Stereochemical aspects of carotenoids

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Abstract: A brief introduction to carotenoid stereochemistry is given for the non-specialist. Stereochemical aspects of carotenoids, of obvious importance for stereoselective total synthesis and structure elucidation of naturally occurring carotenoids, also have an impact on biochemical and biological phenomena, as illustrated by examples. Stereochemical studies are selected from the author's own research, partly in a historical context, but with emphasis on more recent contributions, interdisciplinary aspects of stereochemistry, and the application of different methods comprising spectroscopy (VIS, CD, ^{1}H NMR), HPLC, total and partial synthesis, oxidative degradation, derivatization including partial resolution and enzymic reactions, iodine-catalysed stereomutation and proof of natural occurrence.

INTRODUCTION

Symmetry elements, such as a symmetry plane, axis or centre, are important concepts of stereochemistry, and may be visualized in Nature and art (ref. 1). The stereochemistry of carotenoids, illustrated by a three-dimensional X-ray structure, attainable only for a few carotenoids (ref. 2) by ^{1}H NMR NOE interactions (ref. 3) or from molecular models, governs the shape and size of carotenoid molecules. In spite of the importance of the topic, stereochemical aspects of carotenoids have not been covered in a full lecture in the previous carotenoid symposia.

BASIC CONCEPTS IN CAROTENOID STEREOCHEMISTRY

The selected carotenoid model fucoxanthin (*1*), as shown in Scheme 1, serves to illustrate that complex carotenoids are characterized by the lack of symmetry elements, so that carotenoids are ideal compounds for stereochemical studies, and to point out the different types of stereochemistry of concern. These categories are listed below.
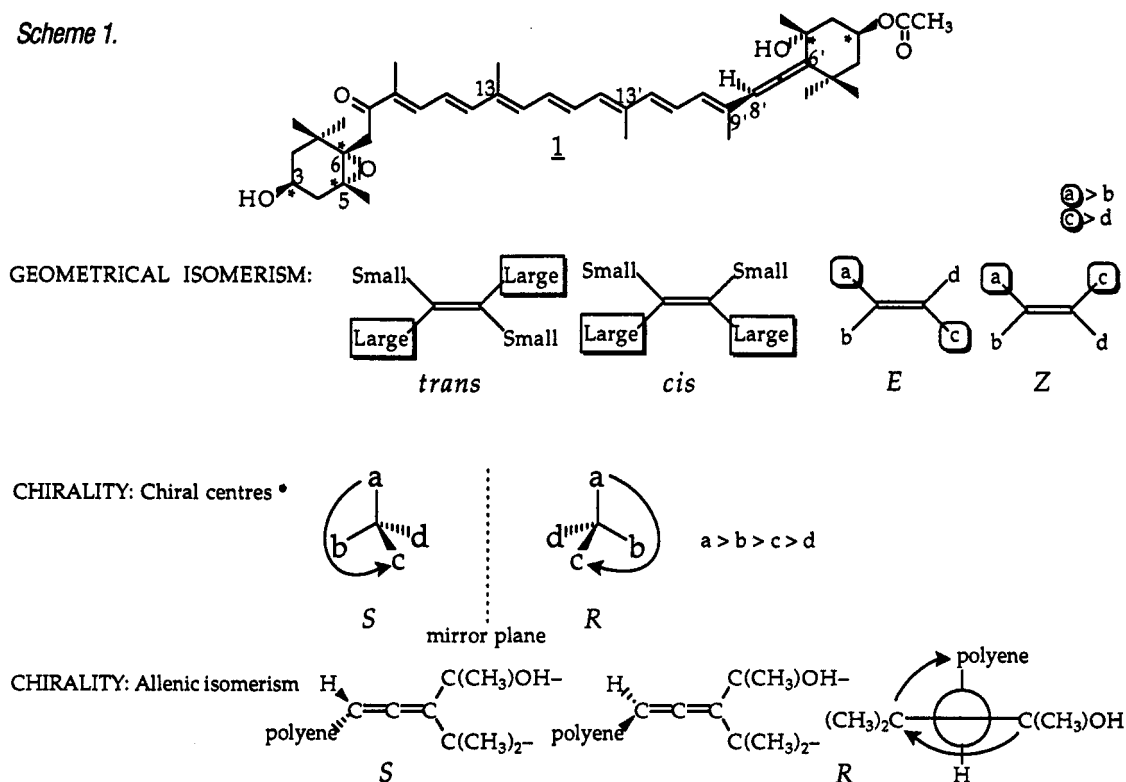
Geometrical isomerism

This is also referred to as *cis-trans* or *E/Z* isomerism. In principle, two alternative configurations may occur around each carbon-carbon double bond in the polyene chain. The *trans/cis* and the unequivocal *E/Z* designations based on the sequence rules (*e.g.* ref. 4), are visualized in Scheme 1. In carotenoids, *trans* and *E* are usually synonymous, except when there are oxygen substituents in the polyene chain. Our model *1* is depicted in the all-*E* configuration. In principle, seven different mono-*Z*, several di-*Z* and poly-*Z* isomers are theoretically possible. However, steric and electronic factors reduce this number. Thus the less stable Δ7 and Δ11 bonds are referred to as sterically hindered *Z*-double bonds.

Chirality

This a property implying the absence of symmetry elements (axis, plane or centre of symmetry). Chiral (stereogenic) centres in carotenoids are carbon atoms with four different substituents; *1* has five chiral

centres. Each chiral centre may have alternative absolute configurations, referred to as R and S by the Cahn-Ingold system including the sequence rules (ref. 4). On this basis our model may exist as $2^4 = 16$ chiral isomers if we allow for the restriction of the epoxy group. Only one of these configurations is encountered in Nature.

Scheme 1.

GEOMETRICAL ISOMERISM:

trans   *cis*   *E*   *Z*

CHIRALITY: Chiral centres •

$a > b > c > d$

S   mirror plane   *R*

CHIRALITY: Allenic isomerism

S   *R*

Allenic isomerism

The trisubstituted allenic group represents another chiral element in our model, namely the chiral axis through C-6',7',8'. Since the π-bonds of allenes are perpendicular to each other, allenes with different substituents at each side of the allenic bond can exist as non-identical mirror images, that is as an allenic R or S isomer. Our model *1* has the (6'R)-configuration. Consequently the total number of chiral isomers of fucoxanthin is doubled from 16 to 32.

Conformation

Different conformations are obtained by rotation of carbon-carbon single bonds, as opposed to different *configurations*, which require breaking and reforming bonds in a different way. Conformers cannot be separated, but certain conformations are energetically favoured. In carotenoids it is the conformation of cyclic end groups and of a partly saturated central chain that is important.

Conclusion

Excluding different conformations the three other types of isomerism may increase the number of known carotenoids from some 700 by a factor of 10-100. Whereas changes in configuration at a chiral centre require a chemical reaction, the three other processes may occur in solution. It is pointed out that (Z)-isomers are the most common artifacts in the carotenoid field (ref. 5). Isomers other than conformers may be separated under appropriate conditions and identified by modern methods.

GENERAL ASPECTS OF CAROTENOID STEREOCHEMISTRY

Stereochemical aspects of carotenoids are of obvious importance for the stereoselective total synthesis of carotenoids (ref. 6), and are also important for the structure elucidation of naturally occurring carotenoids. Since stereochemistry governs the size and shape, including the handedness (chirality) of the carotenoid molecule, it obviously has an impact also on biochemical and biological phenomena. Let us consider some established and some more tentative cases, first those involving *E/Z*-isomerism.

As established by Wald, the vision of mammals requires a *Z* to *E* isomerization of the sterically hindered Δ11 bond in retinal. Nakanishi has characterized (11*Z*)-retinal as a molecule uniquely suited for vision (ref. 7). Work by Koyama's group has demonstrated the existence of the centrally bent (15*Z*)-β,β-carotene in the reaction centre of spinach photosystem II, and of (15*Z*)-spirilloxanthin in the reaction centre of *Rhodospirillum rubrum* (ref. 8), and Lugtenburg and co-workers have presented evidence for the existence of (15*Z*)-spheriodene in the *Rhodobacter sphaeroides* reaction centre (ref. 9).

Z to *E* isomerization is involved in the general biosynthetic pathway of carotenoids where (15*Z*)-phytoene is often the first formed C_{40}-precursor. The isomerization to all-*E* takes place at an early stage of the desaturation sequence (refs. 10,11). Introduction of 9*Z*, 9'*Z* and sterically hindered 7*Z*, 7'*Z* bonds to form prolycopene, and back-isomerization of this to the all-*E* polyene upon subsequent cyclization in *Narcissus* chromoplasts have been studied in Kleinig's laboratory (ref. 11).

(9'*Z*)-Neoxanthin is present in many green photosynthetic tissues. The preformed *Z*-bond is maintained in the metabolic formation of the plant growth regulator abscisic acid, which possesses a *Z*-bond in the relevant position (ref. 12).

Chirality is also important in carotenoid biochemistry and biology. As a general rule most carotenoids occur in Nature in the optically pure state, reflecting stereospecific, enzymic biosynthesis. Consequently biosynthetically related carotenoids have consistent chirality. Deviating chirality and lack of enantiomeric purity are generally a result of secondary metabolic processes. A typical example of the first type is the presence of the (6*S*) ε-end group in carotenoid metabolites established by Schiedt *et al* in egg yolk (ref. 13), and by Matsuno's group in fishes (ref. 14). To the latter type belongs ε,ε-carotene-3,3'-diol, known to exist as several chiral isomers (ref. 15) including the tunaxanthins. These isomers are formed metabolically in fish (ref. 14). Lack of enantioselectivity has been demonstrated for protein binding of the three optical isomers of astaxanthin in crustacyanin (ref. 16) and for absorption of the same enantiomers and *meso* form by salmonids (ref. 17). On the other hand stereoselective esterification of the (3*S*) end group of astaxanthin is observed in salmon skin (ref. 18).

SELECTED STEREOCHEMICAL STUDIES

In the following discussion, stereochemical studies of carotenoids will be illustrated by selected examples from our research group, carried out over a 40-year period, partly in a historical context, but with emphasis on more recent work and interdisciplinary aspects, and the application of different methodology.

Geometrical isomerism

Trisubstituted *Z*-double bonds in the 9 and 13 positions are readily formed in solution. Since this is not the case for unsubstituted diphenylpolyenes studied by Zechmeister (ref. 19), which lack the lateral methyl groups, my suggestion is that the isoprenoid polyene chain is Nature's trick to promote *E/Z* isomerization. For a long time it was assumed, with classical exceptions such as bixin and prolycopene, that the all-*E* carotenoid was the naturally occurring isomer; this is now known not always to be true.

The first example is the C_{50}-carotenoid bacterioruberin (*2*) from halophilic bacteria, which has received our interest over three decades. (2*S*,2'*S*)-Chirality was proved by total synthesis of the tetraanhydro derivative *3*, also obtained from *2* (ref. 20) (Scheme 2). The steric instability of the aliphatic tridecaene chromophore was noted at an early stage (ref. 21). However, HPLC separation and ^{1}H NMR studies of individual geometrical isomers were not effected until the late 1980s in Berne (ref. 22) and Trondheim (ref. 23). Interestingly, the (5*Z*), (9*Z*) and (13*Z*) mono-*Z*-isomers and the (9*Z*,5'*Z*) di-*Z*-isomer were all demonstrated to be naturally occurring, by rapid HPLC analysis after the addition of solvent to the cells. Moreover, the stereoisomeric composition of natural bacterioruberin was surprisingly close to that of an iodine-catalysed equilibrium mixture, which is considered to reflect the thermodynamic equilibrium of an *E/Z* isomerization mixture (ref. 23). Proper demonstration of the natural occurrence of *Z*-isomers requires experiments of this type.

Scheme 2.
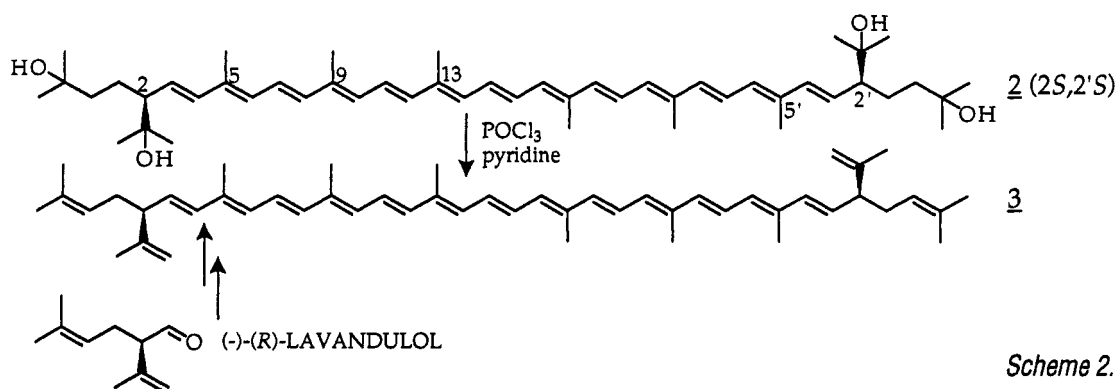
Recently we have demonstrated that the major carotenoid which was isolated from a psycrotrophic, arctic bacterium and exhibited membrane stabilizing properties, and for which a C_{41}-carboxylate structure was previously postulated (ref. 24), is in fact *E/Z*-(2*S*,2'*S*)-bacterioruberin (*2*) (ref. 25). It has been proposed by Ourisson that C_{50}-carotenoids with polar end groups have the correct length to serve as membrane stabilizers (ref. 26). Bent *Z*-isomers obviously cannot serve this purpose, and the dominance of *Z*-isomers calls for a rationalization.

The next example of geometrical isomerism deals with cross-conjugated carotenals, in which a lateral methyl group on the polyene chain has been oxidized to an aldehyde, as in rhodopin-20-al (*4*), first isolated in the 1960s from phototrophic bacteria (ref. 27). We suspected early that the adjacent double bond exhibited the *cis*-configuration (*E*, due to the sequence rules) because of the characteristic *cis*-peak in the UV-region of the spectrum of the corresponding allylic alcohol *5*. Carotenoids with *cis*-bonds near to the centre are known to exhibit strong *cis* peaks in the 350-400 nm region (ref. 19). Such cross-conjugated caroten-20-als exhibit broad absorption spectra in visible light and may facilitate efficient light capture by phototrophic bacteria in restricted wavelength regions.

When we later prepared rhodopinal and related carotenoids by total synthesis (refs. 28, 29), it was confirmed that the preferred configuration of the cross-conjugated system is 13-*cis*. In the total synthesis of renierapurpurin-20-al (*8*) (Scheme 3), a key step was the regioselective allylic bromination of the C_{20}-dial (*6*) at C-20 by NBS, followed by substitution to give the acetoxy C_{20}-aldehyde which, in a Wittig reaction, provided the acetylated C_{40}-carotenol (*7*). Whereas *7* represented a 13-*cis/trans* mixture, the 20-al (*8*) was exclusively 13-*cis* (*E*). More recent NMR studies in collaboration with Englert, including hetero-COSY experiments, have confirmed this conclusion (ref. 30).
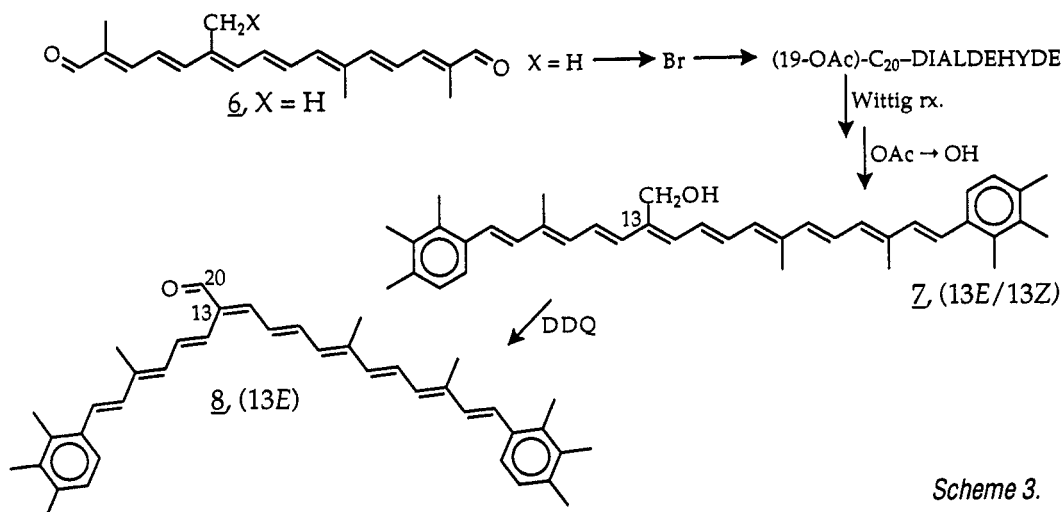
Scheme 3.

Acetylenic carotenoids, typical of diatomaceous phytoplankton, are known to prefer the (9Z)-configuration, for steric reasons as rationalized by Weedon (ref. 31), and confirmed by the composition of iodine-catalysed equilibrium mixtures, where the all-*E* isomer is minor or absent (ref. 32). However, electronic factors are obviously also involved (ref. 32). Recently, stereoselective total syntheses of both (all-*E*)- (ref. 33) and (9Z)-diatoxanthin (*9*) (ref. 34), (Scheme 4), have been carried out in our laboratory. The key C_{15}-intermediate (*10*) which had not previously been prepared, was obtained in several steps by a $C_9+C_6=C_{15}$ Grignard acetylide coupling.
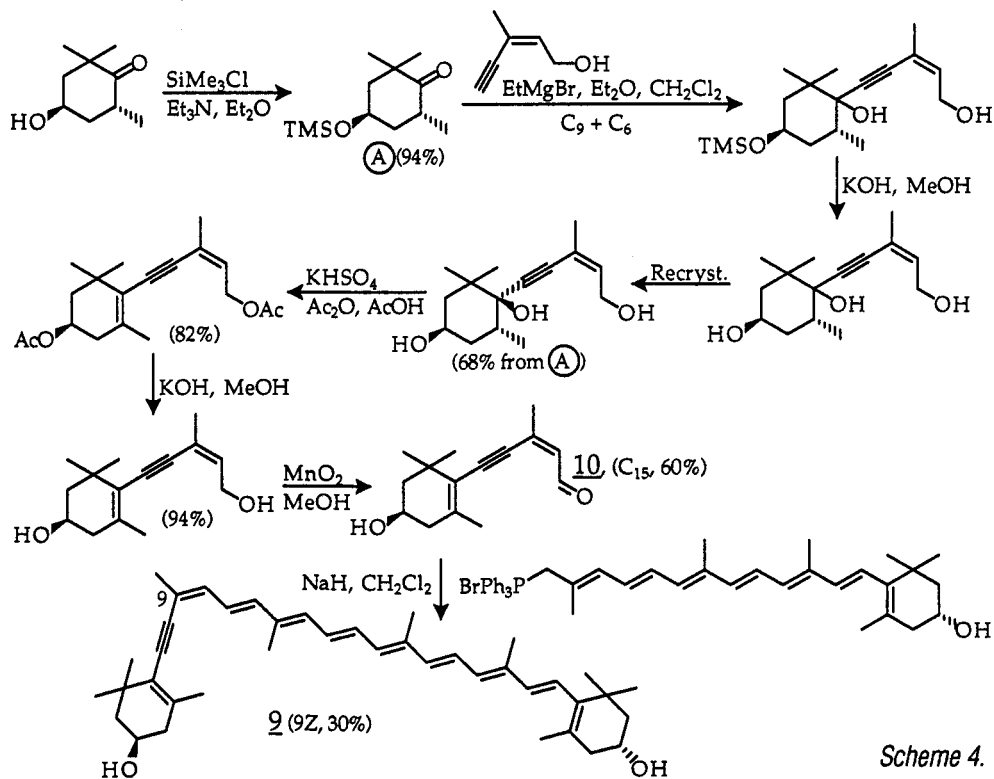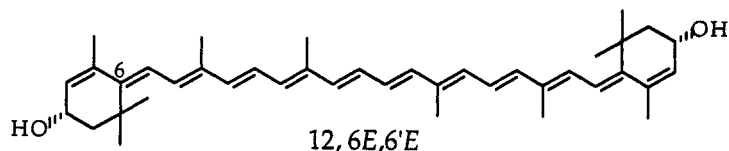

Scheme 4.

Renieracistene (*11*) (Scheme 5), from sponges of the order Hadromerida, first characterized by Japanese workers (ref. 35), possesses a sterically hindered (7*Z*)-bond, possibly formed by metabolic *cis*-hydrogenation of acetylenic carotenoid precursors of dietary origin. The hindered (7*Z*)-isomer, the configuration of which was confirmed by 2D COSY ^{1}H NMR experiments, was surprisingly stable but, in the presence of iodine, was irreversibly transformed to all-*E* and other *Z*-isomers in our isomerization experiments (ref. 36).
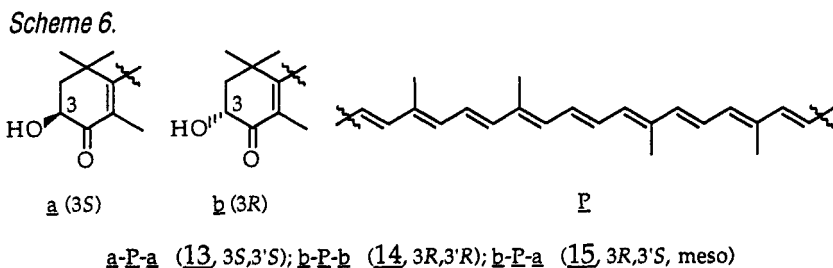
Scheme 5.

Metabolic 7-*cis*-hydrogenation?

I_2, hv

Other isomers   **11**
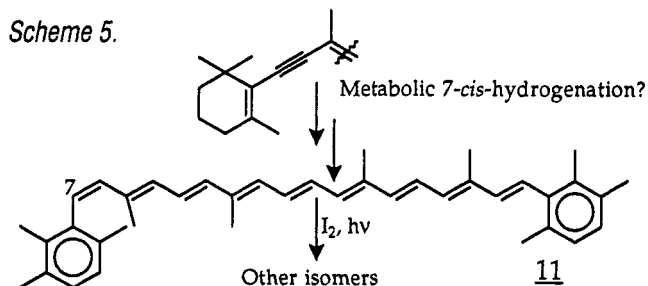
Before leaving geometrical isomerism let us include the *retro*-carotenoid eschscholtzxanthin (*12*) from Californian poppies. The (6*E*,6'*E*) configuration was assigned by NOE experiments including relevant models, in collaboration with Englert (ref. 37), and not the *Z*-configuration as frequently drawn (ref. 14).

12, 6*E*,6'*E*

Chirality

When Hoffmann-La Roche wanted to market astaxanthin as a feed additive it was assumed that the optically active (3*S*,3'*S*)-isomer (*13*) (Scheme 6) was required to satisfy the market for the nature-identical carotenoid. However, as it turned out, the natural feed organisms and the wild salmon itself contained both enantiomers (*13,14*) as well as the *meso* form (15), and this therefore reduced the expense of the industrial synthesis. Astaxanthin was first shown to be partly racemized in lobster (ref. 38) and is fully racemized in shrimps (ref. 39). Such mixtures are analysed as diastereomeric camphanate esters, separated by HPLC, according to the method of Vecchi and Muller (ref. 40).

Scheme 6.

a (3*S*)   b (3*R*)   P

a-P-a (**13**, 3*S*,3'*S*); b-P-b (**14**, 3*R*,3'*R*); b-P-a (**15**, 3*R*,3'*S*, meso)

Another practical example is provided by our isomerization of lutein (*16*) to optically inactive *meso*-zeaxanthin (*17*) promoted by base (Scheme 7), and used as a contribution to confirm the opposite chirality of lutein (*16*) at C-3 and C-3' (ref. 41), as shown independently by Eugster's school (ref. 42). This is now receiving new interest as zeaxanthin is a desired feed ingredient for chickens and laying hens.

Scheme 7.

16

KOMe/MeOH/DMSO   ref. 41
118°C

17, (3R,3'S)-*meso*-zeaxanthin
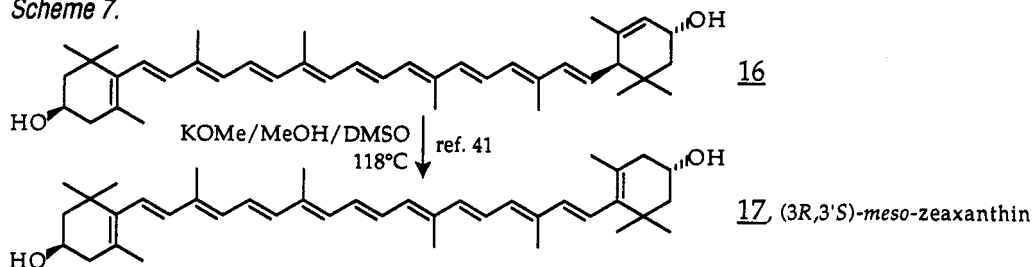
Other partial sytheses have been applied for the determination of absolute configuration. As an example, peridinin *(18)*, the chirality of which was elucidated by oxidative degradation (ref. 43) and NMR studies (ref. 44), and recently confirmed by total synthesis in Ito's laboratory (ref. 45), was, as the diacetate, converted into the acetylenic pyrrhoxanthin *(19)* by reactions not involving the chiral centres (Scheme 8). Upon CD comparison of semisynthetic and natural *19* from dinoflagellates, the same chirality as in peridinin *(18)* was confirmed (ref. 46). This is a biomimetic reaction for observed metabolism in edible mussel (ref. 47) and coral eggs (ref. 48).

Scheme 8.

18

O₃

Ac. anh./pyr.

Biomimetic
conversion

POCl₃/pyr.

19
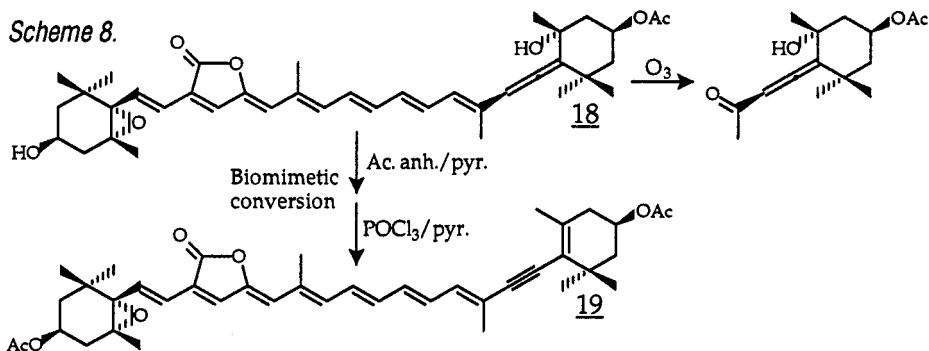
Without going into details, the state of the art is illustrated by the structure of (3*S*,5*R*,6*R*,3'*S*,5'*R*,6'*S*)-13'-*cis*-7',8'-dihydroneoxanthin-20'-al 3'-β-lactoside (P457) *(20)*, where the stereochemistry has been elucidated by derivatization, detailed NMR studies, CD, and synthesis of models (refs. 49,50). By IUPAC nomenclature P457 is 13-*cis*-(3*S*,5*R*,6*S*,3'*S*,5'*R*,6'*R*)-3-(β-D-lactosyloxy)-5,6-epoxy-3',5'-dihydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-β,β-caroten-20-al *(20)*.

20
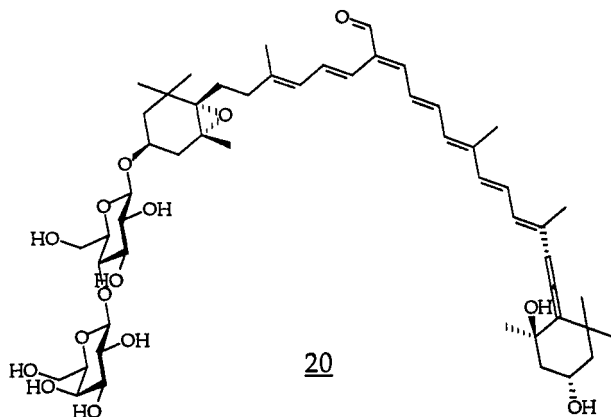
The remaining examples of chirality are chosen in order to illustrate the application of different methodology. In principle, the modified Horeau method is based on the partial resolution of racemic and *meso* α-phenylbutyric anhydride by means of an optically active secondary alcohol which reacts preferentially with the *R* or the *S* acyl group. The unreacted acyl groups are determined quantitatively (ref. 51). (2'*R*)-Chirality for aleuriaxanthin (*21*) was determined by this method (ref. 52).
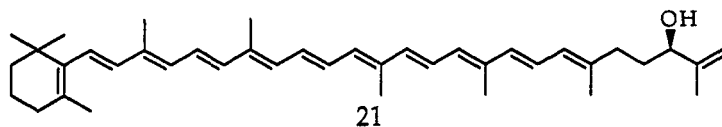
By a similar principle, partial resolution of racemic zeaxanthin (*17,22,23*) was obtained by enantioselective, enzymic esterification, preferentially of the (*R*) end group (ref. 53). Enantiomeric excess was determined by HPLC of the carbamates (ref. 54), prepared after LAH reduction of the esters (Scheme 9).
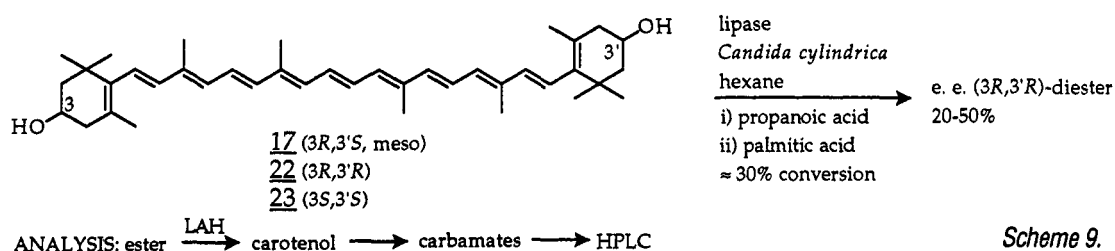

Scheme 9.

Finally the composition of partly racemized β,β-caroten-2-ol (*24,25*) from insects was successfully determined by analysis of the (*S*)-MTPA esters by ^{1}H NMR in the presence of Eu(fod)$_3$ shift reagent (ref. 55).
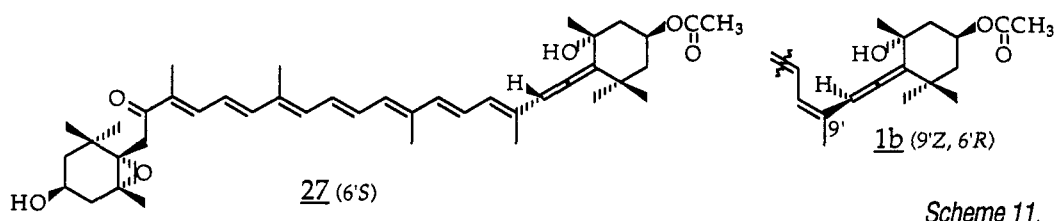
Allenic isomerism

Major allenic carotenoids are fucoxanthin (*1*) and its 19'-acyloxyderivatives, peridinin (*18*), mimulaxanthin, paracentrone and neoxanthin (*26*) (ref. 14), all (6*R*)-allenes. Alternative projections in use are illustrated for neoxanthin (*26*, Scheme 10). The much used projection *a* (ref. 14) gives a false impression of the carbon chain in the encircled region, improved in projection *b*, but here resulting in turning end group B. Projection *c*, employed by Eugster (ref. 56) is a better alternative, although typographically less convenient.


Scheme 10.
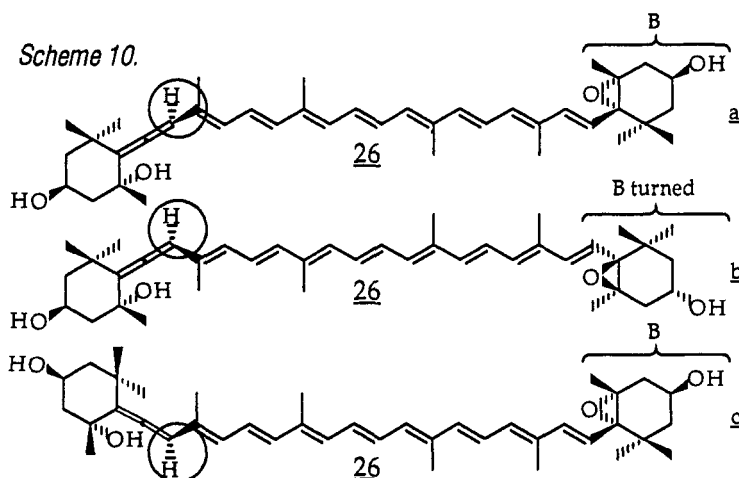
Identical chirality for the allenic end group in all these examples reflects a general biosynthetic pathway. According to Isoe *et al* (ref. 57), the biosynthesis of allenic carotenoids could, by analogy with the photosensitized oxidation of β-ionol, proceed by similar oxidation of zeaxanthin (*22*), which would lead to the (6*S*)-allene, necessitating a subsequent isomerization to the (6*R*)-allene. This hypothesis led to a search for *S*-allenic carotenoids, and in the mid 1970s the natural occurrence of (6'*S*)-fucoxanthin (*27*) was claimed in a brown alga (ref. 58). The conversion of (6'*R*)- into(6'*S*)-fucoxanthin (27) was also reported upon iodine-catalysed stereomutation (ref. 58). However, we later demonstrated that the presumed (6'*S*)-isomer (*27*), with 0.5 ppm downfield shift of the allenic proton relative to the (6'*R*)-isomer (*1*), was misidentified and in fact represented the (9'*Z*,6'*R*) geometrical isomer *1b* (refs. 59,60) (Scheme 11).

Scheme 11.

In an iodine-catalysed stereomutation mixture no allenic *S*-isomer could be detected (ref. 60). Much to our surprise Ito's group later obtained allenic *R/S* isomerization with smaller synthons, as well as with peridinin (*18*) (ref. 61). Reinvestigations in our laboratory demonstrated that isomerization of the allenic bond is condition-dependent and requires high light intensity and/or large quantities of iodine. The daylight at 63° north (Trondheim latitude) is insufficient from October onwards. The configuration of the product, (6'*S*)-peridinin, was confirmed by 2D T-ROESY NMR data (ref. 62). The allenic proton is shifted 0.12 ppm downfield relative to the (6'*R*)-isomer (*18*). We now went back to fucoxanthin (*1*). Indeed, isomerization in bright September sunshine provided an iodine-catalysed stereomutation mixture containing 54% of geometrical isomers with the (6'*R*)-configuration and 46% of geometrical isomers with the (6'*S*)-configuration. The relative ratios of the various geometrical isomers within the allenic *R* and *S* series were the same at quasi-equilibrium. The HPLC purified isomers were characterized by VIS, ¹H NMR and CD spectra. The *R/S* ratio demonstrated no significant difference in thermodynamic stability of the (6'*R*)-allene (ref. 63). This result has been nicely confirmed by AM1 molecular orbital calculations where the heat of formation of the (6'*R*)-isomer was slightly more exothermic, in agreement with a 56:44 *R/S* equilibrium mixture (ref. 64). In experiments with fresh brown algae we have searched for the allenic (6'*S*)-isomer (*27*), which cannot be detected (ref. 65). Two alternative biosynthetic routes to (6*R*)-allenic carotenoids from zeaxanthin (*22*) are depicted in Scheme 12. Route A, proposed by Isoe *et al* (ref. 57) is, in principle, a pericyclic [4+2] reaction with singlet oxygen, providing the (6*S*)-allene and requiring a subsequent isomerization. There is, at present, no evidence in favour of this route, such as the existence in Nature of (6*S*)-allene intermediates or significantly lower thermodynamic stability of the (6*S*)-isomer. Route B is postulated to proceed *via* common 5,6-epoxides. An *anti*-elimination would lead directly to the (6*R*)-allene. The biosynthesis of allenic carotenoids is a challenge for the biochemists.

Concerning the allenic isomerization process we can, after numerous isomerization experiments with fucoxanthin (*1*) and peridinin (*18*) under different conditions, summarize our experience as follows: 1) *R/S* isomerization of the allenic bond in carotenoids may be effected; the process is reversible. 2) Allenic isomerization requires the presence of iodine and is accelerated by strong light. 3) Geometrical isomerization can be effected under milder conditions (no iodine, no light) as a separate process.

Scheme 12.
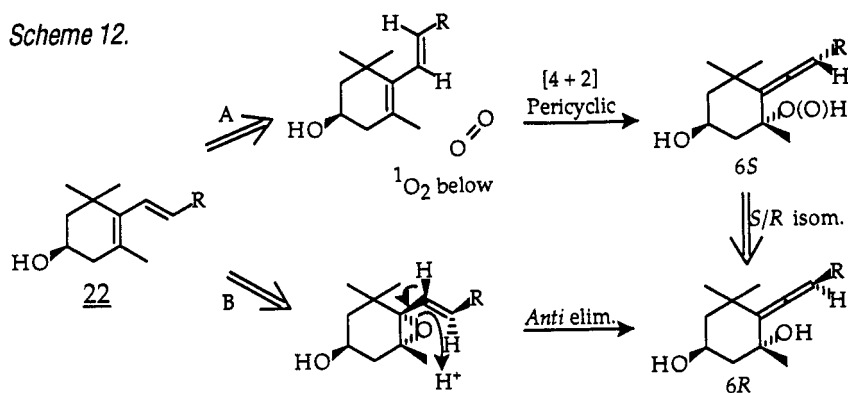
Several matters concerning *E/Z* isomerization and allenic isomerization, including the photochemical event, are not settled. We are currently examining *R/S* allenic isomerization in neoxanthin (*26*) and have a collaboration with Kispert's group on fucoxanthin (*1*). In favour of a cation radical intermediate, an EPR signal for fucoxanthin has been detected on a silica alumina support, and a so far unidentified optical peak around 800 nm has been observed in the presence of iodine (refs. 66,67).
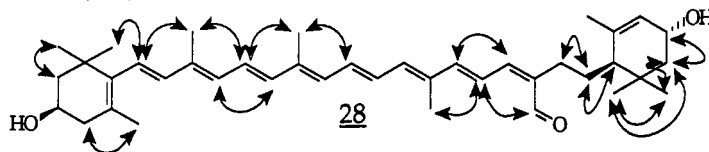
<u>Conformation</u>

The conformation of chiral cyclohexene groups is known to be decisive for the Cotton effect of carotenoids. Conformational analysis is therefore important for the interpretation of carotenoid CD spectra. The so-called conformational rule was used for determining the chirality of (3*S*,3'*S*)-astaxanthin (*13*) after LAH reduction, in collaboration with Snatzke (ref. 68), and for establishing the chirality of (2*R*)-β,β-caroten-2-ol (*24*) (ref. 69). The rule states that it is the conformation of the chiral cyclohexene end group that determines the Cotton effect of the chromophore, perturbed by the chiral end group. The preferred conformation is dictated by large substituents, preferring equatorial positions in the cyclohexene half-chair. Consequently carotenoids with (3*R*)-3-hydroxy-β and (2*R*)-2-hydroxy-β end groups have an opposite Cotton effect, because they adopt opposite chair conformations (Scheme 13).

opposite conformations

Scheme 13.
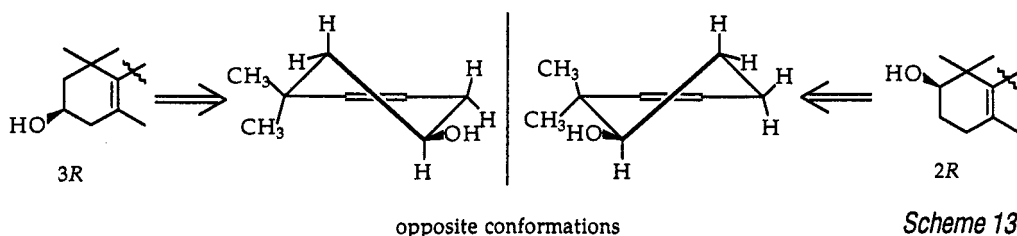
Carotenoids exhibiting conservative CD spectra (ref. 70), with many strong peaks of alternating sign summing up to zero, are known to exhibit inverted CD spectra if a *Z*-bond is introduced in the chromophore (refs. 70-72). This means that only carefully purified geometrical isomers can be used for CD spectra. If not, the wrong conclusion of opposite chirality at chiral centres can be drawn. Micromonal (*28*) from green flagellates exhibited an opposite Cotton effect relative to (3*R*)-cryptoxanthin, consistent with the same (3*R*)-chirality and a (*Z*)-bond in the chromophore. The (9'*Z*)-configuration was proved by ¹H NMR NOE measurements (ref. 73) (Scheme 14).

Scheme 14.

The conformation in the vicinity of the conjugated keto group of the all-*E* chromophore of fucoxanthin (*1*) was demonstrated by NOE effects (ref. 60). Hence fucoxanthin (*1*) should be written as in Scheme 1. Conformational implications of the base-catalysed reaction of fucoxanthin (*1*) have recently been discussed (ref. 74).

ACKNOWLEDGEMENTS

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