

Circular Dichroic Spectra of Mono-*cis* Carotenoids

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CD spectra of the *cis-trans* isomers present in the iodine catalyzed stereomutation mixtures of zeaxanthin (1), diatoxanthin (2) and lutein (3) are reported. The mono-*cis* isomers exhibit opposite Cotton effects relative to the all-*trans* isomers. The intensity of the CD bands in the so-called *cis*-region is enhanced for mono-*cis* isomers, particularly those with near-to-central *cis* bonds.

Chiral centra at C-3 and C-2 in all-*trans*-carotenoids containing ϵ -rings do not contribute significantly to their CD spectra. 19-Hydroxylation appears to influence the chiroptical properties.

Chiroptical properties of carotenoids have been extensively studied in recent years.^{1,2} Useful empirical rules have been developed,^{1,3,4} but theoretical explanations are still lacking.

Inversion of the Cotton effect by introduction of a *cis* double bond in the polyene chain had been noted in the ORD spectrum of 9-*cis*-neoxanthin (6b, Scheme 1)¹ and in the CD spectra of some fucoxanthin (7) stereoisomers⁵ when this study was undertaken. We now document our previous statements² for *cis-trans* isomers of zeaxanthin (1), diatoxanthin (2) and lutein (3).

RESULTS AND DISCUSSION

Zeaxanthin (1). Iodine catalyzed stereomutation of zeaxanthin (1) results in all-*trans* (1a) and two *cis*-isomers referred to as neo A and neo B.⁶⁻⁸ In Zechmeister's nomenclature for *cis*-isomers, neo A, B, etc. should indicate chromatographic position ahead of the all-*trans* isomer (higher R_F -value) and neo U, V, etc. above the all-*trans* (lower R_F -value).⁹ However,

this was not followed in the older literature and the neo A and B isomers of zeaxanthin (1) and lutein (3) are actually neo V and U isomers respectively.

Configurational assignment of neo A as 13-*cis* (1c) and of neo B as 9-*cis* (1b) followed from λ_{\max} shift, *cis*-peak intensities and IR data⁹ and more recently ¹³C NMR evidence.¹⁰

CD spectra of all-*trans* (1a), 9-*cis* (1b) and 13-*cis* (1c) obtained here by iodine catalyzed stereomutation are given in Fig. 1. Opposite Cotton effects are observed for the mono-*cis* isomers (1b, 1c), relative to that of the all-*trans* (1a). The intensity of the negative maximum at 335 nm (*cis*-peak region) for the 13-*cis* isomer (1c) is remarkably enhanced.

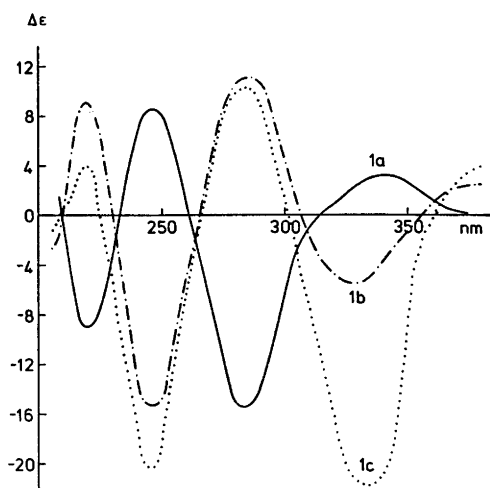
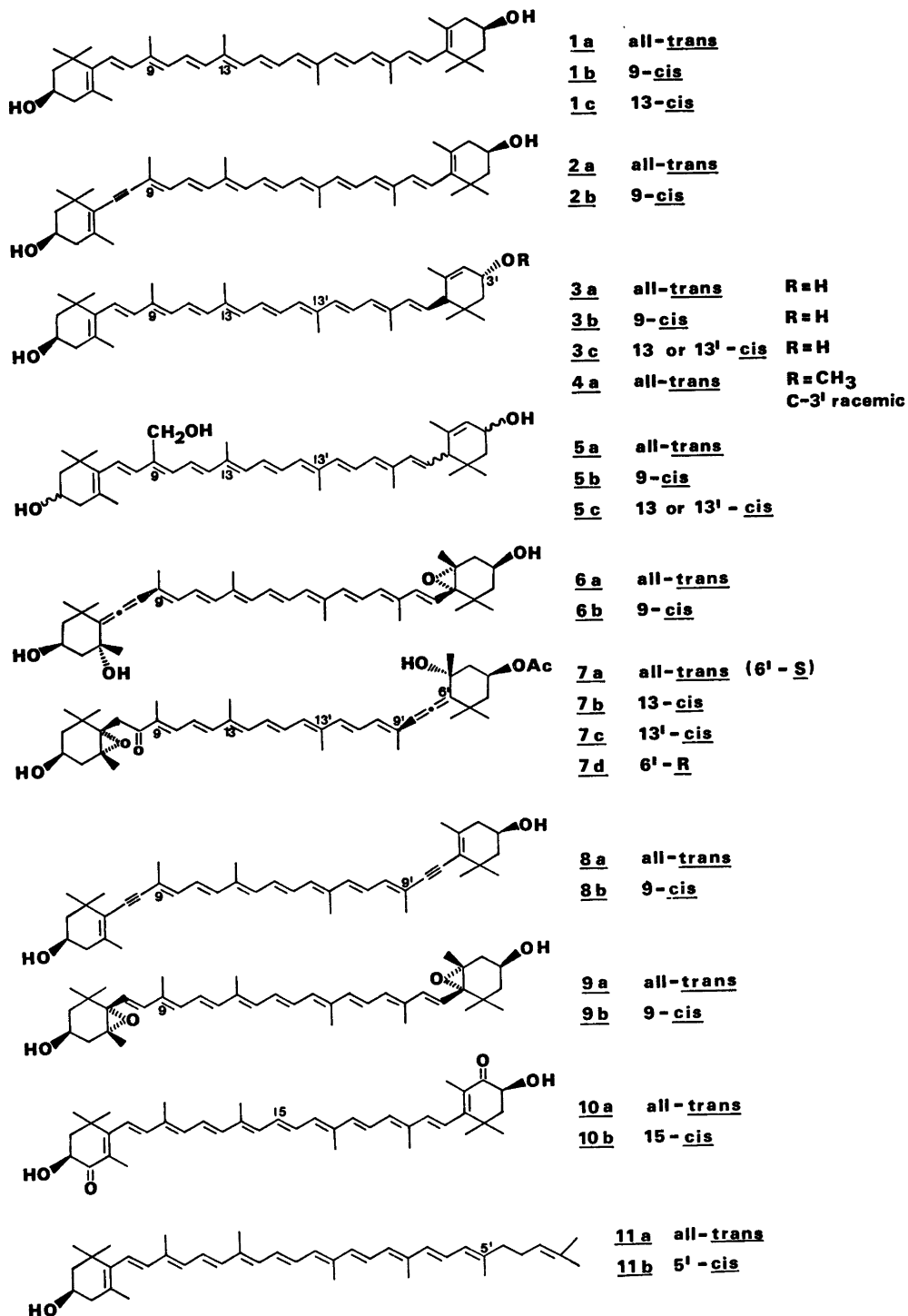


Fig. 1. CD spectra in EPA solution of all-*trans*-zeaxanthin (1a), 9-*cis*-zeaxanthin (1b) and 13-*cis*-zeaxanthin (1c).



Diatoxanthin (2). The stereoisomeric set of the monoacetylenic diatoxanthin (2) has not previously been fully characterized. Iodine catalyzed stereomutation followed by chromatographic separation on calcium carbonate gave all-*trans* (2a) and neo U as major isomers in ratio 47:53. Neo U was tentatively identified as 9-*cis* (2b) from λ_{\max} , small *cis*-peak and increased spectral fine-structure of the main band relative to the all-*trans* isomer. The latter phenomenon is characteristic of 9-*cis* isomers of 7,8-didehydro carotenoids such as alloxanthin (3).^{11,12}

CD spectra of all-*trans* (2a) and 9-*cis* diatoxanthin (2b) are reproduced in Fig. 2, revealing mirror-image relationship.

Lutein (3). Lutein is known to contain all-*trans* (3a), neo A (\equiv neo V) and neo B (\equiv neo U) as major isomers in the iodine catalyzed stereomutation mixture.^{7,9,13} The neo B isomer with low *cis*-peak has recently been identified as 9-*cis* (3b) from oxidative degradation by Szaboles and coworkers.¹⁰ Judged from its high *cis*-peak neo A presumably is 13- or 13'-*cis*.⁹ 15-*cis* bonds appear not to be formed by iodine catalyzed stereomutation.

CD spectra of all-*trans* (3a), 9-*cis* (3b) and 13(13')-mono-*cis*-lutein (3c) are given in Fig. 3. In this case a reversion of the sign of all bands was observed for the *cis*-isomers (3b, 3c) relative to those of the all-*trans* (3a) isomer, except for the shortest wavelength band at 220 nm which remained positive in all three cases. As for the zeaxanthin (1) set the intensity of the

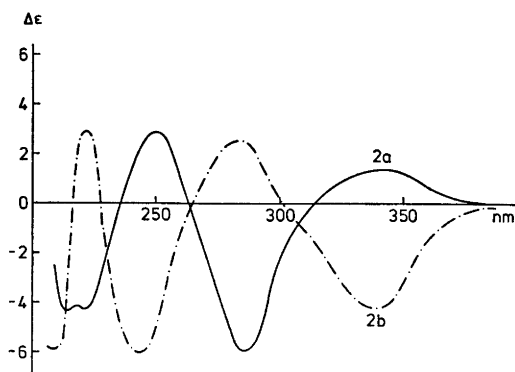


Fig. 2. CD spectra in EPA solution of all-*trans*-diatoxanthin (2a) and 9-*cis*-diatoxanthin (2b).

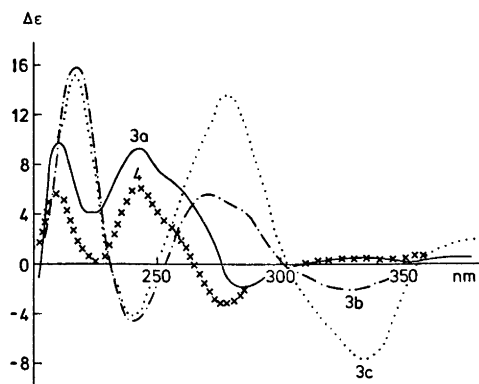


Fig. 3. CD spectra in EPA solution of all-*trans*-lutein (3a) 9-*cis*-lutein (3b), 13 or 13'-mono-*cis*-lutein (3c) and all-*trans* lutein 3'-methyl ether (4).

330 nm band is most enhanced in the 13 (13')-*cis* isomer (3c).

Lutein derivatives. The CD spectrum of lutein 3'-methyl ether (4a) is also included in Fig. 3. The methyl ether (4a) was prepared by treatment with acidified methanol by a presumed S_N1 mechanism, resulting in racemization at C-3'. Since no significant change between the CD spectra of 3a and 4a is observed, it may be concluded that the chirality of the C-3' center does not affect the CD of lutein (3). It has previously been shown that the chirality of C-2 substituents of carotenoids with ϵ -rings has no marked influence on the CD.¹⁴

Loroxanthin (5) is 19-hydroxylutein,¹⁵ but is stereochemically yet undefined. Substitution of in-chain methyl groups is known to cause preference for *cis*-configuration of the neighbouring double bond,^{16,17} and the ^1H NMR spectrum of loroxanthin (5) exhibits singlets at both δ 4.60 and 4.45 for $-\text{CH}_2\text{OH}$ ascribed to *trans* and *cis* 9-double bond.¹⁸ CD-correlation of pure stereoisomers of loroxanthin (5) as triacetate 5' and lutein (3) was expected to provide information about the chirality at C-3 and C-6' in 5. After iodine catalyzed stereomutation of loroxanthin triacetate (5') three stereoisomers tentatively identified as all-*trans* (5'a), 9-*cis* (5'b, neo U) and 13 or 13'-mono-*cis* (5'c, neo A) were isolated. CD spectra of 5, 5'a, 5'b and 5'c indicated that no direct comparison between carotenoids with normal

methylated and in-chain substituted chromophores can be made. This result is compatible with the known out-of-plane bending of the chromophore caused by the oxygen substituent.¹⁷ For loroxanthin (5) this is reflected by features of the electronic spectrum: small hypsochromic λ_{\max} displacement and decreased spectral finestructure relative to 3.

SUMMARIZING DISCUSSION

We have demonstrated that 9-*cis* (1*b*) and 13-*cis*-zeaxanthin (1*c*), 9-*cis*-alloxanthin (2*b*), 9-*cis* (3*b*) and 13(13')-*cis*-lutein (3*c*) exhibit opposite Cotton effects relative to the corresponding all-*trans* isomers (1*a*, 2*a* and 3*a*), except for the 220 nm band of lutein (3) which was not reversed.

The CD bands in the so-called *cis*-peak region are enhanced for the monocis isomers, most for the *cis*-isomers with near-to-central *cis* bonds; cf. $\Delta\epsilon(\text{CH}_2\text{Cl}_2) = -23$ for (3*S*,3'*S*)-15-*cis*-astaxanthin (10*b*) referred to below.¹⁹

Caution must therefore be shown by interpretation of CD spectra of carotenoids that readily undergo *cis*-isomerization, such as acetylenic carotenoids and carotenoids with substituted in-chain methyl groups. In the latter case preliminary data for loroxanthin (5) suggest additional modifications of the CD spectrum ascribed to out-of-plane bending of the polyene chain.

The "*cis*-peak" region of the CD-spectra may be of diagnostic importance when checking *cis-trans* purity.

Similar conclusions are evident from the CD data tabulated by Bernhard *et al.*⁵ for four fucoxanthin (7*a-d*) isomers identified as all-*trans* (6'*R*), 13-*cis*, 13'*cis* and 6'*S* (no 9(9')-mono-*cis*-forms were detected).

Subsequent CD data for all-*trans* violaxanthin (9*a*), violeoxanthin = 9-*cis*-violaxanthin (9*b*), all-*trans* neoxanthin (6*a*) and 9-*cis*-neoxanthin (6*b*) show a similar reversion of the Cotton effect for the 9-*cis* isomers.²⁰

More recent CD data for synthetic (3*S*,3'*S*)-15-*cis*-astaxanthin (10*b*) by Englert *et al.*¹⁹ also reveal reversion of the Cotton effect for the 15-*cis* isomer.

For gazaniaxanthin (11*b*), considered to be the 5'-*cis* isomer of rubixanthin (11*a*) with

terminal *cis* bond,^{21,22} no reversion of the Cotton effect has been observed.²²

CONCLUSION

From the data so far available *cis*-bonds in 9,9',13,13' and 15-positions of the normal methylated polyene chain of mono-*cis*-carotenoids result in reversion of the Cotton effects relative to the all-*trans* isomers. The intensity of the maximum in the *cis*-peak region depends on the location of the *cis*-bond and is most intense for 13- and 15-*cis* isomers.

Attempts to rationalize these findings on a theoretical basis will be made elsewhere.²³

EXPERIMENTAL PART

Materials. Chromatographically purified zeaxanthin (1) *ex Flavobacterium* R1519(=0147); diatoxanthin (2) *ex Prymnesium parvum*.²⁴ lutein (3) *ex* alfalfa and loroxanthin (5) *ex Scenedesmus obliquus*¹⁵ were used. Lutein 3'-ethyl ether (4) was prepared by treatment with acidified ethanol²⁵ and characterized by ¹H NMR and MS data. Loroxanthin triacetate (5') was prepared by standard acetylation.²⁶

Methods. Iodine catalyzed stereomutation was carried out by the general method⁹ in benzene solution in daylight until no further spectral change was recorded; typically 1 (30 mg) in benzene (200 ml) and iodine in benzene (3 ml, 40 $\mu\text{g}/\text{ml}$) were kept in daylight for 5 h. Separation of the stereomutation mixtures of 1 and 3 were affected on a CaCO₃ column developed with a benzene-hexane-acetone mixture (10:4:1), whereas stereoisomerized 2 and 5' were separated on CaCO₃-filled circular paper (Schleicher & Schill No. 290) with 2% acetone in hexane as eluent. The individual isomers were characterized by their electronic spectra, solutions concentrated *in vacuo* at 30 °C and kept at -20 °C until CD spectra were recorded. The true nature of the *cis* isomers formed was checked by reversibility tests (I₂/benzene/daylight). Composition of the iodine catalyzed equilibrium mixtures and spectral characteristics of the individual isomers are given in Table 1. CD spectra of the stereoisomeric sets of zeaxanthin (1), diatoxanthin (2) and lutein (3) and lutein 3'-ethyl ether (4) are given in Figs. 1-3. The CD spectra of "crystallized" loroxanthin (5) and loroxanthin triacetate (5') had the following characteristics (EPA solution = diethyl ether-isopentane-ethanol 5:5:2): 5 220 nm (+), 265 (+), 315 (+), 350 (0), 395 (+); 5'a 210 (+), 245 (+), 265 (+), 300 (0), 320 (-); 5'b 210 (+), 265 (+), 235 (+); 5'c 210 (+), 240 (+).

Table 1. Composition of the iodine catalyzed stereomutation mixtures and spectral characteristics of individual geometrical isomers.

Carotenoid	Stereoisomer	Assignment	% of total	λ_{\max} acetone	% III/II ²⁷	% D _B /D _{II} ²⁷
Zeaxanthin	(1a) All-trans	All-trans	65 (66) ^a	(428) 453 480	14	7
	(1b) Neo U(≡neo B) ^a	9-cis	21 (20)	(345) (425) 449 475	14	22
	(1c) Neo V(≡neo A)	13-cis	14 (10)	343 (425) 448 474	4	53
Diatoxanthin	(2a) All-trans	All-trans	48	(428) 453 480	10	—
	(2b) Neo U	9-cis	52	345 (425) 450 478	43	15
Lutein	(3a) All-trans	All-trans	59 (60) ^a	(425) 448 476	55	7
	(3b) Neo U ^a (≡neo B) ^a	9-cis	26 (23)	334 420 443 472	57	12
	(3c) Neo V(≡neo A)	13 or 13'-cis	15 (16)	334 420 442 470	39	33
Loroxanthin triacetate	5'a All-trans	All-trans	63	(424) 448 475	39	18
	5'b Neo U	9-cis	27	(335) (420) 446 473	37	8
	5'c Neo A	13 or 13'-cis	10	335 (418) 441 468	13	49

^a m.p. 104–105 °C (5 % all-trans).

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