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'-tetradehydroastaxanthin (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 mixture 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.

Steroisomerization 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 isomer is formed, independent of the initial isomer. Isomers with sterically hindered or central double bonds (15-cis) are not encountered in the iodine catalyzed stereomutation mixtures. 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 \(^1\) and acetylenic (7,8-didehydro) carotenoids. \(^1\)

7,8-Didehydro- and 7,8,7',8'-tetradehydrocarotenoids such as (3R,3'R)-dioxanthin (1, Scheme 1), (3R,3'R)-alloxanthin (2), (3S,3'S)-7,8-didehydroastaxanthin (3) and (3S,3'S)-7,8,7',8'-tetradehydroastaxanthin (4) occur in the all-trans form in Nature 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) \(^2\) and 9-cis 5 and 6 only.

Antia and coworkers \(^7\) have reported steresoisomerization studies on alloxanthin (2) and manixanthin using TLC separation. Further detailed studies on the stereormutation of acetylenic carotenoids have not been published.

Scheme 1.

\(\text{HO} \quad \text{RO} \quad \text{O} \quad \text{O} \quad \text{b} \quad \text{c} \quad \text{d} \)

\begin{align*}
1 \text{ a-A-a} & \quad 3 \text{ b-A-b} & \text{ R=H} \\
2 \text{ a-B-a} & \quad 4 \text{ b-B-b} & \text{ R=H} \\
5 \text{ c-A-c} & \quad 3' \text{ b-A-b} & \text{ R=Ac} \\
6 \text{ c-A-d} & \quad 4' \text{ b-B-b} & \text{ R=Ac}
\end{align*}

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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.

<table>
<thead>
<tr>
<th>$t_R$ min</th>
<th>$\lambda_{max}$ nm</th>
<th>% III/II $^a$</th>
<th>% Of total mixture</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Sample A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.0</td>
<td>443</td>
<td>41</td>
<td>4</td>
<td>9,9′-Di-cis</td>
</tr>
<tr>
<td>16.65</td>
<td>454</td>
<td>44</td>
<td>74</td>
<td>All-trans</td>
</tr>
<tr>
<td>17.3</td>
<td>448</td>
<td>43</td>
<td>19</td>
<td>9-Mono-cis</td>
</tr>
<tr>
<td>17.95</td>
<td>450</td>
<td>3</td>
<td></td>
<td>13-Mono-cis</td>
</tr>
<tr>
<td>(b) Sample B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.45</td>
<td>444</td>
<td>72</td>
<td>82</td>
<td>9,9′-Di-cis</td>
</tr>
<tr>
<td>17.5</td>
<td>437</td>
<td>44</td>
<td>18</td>
<td>9,9′,13-Tri-cis</td>
</tr>
<tr>
<td>(c) I$_2$-cat. equilibrium mixture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.6/16.6 $^b$</td>
<td>444/444 $^b$</td>
<td>67/67 $^b$</td>
<td>58.6/58.0 $^b$</td>
<td>9,9′-Di-cis</td>
</tr>
<tr>
<td>17.65/17.65</td>
<td>437/437</td>
<td>47/48</td>
<td>22.9/23.0</td>
<td>9,9′,13′-Tri-cis</td>
</tr>
<tr>
<td>17.95/17.90</td>
<td>448/447</td>
<td>32/23</td>
<td>18.5/19.0</td>
<td>9-Mono-cis</td>
</tr>
</tbody>
</table>

$^a$ % 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 (3R,3′R)-alloxanthin (2) and its 9,9′-di-cis isomer have become available. 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 (3S,3′S)-7,8-didehydroastaxanthin diacetate (3′) and (3S,3′S)-7,8,7′-8′-tetradehydroastaxanthin diacetate (4′) ex Asterias rubens.

RESULTS AND DISCUSSION

Iodine catalyzed stereomutation by light in benzene solution was carried out by standard methods. 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. 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, etc. 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.

<table>
<thead>
<tr>
<th>tR min a</th>
<th>λmax nm</th>
<th>% Of total mixture</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.6</td>
<td>444</td>
<td>47</td>
<td>9,9'-Di-cis</td>
</tr>
<tr>
<td>29.3</td>
<td>442</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>30.0</td>
<td>452</td>
<td>4</td>
<td>All-trans</td>
</tr>
<tr>
<td>30.4</td>
<td>437</td>
<td>15</td>
<td>9,9',13-Tri-cis</td>
</tr>
<tr>
<td>32.1</td>
<td>448</td>
<td>31</td>
<td>9-Mono-cis</td>
</tr>
</tbody>
</table>

Sample B + I₂

<table>
<thead>
<tr>
<th>tR min</th>
<th>λmax nm</th>
<th>% Of total mixture</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.4</td>
<td>444</td>
<td>64</td>
<td>9,9'-Di-cis</td>
</tr>
<tr>
<td>32.1</td>
<td>437</td>
<td>21</td>
<td>9,9',13'-Tri-cis</td>
</tr>
<tr>
<td>32.0</td>
<td>448</td>
<td>15</td>
<td>9-Mono-cis</td>
</tr>
</tbody>
</table>

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

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'-tetra-dehydroastaxanthin as diacetates 3' and 4'. The natural diols 3 and 4 were resolated from Asterias rubens via the carotenoprotein. 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. 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'-tetradehydroastaxanthin diacetate (4') after common isolation and work-up procedures.

<table>
<thead>
<tr>
<th>Sample</th>
<th>tR min</th>
<th>λmax nm</th>
<th>% Of total mixture</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exp. 1</td>
<td>Exp. 2</td>
</tr>
<tr>
<td>3'</td>
<td>16.9</td>
<td>472</td>
<td>trace</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>17.9</td>
<td>478</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>4'</td>
<td>17.4</td>
<td>466</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17.8</td>
<td>475</td>
<td>75</td>
<td>100 a</td>
</tr>
</tbody>
</table>

a Cryst.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>t_R min</th>
<th>λ_max nm</th>
<th>% Of total mixture</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>3'</td>
<td>16.5</td>
<td>460</td>
<td>trace</td>
<td>Di-cis</td>
</tr>
<tr>
<td></td>
<td>17.1</td>
<td>460</td>
<td>trace</td>
<td>Di-cis</td>
</tr>
<tr>
<td></td>
<td>17.4</td>
<td>466</td>
<td>63</td>
<td>9-Mono-cis</td>
</tr>
<tr>
<td></td>
<td>17.8</td>
<td>475</td>
<td>37</td>
<td>all-trans</td>
</tr>
<tr>
<td>4'</td>
<td>16.4</td>
<td>468</td>
<td>55</td>
<td>9,9'-Di-cis</td>
</tr>
<tr>
<td></td>
<td>16.9</td>
<td>472</td>
<td>45</td>
<td>9-Mono-cis</td>
</tr>
<tr>
<td></td>
<td>17.8</td>
<td>407</td>
<td>trace</td>
<td></td>
</tr>
</tbody>
</table>

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 I₂. 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'-tetradehydroastaxanthin 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.

Weedon 11 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'-tetradehydrocarotenoids in the iodine catalyzed stereomutation 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'-tetradehydro series.

EXPERIMENTAL

Materials. All-trans and 9,9'-di-cis alloxanthin (2) were synthetic compounds. 8 The acetylenic astaxanthin derivatives 3 and 4 were isolated from the carotenoprotein preparation of Asterias rubens 12 and the diacetates prepared by a previously described procedure. 9

Methods. Iodine catalyzed stereomutation was carried out by the general procedure. 1 To 2 (0.5 mg) in benzene (5 ml) was added 1 ml I₂ in hexane (10 µg/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 elsewhere 16 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 x 250 mm column of silica type Spherisorb 5 µm and gradient elution with hexane—acetone. During the HPLC separation absorption spectra
in the region 360 – 600 nm were recorded of the components in each peak.

Results are given in Tables 1 – 4. Retention times (tR) 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⁻¹. Data of Table 2 are based on similar conditions, but slower (1% min⁻¹) increase in gradient for better separation of all-trans alloxanthin (2) and its 9,9'-di-cis isomer. tR 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. λmax 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. 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, no attempts are made to correct for this approximation.

Acknowledgement. A. F. was supported by a research grant from The Norwegian Research Council of Science and the Humanities to S. L. J. D. T. was on sabbatical leave from McNeese State University, Lake Charles, Louisiana, USA.

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Received October 26, 1978.