4].

3. *New methods for structure elucidation and separation*

In 1946 about 70 carotenoids of natural occurrence had been characterized[1]. Today the number is nearly tripled, although less than 100 have well-established structures[5]. On the introduction of new spectroscopic methods in the fifties and onwards, carotenoid research entered a new phase. New raw materials, including microorganisms, may now be examined because information can be obtained on small samples. A considerable amount of data has accumulated permitting identification of the chromophoric system by means of the absorption spectrum. IR-spectroscopy gives valuable information about the functional groups

* Based on a lecture delivered at the Paul Karrer Symposium on 18 April 1969 in Zürich. The original paper contained a more detailed review and evaluation of Karrer's direct contribution to carotenoid research.

[1] P. KARRER and E. JUCKER, *Carotinoide* (Birkhäuser, Basel and Stuttgart 1948).

[2] T. W. GOODWIN, *The Biosynthesis of Vitamins and Related Compounds* (Academic Press, London 1963), p. 270; *Chemistry and Biochemistry of Plant Pigments* (Academic Press, London 1965), p. 143. – C. O. CHICHESTER, J. pure appl. Chem. *14*, 215 (1967); Phytochem. *8*, 603 (1969).

[3] P. KARRER and co-workers, Helv. chim. Acta *36*, 828, 1783 (1953); *41*, 1154 (1958).

[4] T. W. GOODWIN, *The Comparative Biochemistry of the Carotenoids* (Chapman and Hall, London 1952); Adv. Enzymol. *21*, 296 (1959); Ann. Rev. Pl. Physiol. *12*, 219 (1961). – J. H. BURNETT, in *Chemistry and Biochemistry of Plant Pigments* (Ed. T. W. GOODWIN; Academic Press, London 1965), p. 381.

[5] J. B. DAVIS, in *Rodd's Chemistry of Carbon Compounds* (Ed. S. COFFEY; Elsevier, Amsterdam 1968), vol. 2, part B, p. 231; see also ref. [24].

present. Fundamental studies on the application of PMR- and mass-spectrometry are reported in more recent years, and these methods are now indispensable in structural studies on carotenoids. In particular PMR-spectra of carotenoids, first studied by JACKMAN and WEEDON with collaborators[6], give information about the number and type of methyl groups present. Analysis of the more complex olefinic region is also gradually being made use of. The first systematic study of carotenoid mass-spectra was published 4 years ago by ISLER's group[7]. Most carotenoids may be studied by mass-spectrometry in the form of a suitable derivative, and the method is today a necessary tool in structural studies on carotenoids. Characteristic losses of 92 and 106 mass units from the molecular ion, explained by SCHWIETER et al.[7] as losses of toluene and xylene by *cis*-oid elimination from the polyene chain (Figure 3), serves to identify the molecular ion. One need no longer assume that a carotenoid is a C_{40}-compound, which is not always true. Information about the characteristic fragmentation pattern of the various structural modifications are continuously accumulating[8]. Stereochemical problems may today be attacked by PMR[9], ORD[10] and CD[11]. Much remains to be done on the stereochemistry of carotenoids in spite of ZECHMEISTER's *cis-trans* isomerization studies[12]. X-

[6] M. S. BARBER, J. B. DAVIS, L. M. JACKMAN and B. C. L. WEEDON J. chem. Soc. *1960*, 2870.
[7] U. SCHWIETER, H. R. BOLLIGER, L. H. CHOPARD-DIT-JEAN, G. ENGLERT, M. KOFLER, A. KÖNIG, C. v. PLANTA, R. RÜEGG, W. VETTER and O. ISLER, Chimia *19*, 294 (1965).
[8] J. BALDAS, Q. N. PORTER, L. CHOLNOKY, J. SZABOLCS and B. C. L. WEEDON, Chem. Commun. *1966*, 852. – C. R. ENZELL, G. W. FRANCIS and S. LIAAEN-JENSEN, Acta chem. scand. *23*, 727 (1969).
[9] M. S. BARBER, A. HARDISSON, L. M. JACKMAN and B. C. L. WEEDON, J. chem. Soc. *1961*, 1625.
[10] P. M. SCOPES, W. KLYNE, A. K. MALLAMS and B. C. L. WEEDON, Abstracts 5th IUPAC. Symp. Chem. Natural Products (London 1968), p. 7.
[11] N. ARPIN and S. LIAAEN-JENSEN, Phytochemistry *8*, 185 (1969).
[12] L. ZECHMEISTER, *Cis-trans Isomeric Carotenoids, Vitamins A and Arylpolyenes* (Springer, Wien 1962).

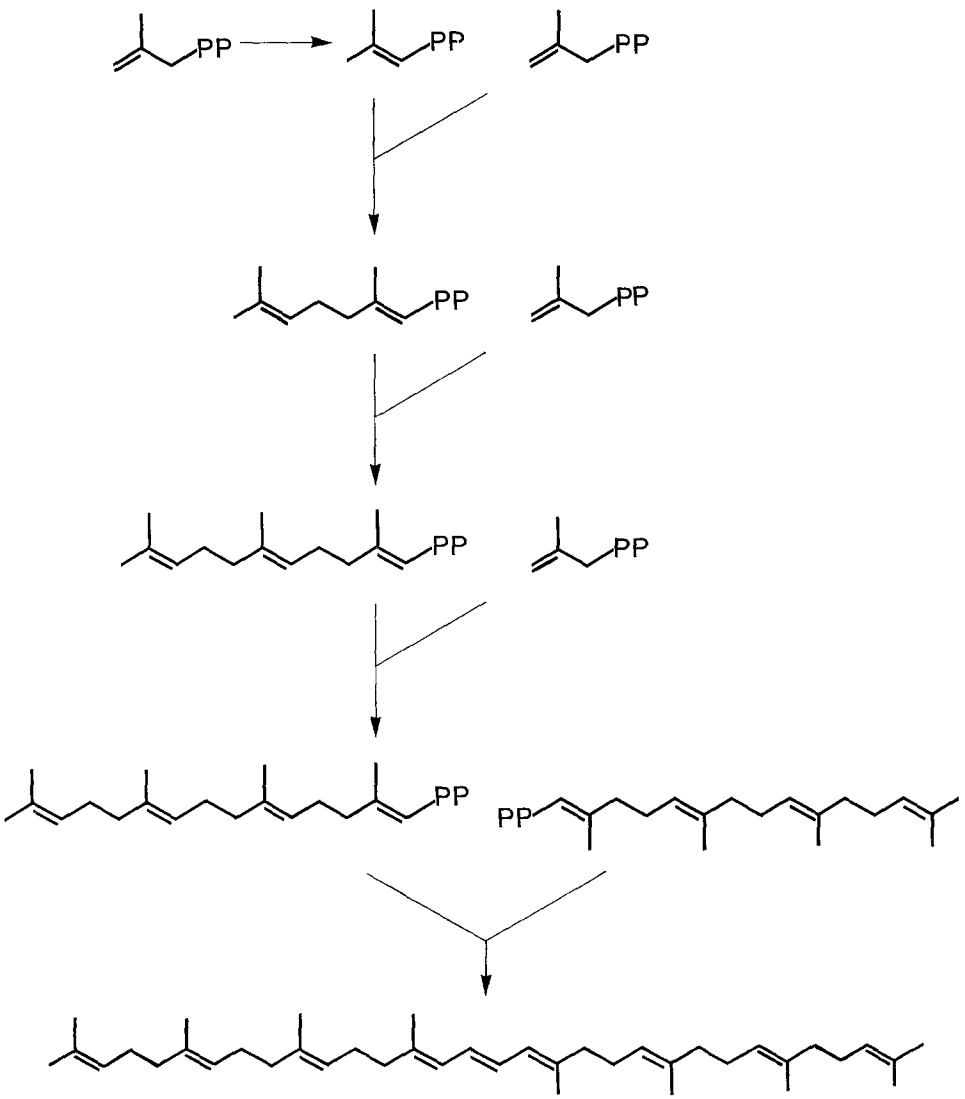

Fig. 1. The biosynthesis of the C_{40}-skeleton of carotenoids from the C_5-precursor, isopentenyl pyrophosphate, derived from mevalonic acid[2].

ray crystallography of carotenoids is still a young science, but represents an important method[13].

In addition to the spectrometric methods now available new reagents are available for derivative preparations, such as various hydrides for reduction of carbonyl groups, reagents like *p*-chloranil, dichlorodicyanobenzoquinone and nickel peroxide for selective allylic oxidation, and new methods such as silylation of tertiary alcohols which are not acetylated by the standard procedure, dehydration of tertiary alcohols by phosphorus oxychloride in pyridine and improved methylation procedures[14].

Improvements in separation techniques, particularly on the micro scale by thin-layer chromatography[15] or paper chromatography[16], have also been made. The potential use of gas chromatography is also available.

The result of the improved facilities is that the structure of a new carotenoid in simpler cases may today be elucidated with 1–10 mg of substance. Some examples of novel structures reported during the last decade by

[13] C. H. STAM and C. H. MACGILLAVRY, Acta Cryst. *16*, 62 (1963). – C. STERLING, Acta Cryst. *17*, 241 (1964).
[14] S. LIAAEN-JENSEN, J. pure appl. Chem. *14*, 227 (1967).
[15] E. STAHL, *Dünnschichtchromatographie* (Springer, Berlin 1962).
[16] A. JENSEN, in *Carotine und Carotinoide* (Ed. K. LANG; Steinkopff, Darmstadt 1963), p. 119.

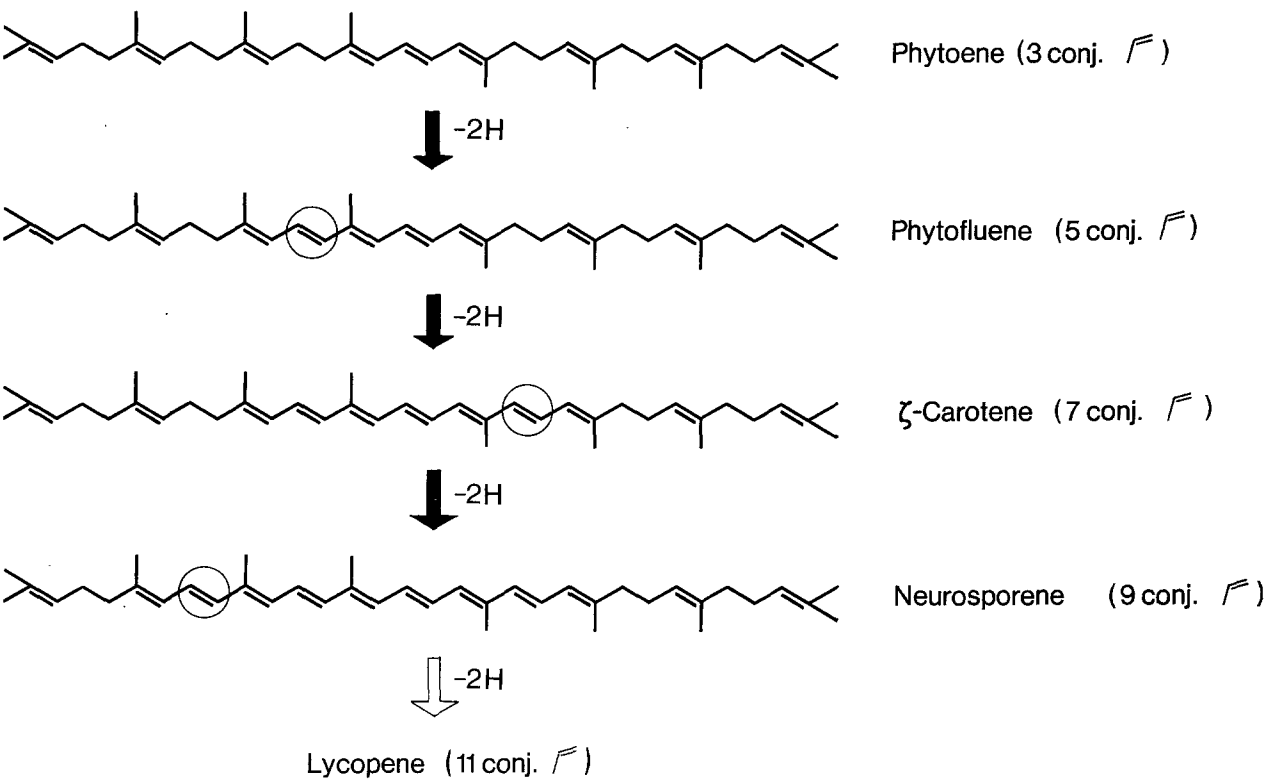

Fig. 2. Biosynthesis of coloured carotenoids from the colourless C_{40}-precursor (Porter-Lincoln series)[2].

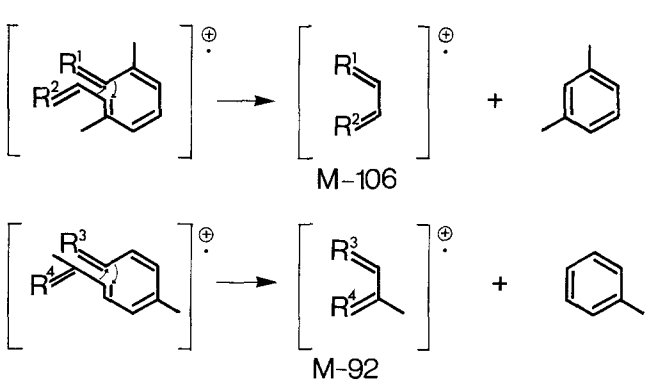

Fig. 3. Mechanism for toluene and xylene formation on electron beam induced fragmentation of carotenes (SCHWIETER et al.[7]).

other workers are given in Figure 4: the allenic fucoxanthin[17], the acetylenic alloxanthin[18], the methyl ketone citranaxanthin[19] and torularhodin[20]. Further examples from our own group will be returned to later.

4. *Total synthesis*

Parallel to this development of sophisticated spectroscopic techniques, great achievements have been made in the field of total synthesis by Swiss, German, English and American schools; the Swiss tradition being extremely well taken care of by ISLER's group[21–23]. Unequivocal structural proof by total synthesis for some 50 natural carotenoids exists today[24]. Four carotenoids are of technical importance, namely β-carotene, β-apo-8'-carotenal, the corresponding ethyl ester and canthaxanthin, all of Swiss production, in spirit and in practice[25].

Figure 5[21] gives the principal schemes for the total synthesis of carotenoids by various alternatives of the $C_x+C_y+C_x=C_{40}$ principle and the $C_x+C_y=C_{40}$ principle. The reaction types employed in each example are included.

The most commonly employed reactions for building up the C_{40}-skeleton have been (a) reaction of carbonyl compounds with acetylene magnesium halogenides or with sodium acetylides in liquid ammonia, (b) enol ether condensation based on acid-catalyzed addition of acetals to enol ethers, (c) Wittig condensation of aldehydes with triphenylalkylidene phosphoranes and (d) Horner reaction of ketones and phosphonate anions (see Figure 6). Other reactions, less extensively used, include aldol condensation, Wurtz reaction, Reformatsky synthesis, Knoevenagel-Doebner condensation, Robinson-Mannich base synthesis and reductive dimerization[21].

Figure 7 gives a total synthesis of the important intermediate β-C_{19}-aldehyde developed by Hoffmann-La Roche from acetone[25]. Acetone and acetylene are

[17] R. BONNET, A. K. MALLAMS, A. A. SPARK, J. L. TEE, B. C. L. WEEDON and A. MCCORMICK, J. chem. Soc. *1969*, 429. – A. JENSEN, Norw. Institute of Seaweed Research, Report No. 31 (Tapir, Trondheim 1966).

[18] A. K. MALLAMS, E. S. WAIGHT, B. C. L. WEEDON, D. J. CHAPMAN, F. T. HAXO, T. W. GOODWIN and D. M. THOMAS, Chem. Commun. *1967*, 301.

[19] H. YOKOYAMA and M. J. WHITE, J. org. Chem. *30*, 2481, 2482, 3994 (1965); Phytochem. *5*, 1159 (1966).

[20] P. KARRER and J. RUTSCHMANN, Helv. chim. Acta *26*, 2109 (1943); *28*, 795 (1945); *29*, 335 (1946). – O. ISLER, W. GUEX, R. RÜEGG, G. RYSER, G. SAUCY, U. SCHWIETER, M. WALTER and A. WINTERSTEIN, Helv. chim. Acta *42*, 864 (1959).

[21] O. ISLER and P. SCHUDEL, in *Advances in Organic Chemistry, Methods and Results* (Eds. R. A. RAPHAEL, E. C. TAYLOR and H. WYNBERG; Interscience, New York 1963), vol. 4, p. 115.

[22] O. ISLER, R. RÜEGG and U. SCHWIETER, J. pure appl. Chem. *14*, 245 (1967).

[23] B. C. L. WEEDON, J. pure appl. Chem. *14*, 265 (1967).

[24] S. LIAAEN-JENSEN and A. JENSEN, in *Progress in the Chemistry of Fats and Other Lipids* (Ed. R. T. HOLMAN; 1965), vol. 8, part 2, p. 133.

[25] O. ISLER, R. RÜEGG and P. SCHUDEL, Chimia *15*, 208 (1961). – O. ISLER, Chim. Ind., Milano *49*, 1317 (1967).

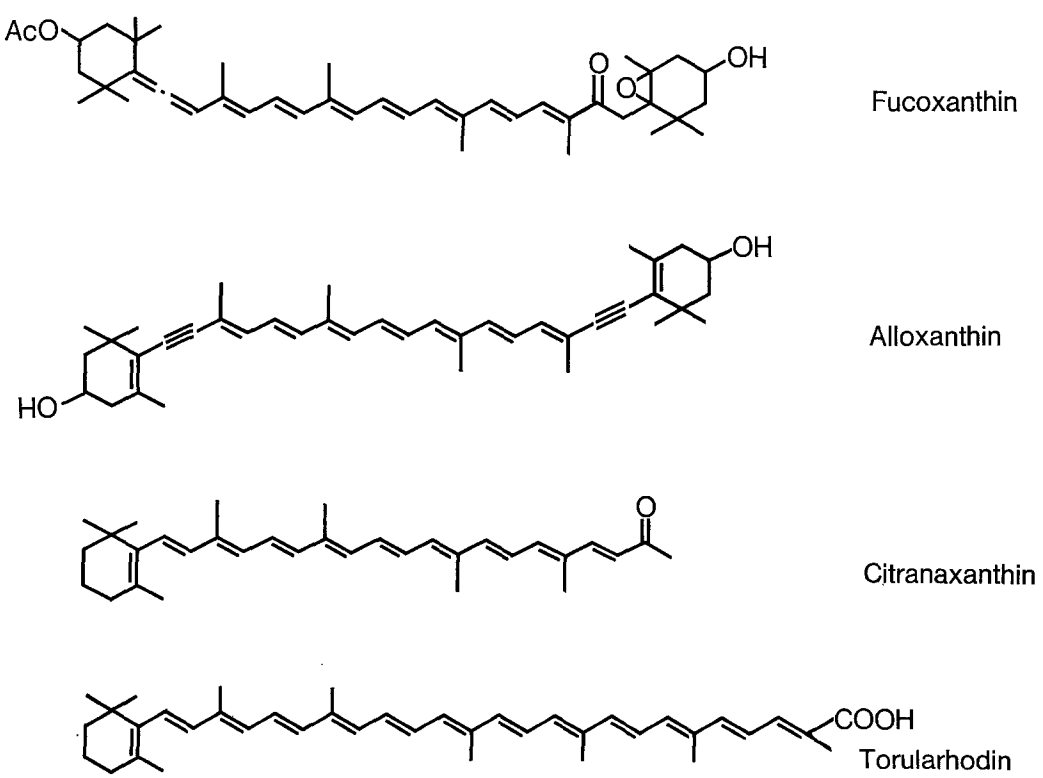

Fig. 4. Examples of novel carotenoid structures (WEEDON and co-workers, A. JENSEN, YOKOYAMA and WHITE, ISLER and collaborators)[17–20].

reacted in a Nef reaction to give methylbutynol, which is partially hydrogenated to methylbutenol. Methylbutenol is treated with isopropenyl methyl ether to yield methylheptenone, which gives dehydrolinalool in a Nef synthesis with acetylene. The latter is converted to pseudoionone in a reaction using again isopropenyl ether. Pseudoionone gives β-ionone on acid-catalyzed cyclization. β-Ionone is transformed into β-C_{14}-aldehyde by glycidic ester synthesis. The carbon chain is successively elongated by vinyl ether synthesis to β-C_{16}-aldehyde and then by propenyl ether synthesis to β-C_{19}-aldehyde.

$C_x+C_y+C_x=C_{40}$	Reaction type
$C_{19}+C_2+C_{19}$	Grignard
$C_{18}+C_4+C_{18}$	Grignard 3 Wittig
$C_{16}+C_8+C_{16}$	Grignard or Nef
$C_{15}+C_{10}+C_{15}$	Wittig
$C_{14}+C_{12}+C_{14}$	Grignard or Nef Enol ether condensation
$C_{13}+C_{14}+C_{13}$	Wittig
$C_{10}+C_{20}+C_{10}$	Wittig Aldol condensation
$C_5+C_{30}+C_5$	Robinson-Mannich base synthesis

$C_x+C_y=C_{40}$	Reaction type
$C_{37}+C_3$	Enol ether condensation Wittig
$C_{35}+C_5$	Wittig Robinson-Mannich base synthesis
$C_{30}+C_{10}$	Wittig Aldol condensation
$C_{25}+C_{15}$	Wittig
$C_{21}+C_{19}$	Grignard or Nef
$C_{20}+C_{20}$	Grignard Wittig Wurtz Reductive dimerization

Fig. 5. Principal schemes for total synthesis of carotenoids[21].

Figure 8, taken from the publication of ISLER et al.[22], shows the use of β-C_{19}-aldehyde as intermediate for synthetic carotenes and carotenoids. The technical synthesis of canthaxanthin from β-carotene, as presently carried out by Hoffmann-La Roche, is actually based on KARRER's work[26].

For the synthesis of aliphatic carotenoids crocetindial, which comprises the 20 central carbon atoms of the carotenoid skeleton, has been a most important intermediate. It has been obtained by linking two C_{10}-units by a Wittig reaction or alternatively by chain extension of the symmetrical C_{10}-dialdehyde by vinyl and propenyl ether synthesis. Condensations with various C_{10}-Wittig compounds lead to the different carotenoids given in Figure 9[22].

A further survey of the many triumphs in recent total synthesis of carotenoids is beyond the scope of this paper.

The successful application of synthetic carotenoids as food colourants is an excellent example of applied carotenoid research[22,25].

5. *Carotenoids of photosynthetic bacteria*

Selecting some examples of what may be considered the after-crop of PAUL KARRER's contribution, namely problems taken up by KARRER or otherwise related to KARRER's work and subsequently pursued in our laboratory during the last decade, first the carotenoids of photosynthetic bacteria shall be mentioned.

Studies on the carotenoids of this class of organisms were started by KARRER around 1934[27]. The carotenoids of all 41 species of photosynthetic bacteria available in pure culture have now been studied. More than 40 different carotenoids have been isolated, and unequivocal or plausible structures are ascribed to 39 of these. Since KARRER's early studies were made on a culture containing *Thiorhodaceae* species, we shall consider the characteristic carotenoids of this family. The

[26] R. ENTSCHEL and P. KARRER, Helv. chim. Acta *41*, 402, 983 (1958).

[27] P. KARRER and co-workers, Helv. chim. Acta *18*, 1306 (1935); *19*, 3, 19 (1936); *21*, 454 (1938); *23*, 460 (1940); *43*, 181 (1960).

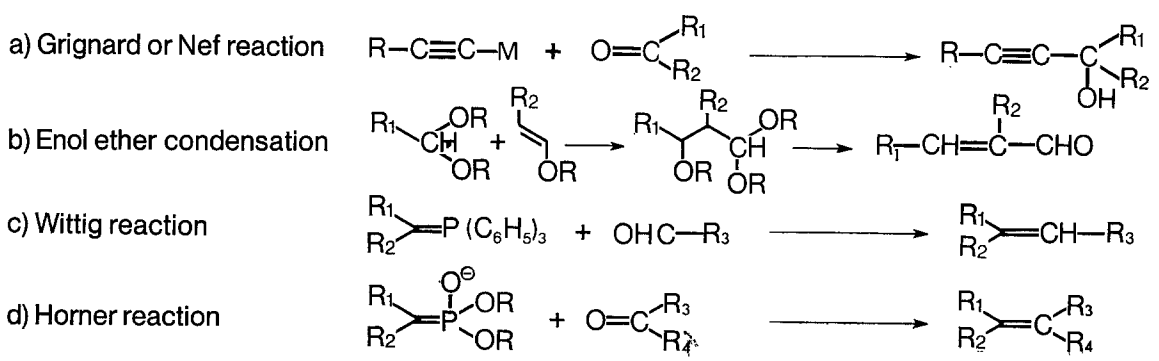

Fig. 6. Main reactions used in total synthesis of carotenoids.

purple sulphur bacteria produces 3 types of carotenoids[28], either (1) of the so-called normal spirilloxanthin series[29], (2) okenone[30] or (3) carotenoids of the rhodopinal type[31].

Figure 10 gives the sequence established by biosynthetic experiments of the first series[29]. The carotenoids involved are aliphatic ones with a long polyene chain containing 11–13 conjugated double bonds and with tertiary methoxy or hydroxy substituents. The di-methoxylated end product spirilloxanthin or rhodoviolascin was characterized independently and simultaneously by KARRER's[27] and VAN NIEL's schools[32].

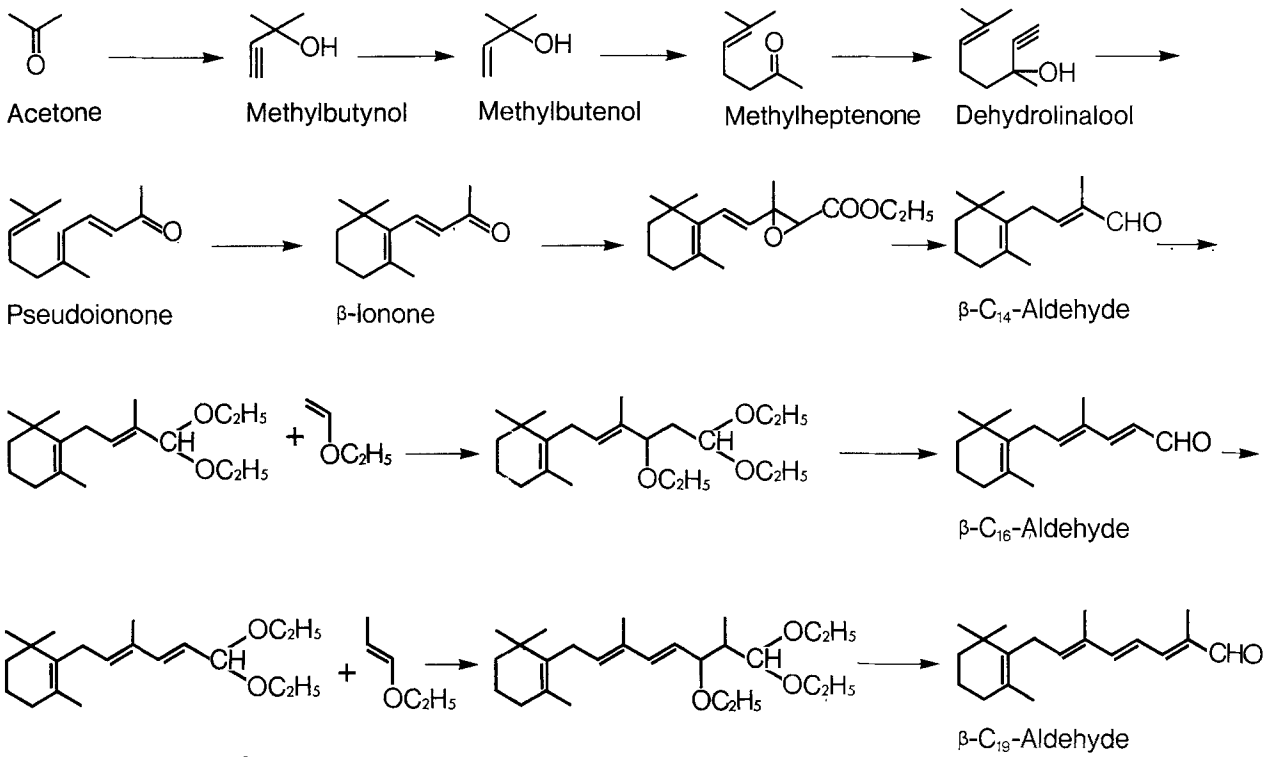

Fig. 7. Total synthesis of β-C_{19}-aldehyde (ISLER et al.[25]).

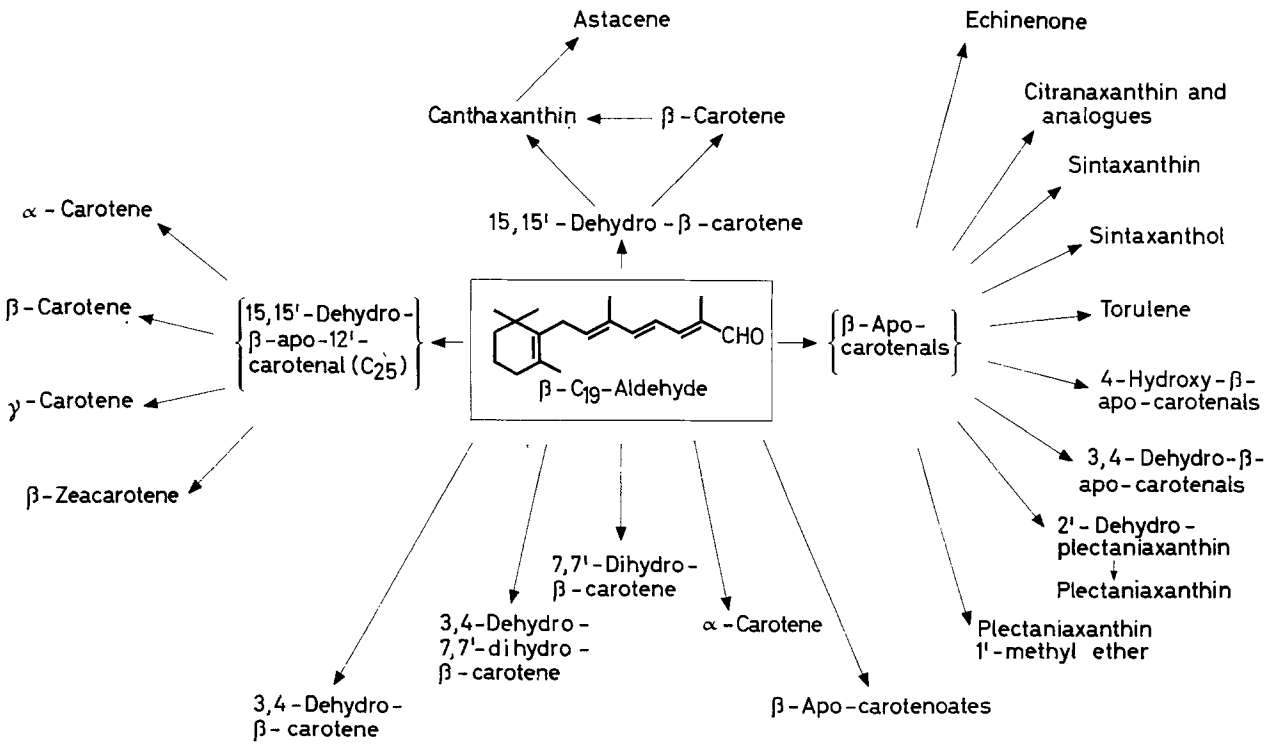

Fig. 8. β-C_{19}-aldehyde as intermediate for synthetic carotenoids[22].

Rhodopin, a hydrated lycopene, and rhodovibrin with dodecaene chromophore, one methoxy and one hydroxy substituent, were also first described by KARRER's school[27].

Figure 11 gives the structure of okenone, the characteristic carotenoid of the second type. Its structure was established[30] on the basis of the spectroscopic properties of okenone itself and the derivatives okenol, okenol acetate and anhydro-okenol. The latter is the dehydration product obtained on treatment of okenol with acidified chloroform, a reaction introduced by KARRER[33]. The methyl and methylene proton signals in the PMR-spectrum of okenone are included in Figure 11. Signals at τ 7.71 (6 H) and 7.79 (3 H) demonstrated the presence of a trimethylphenyl end group, supported by the presence of 2 aromatic protons with identical chemical shift. According to IR data, the aromatic protons must occupy adjacent positions (absorption at 800 cm^{-1}). In order to account for the chromophore of okenone it was necessary to assume 1,2,3-trimethyl substitution of the phenyl group. 1,2,5-Trimethyl substitution would, because of steric conflict with the hydrogen in 8-position which prevents planarity of the phenyl group and the polyene chain, result in absorption maxima at shorter wavelengths in visible light.

The structure of okenone was confirmed by total synthesis[30] via the scheme outlined in Figure 12. Hemimellitene was converted to triphenyl-2,3,4-trimethylbenzylphosphonium bromide in known manner. The ylide of the phosphonium salt was condensed with crocetindial to the C_{30}-aldehyde in a Wittig reaction. In a subsequent Wittig condensation renieral was reacted with the methoxylated ylide to 4'-deoxo-okenone. An allylic acetoxy group was introduced by treatment with N-bromosuccinimide and glacial acetic acid in an ENTSCHEL-KARRER[26] reaction. Hydrolysis of the acetate to okenol and allylic oxidation with *p*-chloranil gave okenone. Synthetic okenone, okenol acetate and anhydro-okenol (which is a by-product in the NBS reaction) were in all respects identical with natural okenone and its corresponding derivatives.

Rhodopinal[31], previously called warmingone, is the characteristic representative of the third type of carotenoids encountered in the *Thiorhodaceae*. Rhodopinal has an unusual absorption spectrum in visible light. PMR-spectroscopy provided the clue to its structure. Mass-spectrometry gave further information[34],

[28] K. SCHMIDT, N. PFENNIG and S. LIAAEN-JENSEN, Arch. Mikrobiol. *52*, 132 (1965).
[29] S. LIAAEN-JENSEN, G. COHEN-BAZIRE and R. Y. STANIER, Nature *192*, 1168 (1961).
[30] A. J. AASEN and S. LIAAEN-JENSEN, Acta chem. scand. *21*, 961, 970 (1967).
[31] A. J. AASEN and S. LIAAEN-JENSEN, Acta chem. scand. *21*, 2185 (1967).
[32] C. B. VAN NIEL and J. H. C. SMITH, Arch. Mikrobiol. *6*, 219 (1935).
[33] P. KARRER and E. LEUMANN, Helv. chim. Acta *34*, 445 (1951).
[34] G. W. FRANCIS and S. LIAAEN-JENSEN, Acta chem. scand., to be published (1970).

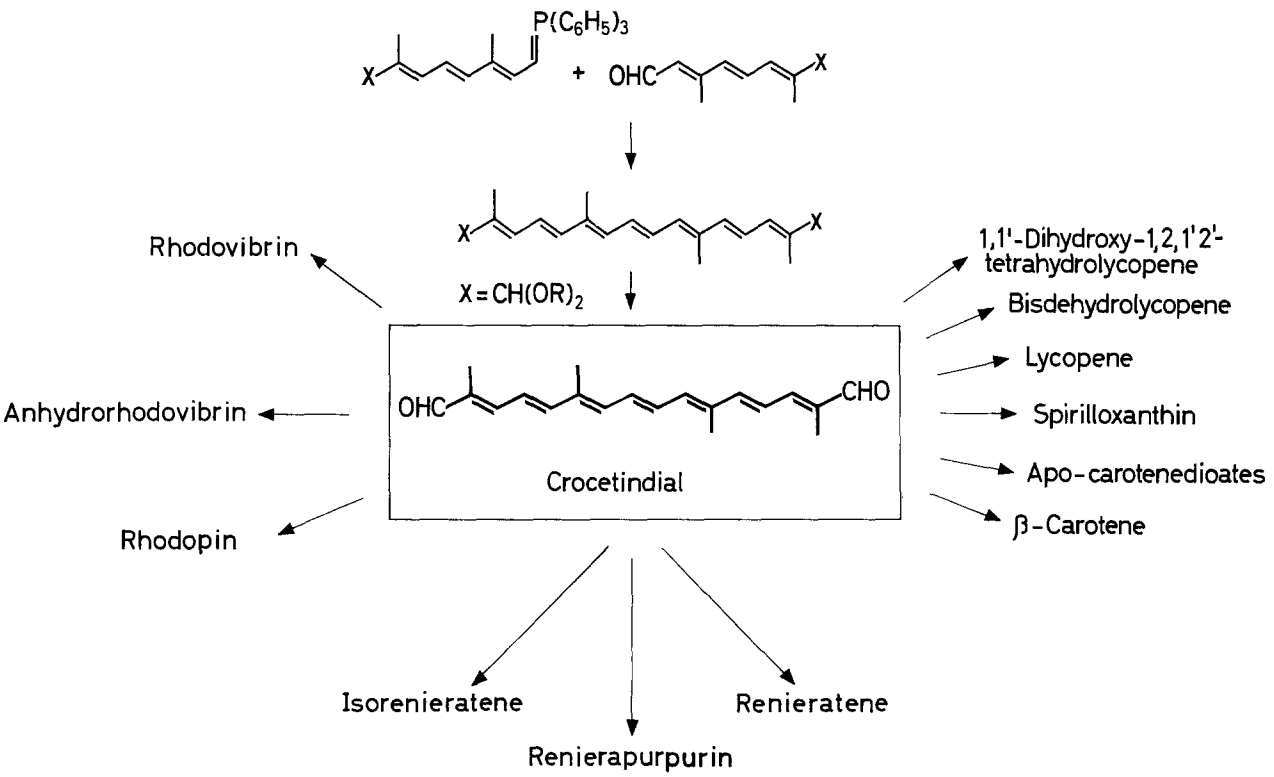

Fig. 9. Crocetindial as intermediate for synthetic carotenoids[32].

and in principle we now consider structures of this type as solved (Figure 13). They are cross-conjugated carotenals where one of the central lateral methyl groups is formally oxidized to an aldehyde. This has been found to cause *cis* configuration of the neighbouring carbon-carbon double bond. The relative position of the 2 end groups, when different, to the oxygen substituent on the polyene chain is not quite clear. Rhodopinal occurs together with the corresponding allylic alcohol rhodopinol, rhodopin and lycopene. Chemically rhodopinal

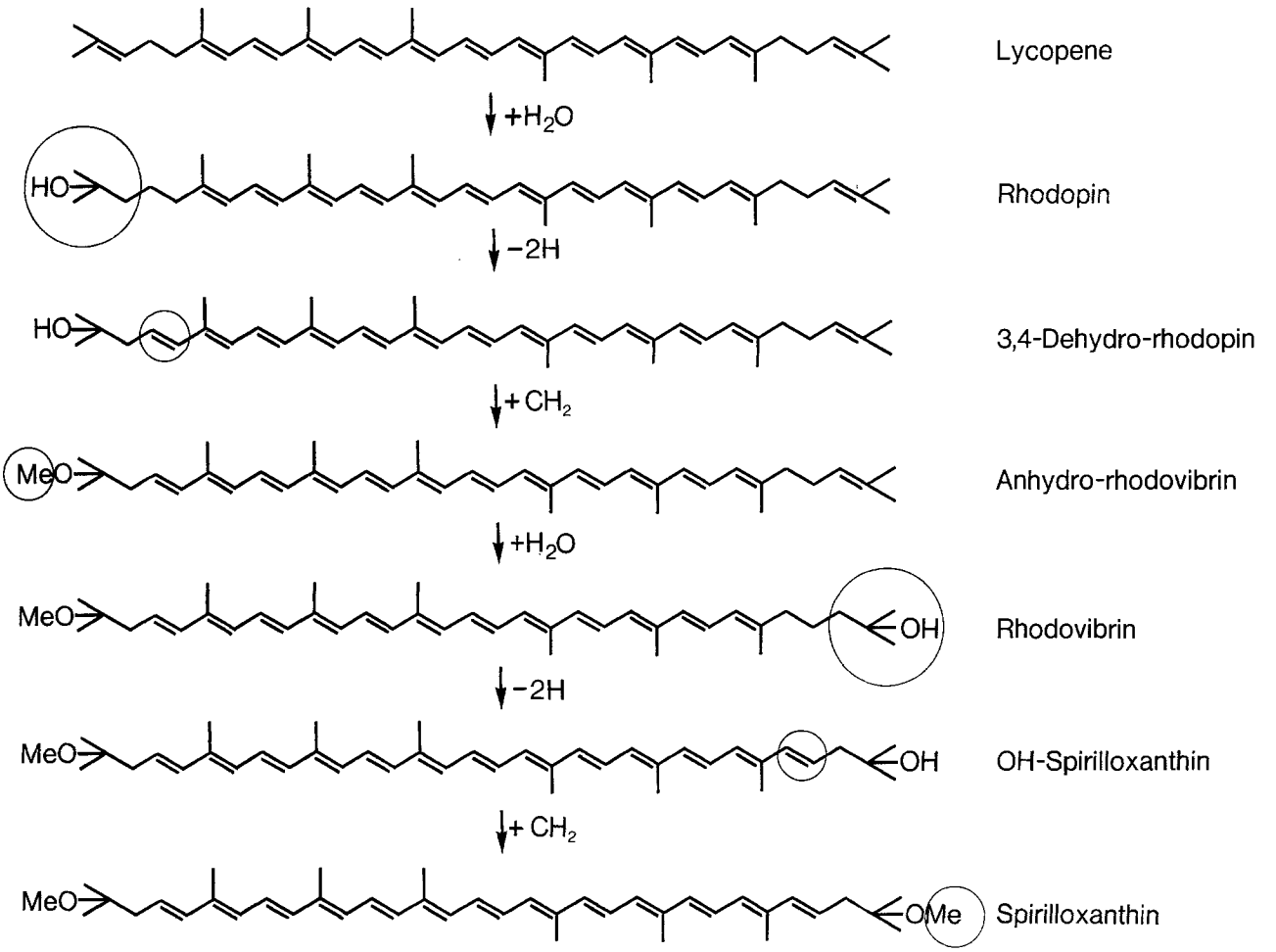

Fig. 10. Biosynthesis and structures of the normal spirilloxanthin series[29].

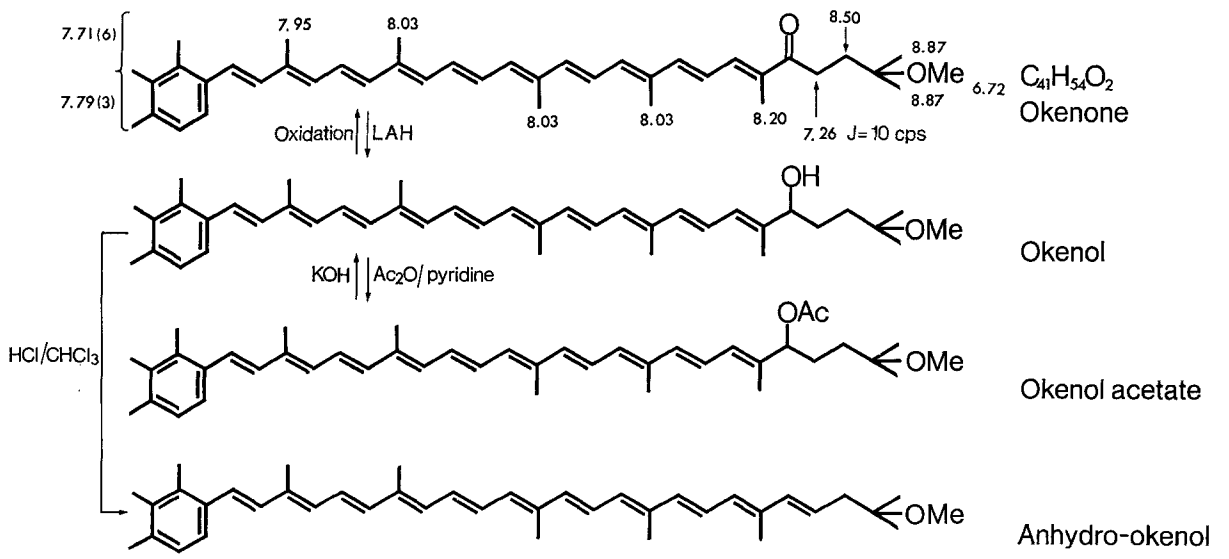

Fig. 11. Structure determination of okenone[30].

was reduced to rhodopinol by lithium aluminium hydride. Rhodopinol tosylate was further converted to rhodopin by hydride reduction, and rhodopin may in turn be dehydrated to lycopene by phosphorus oxychloride in pyridine. The chemical transformation sequence is thus the reverse analogue of the postulated biosynthetic sequence. We have also isolated the corresponding lycopenal[31], and the mono-[34] and dimethoxy[31] derivative. The latter occurred together with tetrahydrospirilloxanthin, the structure of which was proved by total synthesis[35].

6. *C_{50}-carotenoids*

Leaving the photosynthetic bacteria here, we shall turn to the new class of bacterial C_{50}-carotenoids. Until recently carotenoids were considered as compounds with C_{40}-skeletons or apo-carotenoids derived therefrom. The natural C_{50}-carotenoids bear some analogy to KARRER's synthetic decapreno-β-carotene[36] and 2,2'-dimethyl-ε-carotene[37].

Dehydrogenans-P439, isolated from *Flavobacterium dehydrogenans*, was the first representative of this class[38]. Mass-spectrometry showed the molecular composition $C_{50}H_{72}O_2$. Acetylation gave a diacetate which accounted for the 2 oxygen functions as primary or secondary hydroxy groups. A singlet at τ 5.98, integrating for 4 protons, in the PMR-spectrum, suggested that the hydroxy groups were primary and allylic. This was confirmed by selective oxidation with nickel peroxide which gave α,β-unsaturated mono- and dialdehydes with absorption spectra in visible light corresponding to P439 itself. Hence the allylic hydroxy groups were not allylic to the polyene chain. Initially it was tempting to suspect this C_{50}-carotenoid to be a pentaterpene with linearly extended isoprenoid chain analogous to KARRER's synthetic model C_{50}-carotenoid[36]. However, such a structure would require only 12 lateral methyl groups, and the PMR-spectrum revealed the presence of 12 tertiary methyl groups in addition to the primary hydroxy functions considered to be derived from methyl groups. Hence the molecule possessed a more branched structure.

[35] A. J. AASEN and S. LIAAEN-JENSEN, Acta chem. scand. *21*, 371 (1967).
[36] P. KARRER and C. H. EUGSTER, Helv. chim. Acta *34*, 28, 1805 (1951).
[37] C. H. EUGSTER, A. H. TRIVEDI and P. KARRER, Helv. chim. Acta *38*, 1359 (1955).
[38] S. LIAAEN-JENSEN and co-workers, Norwegian J. Chem. Mining, Met. *26*, 130 (1966); Acta chem. scand. *21*, 1972 (1967); *22*, 1171 (1968).

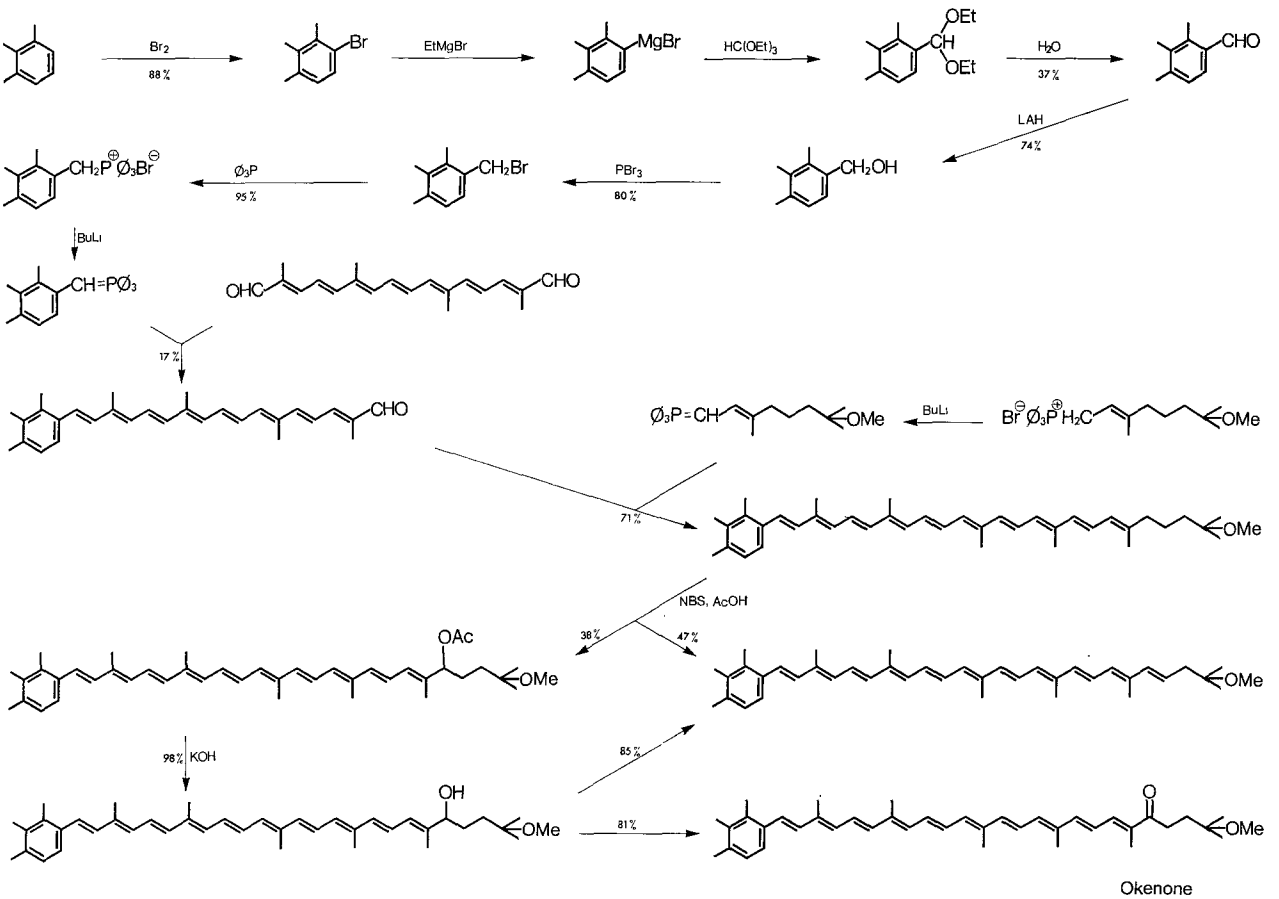

Fig. 12. Synthesis of okenone[30].

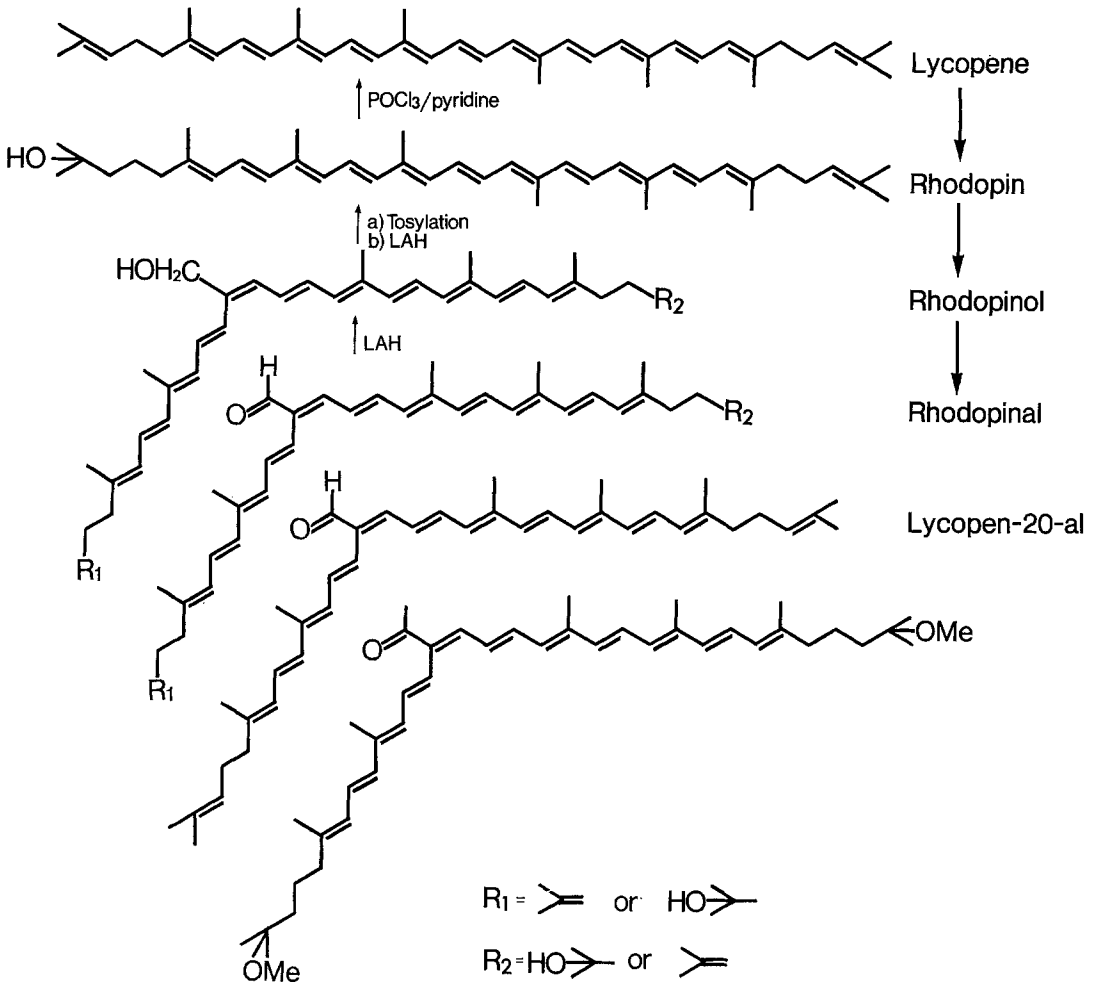

Fig. 13. Structures of the rhodopinal series[31,34].

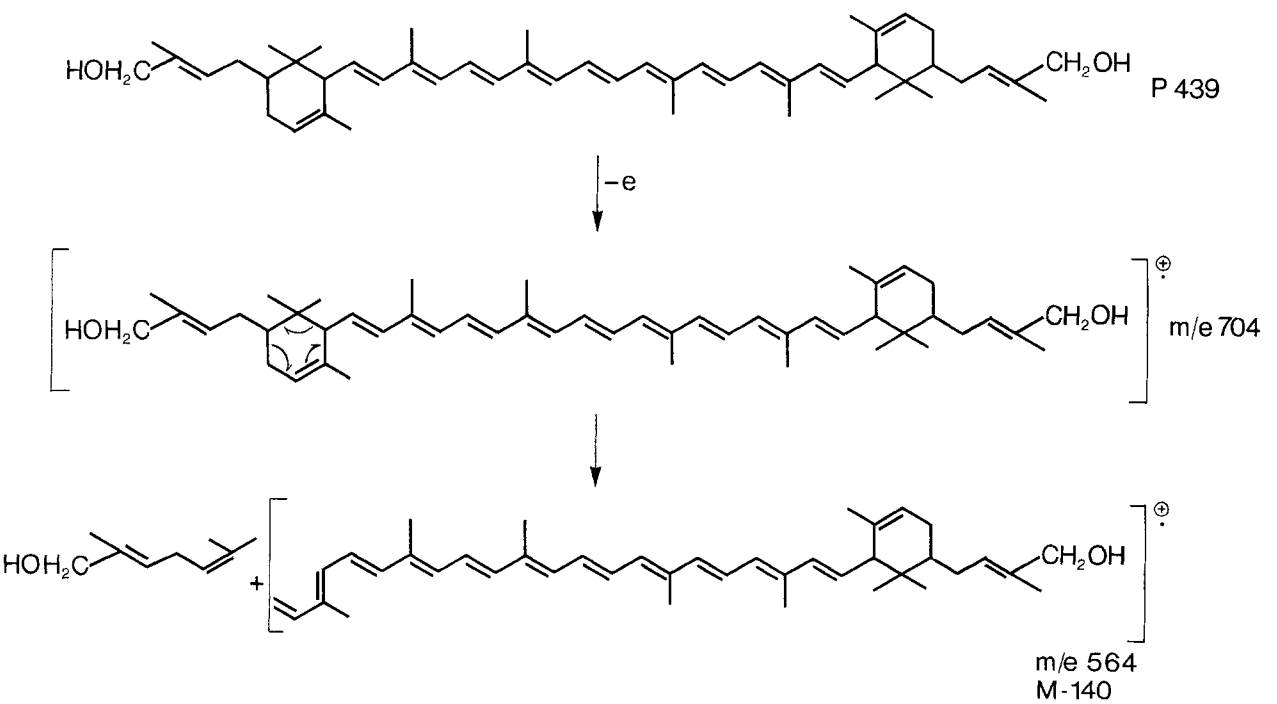

Fig. 14. Structure of *dehydrogenans*-P439[38].

We have now ascribed the structure given in Figure 14 to P439. Carotenoids containing unsubstituted α-rings undergo retro-Diels-Alder fragmentation on electron impact[7]. Loss of 140 mass units from the molecular ion of P439 is explained in analogous manner and supports addition of the extra C_5-units to 2,2'-positions as well as localization of the hydroxy group in the extra C_5-unit. Ozonolytic isopropylidene determination was negative, in accordance with this structure. Two α-rings and the symmetrical nonaene chromophore agree with the PMR-spectrum. The chemical shift position of the aldehyde protons of P439-dialdehyde, compared with that of related *cis* and *trans* α,β-unsaturated aldehydes, supports the given *trans* configuration[39].

Biosynthetically (Figure 15) the P439 skeleton could be formed by addition of isopentenyl pyrophosphate units to the isopropylidene double bond in a C_{40}-skeleton and subsequent cyclization. Elimination of a proton would give the α-ring characteristic of P439. We suspect some other bacterial carotenoids to possess a related end group with β-ring, that is the double bond in 5,6-position. The same line of argument leads to the revised structure for bacterioruberin, if one assumes addition of OH^- to the tertiary carbonium ion and subsequent introduction of a double bond and hydration of the isopropylidene double bond. Bacterioruberin is a tridecaene-tetraol with C_{50}-skeleton[40]. In fact, all

[39] U. SCHWIETER and S. LIAAEN-JENSEN, Acta chem. scand. *23*, 1057 (1969).

[40] S. LIAAEN-JENSEN and M. KELLY, Acta chem. scand. *14*, 950, 953 (1960); *22*, 2578 (1968).

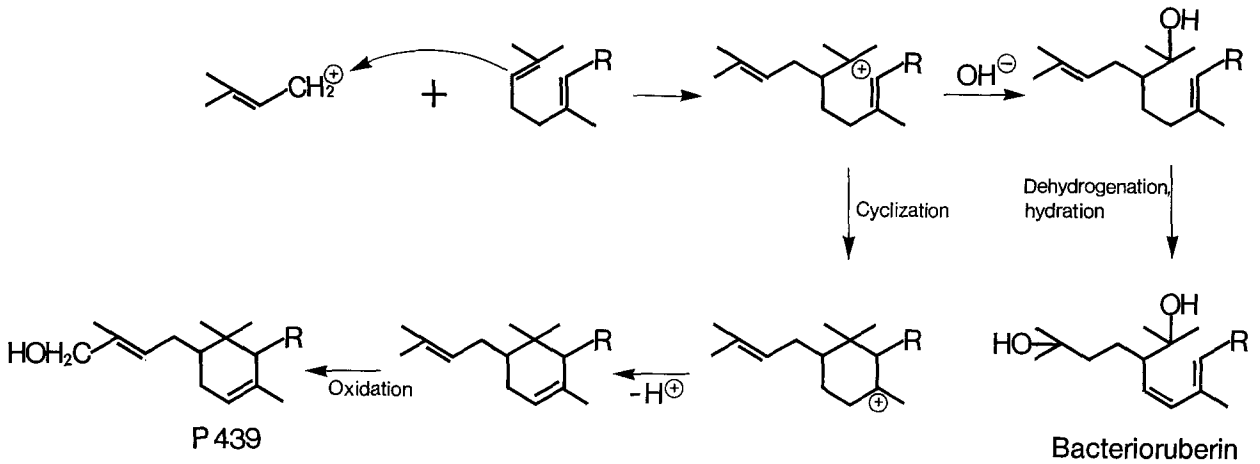

Fig. 15. Postulated biosynthesis of C_{50}-carotenoids.

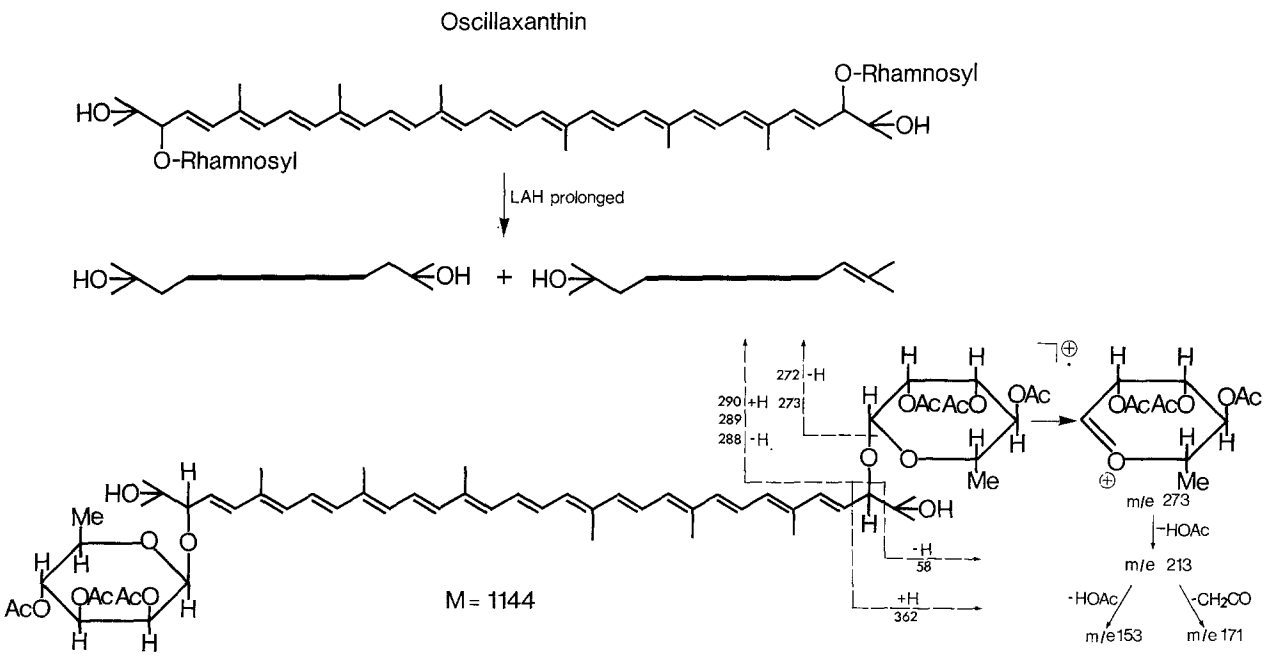

Fig. 16. Structure of oscillaxanthin[44].

C_{50}-carotenoids hitherto studied in our laboratory appear to have the 2 extra C_5-units attached in 2,2'-positions[41].

7. *Glycosidic carotenoids*

The next examples concern glycosidic carotenoids and hence bear a relationship to KARRER's crocin[42]. The compound to be discussed in some detail, oscillaxanthin from the blue-green alga *Oscillatoria rubescens*, was first isolated and named by KARRER[43]. Oscillaxanthin was recently shown to be the di-rhamnoside of a tridecaene-tetraol (Figure 16)[44].

Glycoside hydrolysis gave reducing rhamnose. Quick hydride reduction of its hexaacetate gave oscillaxanthin, whereas prolonged treatment with lithium aluminium hydride in an unusual reaction resulted in elimination of the sugar moiety and gave 2 less polar products, one of which was identified with the tridecaene-diol and one had properties compatible with the tetradecaen-ol structure given in Figure 16.

The electron-beam induced fragmentations of diagnostic value observed for the hexaacetate are indicated on the same Figure. Mass-spectra of acetylated carotenoid glycosides are particularly informative, since acetylation leads to preferential oxonium ion formation on cleavage of the glycoside bond and hence indicates the nature of the sugar unit – in this case a methylpentose.

The PMR-spectrum of oscillaxanthin hexaacetate (Figure 17) is in agreement with the structure indicated – the methyl doublet of the rhamnoside is seen at τ 8.93 and the *gem.* methyl groups are magnetically non-equivalent due to molecular asymmetry of the neighbouring carbon atom. Assuming that oscillaxanthin is an L-rhamnoside a comparison of the protow of triacetylated methyl α- and β-L-rhamnoside and magnetic resonance data of oscillaxanthin hexaacetate appears to be best accommodated with oscillaxanthin being a di-β-L-rhamnoside with 4 heavy equatorial substituents including the polyene chain.

Myxoxanthophyll, another carotenoid studied by KARRER[51,43], accompanies oscillaxanthin in blue-green algae and has recently been ascribed a related structure[48] (Figure 18), again a rhamnoside with one end group common with oscillaxanthin, but a monocyclic aglycone with a 3-hydroxylated β-ring. Other examples of new glycosidic carotenoids are included in Figure 18. Phlei-xanthophyll and 4-keto-phlei-xanthophyll are

[41] S. LIAAEN-JENSEN, J. pure appl. Chem. *17* (1969), in press (1970).
[42] P. KARRER and co-workers, Helv. chim. Acta *12*, 790 (1929); *13*, 268 (1930); *14*, 619 (1931); *15*, 492 (1932). – Arch. Sci. biol. *18*, 36 (1936).
[43] P. KARRER and J. RUTSCHMANN, Helv. chim. Acta *27*, 1691 (1944).
[44] S. HERTZBERG and S. LIAAEN-JENSEN, Phytochem. *8*, 1281 (1969).
[45] P. KARRER and H. SALOMON, Helv. chim. Acta *10*, 397 (1927); *11*, 513, 711 (1928); *16*, 643 (1933).
[46] P. KARRER and co-workers, Helv. chim. Acta *12*, 985 (1929); *13*, 392 (1930); *15*, 1218, 1399 (1932); *16*, 297 (1933).
[47] R. KUHN and co-workers, Helv. chim. Acta *11*, 716 (1928); *12*, 64 (1929); Chem. Ber. *64*, 1732 (1931).
[48] S. HERTZBERG and S. LIAAEN-JENSEN, Phytochem. *8*, 1259 (1969).

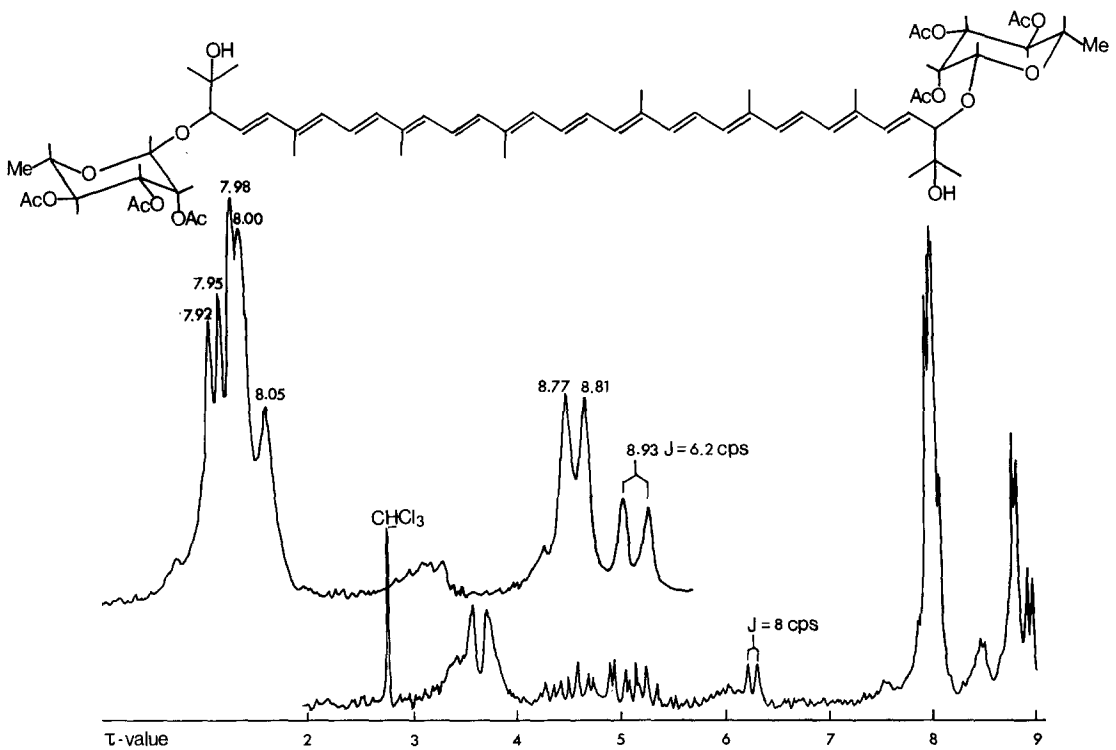

Fig. 17. PMR-spectrum of oscillaxanthin hexaacetate.

tertiary β-D-glucosides[49] and the methyl apo-lycopenoate appears to be a mannoside[50]. The 3 last-mentioned glycosides are of bacterial source.

The last example presents the latest extension of the carotenoid conception. By the previous definition carotenoids were yellow or red pigments[1], but we must now also include blue ones.

8. *Nor-carotenoids*

The carotenoid to be discussed is actinioerythrin[53] from the sea anemone *Actinia equina*. We now consider actinioerythrin to be a 2,2'-bisnorastaxanthin diester with C_{38}-skeleton, and thus the first example of a nor-carotenoid with ring contraction. The structure determination is published elsewhere[52], and the structures of actinioerythrin and its blue alkali conversion product violerythrin are given in Figure 19. Chemical reactions and spectral data (visible light, infrared, proton magnetic resonance and mass) are in agreement with the structures given. The blue violerythrin is the 2,2'-bisnor derivative of astacene, studied by KARRER[54], and the 5-ring structural element in carotenoids was first recognized by KARRER[55] in capsanthin.

[49] S. HERTZBERG and S. LIAAEN-JENSEN, Acta chem. scand. *21*, 15 (1967).
[50] A. J. AASEN, G. W. FRANCIS and S. LIAAEN-JENSEN, Acta chem. scand. *23*, 2605 (1969).
[51] I. M. HEILBRON and B. LYTHGOE, J. chem. Soc. *1936*, 1376.
[52] S. LIAAEN-JENSEN and co-workers, Acta chem. scand. *22*, 1714 (1968); *23* (1969), in press.
[53] E. LEDERER, C. r. Soc. Biol. *113*, 1391 (1933). – I. M. HEILBRON, H. JACKSON and R. N. JONES, Biochem. J. *29*, 1384 (1935).
[54] P. KARRER and co-workers, Helv. chim. Acta *17*, 412, 745 (1934); *18*, 96 (1935); *19*, 479 (1936).
[55] P. KARRER and co-workers, Helv. chim. Acta *43*, 89 (1960); *44*, 1257, 1904 (1961); *47*, 741 (1964).

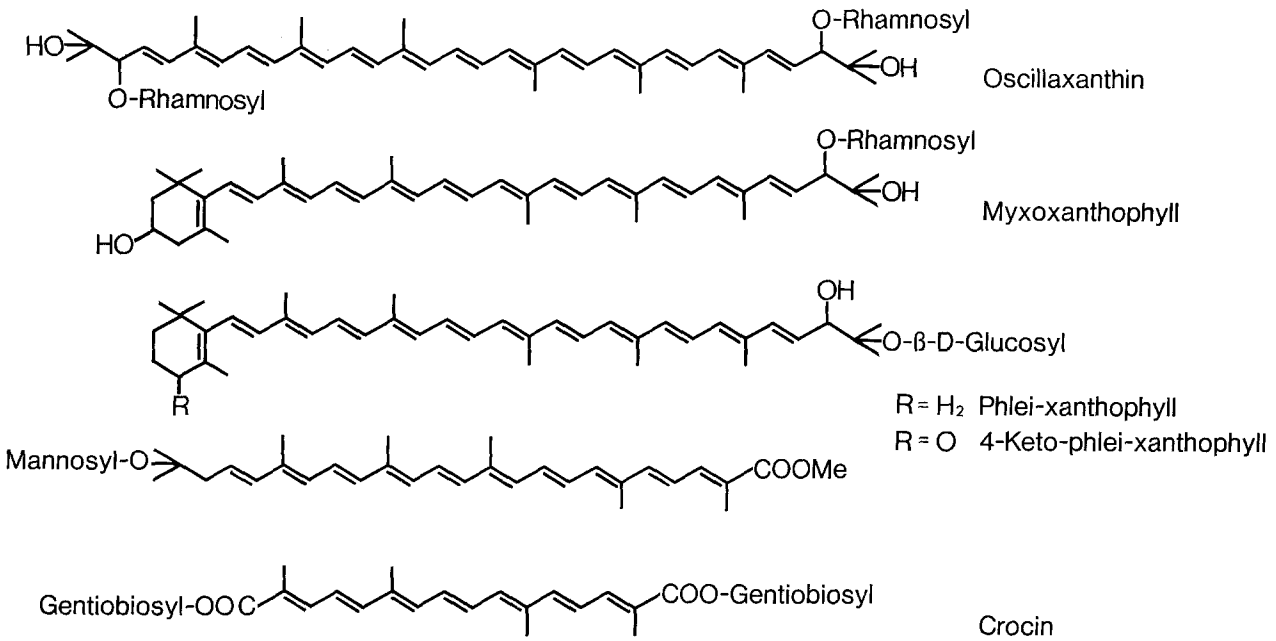

Fig. 18. Known carotenoid glycosides[45–50].

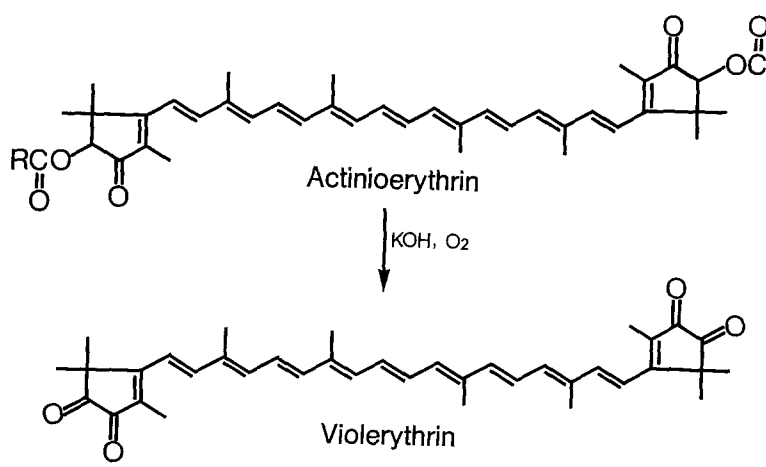

Fig. 19. Structures of actinioerythrin and violerythrin[52].

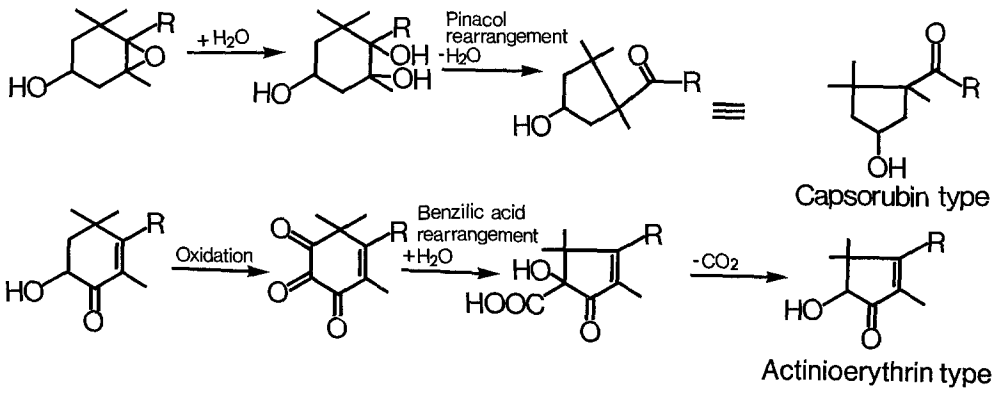

Fig. 20. Postulated biosynthesis of 5-ring type carotenoid end groups[52,55].

Concerning the formation of carotenoids with 5-ring end groups, KARRER[55] assumed that in the case of the capsorubin end group a pinacol rearrangement of an intermediary α-glycol was responsible for the in vivo synthesis from violaxanthin (Figure 20). We suspect that actinioerythrin is formed in vivo from a hypothetic 6-ring triketone by ring contraction (benzilic acid rearrangement) and subsequent decarboxylation. Small amounts of astaxanthin diesters occur together with actinioerythrin in *A. equina*[52].

The last example is typical. So many of the present problems in carotenoid chemistry are somehow linked to PAUL KARRER's work. Every carotenoid chemist has the greatest respect and admiration for his contribution to a field that is continuing to develop on a solid fundament of his work.

Zusammenfassung. Nach einleitender kurzer Würdigung der Höhepunkte der KARRER'schen Carotinoid-Forschung wird zunächst ein knapper Überblick über den gegenwärtigen Stand der Carotinoid-Forschung gegeben. Die jetzige Kenntnis von Biosynthese und Funktion der Carotinoid-Pigmente wird kurz und die organisch-chemischen Aspekte werden etwas ausführlicher erläutert, und zwar Strukturaufklärung einschliesslich neuer Trennmethoden, unentbehrliche spektroskopische Methoden und neue Reaktionen zur Herstellung von Derivaten sowie schliesslich die Totalsynthese.

Anschliessend werden einige ausgewählte Beispiele der Nachlese KARRER'scher Beiträge behandelt, nämlich Probleme, die von KARRER aufgegriffen wurden oder auf andere Weise mit KARRER's Arbeit verbunden sind und später im Laboratorium der Autorin weiter verfolgt wurden. Beispiele von Carotinoiden aus photosynthetischen Bakterien, C_{50}-Carotinoiden, glycosidischen Carotinoiden und Nor-carotinoiden mit Ringverengung werden diskutiert.

SPECIALIA

Les auteurs sont seuls responsables des opinions exprimées dans ces brèves communications. – Für die Kurzmitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed in the authors' brief reports. – Ответственность за короткие сообщения несёт исключительно автор. – El responsable de los informes reducidos, está el autor.

Shock Effects on Plants: Oxygen Evolution of Elodea

A fast-rising pressure pulse, produced as an air blast in a shock tube, inhibited photosynthesis in Elodea 27% after a 20 ψ (pounds per square inch) exposure, 44% after a 40 ψ exposure, and 74% after a 50 ψ exposure (1.21, 2.81, and 3.02 kg/cm² respectively).

The present work stems from an interest in the possibilities for using plants as experimental bio-indicators of underground shock[1–3]. The shock used, defined as a fast-rising pressure pulse of air lasting several seconds, was produced by specially built shock tubes[3]. Since the efficiency of photosynthesis is a good indicator of the oxygen concentration in the immediate atmosphere of the plants[4,5], oxygen evolution in Elodea was measured for 3 shock levels, namely 20, 40 and 50 pounds per square inch (ψ; 1.21, 2.41 and 3.02 kg/cm² respectively).

Materials and methods. An air loader, previously described[6], was used for developing air blasts. The pressure duration for each shock level was approximately 4 sec. Photosynthetic rates were estimated by means of an Audus microburette which measured the amount of oxygen that was produced[7]. Healthy, green, unfractured plants of Elodea were used for both control and test plants. Plants that were fractured by the shock stimulus were discarded. The plants to be shocked were wrapped