

Steric Stability of Acetylenic Carotenoids

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Comparative iodine catalyzed stereomutation studies in light of the diacetylenic all-*trans* alloxanthin (2) and of its 9,9'-*di-cis* isomer, employing separation of geometrical isomers by HPLC, are reported.

The steric stability of all-*trans* 7,8-didehydro- and of 7,8,7',8'-tetrahydroastaxanthin (as diacetates 3' and 4') was also studied. Considerable steric stability for the monoacetylenic 3' and somewhat less stability for the diacetylenic 4' was demonstrated during common isolation procedures.

The composition of the photostationary mixtures produced in the presence of iodine showed the following features: In the diacetylenic series (2 and 4') the all-*trans* isomer was absent and the 9,9'-*di-cis* and 9-mono-*cis* isomers were dominating. In the monoacetylenic series the all-*trans* isomer occurred together with larger amounts of the 9-mono-*cis* isomer.

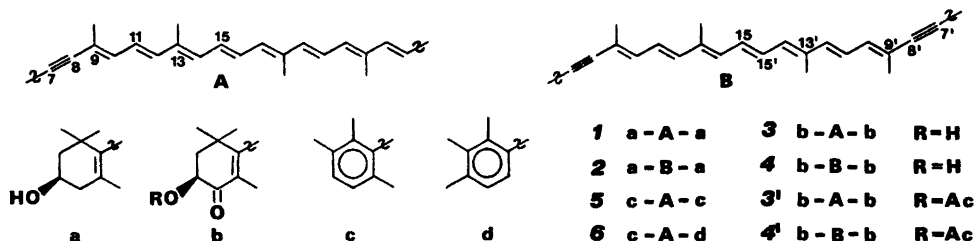
Factors governing the steric stability of acetylenic carotenoids are considered.

Stereoisomerization of double bonds in the polyene chain by light, heat *etc.* is well known in the carotenoid series. In the presence of iodine in light a photostationary mixture of the thermodynamically most stable isomers is formed, independent of the initial isomer.¹

Isomers with sterically hindered or central double bonds (15-*cis*) are not encountered in the iodine catalyzed stereomutation mixtures.² For bicyclic carotenoids *cis*-configuration in 9(9') and 13(13') positions are common. Normally the all-*trans* isomer is the most stable isomer. Exceptions are provided by in-chain substituted^{3,4} and acetylenic (7,8-didehydro) carotenoids.^{3,5}

7,8-Didehydro- and 7,8,7',8'-tetrahydrocarotenoids such as (3*R*,3'*R*)-diatoxanthin (1, Scheme 1), (3*R*,3'*R*)-alloxanthin (2), (3*S*,3'*S*)-7,8-didehydroastaxanthin (3) and (3*S*,3'*S*)-7,8,7',8'-tetrahydroastaxanthin (4) occur in the all-*trans* form in Nature⁶ with the exception of manixanthin which probably is the 9,9'-*di-cis* isomer of alloxanthin (2).⁷ On the other hand previous attempts to synthesize alloxanthin (2) and related aryl carotenoids (5 and 6) have resulted in 9,9'-*di-cis* 2 (optically inactive)⁸ and 9-*cis* 5 and 6⁵ only.

Antia and coworkers⁷ have reported stereo-isomerization studies on alloxanthin (2) and manixanthin using TLC separation. Further detailed studies on the stereomutation of acetylenic carotenoids have not been published.



Scheme 1.

Table 1. Composition of all-*trans* (A) and 9,9'-di-*cis* (B) preparations of alloxanthin (2) after dissolution in benzene, and of the iodine catalyzed stereomutation mixture recorded after 2 h, obtained from A and from B.

t_R min	λ_{max} nm	% III/II ^a	% Of total mixture	Identification
(a) Sample A				
16.0	443	41	4	9,9'-Di- <i>cis</i>
16.65	454	44	74	All- <i>trans</i>
17.3	448	43	19	9-Mono- <i>cis</i>
17.95	450		3	13-Mono- <i>cis</i>
(b) Sample B				
16.45	444	72	82	9,9'-Di- <i>cis</i>
17.5	437	44	18	9,9',13-Tri- <i>cis</i>
(c) I ₂ -cat. equilibrium mixture				
16.6/16.6 ^b	444/444 ^b	67/67 ^b	58.6/58.0 ^b	9,9'-Di- <i>cis</i>
17.65/17.65	437/437	47/48	22.9/23.0	9,9',13'-Tri- <i>cis</i>
17.95/17.90	448/447	32/23	18.5/19.0	9-Mono- <i>cis</i>

^a % III/II is a measure of the vibrational fine-structure of the electronic spectrum, see Experimental.

^b Values before the stroke refer to the all-*trans* preparation (A) as starting compound, whereas values after the stroke refer to the 9,9'-di-*cis* preparation (B) as starting compound.

Recently synthetic, optically active all-*trans* (3*R*,3'*R*)-alloxanthin (2) and its 9,9'-di-*cis* isomer have become available.⁸ A comparative study of the stereomutation of the all-*trans* and 9,9'-di-*cis* isomers is now reported. We have also examined the steric stability of all-*trans* (3*S*,3'*S*)-7,8-didehydroastaxanthin diacetate (3') and (3*S*,3'*S*)-7,8,7'-8'-tetrahydroastaxanthin diacetate (4') *ex Asterias rubens*.⁹

RESULTS AND DISCUSSION

Iodine catalyzed stereomutation by light in benzene solution was carried out by standard methods.¹ Chromatography was effected by HPLC in a recently developed system, offering efficient separation and the possibility of recording visible spectra and calculation of concentration before reversible isomerization of the individual geometrical isomers occurred.¹⁰ The HPLC system is thus superior to conventional separation of *cis-trans* mixtures by column, thin layer or paper chromatography where some reversible isomerization cannot be avoided during subsequent elution and concentration procedures prior to spectral characterization.

The results for all-*trans* alloxanthin (2) and its 9,9'-di-*cis* isomer are compiled in Table 1. The steric purity of the samples used after dissolution in benzene, is given in Table 1a, b. The iodine catalyzed equilibrium mixture obtained while starting from (i) the all-*trans* preparation A and (ii) the 9,9'-di-*cis* preparation B is characterized in Table 1, c. Excellent agreement was obtained for the qualitative and quantitative composition of the equilibrium mixture obtained in either case. Separate experiments (Table 2) revealed that photostationary conditions were nearly reached after only 3 min exposure in the presence of iodine. Upon catalytic isomerization of the all-*trans* isomer only *ca.* 4 % of this isomer remained after 3 min exposure, demonstrating exceptional steric lability under these conditions. Identification of the isomers present in the various mixtures is based on retention times and spectral characteristics in visible light for the two authentic isomers and tentative conclusions for the additional isomers based on spectral criteria and their occurrence in the starting material. Thus the presence of a certain amount of the 9-mono-*cis* and the 9,9'-di-*cis* isomers was indicated in the all-*trans* prepara-

Table 2. All-*trans* (A, Table 1) and 9,9'-*di-cis* alloxanthin (B, Table 1) after 3 min isomerization with iodine in light.

t_R min ^a	λ_{max} nm	% Of total mixture	Identification
Sample A + I ₂			
28.6	444	47	9,9'-Di- <i>cis</i>
29.3	442	3	?
30.0	452	4	All- <i>trans</i>
30.4	437	15	9,9',13'-Tri- <i>cis</i>
32.1	448	31	9-Mono- <i>cis</i>
Sample B + I ₂			
29.4	444	64	9,9'-Di- <i>cis</i>
32.1	437	21	9,9',13'-Tri- <i>cis</i>
32.0	448	15	9-Mono- <i>cis</i>

^a Note different gradient rate vs. Table 1 specified under Results.

tion. No all-*trans* or 9-mono-*cis* but some 9,9',13-tri-*cis* in the 9,9'-*di-cis* preparation is compatible with the relative steric stability of the 9-*trans* and 9-*cis* double bonds reflected by the isomerization studies. The absence of all-*trans* and dominance of 9,9'-*di-cis* together with 9,9',13-tri-*cis* and 9-mono-*cis* (Table 1) in the iodine catalyzed equilibrium mixture is noteworthy.

The presence of a certain amount of the 9-mono-*cis* isomer (19%) is compatible with results for the monoacetylenic diatoxanthin (1) where the all-*trans* isomer indeed represented 48% besides the 9-mono-*cis* isomer (52%) in the photostationary mixture produced in the presence of iodine.¹¹ Thus in the 7,8,7',8'-tetrahydro series the 9,9'-*di-cis* configuration appears to represent the thermodynamically preferred configuration besides the 9-mono-*cis*. These results are consistent with previous reports.^{3,7,12} Amongst other criteria⁸ the higher

vibrational fine-structure in the electronic spectra of the 9-*cis* and 9,9'-*di-cis* isomers of 2 is noteworthy.⁷

The above findings for alloxanthin (2) are supplemented by studies on the steric stability of all-*trans* 7,8-didehydro- and 7,8,7',8'-tetrahydroastaxanthin as diacetates 3' and 4'. The natural diols 3 and 4 were reisolated from *Asterias rubens* via the carotenoprotein.¹³ Table 3 gives the steric composition of the monoacetylenic diacetate 3' and the diacetylenic diacetate 4' after common work-up procedures, demonstrating the steric stability of all-*trans* 3' and 4' under conditions preventing isomerization, in agreement with previous results.⁹ Compositions of the iodine catalyzed stereomutation mixture of the monoacetylenic diacetate 3' and the diacetylenic diacetate 4' are given in Table 4. The isomerization in the presence of iodine occurred, in our experience, considerably

Table 3. Composition of 7,8-didehydroastaxanthin diacetate (3') and of 7,8,7',8'-tetrahydroastaxanthin diacetate (4') after common isolation and work-up procedures.

Sample	t_R min	λ_{max} nm	% Of total mixture		Identification
			Exp. 1	Exp. 2	
3'	16.9	472	trace	2	9-Mono- <i>cis</i>
	17.9	478	100	98	All- <i>trans</i>
4'	17.4	466	25	—	9-Mono- <i>cis</i>
	17.8	475	75	100 ^a	All- <i>trans</i>

^a Cryst.

Table 4. Composition of iodine catalyzed stereoisomerization mixtures of 7,8-didehydroastaxanthin diacetate (3') after 14 h irradiation and of 7,8,7',8'-tetrahydroastaxanthin diacetate (4') after 12 h irradiation.

Compound	t_R min	λ_{max} nm	% Of total mixture	Identification
3'	16.5	460	trace	Di- <i>cis</i>
	17.1	460	trace	Di- <i>cis</i>
	17.4	466	63	9-Mono- <i>cis</i>
	17.8	475	37	all- <i>trans</i>
4'	16.4	468	55	9,9'-Di- <i>cis</i>
	16.9	472	45	9-Mono- <i>cis</i>
	17.8	467	trace	?

slower than that of all-*trans* alloxanthin (2). The photostationary stage was reached for the diacetylenic diacetate 4' before 4 h, whereas the relative concentrations of all-*trans* and 9-mono-*cis* 3' were still changing slightly in favour of the 9-mono-*cis* isomer after 14 h irradiation with repeated additions of I_2 . However, the composition of the photostationary mixture for the monoacetylenic 3' resembles that of diatoxanthin (1)¹¹ cited before, and that of the diacetylenic diacetate 4' resembles that of alloxanthin (2) in its absence of all-*trans* and dominance of 9,9'-di-*cis* and 9-mono-*cis*. This reflects similar relative stability of the all-*trans*, 9-mono-*cis* and 9,9'-di-*cis* isomers of the diacetylenic alloxanthin (2) and 7,8,7',8'-tetrahydroastaxanthin diacetate (4'), as well as of the all-*trans* and 9-mono-*cis* isomers in the monoacetylenic series; diatoxanthin (2) and 7,8-didehydroastaxanthin diacetate (3'). The results further demonstrate greater steric lability of the all-*trans* isomers in the diacetylenic than in the monoacetylenic series. Also for 3'

and 4' increased vibrational fine-structure in the electronic spectra of all-*trans*→9-mono-*cis*→9,9'-di-*cis* was observed.

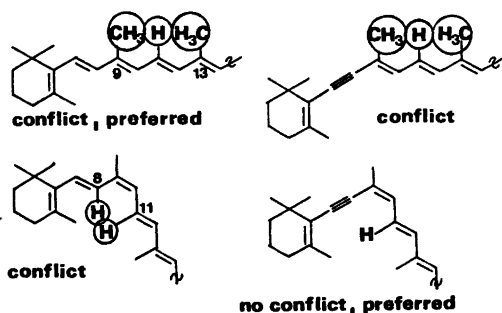
Weedon¹² has rationalized the preference for 9-*cis* configuration in 7,8-didehydro carotenoids by a steric argument visualized in Scheme 2. However, the results presented here, demonstrating a high percentage of all-*trans* 7,8-didehydro- and of 9-mono-*cis* 7,8,7',8'-tetrahydrocarotenoids in the iodine catalyzed stereoisomerization mixtures and also a lower rate of isomerization of all-*trans* in the 4,4'-diketo series, suggest that a combination of steric and electronic factors govern the steric stability of acetylenic carotenoids in the 7,8-didehydro- and 7,8,7',8'-tetrahydro series.

EXPERIMENTAL

Materials. All-*trans* and 9,9'-di-*cis* alloxanthin (2) were synthetic compounds.⁹ The acetylenic astaxanthin derivatives 3 and 4 were isolated from the carotenoprotein preparation of *Asterias rubens*¹³ and the diacetates prepared by a previously described procedure.⁹

Methods. Iodine catalyzed stereoisomerization was carried out by the general procedure.¹ To 2 (0.5 mg) in benzene (5 ml) was added 1 ml I_2 in hexane (10 $\mu\text{g}/\text{ml}$) followed by illumination between six 40 W fluorescent lamps at 60 cm distance from the sample. The acetates 3' and 4' were isomerized in the same manner.

HPLC was carried out as described elsewhere¹⁰ on a Du-Pont 830 Liquid Chromatograph supplied with a Varian Series 634 double-beam spectrophotometer as detector (set at 450 nm for 2 and 470 nm for 3' and 4') using a 4.6 × 250 mm column of silica type Spherisorb 5 μm and gradient elution with hexane-acetone. During the HPLC separation absorption spectra



Scheme 2.

in the region 360–600 nm were recorded of the components in each peak.

Results are given in Tables 1–4. Retention times (t_R) cited in Table 1 refer to the following conditions: Pressure 2 MPa, flow rate 1.4 ml min^{-1} , injected carotenoid 0.8 μg , eluent 0–50 % acetone in hexane, gradient rate 3 %/min. Data of Table 2 are based on similar conditions, but slower (1 %/min) increase in gradient for better separation of all-*trans* alloxanthin (2) and its 9,9'-*di-cis* isomer. t_R is, however, less reproducible with the lower increase in gradient. For experiments cited in Tables 3–4 the latter conditions (gradient rate 1 % min^{-1}) were used. λ_{max} is the middle main absorption maximum recorded in the actual eluent. Depending on acetone concentration the figures are shifted bathochromically *ca.* 0–2 nm relative to hexane values. % III/II is a measure of the vibrational fine-structure of the electronic spectrum and gives the extinction of the longest wavelength band in percentage of the extinction of the middle main band with the bottom between these bands as base line.¹⁴ Calculation of percentage composition is based on identical extinction coefficients of each geometrical isomer at the wavelength of peak detection. Since the extinction coefficients of only all-*trans* 2 and its 9,9'-*di-cis* isomer are known,⁸ no attempts are made to correct for this approximation.

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