195. Conformation and Optical Activity of all-*trans*, mono-*cis*, and di-*cis* Carotenoids: Temperature Dependent Circular Dichroism

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Summary

The CD. spectra of carotenoids with an asymmetric centre at C(3) have the following unusual features: 1) All-*trans* and di-*cis* compounds with two end-rings, at least one of which possesses an asymmetric C-atom, have very similar CD. spectra, whereas the corresponding mono-*cis* compounds give mirror-image CD. spectra; 2) In carotenoids or apocarotenoids with only one end-ring all-*trans* and mono-*cis* compounds have the same CD. spectra; 3) The CD. spectra of such carotenoids are strongly temperature dependent either increasing in magnitude or completely changing in sign upon cooling.

These properties have been rationalized with the aid of a model with takes the total chromophore of the carotenoid as being intrinsically chiral with symmetry C_2. It seems that the chirality arises not only from the presence of the hydroxyl group of an asymmetric carbon atom, C(3), which occupies an equatorial position thereby locking the conformation of the end-ring, but also from the steric hindrance across the formal single bond C(6),C(7), linking the end-ring to the chain and thus creating a chiral π-system. (The twist about the C(6),C(7)-bond acquires a handedness because of the predominance of one conformational form of the end-ring. In this way, the double bonds of the end-ring become twisted out of the plane of the chain with one hand predominating. Thus the whole conjugated system becomes chiral). The reversal of sign between the *trans* (and di-*cis*) and mono-*cis* compounds is due to a tilt of the 2-fold symmetry axis and thereby a change of chirality. The temperature dependence stems from the varying population of forms of different twist of the end-group relative to the chain.

Compounds with 7,8-triple bonds also show distinct CD. spectra and a sign change between all-*trans* and mono-*cis* isomers in addition to temperature dependence. The latter property demonstrates that some steric hindrance between the end-ring and the main chain is present in these compounds.

Some suggestions for the origins of the sign patterns and band intensities of the CD. and absorption spectra are included.

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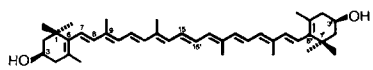
In recent years a number of reports have appeared regarding the circular dichroism (CD.) spectra of optically active carotenoids [1–7]. Attempts have been made to deduce absolute configurations from these data. Although some empirical rules have been proposed a full theoretical understanding of the origin of the optical rotation is lacking. The first attempt to systematise the field was made by *Bartlett et al.* [1] who measured the optical rotatory dispersion (ORD.) of over 40 carotenoids in the range 400–200 nm. In every case the chromophore was a conjugated polyene chain linked to one or two chiral end-groups. The curves could be analyzed in terms of the simple additivity of the effects of the end groups. This enabled a provisional assignment of absolute configurations to be made. *Cis*-carotenoids were largely excluded from this survey. From the CD. spectra of zeaxanthin and astaxanthin it was proposed that the signs of the bands are determined solely by the helicity of the half-chair conformation of the end-ring when it is a cyclohexene ring [3]. This was based upon *Mills* rules [8] for the CD. of conjugated dienes and enones which states that axial substituents, including hydrogen, allylic to the diene or in the α-position to the conjugated ketone determine the sign of the *Cotton* effects. This hypothesis, coupled with conformational analysis to predict the preferred conformation of the cyclohexene ring, led to the suggestion that the CD. of zeaxanthin and 3,4-diols should be determined mainly by the absolute configuration at C(3) (or C(3′)) [3].

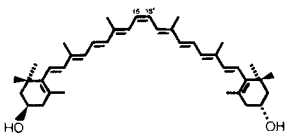
However an important new observation has shown the inadequacy of these early rationalizations. The introduction of one *cis* double-bond into the polyene chain can cause a complete inversion in the CD. spectrum of a carotenoid [4–6]. It was first observed in the ORD. spectrum of 9-*cis*-neoxanthin and then in the CD. spectrum of some fucoxanthin stereoisomers [6]. The CD. spectra of (3*S*,3′*S*)-astaxanthin and the mono-15-*cis*-isomer are almost perfect mirror images [5]. The CD. spectra of the mono-*cis*-isomers of zeaxanthin, diatoxanthin and lutein also exhibit the sign inversion [4].

Since it is unlikely that *cis-trans* isomerism at the central C(15),C(15′)-double-bond of the polyene chain can affect the conformation of the end-rings a reappraisal of the origin of the optical activity in the absorption bands of the polyene chain is clearly needed. We present new experimental results which throw light on this question and we outline a possible theoretical interpretation which should be generally applicable to the understanding of the conformational properties of carotenoids.

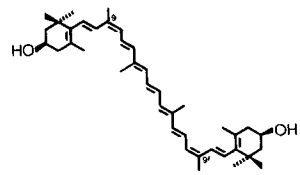
The CD. spectra of the following compounds which all are optically active by virtue of the hydroxy substituent at C(3) (some also at C(3′)) have been studied.



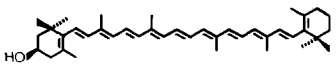
(3*R*,3′*R*)-zeaxanthin



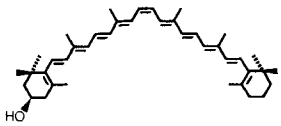
15-*cis*-(3*R*,3′*R*)-zeaxanthin



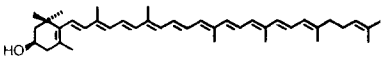
9,9′-di-*cis*-(3*R*,3′*R*)-zeaxanthin



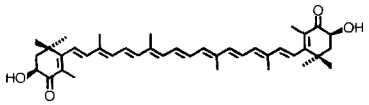
(3*R*)-cryptoxanthin



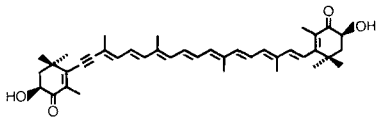
15-*cis*-(3*R*)-cryptoxanthin



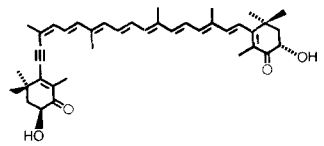
(3*R*)-rubixanthin



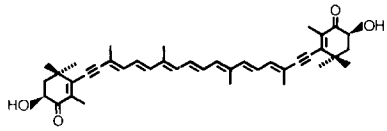
(3*S*,3′*S*)-astaxanthin



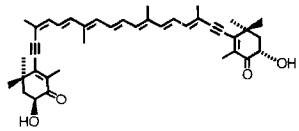
(3*S*,3′*S*)-7,8-didehydro-astaxanthin



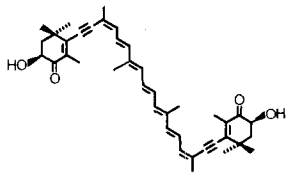
(3*S*,3′*S*)-9-*cis*-7,8-didehydro-astaxanthin



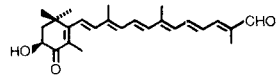
(3*S*,3′*S*)-7,8,7′,8′-tetradehydro-astaxanthin
[(3*S*,3′*S*)-asterinic acid]



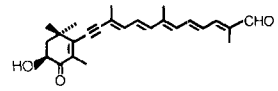
(3*S*,3′*S*)-9-*cis*-asterinic acid



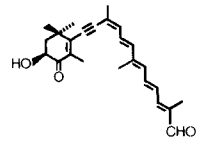
(3*S*,3′*S*)-9,9′-di-*cis*-asterinic acid



(3*S*)-apo-astaxanthin



(3*S*)-7,8-didehydro-apo-astaxanthin



(3*S*)-9-*cis*-7,8-didehydro-apo-astaxanthin

This set of compounds, comprising all-*trans*, mono- and di-*cis* isomers provides examples to examine the effects on the CD. spectrum of changing (i) the isomeric form of the chain, (ii) the nature of the end-ring and (iii) the nature of the linkage between the ring and the side chain. In addition, we have studied the CD. spectra as a function of temperature down to $-180°$.

Carotenoids are conformationally mobile molecules with two features of particular interest, namely, the conformation of the cyclohexene end-ring and the angle between the polyene chain and the end-ring. Hence, if either of these features were significant in controlling the nature of the CD. spectrum, then a temperature-dependent study should provide evidence. This turns out to be the case.

One further aspect of the CD. spectra of carotenoids has also been examined. All the reliable CD. and ORD. data in the literature refer only to the absorption bands higher in energy than the long wavelength band. The optical activity under the intense longest wavelength absorption band of carotenoids is exceedingly weak. Several attempts to measure the CD. curve have given unreliable results because the absorbance of the samples in this spectral region were too high. It is always important to establish that any signals observed obey *Beer*'s law before they can be accepted as reliable. We report examples of CD. spectra at the longest wavelength absorption which we judge to be the first reliable measurements. The spectra have been obtained by the accumulation of many spectra to improve S/N ratios. In spite of this the spectra are noisy. However, optical antipodes have been used to establish the reliability of the signals reported here. In an earlier paper [5], we had only one of the antipodes available. In spite of many precautions, the CD. spectra under the main absorption given there were artifacts (see details in the experimental part).

Results. – *Figure 1* shows the CD. spectrum of (3*R*,3′*R*)-zeaxanthin in EPA (ethanol/isopentane/ether 2:5:5) measured at +20°, −60° and −180° over the spectral range 200–400 nm. The CD. spectra from 370–550 nm of the enantiomorphs (3*R*,3′*R*)- and (3*S*,3′*S*)-zeaxanthin are shown at −150°. The absorption spectrum at +20° and −180° is also given. The values of $\Delta\varepsilon$ and ε shown in this and all subsequent figures have been corrected for the effect of the thermal contraction of the solvent.

Figures 2 and *3* show the CD. spectra as a function of temperature of 15-*cis*-(3R,3′*R*)-zeaxanthin and 9,9′-di-*cis*-(3*R*,3′*R*)-zeaxanthin respectively. As in the case of the *trans*-isomer there is a 4–5-fold increase in the $\Delta\varepsilon$ value upon cooling

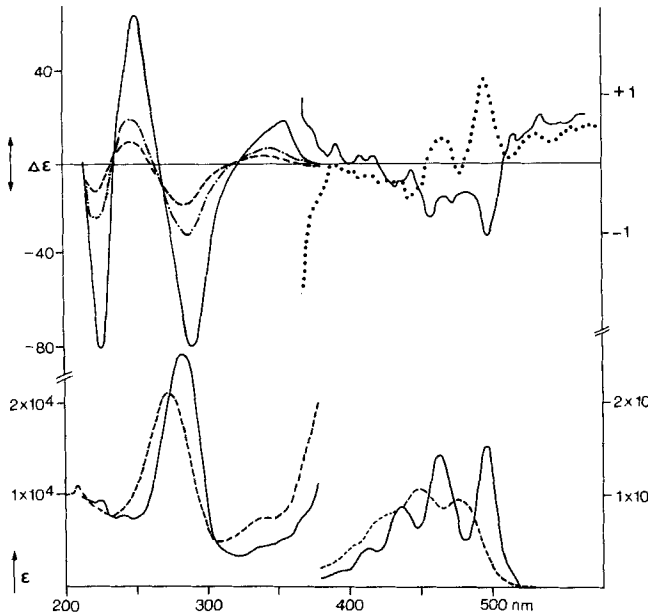


Fig. 1.
CD. and absorption spectrum of (3R,3′R)-zeaxanthin
---- *+20°*
-·-·- *−60°*
—— *−180°*
CD. 580–380 nm:
—— *(3R,3′R)-zeaxanthin*
···· *(3S,3′S)-zeaxanthin, both at −150°* (solvent: EPA)

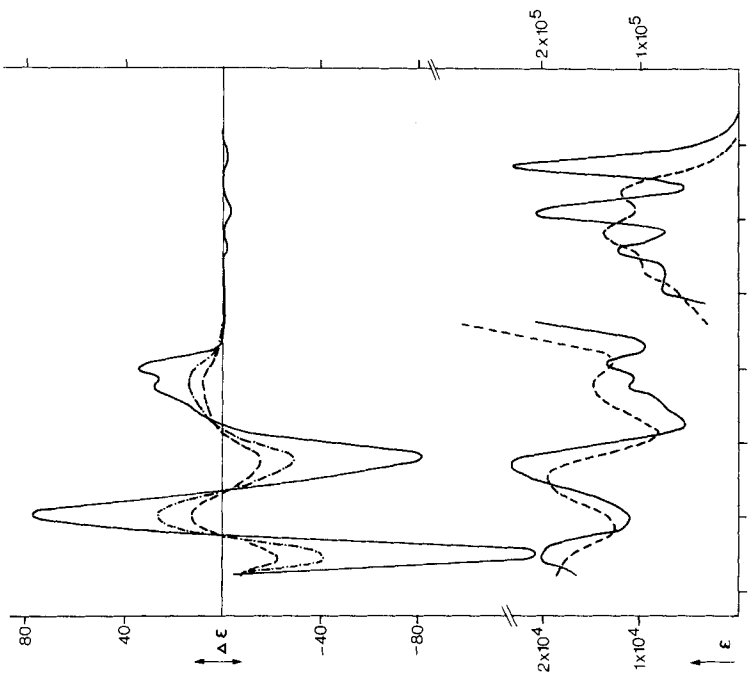


Fig. 3. *CD. and absorption spectrum of (3R,3'R)-9,9'-di-cis-zeaxanthin* ---- *+ 20°,* ·-·-· *– 60°,* —— *– 180°* (solvent: EPA)

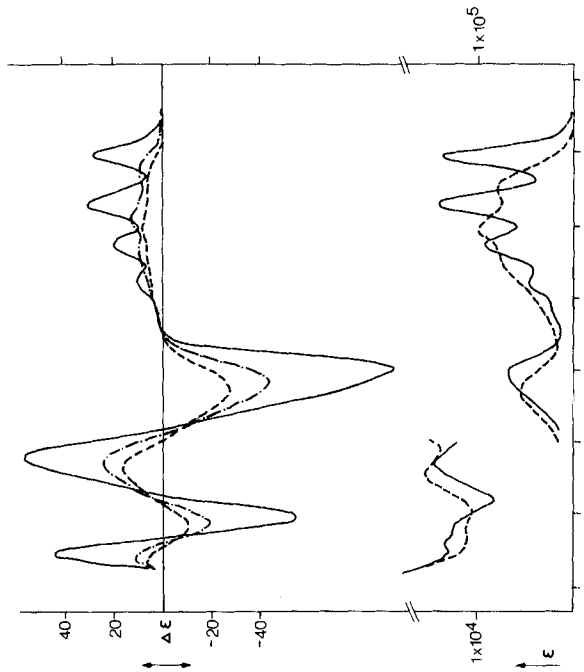


Fig. 2. *CD. and absorption spectrum of (3R,3'R)-15-cis-zeaxanthin* ---- *+ 20°,* ·-·-· *– 60°,* —— *– 180°* (solvent: EPA)

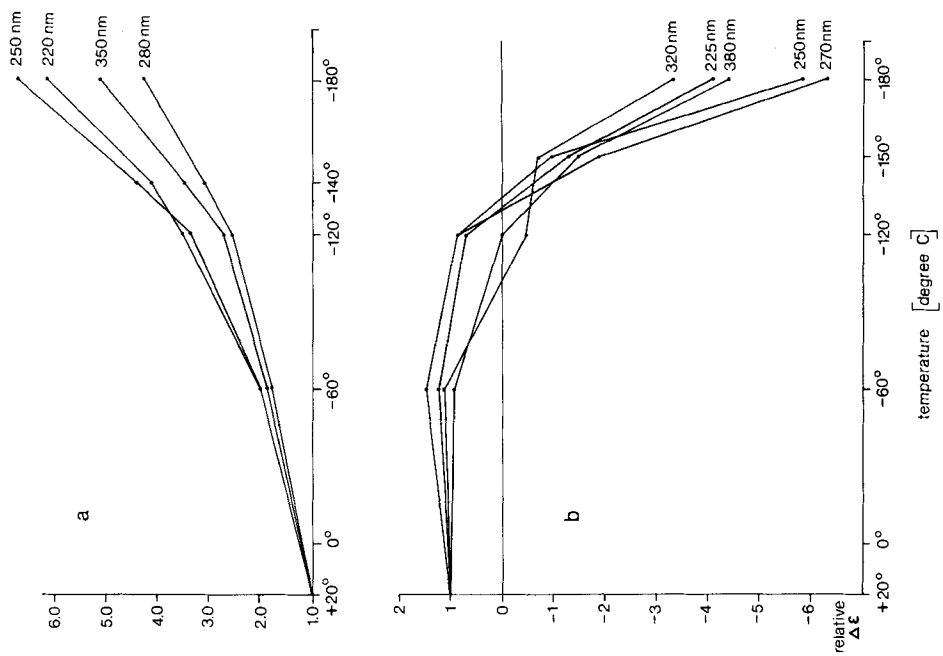


Fig. 5. *Relative $\Delta\varepsilon$-values of all CD. maxima vs. temperature, referred to $\Delta\varepsilon$ at + 20° for (3R, 3'R)-zeaxanthin (a), and (3S, 3'S)-astaxanthin (b)*

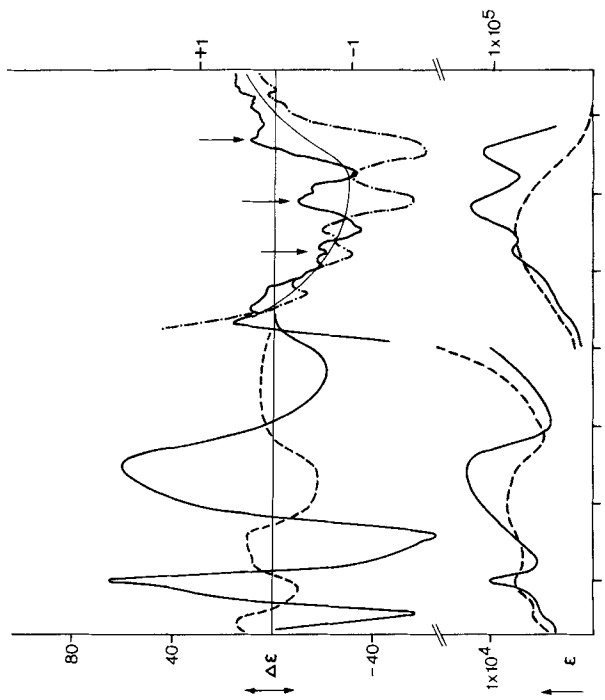


Fig. 4. *CD. and absorption spectrum of (3S, 3'S)-astaxanthin* ---- + 20°, —— – 180°.
CD. 580–400 nm: ——, *(3S, 3'S)-astaxanthin* - · - · -, *(3R, 3'R)-astaxanthin at – 180°* (positions of main bands marked with ↓)

from +20° to −180°. The signs of the CD. maxima are unchanged. Only in the case of the mono-*cis* compound is a CD. signal detectable at RT. under the longest wavelength absorption band. In the di-*cis* and all-*trans*-compounds there is no CD. detectable at RT., but it can be detected at −180°. The spectra of the enantiomorphs of all-*trans* zeaxanthin at −150° definitely show peaks in the CD. spectrum between 450 and 500 nm which correspond closely with peaks in the absorption spectrum at −180°. In the spectra of all three compounds the CD. maxima correspond clearly to peaks or shoulders present in the absorption spectra. The CD. spectrum of the 15-mono-*cis*-zeaxanthin is approximately the mirror-image of that of the all-*trans* and the 9,9′-di-*cis*-compounds, a most important conclusion.

Astaxanthin and its stereoisomers show different behaviour upon cooling. *Figure 4* shows the CD. spectrum of all-*trans*-(3*S*,3′*S*)-astaxanthin at +20° and −180°, over the range 220–400 nm. The CD. maxima first become stronger on cooling to −60° but upon further cooling there is a complete sign reversal of all the CD. maxima and also a very strong (about 5-fold) increase in the $\Delta\varepsilon$-values. The details of the temperature dependence are shown in *Figure 5*, where the $\Delta\varepsilon$-values of the five maxima between 220 and 400 nm are plotted against temperature.

As in all-*trans*-zeaxanthin, CD. has been detected under the longest wavelength band only at −180°. Spectral accumulation was carried out to improve the signal-to-noise ratio but even so considerable noise and a strongly curved base-line is evident in the spectrum, *Figure 4*. However, by comparison of the results from the two enantiomorphs (3*S*,3′*S*)- and (3*R*,3′*R*)-astaxanthin the main peaks are identified unambiguously. Further confirmation is provided by the good correspondence between the main peaks in absorption and CD. spectra measured at −180°.

A sign inversion upon cooling, similar to that in all-*trans*-(3*S*,3′*S*)-astaxanthin, is exhibited also by the following compounds: (3*S*,3′*S*)-15,15′-didehydroastaxanthin [9], (3*S*,3′*S*)-7,8-didehydroastaxanthin and (3*S*,3′*S*)-9-*cis*-7,8-didehydroastaxanthin (see *Fig. 6*). The latter compound also has CD. bands of opposite sign to the corresponding all-*trans* compound. In an earlier account, one of us reported that at RT. the CD. spectrum of all-*trans*-astaxanthin in the region 200–400 nm exhibits a CD. spectrum of opposite sign to the 15-*cis* isomer [5].

Figures 7 and *8* show the temperature dependent CD. and absorption spectra of all-*trans*- and 9,9′-di-*cis*-asterinic acid. The di-*cis*-compound shows much more change on cooling than the *trans* compound.

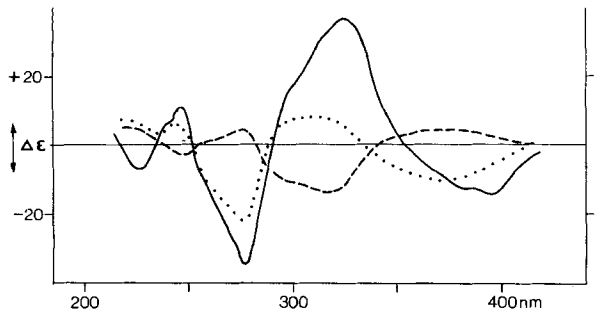


Fig. 6.
CD. spectra of (3S,3′S)-7,8-didehydroastaxanthin ---- at +20°, —— at −180°, and of (3S,3′S)-9-cis-7,8-didehydroastaxanthin at +20° ····

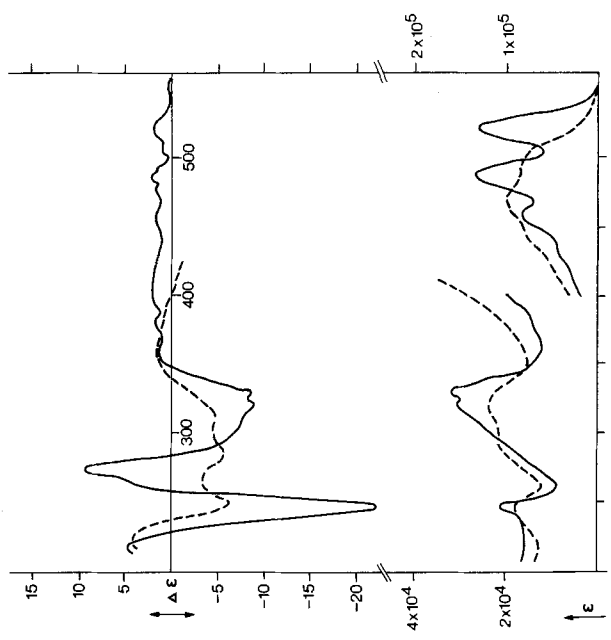


Fig. 8. *CD. and absorption spectra of (3S, 3'S)-9, 9'-di-cis-asterinic acid*
---- *+ 20°*, —— *– 180°* (solvent: EPA)

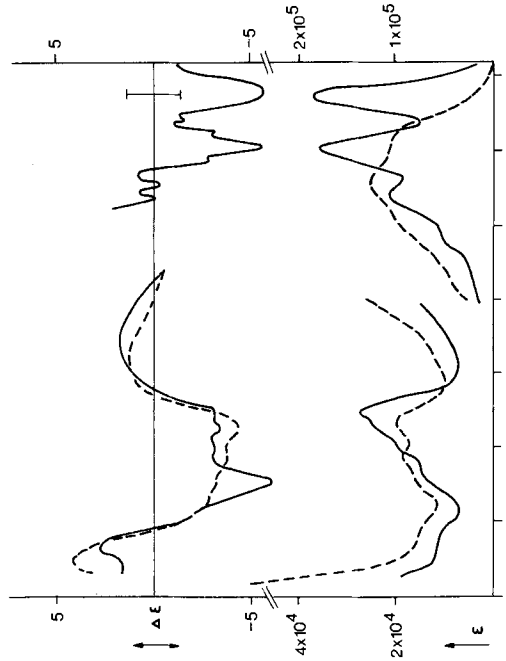


Fig. 7. *CD. and absorption spectra of (3S, 3'S)-asterinic acid*
---- *+ 20°*, —— *– 180°* (solvent: EPA)

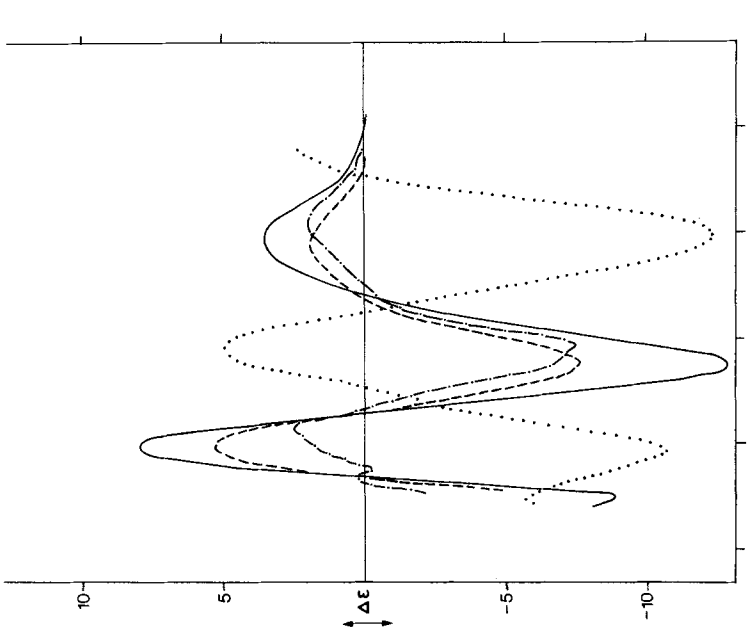


Fig. 10. *CD. spectra of (3R,3'R)-zeaxanthin (——), (3R)-rubixanthin (-·-·-), and (3R)-cyptoxanthin (----) at RT., solvent: dioxan; (····) (3R)-15-cis-cryptoxanthin at −30° (solvent: CH_2Cl_2)*

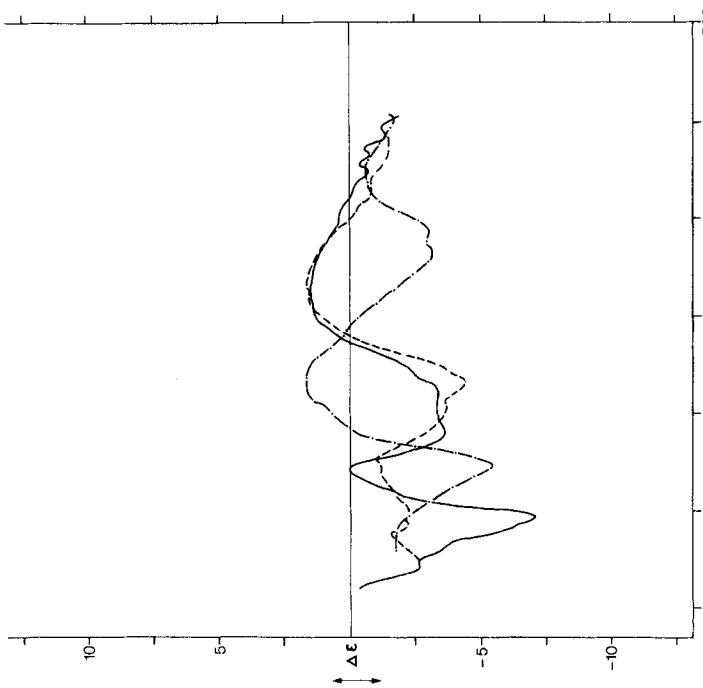


Fig. 9. *CD. spectra of all-trans (----), 9-mono-cis (-·-·-), and 9,9'-di-cis (——) (3S,3'S)-asterinic acid diacetate at RT.* (solvent: dioxan)

Figure 9 compares the CD. spectra at RT. of all-*trans*, 9-mono-*cis*- and 9,9′-di-*cis* asterinic acid diacetates. Those of the all-*trans* and di-*cis* compounds are almost identical with the free hydroxy compounds of *Figures 7* and *8* and also very similar to each other. The mono-*cis* compound has the signs of all CD. bands inverted as compared to the other two.

Figure 10 shows a comparison of the CD. spectra of (3*R*,3′*R*)-zeaxanthin, all-*trans* (3*R*)-cryptoxanthin and (3*R*)-rubixanthin, all of which possess one identical end-group; cryptoxanthin having as its second end-group a β-ionone ring without an asymmetrical C-atom and rubixanthin an open-chain end. In addition, the CD. spectrum of (3*R*,3′*R*)-15-mono-*cis* cryptoxanthin is given to show the sign inversion from the corresponding all-*trans* compound. The spectrum of *cis*-cryptoxanthin was measured in CH_2Cl_2 at −30°, so the $\Delta\varepsilon$-values are greater than in the *trans* compound.

Figure 11 shows the temperature dependence of the CD. spectrum of (3*S*)-apo-astaxanthin. On cooling the CD. bands change sign.

The CD. spectra at RT. of all-*trans* and 9-*cis*-7,8-didehydroastaxanthin are given in *Figure 12*. They show no sign inversion although they are all-*trans* and mono-*cis* isomers, respectively.

Discussion. – We summarise the data from this and related work by means of a set of rules.

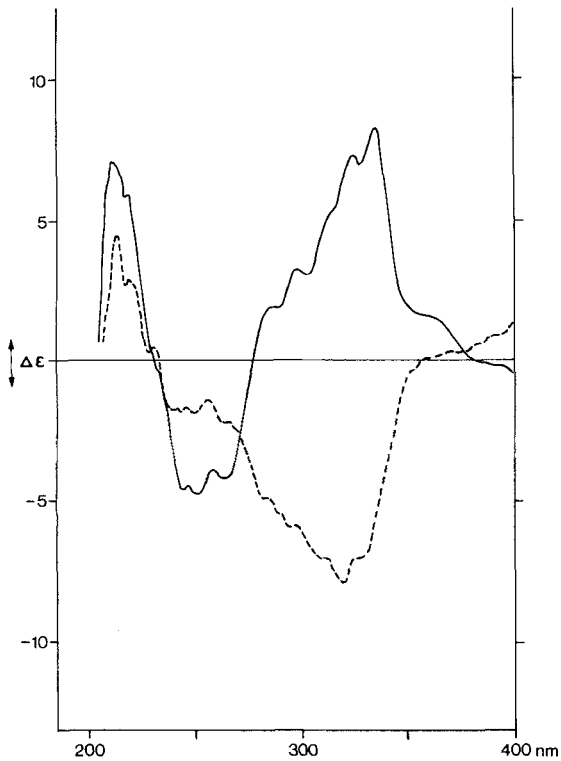


Fig. 11. *CD. spectra of* S-*apoastaxanthin* ---- *+20°*, —— *−150°* (solvent: EPA)

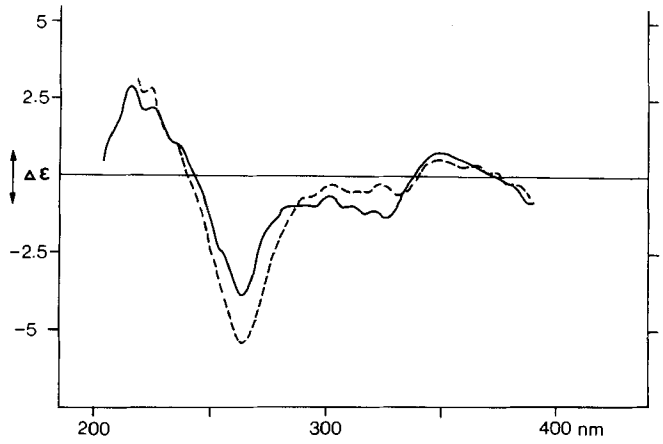


Fig. 12.
CD. spectra of (S)-7,8-dide-hydroapoastaxanthin ——, *and (S)-7,8-didehydro-9-*cis *apoastaxanthin* ---- *at RT.* (solvent: EPA)

Rule 1. *The bands in the CD. spectra of all-*trans *and di-*cis *isomers are opposite in sign to the mono-*cis *isomers of the same carotenoid.*

This rule has been verified in the present study for *trans*, 9,9′-di-*cis* and 15-*mono-cis*-zeaxanthin and for 9 and 13-*cis*-zeaxanthin by *Hertzberg et al.* [4]; for *trans* and 15-mono-*cis* astaxanthin [5]; for the *trans*, 9,9′-di-*cis* and 9-mono-*cis*-isomers of 7,8,7′,8′-tetradehydroastaxanthin diacetate (this study); for *trans* and 9-*cis*-diatoxanthin (7,8-didehydrozeaxanthin) [4]; for *trans* and 9-mono-*cis*-dide-hydroastaxanthin (this work). The last two compounds and the diatoxanthins possess two dissimilar optically active halves, whereas all the other compounds have two identical optically active rings. This rule also holds in the case of a carotenoid having two end-rings, only one of which is optically active, as in cryptoxanthin.

Rule 2. *In pairs of all-*trans *and mono-*cis *isomers of half-carotenoids or carotenoids with only one end-ring the CD. spectra have the same sign.*

This rule has been verified in the cases of *trans* and mono-*cis* apo-7,8-di-dehydroastaxanthin *(Figure 12)*, and *trans* and 5′-mono-*cis*-rubixanthin [10].

Rule 3. *The CD. spectra of carotenoids possessing at least one optically active end-group are strongly temperature dependent.*

The CD. spectra of all the zeaxanthin isomers examined increase in magnitude with decreasing temperature, whereas the CD. spectra of the stereoisomers of astaxanthin and the 7,8-didehydroastaxanthin *(Fig. 6)* invert in sign and then strongly increase on cooling. This sign inversion appears to be general for a carot-enoid having either one or two end-groups of the astaxanthin type, i.e. a 3-hydroxy-4-oxo-ionone. For example the CD. spectrum of the half-carotenoid 8-apo-asta-xanthin inverts in sign on cooling from 20° to − 150° *(Fig. 11)*.

Previous attempts to account for the CD. spectra of polyenes have assumed that the polyene chain, which is the chromophore, is not intrinsically chiral [3]. An attempt was made to explain the spectra in terms of the conformation and asym-metric substitution of the cyclohexene rings using *Mills* rules [8]. These are really a set of octant rules for the cyclohexene ring in which the spatial orientation of the asymmetric substituent relative to the chromophore accounts for the sign of the

optical activity. However, it is difficult to see how such a model can account for the almost perfect inversion of the CD. spectra on forming a mono-*cis*-isomer of the polyene chain *(Rule 1)*. Thus it is necessary to examine more closely the conformational properties of optically active carotenoids.

Conformation of carotenoids. A carotenoid possessing a β-ionone end-ring is an intrinsically chiral chromophore by virtue of the twist about the C(6),C(7) formal single-bond. Steric hindrance between the H-atoms on C(7) and on C(8) and the methyl groups on C(5) and C(1) prevents the C-atoms C(5), C(6), C(7) and C(8) from being coplanar. There is a compromise between the steric hindrance just mentioned and the tendency of the four C-atoms to lie coplanar thereby maximising conjugation between the C(5),C(6) double-bond and the polyene chain. The twist about the C(6),C(7) single-bond is now well documented in a number of carotenoids and vitamin A derivatives. The X-ray structures of several polyenes reveal a wide range of angles of twist in the crystalline state. *Figure 13* and *Table 1* summarize all the data.

The data can be placed into two groups. The conformation of the ring is fixed in the half-chair form shown in *Figure 13*. The angle D measures the rotation about the C(6),C(7) single-bond. (Strictly speaking, D in *Table 1* are the angles between the best plane through the chain and the flat part of the ring. They are not very different from the torsion angles of the bonds 5(6,7)8; see discussion in [e]). Thus D = 0° is the planar-*s-trans*, D = 180° the planar-*s-cis* conformation. The first two groups of molecules have values of D such that the double bond C(7),C(8) is lying in a range between 28° and 62° away from the *s-cis* position. These molecules constitute two groups because C(8) can lie either above the plane C(1), C(5), C(6) or below this plane (that is, C(8) and C(2) on the same or on the opposite sides of that plane). The two sides of this plane are non-equivalent because of the conformation of the end-ring. The molecules of the third group have values of D very close to 0° which gives them an *s-trans* configuration about C(6),C(7). These results have been nicely rationalized by the computation of a semiempirical potential for the ring torsional angle D [11]. The potential contains two minima between 0° and 180°, one ($60° \leqslant D \leqslant 140°$) being rather broad and flat and the other ($D \cong 0°$) narrow and sharp. They correspond respectively to a non planar *s-cis* conformation around the C(6),C(7) single-bond that minimizes the repulsion between the methyl group at C(5) and the H-atom at C(8) and an almost planar *s-trans* conformation with H–C(8) between the *gem.* methyl groups at C(1). The potential curve repeats itself for value of D = 181–360°, and the second half is identical to the first as no account is taken of the conformation of the cyclohexene ring. Hence in this model there appear two broad minima at $60° \leqslant D \leqslant 140°$ and $220° \leqslant D \leqslant 320°$ and a sharp minimum at D = 0° (= 360°). As the conformation of the ring flips the two CH_3–C(1) wag up and down. Thus the

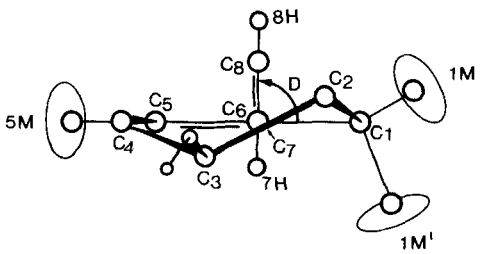


Fig. 13. *Carotenoid with 3-hydroxy-β-ionone end-ring viewed along C(6)–C(7)-bond from one end across the ring.* The fixed conformation of the ring makes the 4 quadrants $0 < D < 90°$, $91° < D < 180°$, $180° < D < 270°$ and $271° < D < 360°$ all inequivalent. We call these quadrants Q_1 to Q_4 respectively.

Table 1. *Conformations of carotenoids from crystal structures.*

Compound	Angle D	
15,15′-Dehydro-β-carotene[a])	224° 44° from *s-cis*	C(2) and C(8) on opposite sides of C(1,5,6) plane, C(8) in Q_3
β-Carotene[b])	215° 35° from *s-cis*	
Vitamin A acid (triclinic form)[c])	215° 35° from *s-cis*	
15,15′-Dehydrocanthaxanthin[d])	152° 28° from *s-cis*	C(2) and C(8) on same side of C(1,5,6) plane, C(8) in Q_2
Canthaxanthin[e])	137° 43° from *s-cis*	
11-*cis*-Retinal[f])	139° 41° from *s-cis*	
trans-Retinal[g])	118° 62° from *s-cis*	
trans-Vitamin-A acetate[h])	58° from *s-cis*	
trans-β-Ionylidene γ-crotonic acid[i])	3° 3° from *s-trans*	
Vitamin A acid (monoclinic form)[j])	6° 6° from *s-trans*	
A carotenoid nitrile[k]) (1,14-bis(2′,6′,6′-Trimethylcyclohex-1′-enyl)-3,12-dimethyltetradeca-1,3,5,7,9,11,13-heptalene-6,9-dinitrile)	17° 17° from *s-trans*	

[a]) *W.G. Sly*, Acta crystallogr. *17*, 511 (1964). [b]) Quoted in ref. e). [c]) *C.H. Stam & C.H. MacGillavry*, Acta crystallogr. *16*, 62 (1963). [d]) *J.C.J. Bart & C.H. MacGillavry*, Acta crystallogr. B*24*, 1569 (1968). [e]) *J.C.J. Bart & C.H. MacGillavry*, Acta crystallogr. B*24*, 1587 (1968). [f]) *R.D. Gilardi, I.L. Karle & J. Karle*, Acta crystallogr. B*28*, 2605 (1972). [g]) *J. Hamanaka, T. Mitsui, T. Ashida & M. Kakudo*, Acta crystallogr. B*28*, 214 (1972). [h]) Torsion angle; *W.E. Oberhänsli, H.P. Wagner & O. Isler*, Acta crystallogr. B*30*, 161 (1974). [i]) *B. Koch*, Acta crystallogr. B*28*, 1151 (1972). [j]) *C.H. Stam*, Acta crystallogr. B*28*, 2936 (1972). [k]) *P.B. Braun, J. Hornstra & J.I. Leenhouts*, Acta crystallogr. B*27*, 90 (1971).

s-trans position will move a few degrees up and down. This may account for the slight variation in the *s-trans* bond angle seen in *Table 1*.

In *Table 1* a distinction has been made between *s-cis* carotenoids with C(8) lying in the quadrant with $118° < D < 152°$ and those in the quadrant with $215° < D < 224°$. There is a slight energy difference between these two forms because of the conformation adopted by the cyclohexene ring. The form seen in the crystal lattice may well be dictated by packing forces. In some cases the position of C(2) especially is uncertain because of disorder in the structure. This could imply that the crystal contains molecules with both conformations of the end-ring. When C(2) and C(3) carry only H-atoms then the ring conformation can flip very rapidly in solution. On the NMR. time-scale, ring flipping is known to be fast since in canthaxanthin the pairs of protons on C(2) and C(3) give rise to single signals (H–C(2) and H–C(3) are split by coupling to one another) [12]. If the ring can flip and rotate readily around C(6)–C(7) from one mirror image form to the other then the solution will contain an equal population of forms with a right and left hand twist about C(6)–C(7). However, if C(2) or C(3) carries a group such as OH, one conformation of the ring can predominate. The NMR. spectrum of astaxanthin shows clearly that HO–C(3) occupies the equatorial position thereby preventing the ring from flipping from one half-chair to the other [5]. A similar result for zeaxanthin has been obtained with ^{13}C-NMR. [13]. In this case significant energy differences should arise between those conformations with $60° \leq D < 140°$ and those with $220° < D < 300°$. In this way one of the twist forms about C(6)–C(7) will be

favoured over the other. By varying the temperature it should be possible to change the relative proportions of the two forms of different handedness.

In summary the presence of an asymmetric C(3) (or presumably also C(2)) in the ring will cause one of the two twist forms about C(6)–C(7) to predominate and thereby make the polyene chain become intrinsically chiral ('polyene chain' in this context always means the *whole* conjugated system, including the C,C double-bonds of the end-rings).

We now show that the presence of a twist in the polyene chain of one predominant handedness can account for the characteristic features of the CD. spectra of the optically active carotenoids discussed in this paper.

CD. spectra of twisted carotenoids. Applying the arguments about ring conformation to the case of zeaxanthin it can be concluded that the polyene chain will adopt a twist at each end, *Figure 14*, and a given hand will predominate. It is assumed that the end-rings of zeaxanthin will adopt the conformation of β-carotene, C(2) and C(8) lying on opposite sides of the C(1),C(5),C(6)-plane. Since HO–C(3) adopts the equatorial position this confers the twist shown in *Figure 14* at both ends of the carotenoid chain.

The signs of the CD. bands of the first two transitions of twisted conjugated dienes as a function of the twist angle have been calculated by *Charney* [14] and by *Wagnière & Hug* [15]. A twisted *s-trans* butadiene gives a negative CD. for the longest wavelength band, assigned $B' \leftarrow A'$, and a positive CD. for the second ($A' \leftarrow A'$) band when the angle is as shown in *Figure 15*. Of course opposite signs are observed for opposite skew angles as in the figure. This picture can be extended to the twisted carotenoids, the double bonds in the end-rings replacing the double bonds of butadiene, and the connecting conjugated chain taking the place of the C,C single-bond of butadiene. Naturally, the positions of the bands will depend upon the number of conjugated double-bonds in the chain. In the carotenoids the bands are considerably shifted towards lower energy from the positions of the bands of butadiene. This comparison implies that the chirality and therefore the signs of the first and second CD. bands of zeaxanthin are as in butadiene.

The molecules of all-*trans* and the symmetrical 9,9'-di-*cis*-zeaxanthin possess as the only symmetry element a two-fold axis of symmetry perpendicular to the plane of the main chain. The twist of the end-groups with respect to each other is the same in both isomers. Therefore the same sign pattern in the CD.

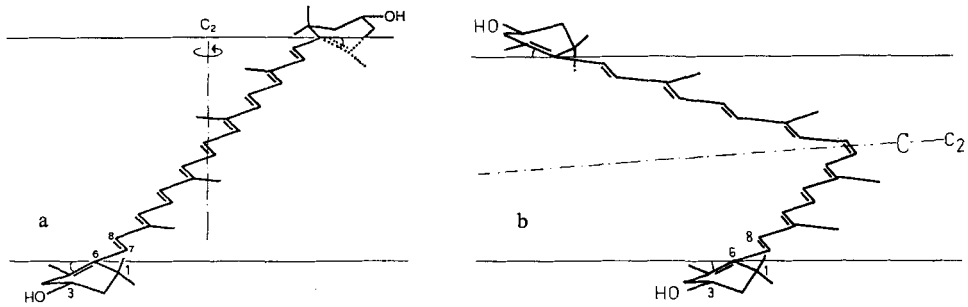


Fig. 14. *Conformations of twisted* trans-*(a) and 15*-cis-*zeaxanthin (b)*

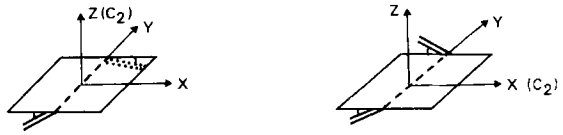


Fig. 15. *Skewed s*-trans *and s*-cis-*butadiene*

spectrum is expected and indeed observed. But for the 15-mono-*cis*-zeaxanthin the situation is quite different. The two-fold axis of symmetry is now swung by 90° into the plane of the conjugated chain. The end-groups are now as in a twisted *s-cis* butadiene (see *Fig. 14*). This, according to *Charney* [14], gives a positive CD. for the first (B′←A′) and a negative CD. for the second (A′←A′) transitions. This sign inversion (as compared with the all-*trans* and di-*cis* compounds) corresponds with our observation. It has been shown earlier that at RT. all-*trans*-astaxanthin and 15-mono-*cis*-astaxanthin show approximately mirror-image CD. spectra [5].

Thus this change in the conformation of carotenoids with two optically active end-groups provides an explanation for the otherwise puzzling observation that the CD. spectra of mono-*cis*-carotenoids are the mirror-image of the all-*trans* and di-*cis* spectra.

A further prediction from this argument is that optically active carotenoids which possess only one end-ring and an open chain at the other end should not show a sign inversion in their CD. spectra in going from the all-*trans* to a mono-*cis* form. In carotenoids with two end-rings the isomerism alters the relative disposition of the twisted end-groups to reverse the direction of the helix. This cannot occur for a compound possessing only one end-ring. The prediction accounts for the observations stated in *Rule 2.*

For compounds having two end-rings, only one of which bears an asymmetric carbon, as for instance cryptoxanthin, (3-hydroxy-β-carotene), the situation is as follows. For the asymmetric end of the molecule the same considerations apply as for zeaxanthin, but at the 'β-carotene' end, the two conformations of the ring are energetically equivalent. Therefore both twists around C(7′)–C(8′) will be equally populated, since a direct influence from the other end of the chain can be neglected. There is one twist at the optically active end, say *R*, and two equally populated twists at the β-carotene end (*R′* and *S′*). The whole ensemble consists therefore of an equal mixture of *R, R′* and *R, S′* forms. The second form is internally compensated ('*meso* form'). Although the end-groups are not identical, this compensation holds to a very high degree of approximation since it is the twist around C(6)–C(7) and not the detailed nature of the end-groups which determines the CD. The other form in the mixture is optically active. The total optical activity is therefore expected to be half that of zeaxanthin, which is exactly what one observes (see *Fig. 10*). Furthermore, a reversal in sign as in the zeaxanthin isomers is expected in going from all-*trans* (or di-*cis*) to mono-*cis* and indeed is observed *(Fig. 10).*

Temperature dependence of the CD. spectra. Turning now to a discussion of the origin of the temperature dependence of CD. spectra, it is clear that this provides

conclusive evidence for considerable conformational mobility of the carotenoids. Mobility either of the ring conformation or about the C(6),C(7) bond is expected, on the model proposed, to affect very significantly the magnitude and even the sign of the CD. spectra. Both effects were observed in the CD. spectra of optically active carotenoids, thereby providing strong support of the theoretical model.

Measurements of nuclear *Overhauser* enhancements (NOE) in β-ionone and the retinals have led to estimate of the bond angles about the C(6),C(7) bond of these compounds in solution [11]. In agreement with the extensive X-ray studies on the crystalline state the NOE show a non planar *s-cis* conformation about C(6)–C(7) which is strongly temperature dependent. The angle D is in the region of 110° at −50° with a somewhat smaller angle (*i.e.* larger twist) at 32°. Values of long-range coupling constants suggest a *s-cis* conformation with an angle of approximately 150° at 32°. These results were consistent with the potential function, which predicts a broad flat minimum permitting angles over a range of about 80° at RT. In β-ionone it is possible that an equilibrium exists between *s-cis* and *s-trans* conformers.

In summary the NMR. data suggest that at higher temperature a wider range of *s-cis* bond angles can persist, and population of the *s-trans* forms can be found. In *Charney*'s early work he calculated the rotational strength as a function of the torsion angle about the central single bond of butadiene [14]. This treatment predicts that neither *s-trans* nor planar *s-cis* butadiene will have rotational strength (this must of course be so, since both possess planes of symmetry). The activity will increase as the angle of twist increases, being a maximum at about 90°. However, *Charney* questions the validity of his own treatment for really large torsion angles, say greater than 25°, since at 90° the system is no longer a conjugated diene.

The results for zeaxanthin show an increase in magnitude, but no change in sign of the CD. spectra on cooling. At RT. a range of twist angles is expected to be present, each conformer contributing a CD. spectrum of different magnitude and possibly also of different sign if the angle D lies in different quadrants. As the temperature is lowered the range of ring angles will decrease and one form will finally predominate. It is not possible to predict whether the overall CD. signal will increase or decrease or even change in sign as this process takes place. Such a prediction would only be possible if the variation of the rotational strength with angle D were known and also if the detailed shape of the potential function were also known. Certainly any population of the *s-trans* form or of forms with D in a different quadrant must lead to a reduction in the CD. signal intensity.

The temperature dependence of the CD. spectrum of astaxanthin is quite different showing little change with temperature until −120° then a steep decrease and change of sign to −180°. This behaviour seems to be characteristic of a carotenoid with a cyclohexenone end-ring. The exo C=O bond is expected to stiffen the ring so that release from angular strain within the ring is effected through C(2) moving out of the plane. On the other hand in a cyclohexene ring there are more degrees of freedom for release from strain.

The inversion of sign with changing temperature can only be accounted for on our model by an initial population of conformers with C(8) in different quadrants and which therefore give opposite contributions to the optical rotation at RT. At

low temperature one form predominates. Since the maximum population of the less stable form at high temperature can never be higher than 50%, this form must have a stronger intrinsic CD. than the more stable one in order to dominate the overall CD. at RT. At low temperature the other form increases in proportion and will then dominate the CD. and hence bring about the change of sign. It is of course very probable that more than two forms participate in such a temperature dependent equilibrium although this does not change the essential features of the picture.

Now it is possible to predict the preferred low temperature conformers of *trans*-zeaxanthin and *trans*-astaxanthin. *Charney*'s rules [14] for the sign of the CD. of the first and second transitions predict for (3*R*,3'*R*)-zeaxanthin a left handed helix (negative CD. for the first and positive CD. for the second band). Assuming that the OH-groups are equatorial and knowing the absolute configuration being *R*, it follows that the preferred conformation of zeaxanthin about the C(6),C(7) bond is the same as that of β-carotene in the crystalline state, that is C(2) and C(8) on opposite sides of the cyclohexene ring plane (plane through C(1), C(6) and C(5)). The preferred conformation of astaxanthin at low temperature is the same as that of canthaxanthin, that is C(2) and C(8) on the same side of the cyclohexene ring plane. We have then for (3*R*,3'*R*)-zeaxanthin the twist shown in *Figures 14* and *15*. (3*S*,3'*S*)-Astaxanthin at low temperature has the opposite twist to (3*R*,3'*R*)-zeaxanthin.

Asterinic acid isomers. It has been argued here that the CD. spectrum of a carotenoid and also its remarkable sign inversion between the all-*trans* and the mono-*cis* form arises from the presence of a hindered C(6),C(7)-bond linking the optically active end-ring to the remainder of the carotenoid chain. In the carotenoids which have a 7–8 triple bond, such as asterinic acid (7,8,7',8'-tetradehydroastaxanthin), the conjugated chain appears to be much less strongly sterically coupled to the end-ring. However, all 3 isomers, the all-*trans*, 9-mono-*cis* and 9,9'-di-*cis* show very distinct CD. spectra. Furthermore there is a sign reversal between all-*trans* and di-*cis* isomers on the one hand and the mono-*cis* on the other. No X-ray structural studies have been carried out on such compounds with a C(7),C(8) triple-bond. The structures of 15,15'-dehydrocanthaxanthin [16] and 15,15'-didehydro-β-carotene [17] show that the triple bond is somewhat shorter (1.19 Å) than the formal double bond ($\sim$ 1.35 Å). The shortening of the C(7),C(8)-bond may bring, in *trans*-asterinic acid, $H_3C-C(9)$ and H–C(10) close enough to the atoms of the end-ring to give some steric repulsion. Molecular models suggest, however, that there is not much steric hindrance in the *trans*-isomer, neither in the coplanar *'s-trans'* nor in the *'s-cis'* form. In the 9-*cis* compounds the *van der Waals* radius of H–C(11) overlaps in the planar *'s-cis'* form with one of the H-atoms of $CH_3-C(5)$, whereas in the planar *'s-trans'* form the overlap is with the equatorial $H_3C-C(1)$.

The temperature dependence of the CD. spectra bears out these points and supports the contention that some steric hindrance is present. The CD. spectrum of the all-*trans* isomer, in which the chain and end-rings are far apart, changes very little on cooling *(Fig. 7)*, whereas in the CD. spectrum of the di-*cis* compound, in which the planar form appears from models to be somewhat hindered,

there is a considerable increase of the $\Delta\varepsilon$-values on cooling *(Fig. 8)*. However, the absolute values of $\Delta\varepsilon$ are much smaller than in astaxanthin. It is now of great importance to have an X-ray structural determination of some asterinic acid isomers.

Signs and magnitudes of carotenoid CD. bands. There are two other striking features of the CD. spectra of the optically active carotenoids discussed in this paper besides the unusual sign inversion and the marked temperature dependence of the signal intensities: 1) the regular alternation in sign of the CD. bands in ascending order of their energies and 2) the exceedingly weak natural CD. under the lowest energy absorption band of the all-*trans* carotenoids. In the mono-*cis* compounds the natural CD. of the longest wavelength band is stronger than in its all-*trans* counterpart but even so it is very weak. Although a complete explanation of these features must await a complete calculation of rotational strengths, it is worthwhile initially to see whether simple symmetry arguments can give a guide to the origin of these characteristics.

Taking into account the twist about the C(6), C(7) single-bond the complete polyene chain has the symmetry C_2. However, the major part of the chromophore belongs to the point group C_{2h} and C_{2v} in the case of the all-*trans* and mono-*cis* isomers, respectively. The deviation from planarity will have only a small effect on the electronic spectrum of the polyene chain although the twist remains important as the source of the rotational strength. Therefore the selection rules for electric and magnetic dipole processes under these two point groups C_{2h} and C_{2v} must be examined. *Table 2* lists the symmetry properties of the ground and excited electronic states of a *trans* and a mono-*cis* polyene. Also shown are the selection rules for transitions from the ground state to each of the excited states both by an electric and by a magnetic dipole process. These rules provide the basis for the usual interpretation of the electronic spectra of polyenes. For example, the lowest energy intense band arises from a transition to a B_u (or B_1) state, which is electric dipole allowed and polarized along y, the long axis of the polyene, both in the *trans* and mono-*cis* isomers. The so-called *cis*-band is the transition to an excited A_g state in the *trans*-isomer and hence electric dipole forbidden. But in the *cis*-isomer the transition is to an A_1 state, now electric dipole allowed along x under the C_{2v} point group. This fact provides an explanation for the well-known intensification of the *cis*-band when a carotenoid isomerises from the *trans* to the mono-*cis* form.

Charney has shown in his calculations on twisted butadienes that transitions to B and A states have CD. bands of opposite sign [14]. If this conclusion can be extended to polyenes as long as the carotenoids, then the regular alternation in sign strongly suggests an assignment of the higher lying carotenoid states as alternately A and B, the *cis*-band being A and those with bands of opposite sign being of B symmetry. This conclusion was reached for the case of lycopene and other carotenoids by showing that alternate bands gain intensity on isomerising with iodine and light [18].

To exhibit non-zero rotational strength an electronic transition must have both a magnetic and an electric dipole transition moment. Furthermore components of these two transitions must be co-linear. The selection rules of *Table 2* show that

Trans, C_{2h} Mono-*cis*, C_{2v}

Fig. 16. *Co-ordinate system for* Table 2

Table 2. *Selection rules for* $\pi - \pi^*$ *transitions of (a)* trans *(b) mono-*cis *polyene*

Ground state	Excited state	Selection rule
a) *trans*, C_{2h}		
A_g	B_u	electric dipole allowed along y magnetic dipole forbidden
	A_g	electric dipole forbidden magnetic dipole allowed along x
b) mono-*cis*, C_{2v}		
A_1	B_1	electric dipole allowed along y magnetic dipole allowed along z
	A_1	electric dipole allowed along x magnetic dipole forbidden

Electric dipole moment transforms as x, y and z.
Magnetic dipole moment transforms as R_x, R_y and R_z.
See *Figure 16* for coordinates.

the longest wavelength band of a *trans*-carotenoid is magnetic dipole forbidden. Hence its rotational strength will be weak. However, a transition to an A_g state is magnetic dipole allowed although electric dipole forbidden. This transition might readily gain electric dipole intensity by borrowing especially from the intense long wavelength band through the effect of vibrations of B_u symmetry. Indeed, the CD. spectra of the *trans*-carotenoids show the presence of a distinct and reasonably intense band where the *cis*-band is expected to lie although it is exceedingly weak in the absorption spectrum. But the long wavelength band is magnetic dipole forbidden in the *trans*-isomer. Perhaps for this reason it has such a low rotational strength. In the mono-*cis* isomer the transition to the B_1 state becomes magnetic dipole allowed and therefore is expected to increase in rotational strength. This is borne out by experiment as we have demonstrated. It remains to see whether these simple selection rule arguments can be made quantitative. Our spectra have provided no clear evidence for the whereabouts of the low energy forbidden A_g state in carotenoids although there has been a flood of activity, both theoretical and experimental, on the retinal polyenes in the search for a transition to this state [19].

Experimental

All compounds are from the Vitamins Research Group of *Hoffmann-La Roche*. We thank Drs. *H. Mayer, R.K. Müller, F. Kienzle, K. Bernhard,* and *A. Rüttimann* for the samples. For description of the synthetic procedures see [20].

The CD. spectra were measured on a dichrographe Mark II *(Jobin-Yvon)* fitted with a 450 watt xenon arc and an automatic scan control for repetitive scanning and accumulation of spectra on paper tape. The data reduction (averaging, smoothing, background subtraction and plotting) was done off line on a IBM 1130 computer.

The solvent used in most cases was EPA (ether/isopentane/ethanol 5:5:2 by volume). The low temperature CD. and absorption spectra are corrected approximately for solvent contraction, a linear contraction of 0.1% per °C being assumed. The variable temperature cell was of a commercial type *(Jobin-Yvon)*. The absorption spectra were measured on a *Cary* Model 14 spectrophotometer with the same variable temperature cell.

Special care was taken for the measurement of the CD. under the main absorption band of zeaxanthin and astaxanthin. The CD. is so small that irregular drift of the base-line can severely compromise the result. Only if mirror-image spectra are obtained with both optical antipodes can the results be trusted. The $\Delta\varepsilon$-values found [5] for (3*S*,3′*S*)-astaxanthin, (3*S*,3′*S*)-15,15′-didehydro-astaxanthin and (3*R*,3′*R*)-zeaxanthin without taking that precaution are unfortunately due to uncontrolled artifacts. The detailed procedure of the new measurement was as follows: OD ~ 0.8–1.0, time constant 10 s, 3 to 6 CAT for solvent filled cell and for solution, both for the *S,S* and the *R,R*-compounds. The spectra of *Figures 1* and *4* show, that even with these precautions a rolling baseline is obtained. However, the mirror-image character of the CD. maxima and the coincidence with absorption maxima gives us confidence in these spectra at low temperature. The sample used for CD. measurements was tested for the presence of linear dichroism by showing that the CD. signal was invariant by inverting the sample about a vertical axis. At RT. the baseline instability was greater than the CD. signal, so only a maximum upper value for the CD. can be given ($\Delta\varepsilon \leqslant 0.5$). The error for the low temperature CD. is also of this order of $\Delta\varepsilon \pm 0.5$. For the *cis*-compounds the CD. under the main absorption band was considerably stronger, reliable values could therefore be obtained on one enantiomer only.

REFERENCES

[1] *L. Bartlett, W. Klyne, W.P. Mose, P.M. Scopes, G. Galasko, A.K. Mallams, B.C.L. Weedon, J. Szabolcs & G. Tóth,* J. chem. Soc. (C) *1969*, 2527.

[2] *R. Buchecker & C.H. Eugster,* Helv. *56*, 1124 (1973).

[3] *A.G. Andrewes, G. Borch, S. Liaaen-Jensen & G. Snatzke,* Acta chem. Scand. B*28*, 730 (1974).

[4] *S. Hertzberg, G. Borch & S. Liaaen-Jensen,* Acta chem. Scand. B*33*, 42 (1979).

[5] *G. Englert, F. Kienzle & K. Noack,* Helv. *60*, 1209 (1977).

[6] *K. Bernhard, G.P. Moss, G. Tóth & B.C.L. Weedon,* Tetrahedron Letters *1974*, 3899.

[7] *A.G. Andrewes, S. Liaaen-Jensen & G. Borch,* Acta chem. Scand. B*28*, 737 (1974).

[8] *J.A. Mills,* J. chem. Soc. *1952*, 1916.

[9] *K. Noack,* unpublished.

[10] *N. Arpin & S. Liaaen-Jensen,* Phytochemistry *8*, 185 (1969).

[11] *B. Honig, B. Hudson, B.D. Sykes & M. Karplus,* Proc. Nat. Acad. Sci. USA *68*, 1289 (1971).

[12] *B.H.S. Lienard & A.J. Thomson,* J. chem. Soc. Perkin II *1977*, 1390.

[13] *G.P. Moss,* Pure appl. Chemistry *47*, 97 (1976).

[14] *E. Charney,* Tetrahedron *21*, 3124 (1965).

[15] *G. Wagnière & W. Hug,* Tetrahedron Letters *55*, 4765 (1970).

[16] *J.C.J. Bart & C.H. MacGillavry,* Acta crystallogr. B*24*, 1569 (1968).

[17] *W.G. Sly,* Acta crystallogr. *17*, 511 (1964).

[18] *L. Zechmeister, A.L. LeRosen, W.A. Schroeder, A. Polgár & L. Pauling,* J. Amer. chem. Soc. *65*, 1940 (1943).

[19] *B. Hudson & B. Kohler,* J. chem. Physics *59*, 4984 (1973).

[20] *H. Mayer,* Pure appl. Chemistry *51*, 535 (1979). See also: Abstracts of contributed papers, 5th International Symposium on Carotenoids, Madison, Wisc. USA, July 23–28, 1978, paper by *K. Bernhard, F. Kienzle, H. Mayer & R.K. Müller.*