STRUCTURAL ELUCIDATION OF CAROTENOIDS— A PROGRESS REPORT

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ABSTRACT

Selected topics of the research carried out in the author's laboratory during the past three years are surveyed.

Specific examples of the structural elucidation of new carotenoids are discussed. These include carotenes with terminal methylene, cyclic 2-hydroxy-carotenoids, C_{50}-carotenoids, several glycosides, cross-conjugated carotenals and C_{43}-artefacts thereof, and nor-carotenoids such as roserythrin and peridinin.

Some progress in stereochemistry is discussed: the stereochemistry of carotenoid glycosides, of lycoxanthin and stereochemical conclusions based on circular dichroism are considered.

General results in applied spectroscopy are reviewed, including the use of electronic absorption spectroscopy at liquid nitrogen temperature, general fragmentation mechanisms in mass spectrometry and the application of lanthanide proton magnetic resonance shift reagents to the carotenoid field.

Included in these topics are total syntheses of several carotenes (also deuterated ones) and of lycoxanthin and lycophyll.

SPECIFIC EXAMPLES OF STRUCTURAL ELUCIDATION

Carotenoids with terminal methylene

Terminal methylene groups have been considered to be rare structural elements in the carotenoid field, in contrast to the situation in other terpenoid classes. Until recently the fungus *Aleuria aurantia*, containing aleuriaxanthin (*Figure 1*), was the only known natural source of such carotenoids[1, 2]. The preference for 2′-hydroxy substitution[3] needs confirmation. Aleuriaxanthin together with the partially synthetic carotene[4], obtained as a minor product on dehydration of the tertiary mono-ol (*Figure 1*), were until recently the only carotenoids with terminal methylene that had been described. The cyclic end-group that is involved is not included in the new carotenoid nomenclature rules[5], and we have taken the liberty of denoting it γ[6] by analogy with γ-ionone.

In his thesis, Arpin[7] described a new carotene from the discomycete *Caloscypha fulgens* which represented 8 per cent of the total carotenoids. This carotene exhibited an electronic absorption spectrum identical with that of β,ε-carotene, but with a somewhat lower R_f value. This new carotene

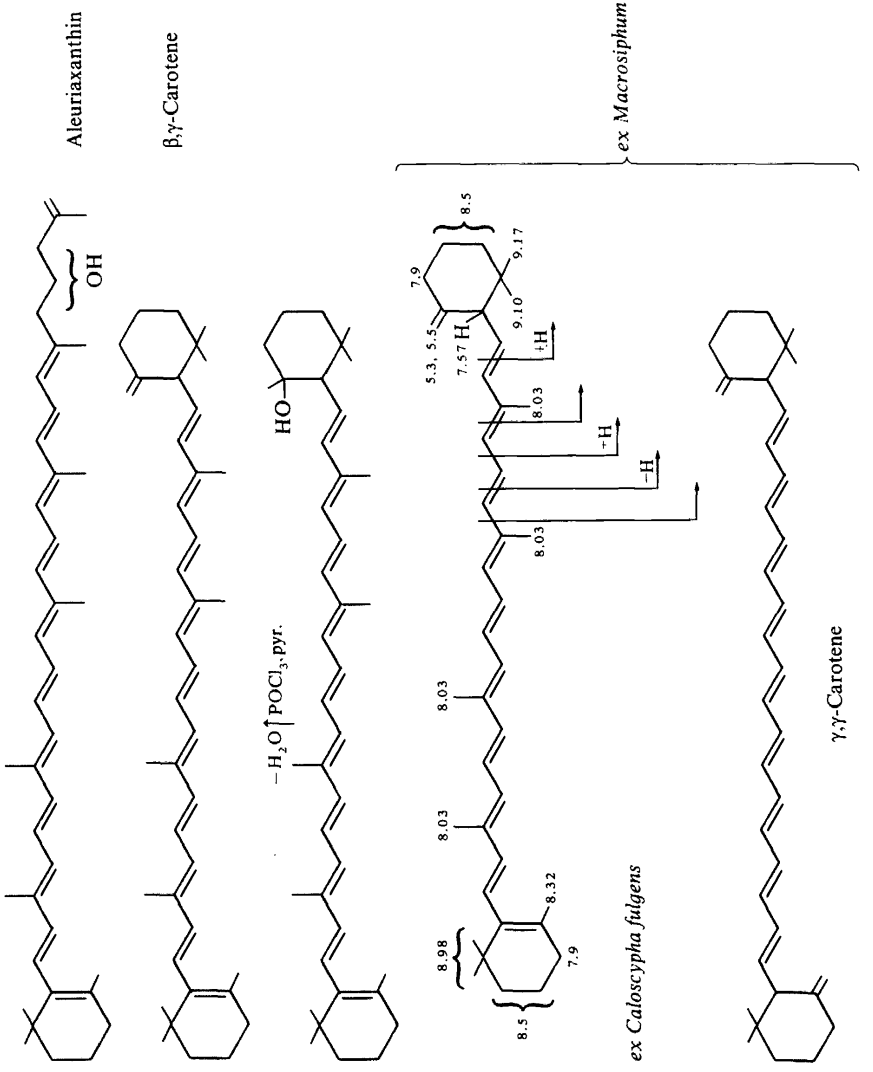


Figure 1. Carotenoids with terminal methylene groups

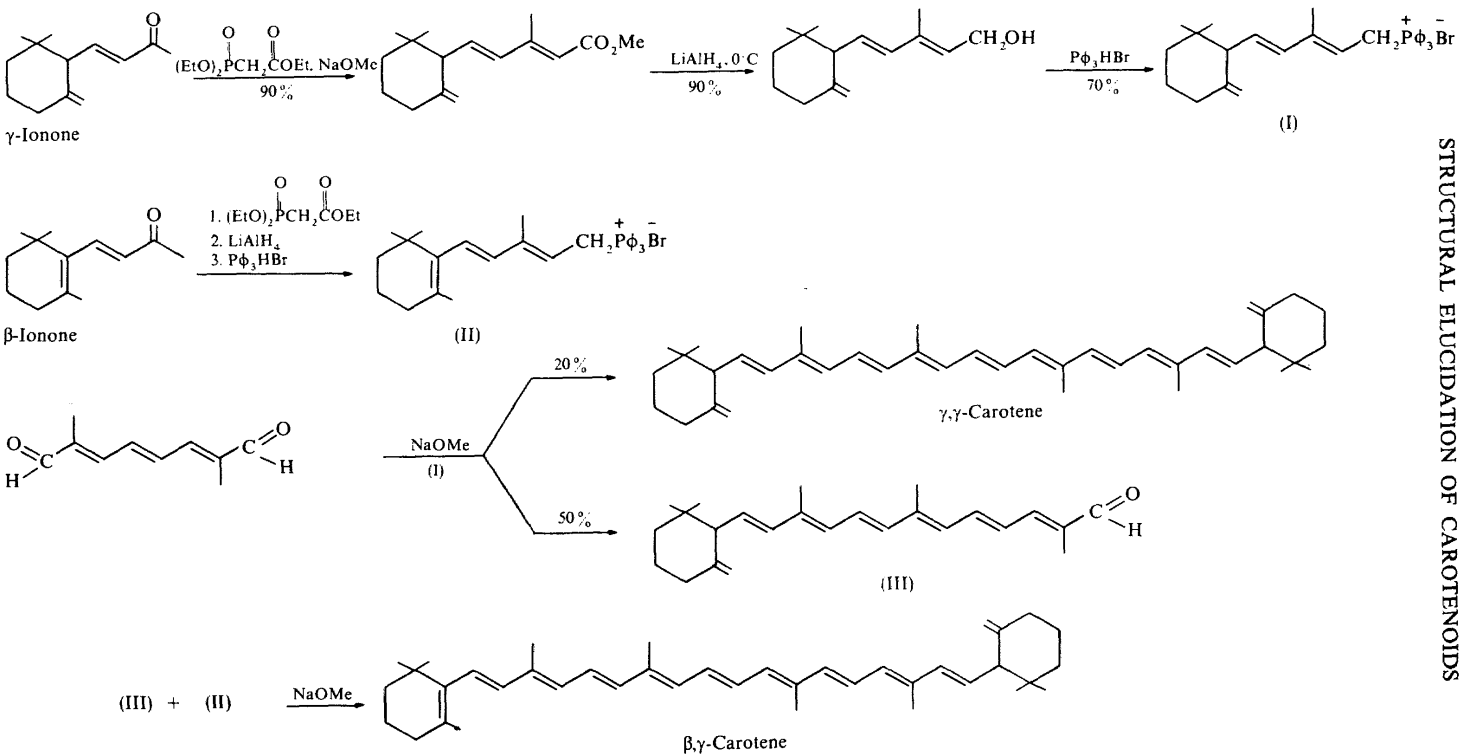


Figure 2. Total synthesis of β,γ-carotene and γ,γ-carotene

was subsequently assigned the same structure (disregarding stereochemistry) as the semi-synthetic carotene, on the basis of full spectral characterization[6]. The positions of the p.m.r. signals are included on the structure (*Figure 1*). A double doublet at τ 5.3, 5.5 and relatively strong i.r. absorption at 889 cm^{-1} revealed the terminal methylene group, and a doublet at τ 7.57 (1H, $J = 8$ Hz) was attributed to the methine proton at C-6′. Cleavages observed on electron impact, in addition to the common in-chain eliminations are also indicated.

This same carotene was later found, in collaboration with Dr. Weiss' group in Bethesda, in the green variety of the aphid *Macrosiphum liriodendri* Another new carotene present in this aphid was tentatively identified as γ,γ-carotene[8,9].

Total synthesis of optically inactive β,γ-carotene and γ,γ-carotene by Andrewes[10], starting from synthetic γ-ionone, by the scheme outlined in *Figure 2* subsequently confirmed both assignments[9]. The carbon chain was elongated in a Horner reaction, and the carbomethoxy group reduced to the corresponding allylic alcohol and converted to the phosphonium salt, which in a Wittig reaction gave γ,γ-carotene. β,γ-Carotene was obtained by an analogous sequence; β-ionone providing the β-end of the molecule.

The stereochemistry at C-6 (6′) in the naturally occurring carotenes (they are optically active[11]) is not yet defined. With the methods now available isolation of further carotenoids with terminal methylene is anticipated.

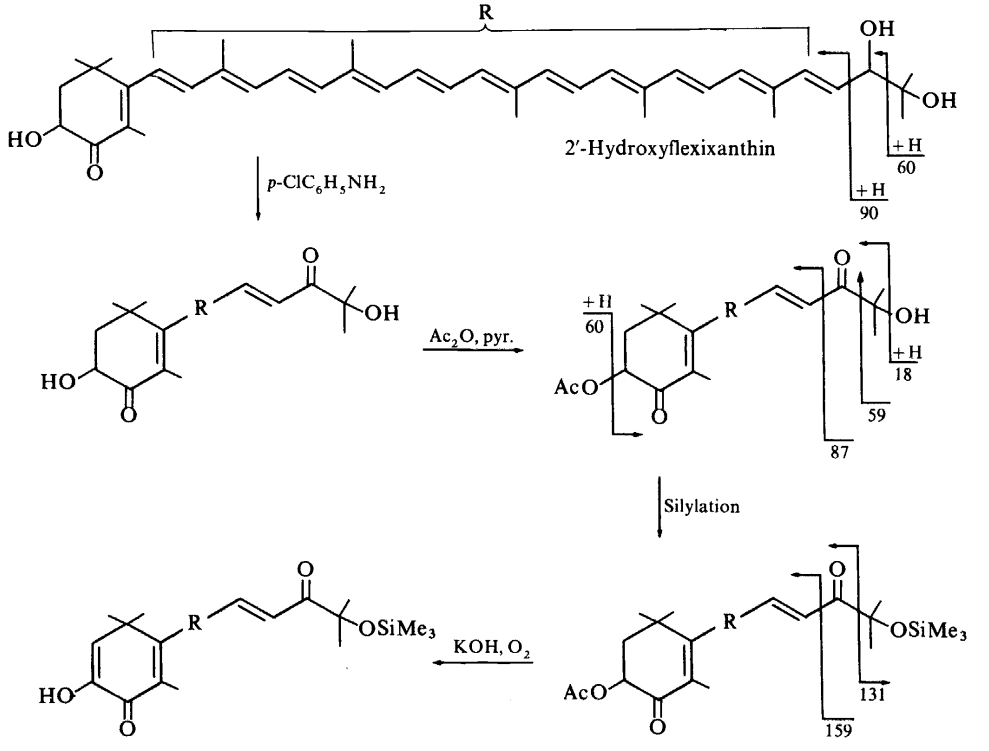


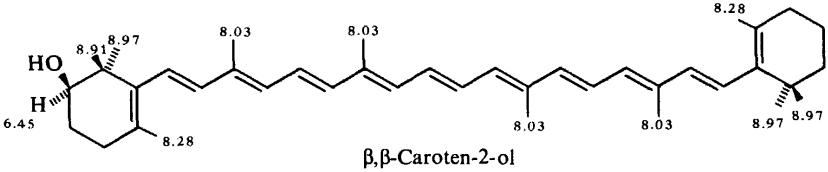
Figure 3. 2′-Hydroxyflexixanthin

2-Hydroxycarotenoids

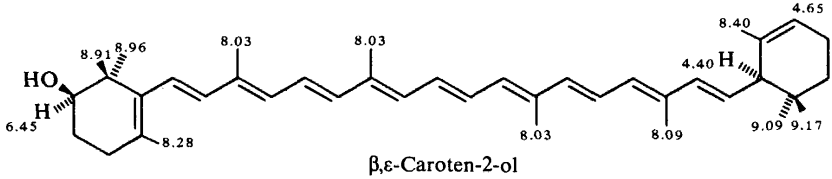
Carotenoids hydroxylated in the 2-position in the aliphatic termini are frequently encountered. One new representative is 2′-hydroxyflexixanthin (*Figure 3*) studied with Aguilar-Martinez[12]. Key reactions together with diagnostically important mass spectrometric fragmentations of the derivatives are given in *Figure 3*. The ω,ω′-ketone provided a monoacetate, which subsequently gave a mono(trimethylsilyl) ether which on alkali treatment gave an acidic diosphenol. 2′-Hydroxyflexixanthin occurs together with flexixanthin, a carotenoid so far peculiar to the gliding Flexibacteria[13]. The carotenoid composition may contribute to the classification of the so far unclassified non-gliding bacterium from which 2′-hydroxyflexixanthin was isolated.

The green alga *Trentepholia iolithus*, previously stated to contain traditional carotenoids[14], offered a surprise. Carotenoids with 2-hydroxy-substituted β-rings have been unknown until recently, and their predicted properties have been used in structural arguments.

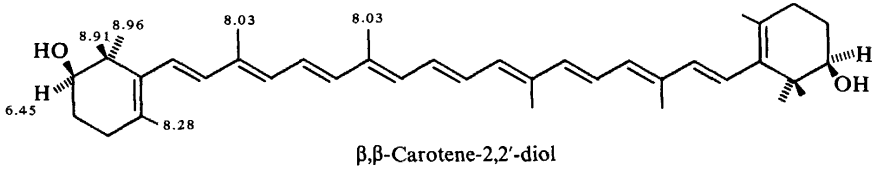
Trentepohlia iolithus, investigated with Kjøsen and Arpin[15], was found to contain β,β-caroten-2-ol, β,ε-caroten-2-ol and β,β-carotene-2,2′-diol, all esterified with fatty acids. These structures with defined stereochemistry were assigned by means of full spectral characterization. Some important p.m.r. signals are included in *Figure* 4. As expected the 2-hydroxylated analogues are less strongly adsorbed than the 3- or 4-hydroxylated β-type carotenoids and less prone to undergo acetylation. The chemistry of these compounds is discussed separately[16].



β,β-Caroten-2-ol

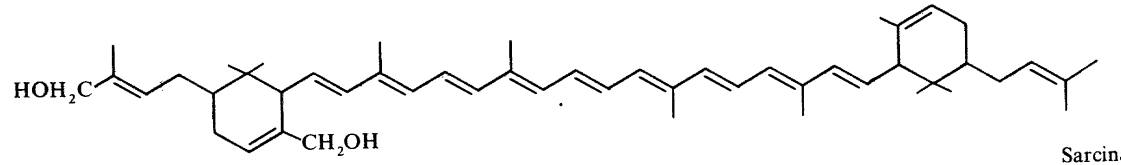


β,ε-Caroten-2-ol



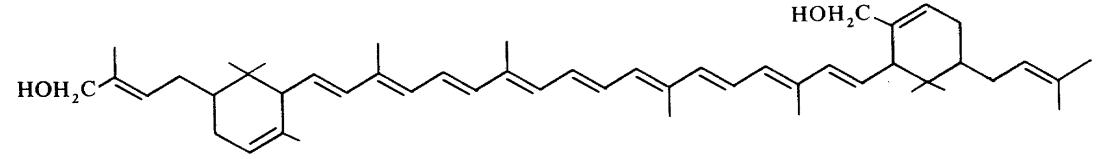
β,β-Carotene-2,2′-diol

Figure 4. Carotenoids with 2-hydroxylated β-rings

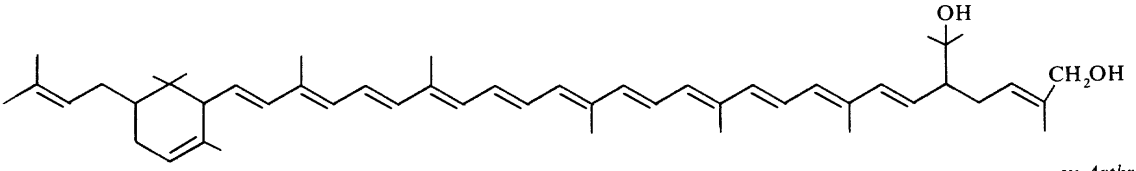


(IV)

Sarcinaxanthin
ex Sarcina lutea

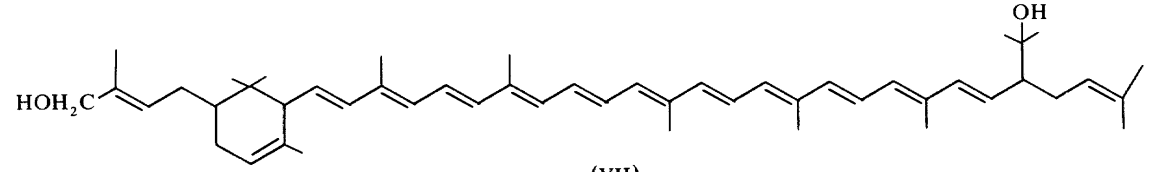


(V)



A.g. 471
ex Arthrobacter glacialis

(VI)



(VII)

Figure 5. Alternative structures of C_{50}-carotenoids

C_{50}-carotenoids

Considering the C_{50}-carotenoids, we have resigned from isolating sufficient sarcinaxanthin[2,17] for the required differentiation between (IV) and (V) (*Figure 5*); (V) being more likely from the occurrence of its D-glucoside[18]. From *Arthrobacter glacialis*, studied in collaboration with Arpin, Fiasson and Norgård[19], decaprenoxanthin, bisanhydrobacterioruberin and a new monocyclic C_{50}-diol, *A.g.* 471, have been isolated. Again there is some uncertainty concerning the location of the primary, allylic hydroxy group (VI or VII, *Figure 5*).

Glycosides

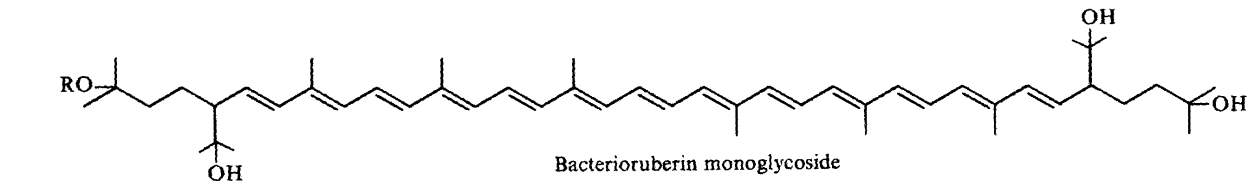
New glycosides of C_{50}-carotenoids are bacterioruberin mono- and diglycoside from a moderately halophilic bacterium, and decaprenoxanthin diglucoside from an *Arthrobacter* sp. (*Figure 6*) studied with Arpin[20,21]. The studies include p.m.r. and mass spectroscopy of various derivatives, and hydrolysis with subsequent identification of the sugars involved.

Aphanizophyll from blue-green algae, studied with Hertzberg[22], has been ascribed the structure 4-hydroxymyxoxanthophyll, *Figure 7*. On acetylation aphanizophyll gives a pentaacetate which provides a mono(trimethylsilyl) ether on silylation. Prolonged treatment of the pentaacetate with $LiAlH_4$ gave two products considered to be 4-hydroxysaproxanthin and 3,4-dihydroxytorulene as predicted for this compound on the basis of previous experience with allylic carotenoid glycosides with the same type of end-group[23,24]. Mass spectrometry of the pentaacetate supported this assignment. Diagnostically important cleavages are indicated in *Figure 7*. In this case restricted quantity rendered glycoside hydrolysis impossible.

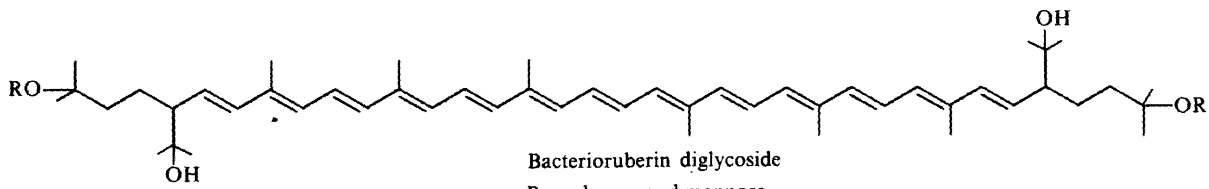
Even photosynthetic bacteria produced glycosidic carotenoids. In work with Schmidt and Francis[25], rhodopin-β-D-glucoside has been isolated from *Rhodopseudomonas acidophila.* P.m.r. signal assignments and characteristic fragmentation of the tetraacetate on electron impact are indicated in *Figure 8*. The tetraacetate formed no silyl ether on silylation and thus contains no tertiary hydroxy groups. Sugar hydrolysis provided glucose; β-D-glucoside assignment follows from p.m.r. evidence of the tetraacetate and analogy with the β-D-glucoside described below.

From the same bacterium the remarkable C_{43}-carotenoids, depicted in *Figure 9*, were subsequently isolated and, as an anticlimax, they were shown to be artefacts quantitatively produced during the saponification step in the presence of small amounts of acetone; the native carotenoids being rhodopinal, rhodopinal-β-D-glucoside, lycopenal and a methoxylated dodecaenal[25]. The facile aldol condensation of carotenals with acetone is a warning concerning the identification of naturally occurring methyl ketones if acetone has been employed in the isolation procedure.

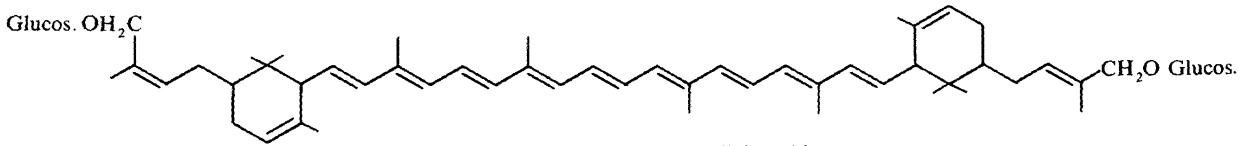
Key steps in the structural elucidation of the C_{43}-β-D-glucoside were as follows. Polarity properties, i.r. spectrum and acetylation data were indicative of a glycoside, and D-glucose was isolated after hydrolysis and oxidation with D-glucose oxidase. Mass spectrometry of the free glucoside and of the peracetate revealed that the peracetate was a tetraacetate. The tetraacetoxyoxonium ion (*m/e* 331), characteristic of hexosides and fragment ions thereof[26], confirmed the glucoside formulation, and from the mass spectrometric data it



Bacterioruberin monoglycoside
R = glucose and mannose

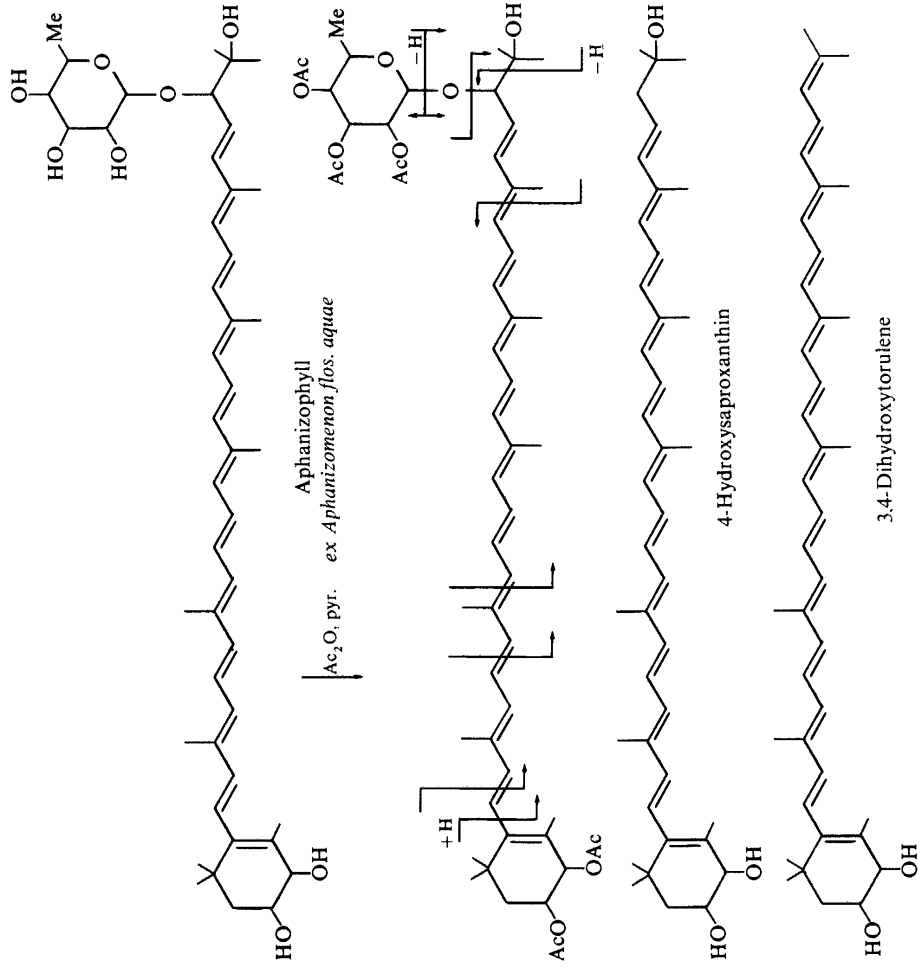


Bacterioruberin diglycoside
R = glucose and mannose



Decaprenoxanthin diglucoside

Figure 6. New glycosides of C_{50}-carotenoids



Fiaure 7. Aphanizophyll

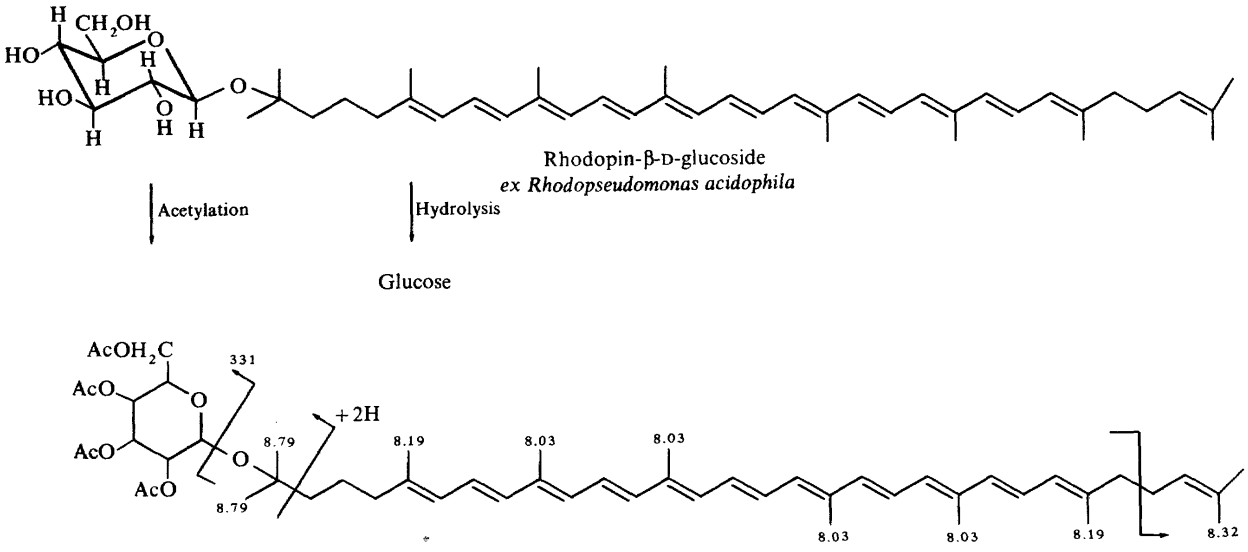


Figure 8. Rhodopin-β-D-glucoside

Figure 9. C_{43}-carotenoids

was further inferred that the aglucone had the molecular composition $C_{43}H_{60}O_2$. Since the second oxygen function was shown by hydride reduction to represent a conjugated carbonyl group, the aglucone possessed a C_{43}-skeleton. The round-shaped absorption spectrum in visible light and the spectral change on hydride reduction indicated cross conjugation, and from the mass spectrometric fragmentation pattern it was evident that an extra C_3H_2O substituent was connected with one of the lateral methyl groups (cf. general discussion below). In short all data obtained were in agreement with the rhodopin-20-al-β-D-glucoside structure, including in-chain fragmentations of the tetraacetate. β-D-Configuration was defined by a p.m.r. signal at τ 5.35 with a coupling constant of 8 Hz for axial–axial coupling.

Also the C_{43}-aglucone was isolated and its structure was confirmed by partial synthesis from rhodopinal and triphenylacetylmethylenephosphorane in a Wittig reaction, and by *retro*-aldol cleavage of the C_{43}-aglucone to rhodopinal on alkali treatment.

Nor-carotenoids

The only nor-carotenoid known until recently was actinioerythrin[27] (*Figure 10*) and its derivatives. From the sea anemone *Actinia equina*, the source of actinioerythrin, we have isolated another nor-carotenoid, referred to as ester X. With Francis, Hertzberg and Upadhyay[28], ester X has been indirectly shown to have the C_{39}-structure given in *Figure 10* by studies of the alkali product roserythrin, which reacted in the manner predicted from our experience with actinioerythrin[28]. Thus roserythrin gave the mono-quinoxaline derivative in a fast reaction and then more slowly the bisquinoxaline derivative. Hydride reduction gave roserythrol. Some difficulties were encountered because of the incomplete diosphenol formation during weak alkali treatment resulting in a mixture of roserythrin and dihydroroserythrin, inseparable in our chromatographic systems, and causing some discrepancy in the predicted spectral data of various derivatives.

Roserythrin was subsequently prepared by partial synthesis from astacene using the method published, by Holzel *et al.*[29], for violerythrin. Heterogeneous oxidation with MnO_2 at optimum conditions gave roserythrin, an expected intermediate in violerythrin formation, as 20 per cent of the recovered carotenoid. Roserythrin thus prepared compared favourably with roserythrin obtained from ester X, and its quinoxaline derivative exhibited the predicted in-chain cleavages[28] expected from the mass spectra of violerythrin and astacene bisquinoxaline derivatives.

Undoubtedly the most fascinating carotenoid studied in this period is peridinin, the characteristic carotenoid of dinoflagellates. The structural elucidation of peridinin[30–34] has been a joint project between three American groups, with Professor Haxo at La Jolla, Dr. Strain at Argonne and Professor Rapoport in Berkeley in charge, and ourselves—a collaboration initiated during the last carotenoid symposium in Las Cruces.

In early work by Heilbron[35], the elemental analysis indicated a molecular formula $C_{40}H_{52}O_8$ for peridinin, which was corrected to $C_{39}H_{50}O_7$ by high-precision mass spectrometry[32]. The molecular formula consequently revealed the presence of fifteen double-bond equivalences. The visible spectrum

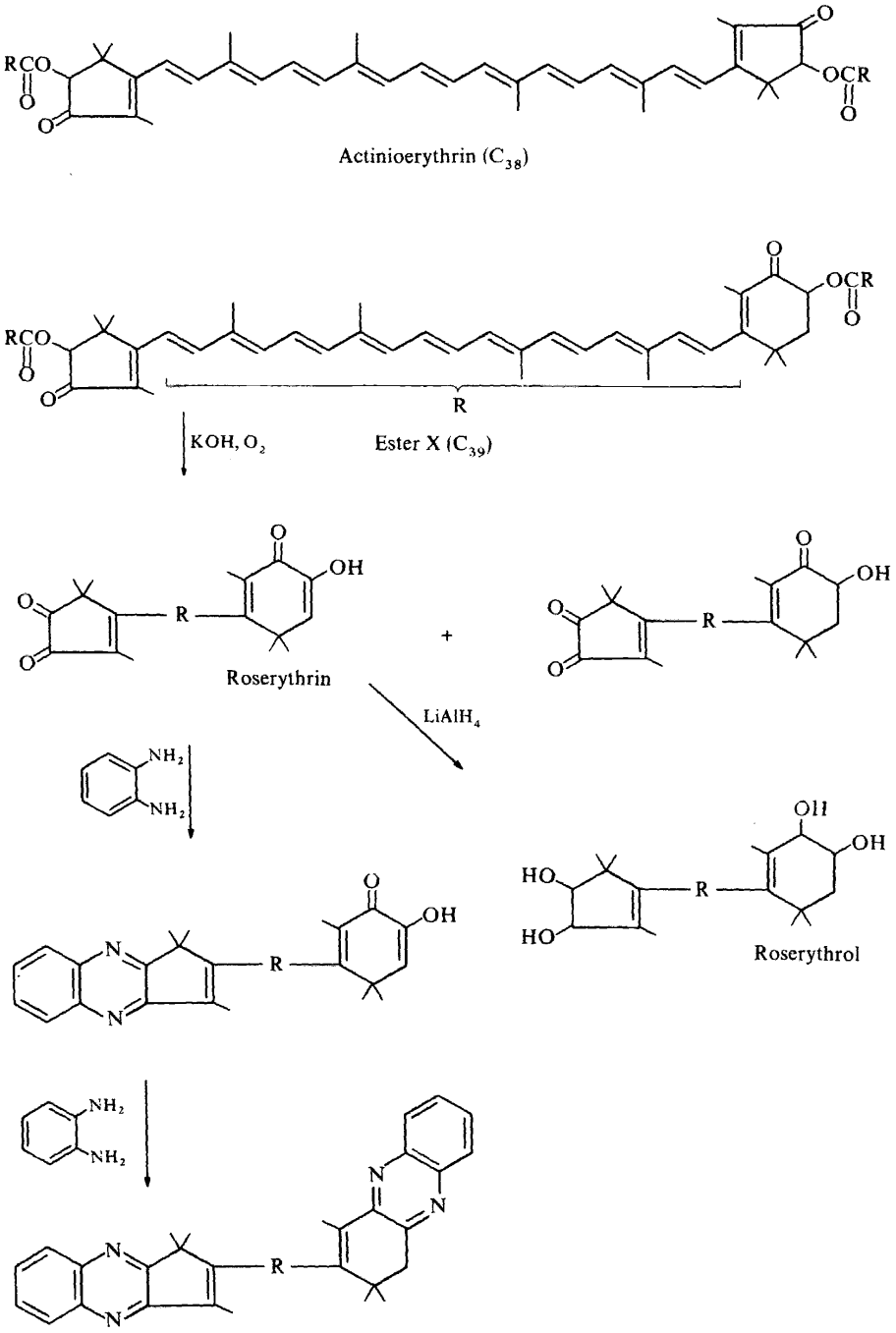


Figure 10. Roserythrin

exhibited the general solvent effect of conjugated carbonyl functions—fine structure in hexane and rounded spectrum shifted towards longer wavelengths in methanol. The i.r. spectrum revealed the presence of an allene grouping (1930 cm^{-1}) and exhibited broad absorption at 1750 cm^{-1}, but no absorption in the 1700–1600 cm^{-1} region, expected for conjugated carbonyl functions. The proton magnetic resonance spectrum showed nine tertiary methyl groups, one of which (correlated with the 1750 cm^{-1} absorption in the infrared spectrum) was ascribed to an acetate group, subsequently chemically proved by alkaline hydrolysis. Peridinin thus had a C_{37}-skeleton and eight lateral methyl groups. Two of the seven oxygen functions were accounted for by the acetate. The presence of one primary or secondary hydroxy group was shown by acetylation giving peridinin monoacetate (actually a diacetate, since peridinin itself is a natural acetate). Silylation of the acetylated product gave a mono (trimethylsilyl) ether, demonstrating the presence of one tertiary hydroxy group in peridinin. Treatment of peridinin with weak mineral acid in ether caused a hypsochromic shift of the absorption spectrum in visible light of *c.* 20 nm, which was ascribed to an epoxide–furanoid rearrangement, supported by mass spectral evidence for the furanoid product. Peridinin thus contains one epoxy group. A carbonyl group had to be present to account for the visible absorption spectrum and the spectral effect of hydride reduction giving products with pentaene chromophore. A conjugated lactone arrangement was compatible with the i.r. absorption at 1750 cm^{-1}. Thus the seven oxygen functions were assigned.

The p.m.r. spectrum showed that six of the methyl groups were present in saturated environments, and that only two of them could be of in-chain type. It was thus clear that the C_{37}-skeleton of peridinin was formed by some kind of modification of the central polyene chain rather than of the end-groups.

The evidence discussed permitted the formulation of the partial structure given in *Figure 11*—the central part representing a $C_{14}H_{13}O_2$ unit with eight double-bond equivalences and containing one lactone and one methyl group. To make a long story short peridinin was finally formulated as in *Figure 11*; tetrenolin[37] (an antibiotic from *Micropolyspora venezuelensis*) represents a useful model for spectral assignments. Furthermore three singlets at τ 2.98, 3.95 and 4.25 were ascribed to particular olefinic protons, *Figure 11*, and the AB system caused by the olefinic protons flanked by the epoxy and butenolide groups could be demonstrated by means of the lanthanide-shift technique.

Products obtained on ozonolysis[33] (the allenic ketone has also been obtained[38, 39] from fucoxanthin and fully confirms the end-group in common with fucoxanthin) and on borohydride reduction, as well as by acid and alkali treatment[31] are compatible with the assigned structure. The structures of some important derivatives of peridinin are given in *Figure 12*.

The mass spectrometric fragmentation further confirmed the assignment. Losses of water and acetic acid are observed. The loss of carbon dioxide, not earlier encountered in carotenoid mass spectra, is explained by the lactone formulation. Loss of toluene, but not xylene, is observed. This reflects the scarcity of methyl groups on the polyene chain. Ions observed in the lower region confirm in particular the lactone-containing half of the molecule.

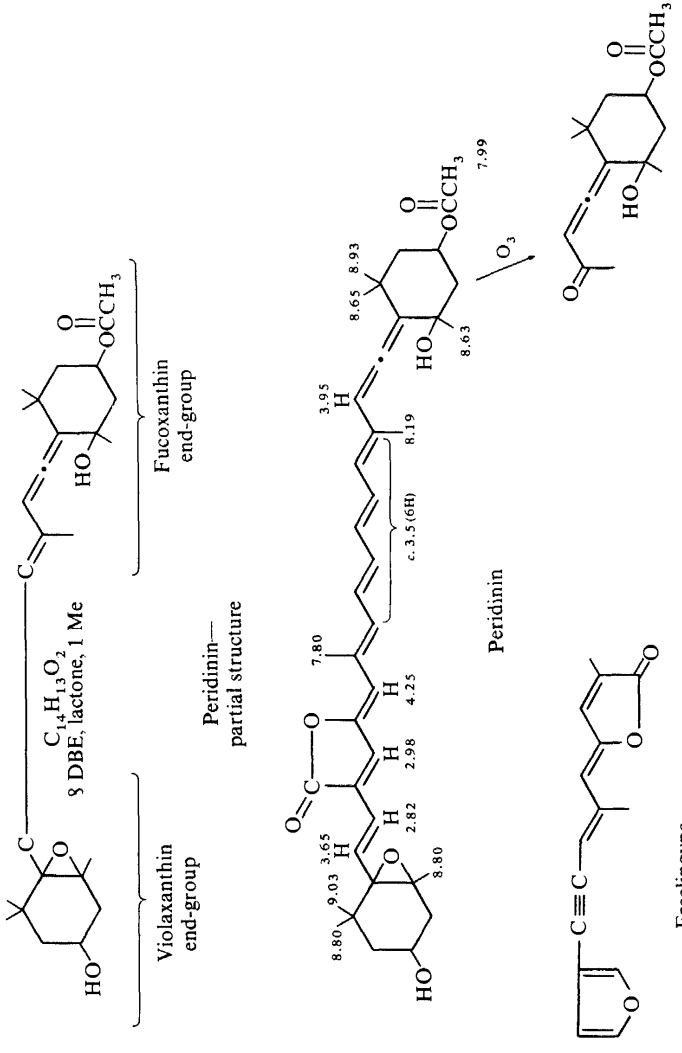


Figure 11. Peridinin and related compounds

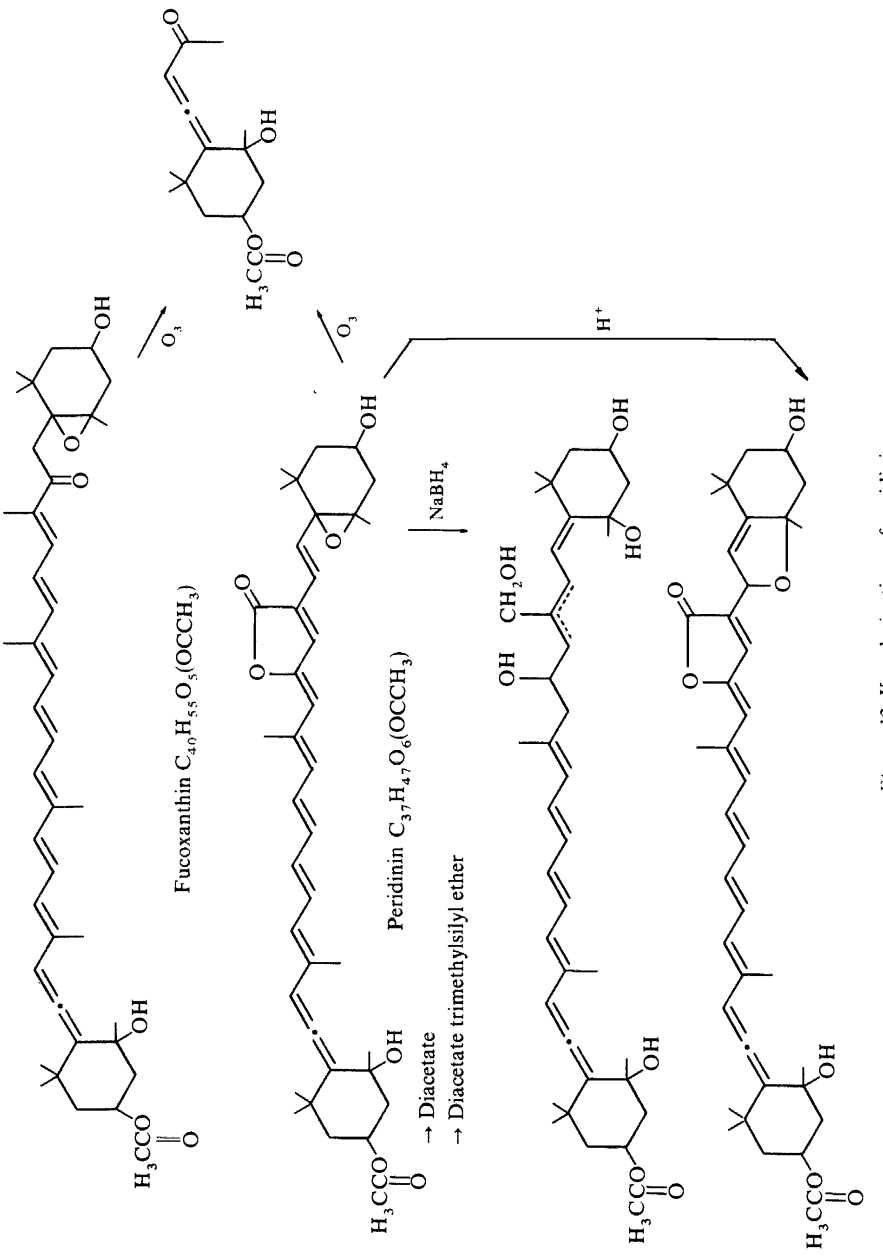


Figure 12. Key derivatives of peridinin

Mechanisms have been invoked to explain the formation of these ions. The presumed origin of the ions designated (A) and (B) is given in *Figure 13*. The base peak *m/e* 181 is ascribed to ion (A), which earlier[40] was found typical for carotenoid epoxides. The appropriate mass shift is seen for various substitutions of the hydroxy group. The ions *m/e* 234 (B) and *m/e* 207 (C) are ascribed to homopyrylium ions. Moreover, some ions *m/e* 197, 212, 221 and 275, also observed for fucoxanthin and isofucoxanthin, have been rationalized[32].

It was mentioned that the structurally related tetrenolin possessed antibiotic activity against gram-positive bacteria[37]. We have tested peridinin from *Sarcina lutea* (gram +) and *Escherichia coli* (gram −) for such activity, but have not observed growth-inhibiting effects[31].

Curiously enough, peridinin, (which lacks one of the central methyl groups) in spite of its carotenoid-type structure, is not a carotenoid according to the new definition[5]. Peridinin occurs together with other carotenoids

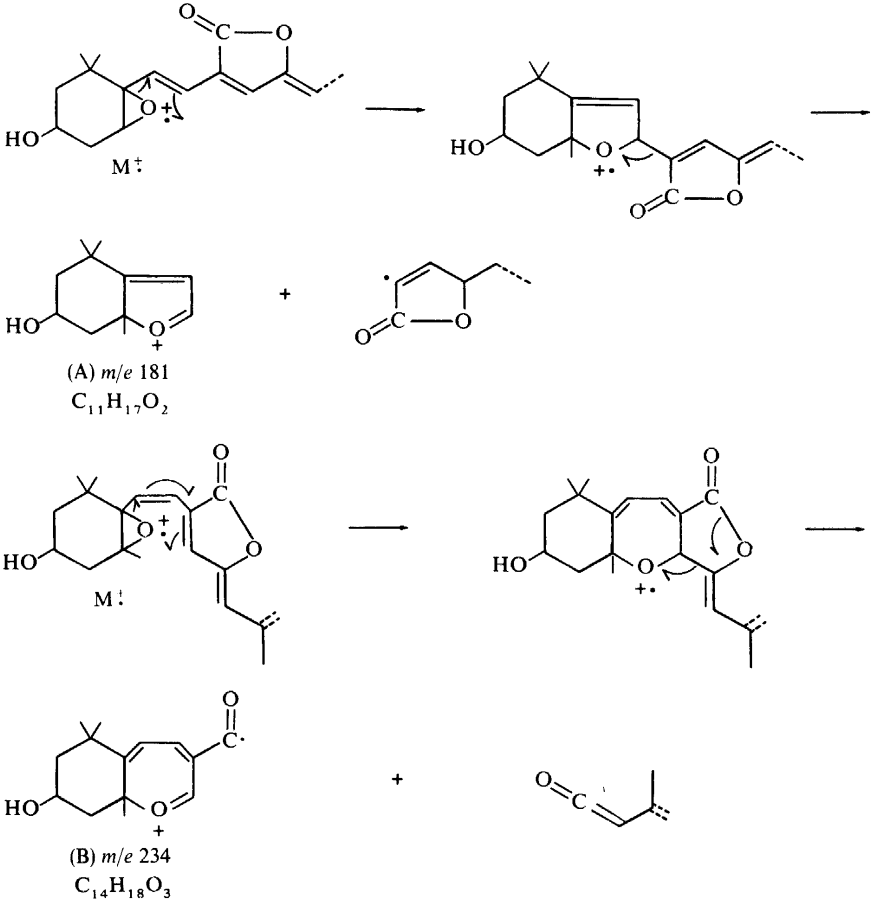


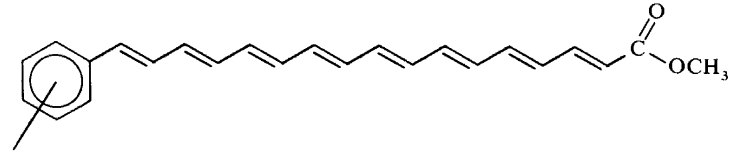
Figure 13. Rationalization of the formation of some fragment ions of peridinin on electron impact

possessing ordinary isoprenoid C_{40}-skeletons. Loss of three carbon atoms from the central part of the molecule may be rationalized in terms of oxidation of the 20-methyl group of a traditional C_{40}-carotenoid skeleton, which by analogy with carotenoids of the rhodopinal series would result in isomerization of the adjacent double bound to *cis* configuration, thereby facilitating rearrangement and elimination of a C_3-acetylenic moiety, perhaps by an electrocyclic type of reaction. The butenolide group could be formed by oxidation of the 19′-methyl group to carboxyl, followed by lactonization via addition to a triple bond, by analogy with examples from acetylenic chemistry.

Fucoxanthin and peridinin, which have similar end-groups, never occur together. A precursor relationship, fucoxanthin → peridinin, or more likely a common precursor relationship, may be considered.

Non-carotenoid polyenes

A final interesting problem to be mentioned is the structure of the so-called '*Xanthomonad* carotenoids'[41] studied in collaboration with Andrews, Hertzberg and Starr[42]. These pigments are, in spite of the carotenoid-like electronic spectrum, not carotenoids, but bromine-containing polyenes for which the structures given in *Figure 14* are considered.



(*i*) 2Br + OCH_3
(*ii*) 1Br + OCH_3
(*iii*) 2Br + OH
(*iv*) 1Br + OH

Figure 14. Xanthomonas 'carotenoids'—tentative structures

SOME PROGRESS IN STEREOCHEMISTRY

The structural elucidation of a new carotenoid is not complete until the stereochemistry is adequately defined. The stereochemical implications of some p.m.r. and c.d. data will be discussed here.

Carotenoid glycosides

For carotenoid glucosides the configuration of the glucose involved (D or L) may be determined after hydrolysis and treatment of the liberated glucose with D-glucose oxidase[18,43].

The stereochemistry of the glycosidic linkage of glucosides is readily revealed by the p.m.r. spectrum. Acetylated β-D-glucosides exhibit a characteristic doublet for the anomeric proton at *c.* τ 5.3 ($CDCl_3$) with an axial–axial coupling constant[44], a feature allowing definition of the stereochemistry of the glucosidic linkage in rhodopinal-β-D-glucoside[25], bacterioruberin-

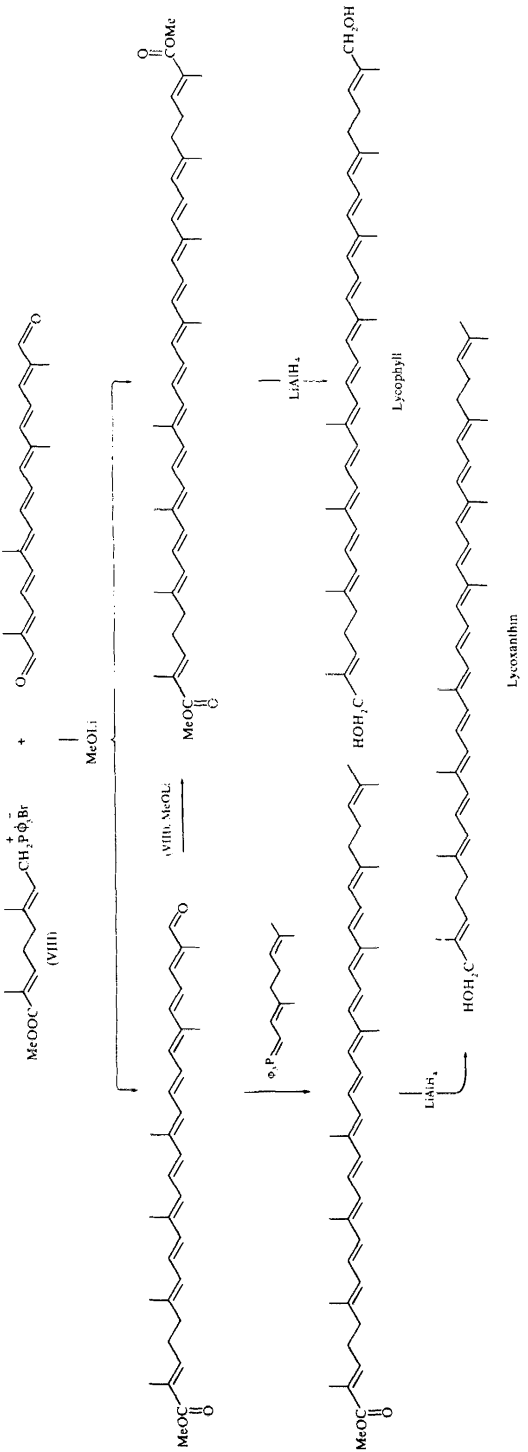


Figure 15. Total synthesis of lycoxanthin—Part 2

β-D-diglucoside[20] and decaprenoxanthin monoglucoside[21] *ex Arthrobacter* M3, as β-L (or α-D).

In the case of rhamnosides, oscillaxanthin[24] and myxoxanthophyll[23] are prototypes; L-rhamnose is considered to be involved by analogy with all known rhamnosides. A p.m.r. study of methyl triacetyl-α- and -β-L-rhamnosides has been carried out[45] in order to facilitate the interpretation of the p.m.r. spectrum of oscillaxanthin octaacetate, which is compatible with the 1C(L)-conformation of the rhamnose moiety. Examination of the coupling constant of the anomeric proton is in this case not very informative (ax.ax. or ax.eq.). The previously suggested β-L-configuration[24], where the large aglycone occupies equatorial position, seems most plausible.

Lycoxanthin and lycophyll

For lycoxanthin, from berries of *Solanum dulcamara*, *trans* configuration of the hydroxy-substituted isopropylidene group is concluded from combined p.m.r. and isomerization studies[46].

It had previously been observed that on iodine-catalysed stereomutation of *trans*-lycoxanthin (for structure see *Figure 15*) an isomer with unchanged electronic spectrum was produced[47]. In view of the revised lycoxanthin structure[48,49] this could be ascribed to *cis* configuration around one of the terminal double bonds of the polyene chain (rubixanthin-gazaniaxanthin type isomerism[50]) or alternatively around the hydroxy-substituted isopropylidene bond. Examination of the p.m.r. spectrum of the stereomutation mixture revealed a new signal at τ 5.86 assigned to $C\underline{H}_2OH$ in *cis* position to the latter bond, in agreement with data for relevant model compounds, where *cis* configuration invariably causes resonance of the CH_2OH group at lower field than with *trans* configuration of the trisubstituted double bond. The integral of the τ 6.00 (*trans*) and 5.86 (*cis*) $C\underline{H}_2OH$ signals further agreed with the proportions of these isomers established by circular paper chromatography. From the chemical shift of the $C\underline{H}_2OAc$ signal of the diacetate of natural lycophyll and of lycoxanthin acetate it was inferred that lycophyll is the corresponding di-*trans* compound[46].

All-*trans* lycoxanthin has recently been totally synthesized by Kjøsen[51], according to the scheme given in *Figure 15*, and found to be identical with natural lycoxanthin. The corresponding phosphonium salt of the key intermediate, 7-carbomethoxy-3-methyl-octa-2,6-dien-1-ol, was condensed with crocetindial in a Wittig reaction to the C_{30}-ester, which with geranylidenetriphenylphosphorane in another Wittig reaction gave the C_{40}-ester. The ester function was reduced with $LiAlH_4$ in the final step to give lycoxanthin. Lycophyll was obtained from the C_{30}-ester as shown in *Figure 15*. The earlier steps are given in *Figure 16*. These are the synthesis of the phosphonium salt from acrolein via acrolein acetal, conversion to levulinic aldehyde acetal, the carbon chain of which was extended in a Horner reaction to the α,β-unsaturated ester, reduction to the allylic alcohol which was acetylated to permit hydrolysis of the acetal, condensation of the aldehyde with α-carbomethoxyethylidenetriphenylphosphorane, hydrolysis of the acetoxy group accompanied by some unwanted hydrolysis of the methyl ester, recycling by methylation with diazomethane, and finally phosphonium salt formation of the desired product with triphenylphosphine hydrobromide.

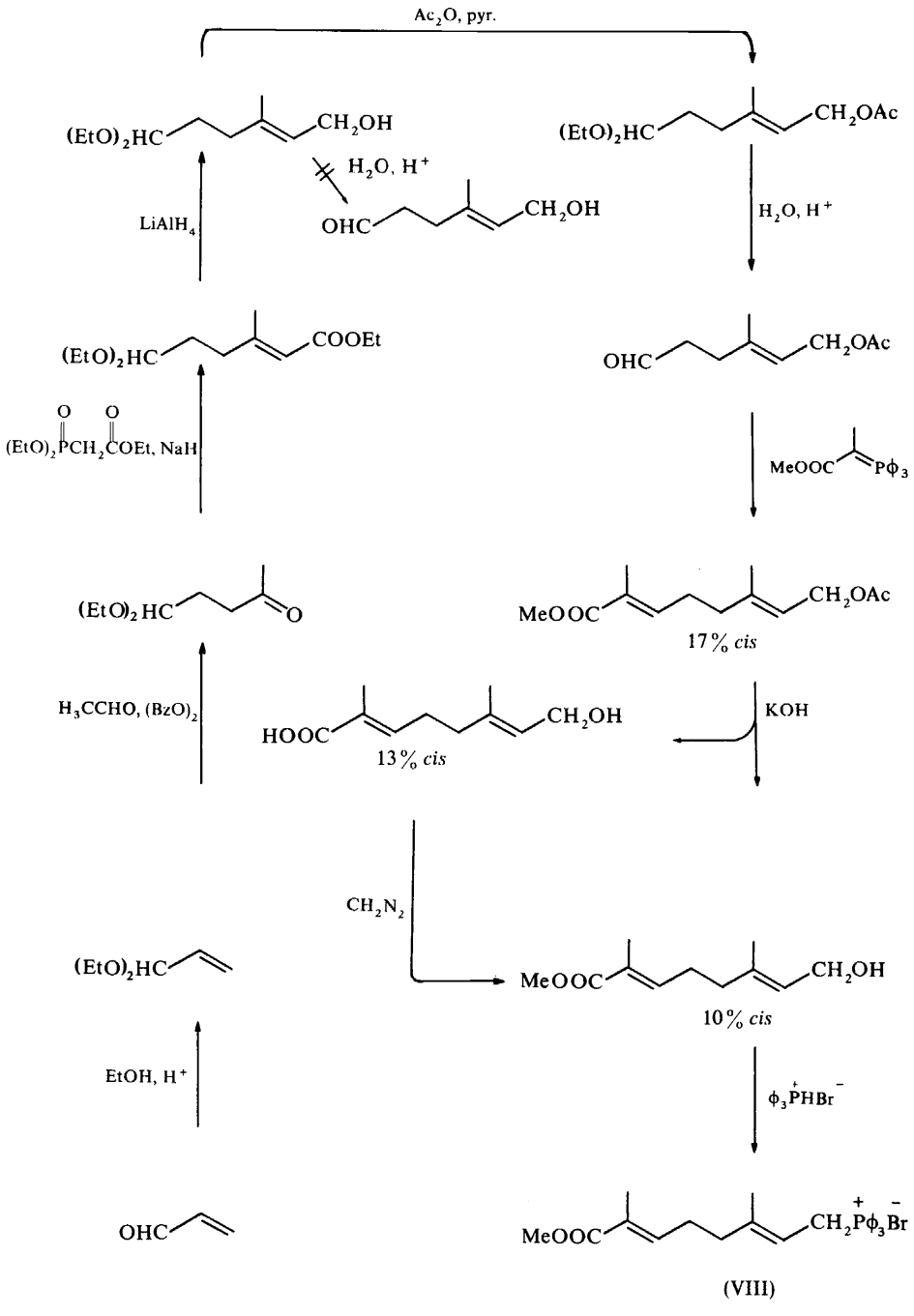


Figure 16. Total synthesis of lycoxanthin—Part 1

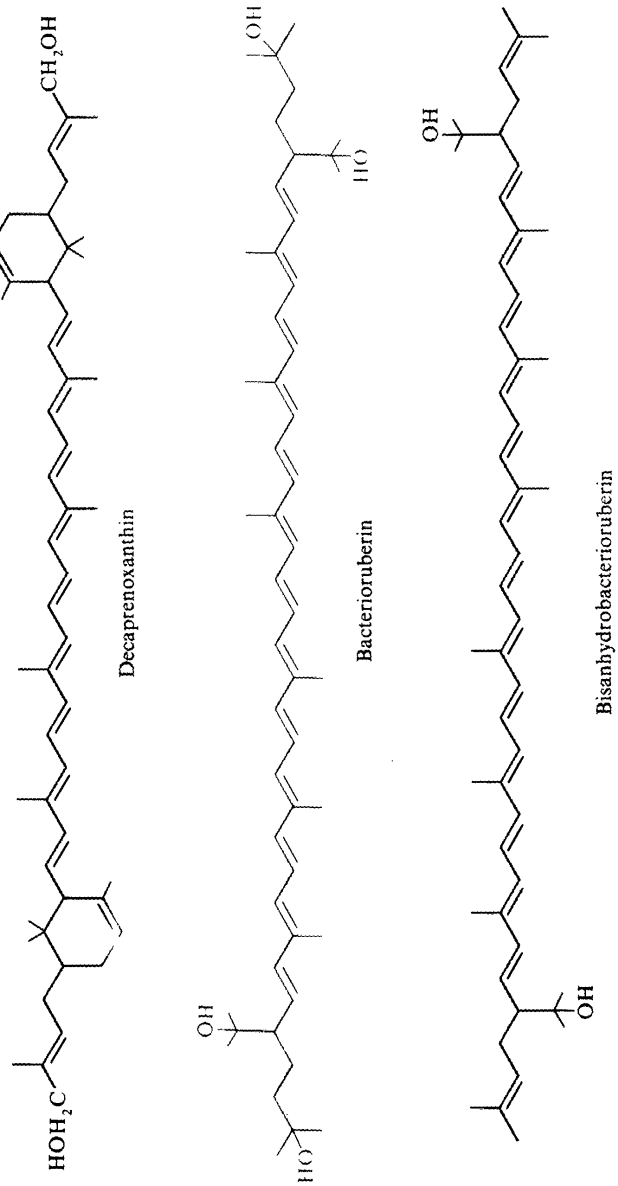
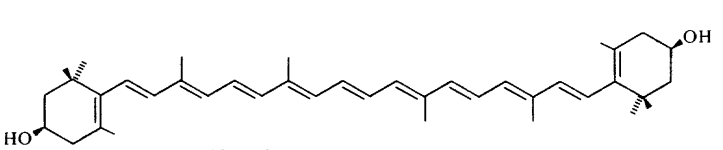
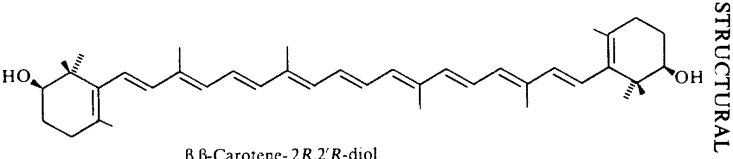


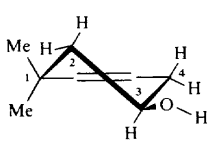
Figure 17. Related C_{50}-carotenoids



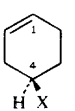
Zeaxanthin (3, 3′*R*)

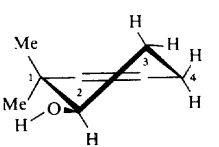


β,β-Carotene-2*R*,2′*R*-diol



(3*R*)





(2*R*)

Figure 18. Absolute stereochemistry of zeaxanthin and β,β-carotene-2,2′-diol

The percentage *cis* isomer in these intermediates, as determined by p.m.r. spectroscopy is indicated in *Figure 16* (predominance of *trans*). In the final steps only all-*trans* lycoxanthin and all-*trans* lycophyll were isolated; this may be due to preferential crystallization of the *trans* isomer.

Lanthanide-shift technique

The lanthanide-shift technique[52], recently applied to carotenoid p.m.r. spectra[53], is expected to provide stereochemical information in certain cases, e.g. for cross-conjugated carbonyl carotenoids and carotenoids with *cis* double bonds, because of angular variation influencing the induced shifts.

Circular dichroism

Optical rotatory dispersion studies have been performed by Klyne's and Weedon's schools[54] and have provided much information on the stereochemistry of asymmetric centres in conjunction with x-ray studies. More recent work by Eugster's school on the optical properties of carotenoids and derivatives has led to further elegant stereochemical correlations[55, 56]. However, a satisfactory theoretical interpretation of the carotenoid o.r.d. and c.d. spectra is still required.

Together with Borch in Copenhagen we have examined c.d. spectra of various carotenoids. A comparison of the c.d. spectra of bisanhydrobacterioruberin *ex Corynebacterium poinsettiae* and of bacterioruberin *ex Halobacterium salinarium* (*Figure 17*) supports an identical absolute configuration of the two compounds and a common biosynthetic formation as well as stereospecific addition of the two extra C_5-units to the traditional C_{40}-carotenoid skeleton[57]. Correlation with the c.d. spectrum of the bicyclic decaprenoxanthin (*Figure 17*), which has two, presumably identical, additional asymmetric centres, is not possible at present[57].

Perhaps brave conclusions have been drawn from the opposite c.d. spectra of zeaxanthin, of known absolute stereochemistry (3,3′*R*[58]), and of β,β-carotene-2,2′-diol[15] (see *Figure 18*). Mill's rule[59], elaborated by Eliel[60], states that in cyclohexene systems with an asymmetric carbon in the 4-position, the nature of the substituent at this carbon atom is irrelevant, since the optical rotation is caused by a preferred (asymmetric) conformation of the cyclohexene ring. As shown here both the 2-hydroxy-β-end-group and the 3-hydroxy-β-end-group may be regarded as 4-substituted cyclohexene systems. Assuming that the hydroxy group in both cases prefers the equatorial position (supported by p.m.r. evidence[15]), the absolute configurations 2-*R* and 3-*R* will result in opposite half-chair conformations, which would be expected to cause opposite optical activity. Work aimed at checking the 2,2′*R* assignment of β,β-carotene-2,2′-diol is in progress[61].

C.d. correlations have further enabled stereochemical assignments to β,ε-caroten-2-ol as 2*R*,6′*R* and to β,β-caroten-2-ol as 2-*R*[15].

SOME GENERAL RESULTS IN APPLIED SPECTROSCOPY

Low-temperature spectra

Through a contact made with Dr. Ke of Kettering Research Laboratory at the last carotenoid symposium, we have examined the electronic absorption

spectra of selected carotenoids at liquid nitrogen temperature (77 K) in order to investigate the scope and limitation of low-temperature spectroscopy in the carotenoid field[62]. Relative to room-temperature spectroscopy the technique offers possibilities for accentuating *cis* peaks, for the determination of steric conflict between the polyene chain and terminal rings containing double bonds in conjugation with the polyene chain, for the determination of conjugated carbonyl chromophores (with round-shaped spectra at room temperature) and for gaining information about the stereochemistry of cross-conjugated chromophores.

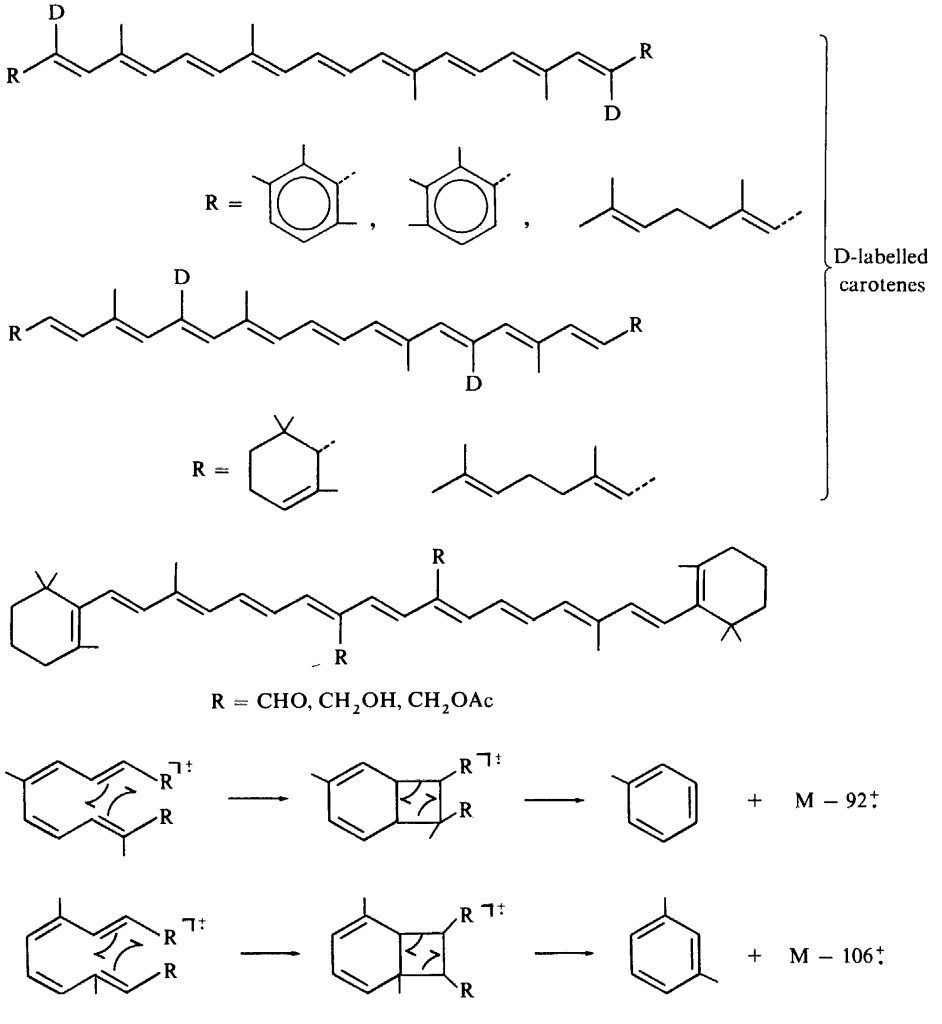


Figure 19. Deuterated carotenes prepared by total synthesis and the Edmunds–Johnstone mechanism for in-chain eliminations on electron impact

Mass spectrometry

In collaboration with Enzell (in Stockholm), Francis and Kjøsen, further mass spectrometric studies of carotenoids have been carried out.

Carotenoids with deuterium labels in the 7,7′-position, synthesized by Kjøsen[63], in the 11,11′-position, synthesized by Eidem[64], and synthetic carotenoids with the two central methyl groups shifted from the 13,13′- to the 14,14′-position and oxygenated[65,66], have been examined (*Figure 19*). The fragmentation pattern on electron impact is in each case[64–66] consistent with the Edmunds–Johnstone mechanism[67] advanced by Schwieter *et al.*[68] for eliminations from the polyene chain. This mechanism involves a four-membered transition state and rupture of double bonds.

Steric effects in in-chain elimination reactions based on this mechanism (*Figure 19*) have been discussed with Enzell[66]. Assuming that steric interaction may prevent formation of the cyclic intermediate, the previously observed variation in the M − 92 : M − 106 ratio[69] may be rationalized. Such considerations have been further refined by Schwieter *et al.*[70] and by Francis[71] using the Woodward–Hoffmann rules.

The origin of toluene and xylene from the polyene chain, as established for aliphatic and bicyclic carotenes by means of deuterium labelling by Schwieter's group[68] and ourselves[63], is given in *Figure 20*. It has been observed in a large number of cases[25,72,73] that carotenoids, in which one of the four lateral methyl groups is replaced by another substituent, fragment in an analogous manner.

Let us refer to the common M − 92, M − 106 and M − 158 ions as P, Q and T ions respectively, and the corresponding ions shifted in mass due to the extra substituent as P′, Q′ and T′ ions. From the appearance of such P′, Q′ and T′ ions one can tell for a bicyclic cartenoid which pair of the in-chain methyl groups carry the extra substituent. This follows from the origin of the P,P′ and Q,Q′ ions[66]. If we observe Q′ and P′ ions it is one of the central methyl groups which is substituted. If only Q′ ions are observed it is the 19 or 19′ methyl group which is substituted. Unfortunately the situation is not so simple in the aliphatic series and no distinction between the 19,19′- and 20,20′-positions can be made on this basis[72].

Whereas Retro-Diels–Alder fragmentations are common in carotenoids containing α-rings[74,75], this fragmentation does not appear to be important in carotenoids containing unsubstituted β-rings where ethylene should be eliminated. However, RDA fragmentation appears to be operative in 2-substituted β-ring compounds such as the C_{50}-diol[76], *Figure 21*.

Examples of double rearrangement processes are rare in carotenoid mass spectrometry, but appear to occur in the C_{43}-carotenoids[25]. The observed peaks, measured by high precision, may be rationalized by in-chain eliminations involving double hydrogen transfer and cleavages (*Figure 22*), although other mechanisms may be formulated. In any case these cleavages involve transfer of two hydrogen atoms and hence are considered the result of double rearrangement processes.

P.m.r. spectroscopy—lanthanide-shift reagents

In the p.m.r. field we[53] have studied the effect of lanthanide-shift reagents on carotenoid spectra. The method has proved to be extremely useful for

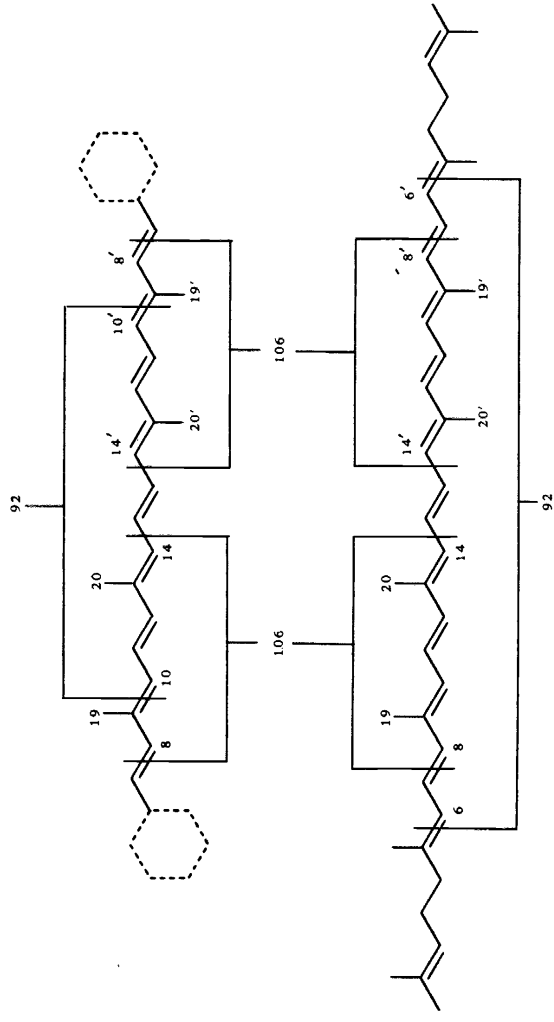


Figure 20. Sites of toluene and xylene elimination on electron impact

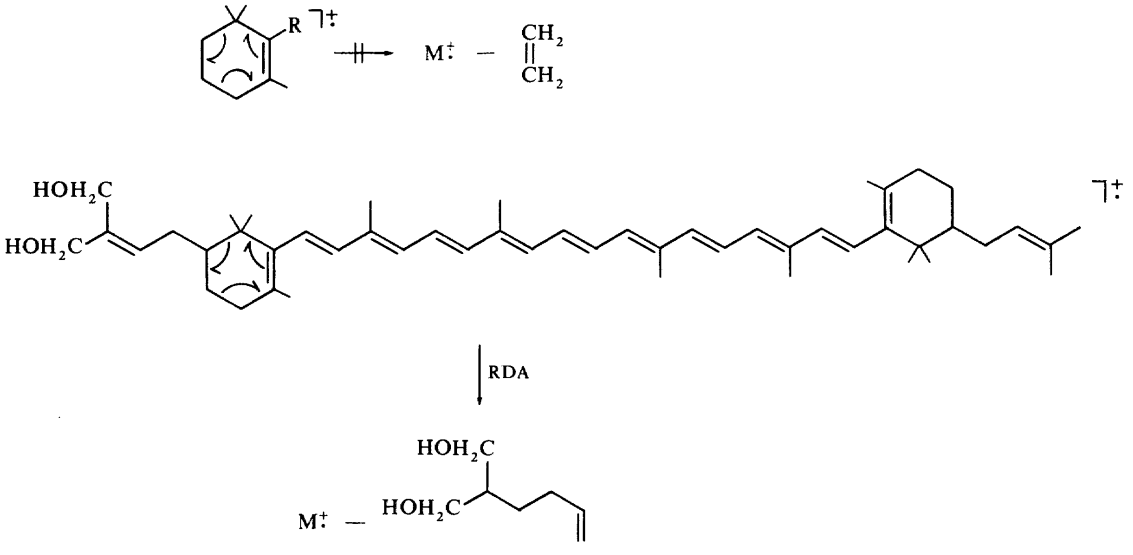


Figure 21. Retro-Diels–Alder fragmentation

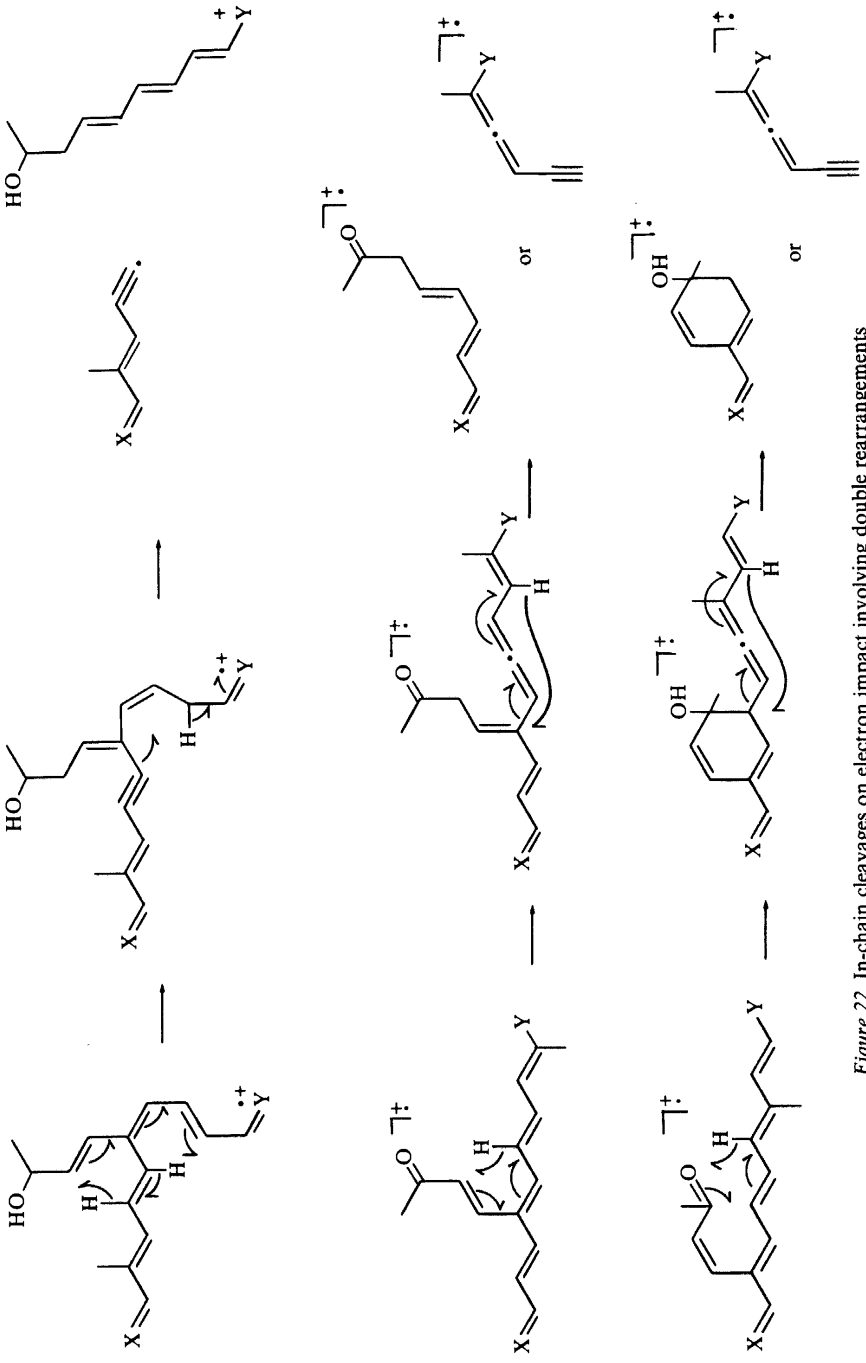


Figure 22. In-chain cleavages on electron impact involving double rearrangements

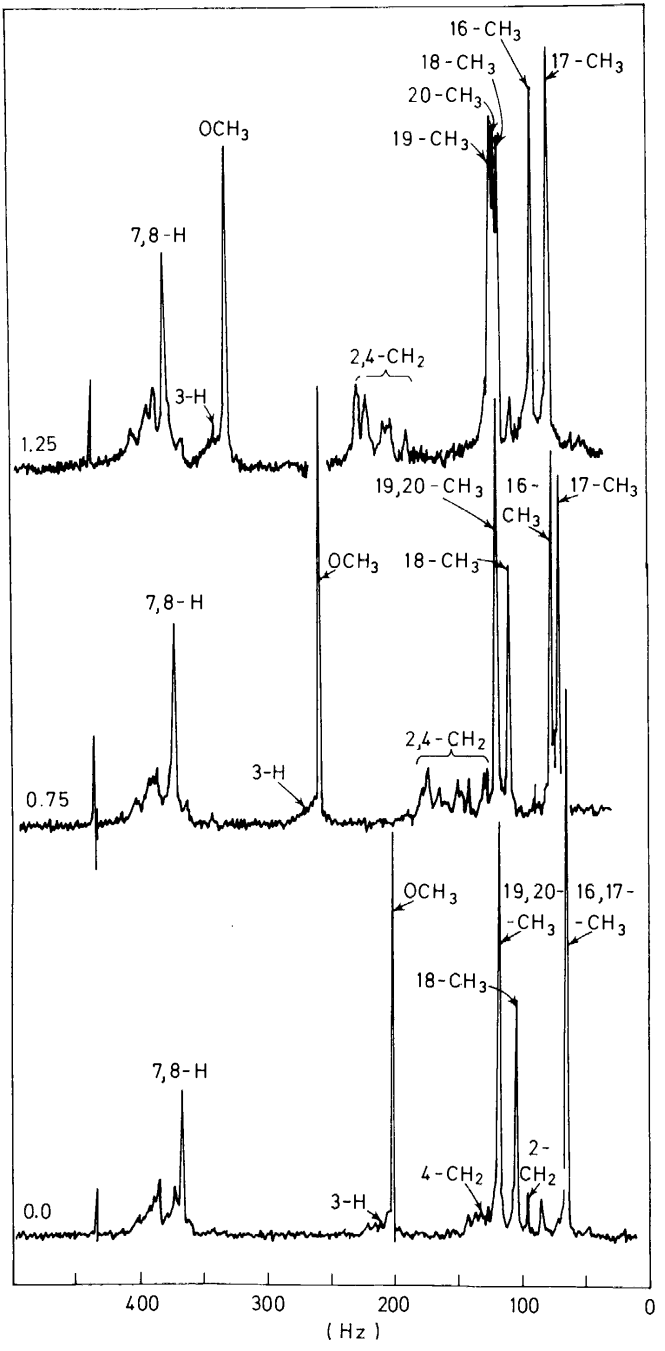


Figure 23. Proton magnetic resonance spectra of zeaxanthin dimethyl ether at 0.0, 0.75 and 1.25 molar ratios of $Eu(dpm)_3$:carotenoid

assigning the position of single oxygen functions, or two identical, symmetrically located oxygen functions, in carotenoids. Because of the paramagnetic shift induced by tris(dipivalomethanato)europium(III) on pseudocontact complexation with the oxygen functions[52] in carotenoids, the signals caused by neighbouring protons are moved downfield. Methylene and methine proton signals usually hidden in the methyl-methylene-methine envelope may thus be made distinguishable. This is exemplified (*Figure 23*) in the p.m.r. spectrum of zeaxanthin dimethyl ether with and without the addition of tris(dipivalomethanato)europium(III). Useful results can be obtained for complex molecules such as peridinin[30, 32] provided suitable model substances are available.

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