Animal Carotenoids. 12.* Chirality of Asterinic Acid

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The chirality of monoacetylenic asterinic acid [(3S,3'S)-7,8-didehydroastaxanthin, 1a] has been established by NaBH₄-reduction and hydrolysis of the corresponding diesters 1b and 1c providing the tetrol 9 and CD-correlation with diatoxanthin [(3E,3'R)-7,8-didehydro-β,β-carotene-3,3'-dil, 10].

Diacylenic asterinic acid [(3S,3'S)-7,8,7',8'-tetradehydrō-β,β-carotene-3,3'-dil, 2a] was assigned the same absolute configuration by similar conversion to the diacylenic tetrol 11 and CD-correlation with alloxanthin [(3R,3'R)-7,8,7',8'-tetradehydrō-β,β-carotene-3,3'-dil, 12].

IR and CD properties of the diacetates 1c and 2c of the naturally occurring α-ketols are reported.

Asterinic acid (asterinsäure), first isolated by von Euler and Hellström ⁴ from the starfish Asterias rubens (Linné), has also been encountered in the soft coral Alcyonium digitatum (Linné) ⁶ and recently in lobster roe.⁸

Asterinic acid occurs as a protein complex in Asterias rubens, presumably also in the lobster eggs,¹ ¹⁸ and is known to be a mixture of 7,8-didehydro- and 7,8,7',8'-tetradehydroastaxanthin (1 and 2, Scheme 1) from chromatographic and spectroscopical (electronic, IR, ¹H NMR and mass spectra) evidence.⁵,⁸

We now report on the absolute configuration of these acetylenic derivatives of astaxanthin (3).

RESULTS AND DISCUSSION

The chirality of astaxanthin (3) in lobster was assigned in our laboratory by conversion to a diastereomeric mixture of tetrols (4) by LiAlH₄-reduction. Conformational analysis of the tetrols (4) revealed that the chirality at C-3(3') was decisive for the preferred half-chair conformation of the cyclohexene end groups, dictating the sign of the Cotton effect. Since the CD of the tetrol mixture (4) was identical with that of zeaxanthin (6) of known 3R,3'R configuration,⁹ the same chirality at C-3,3' of the tetrols 4 and zeaxanthin (6) and consequently also in astaxanthin (3) was concluded. The chirality of astaxanthin (3) has since been confirmed by Kienzle ¹⁰ by total synthesis of (3S,3'S)-astaxanthin (3), thus verifying the validity of the arguments used in our configurational assignment.

It is therefore evident that the chirality of the mono- and diacylenic derivatives (1 and 2) of astaxanthin (3) could be solved by the same approach.

A mixture of astaxanthin (3) and the monoaacetylenic (1) and diacylenic derivative 2 were reisolated from Asterias rubens via the crude protein complex. Also present were fatty acid diesters 1b, 2b and 3b of 1, 2 and 3, respectively.

The mixed α-ketols 1, 2 and 3 were converted to the diacetates 1c, 2c and 3c to effect better chromatographic separation and less facile conversion to the corresponding diosphenols 6, 7 and 8. The monoaacetylenic diacetate 1c and the diacylenic diacetate 2c were characterized by electronic, IR and mass spectra.

To simplify the isolation the monoaacetylenic diesters 1b and 1c were treated with NaBH₄, rather than LiAlH₄, resulting in reduction of the keto groups, followed by alkaline hydrolysis of the ester functions to provide the monoaacetylenic tetrol 9 with retention of configuration at C-3,3' and mixed configuration at C-4,4'. After TLC purification the tetrol 9 was characterized

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by electronic, mass and CD spectra. The CD spectrum (Fig. 1) showed agreement with that of authentic all-trans diatoxanthin (10) of known 3R,3’R-configuration. By the same argumentation as used in the configurational assignment of astaxanthin (3), the diastereomeric mixture of monoacetylenic tetrols 9 must have the same chirality at C-3,3’ as diatoxanthin (10). Consequently the monoacetylenic astaxanthin from which the tetrol 9 was prepared has the same absolute configuration and is (3S,3’S)-7,8-didehydroastaxanthin (1a).

One point must be commented on. It is known that triple bonds in 7,8-position of carotenoids favour cis configuration of the adjacent double bond. Moreover, a cis bond in the polylene chain reverses the sign of the Cotton effect. However, the spectral characteristics (λmax and insignificant cis peak) in the electronic spectrum of the tetrol 9 for which the CD was recorded, are incompatible with dominance of a 9-cis tetrol.

The natural diacetylenic diesters 2b, were converted by NaBH₄ followed by alkaline hydrolysis to the diacetylenic tetrol 11, the CD of which was correlated with that of the corresponding diacetylenic 3,3′R-diol alloxanthin (12) of known 3R,3′R-configuration, Fig. 2. As references are used the CD spectrum of all-trans alloxanthin (12) calculated by a Koenig-Kramers transformation of the published ORD spectrum and the measured CD spectra.
of alloxanthin ex *Asterias rubens* and ex the sponge *Microciona prolifica*. In all cases a negative Cotton effect is observed below 310 nm. It is concluded that the diacetylenic tetrol *II* has the same chirality at C-3,3' as alloxanthin (12) and thus that the diacetylenic asterinic acid is (3S,3'S)-7,8,7',8'-tetradehydroastaxanthin (2a).

In conclusion it is here shown that the naturally occurring 7,8-didehydro- and 7,8,7',8'-tetradehydro derivatives of astaxanthin from starfish have the same chirality as astaxanthin (3), ex lobster, zeaxanthin (5), diatoxanthin (10), and alloxanthin (12). The stereochemical result is consequently compatible with a biosynthetic precursor relationship to any of these 3,3'-diols. It should be mentioned that astaxanthin occurs with 3S,3'S chirality (3) in all sources studied hitherto, except a red yeast which produces the enantiogenic (3R,3'R)-astaxanthin.

ORD spectra of zeaxanthin (5), diatoxanthin (10) and alloxanthin (12) are published by Bartlett et al. CD spectra of the same series are reported elsewhere and in the present work. In Fig. 3 are compiled the CD spectra of (3S,3'S)-astaxanthin (3), (3S,3'S)-7,8-didehydroastaxanthin diacetate (1c) and (3S,3'S)-7,8,7',8'-tetradehydroastaxanthin diacetate (2c). It is obvious that triple bonds in 7,8(7',8')-positions change drastically the chiroptical properties of carotenoids. Introduction of such triple bonds cause a gradual “flattening” of the CD spectra ascribed to chromophoric changes.

**EXPERIMENTAL**

**Materials and methods** were those commonly employed in the Trondheim laboratory. Chromatography was effected by TLC System A (kieselgel G; 0.75 or 1 mm layers) or System B (kieselgel 30 g, MgO 9 g, Ca(OH)$_2$ 12 g, CaSO$_4$ 3 g, and water 93 ml; 0.75 or 1 mm layers) and circular kieselguhr paper Schleicher & Schüll No. 237 (System C), using mixtures of acetone in hexane (A-hex) for development.

CD spectra were recorded on a Roussel-Jouan Dicrograph. $\Delta\varepsilon$ Values are based on calculated concentrations. The following $B(1\%, 1$ cm) values at $\lambda_{\text{max}}$ in acetone were used for 1c and 2c 2100, for 9 and 11 2250, and for 10 and 12 2350.

**Biological material.** Bluish-violet starfishes *Asterias rubens* (190 specimens, 7.3 kg live weight) were collected near Røskje, Nord-Triendelag, August 1974.

**Fugment isolation.** The coloured parts of the back skin were cut out (rubber gloves) and rinsed quickly in cold water. The residue (1.2 kg wet weight) was minced in a Waring blender with water, and the suspension extracted with water (5 l + 3.5 l) at room temperature for 2 days, followed by decantation and filtration.

Addition of aqueous, saturated *NH$_4$Cl* solution to aliquot of the aqueous extract in various proportions failed to precipitate the protein complex. The crude protein complex in the aqueous extract was therefore split by solvent extraction using at optimum conditions aqueous extract—acetone—diethyl ether 1:1:2:1, providing 22.7 mg carotenoids $B(1\%, 1$ cm) = 2500 after transfer to ether. Lipids were removed from the crude extract by repeated precipitation from acetone at low temperature.

**Fugment separation** was effected by preparative TLC. The results are given in Table 1.
Table 1. Pigment separation by preparative TLC (System A, 20 % A-hex). u, unidentified.

<table>
<thead>
<tr>
<th>Zone</th>
<th>$R_F$</th>
<th>Yield (mg)</th>
<th>% of total</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.96</td>
<td>0.12</td>
<td>0.5</td>
<td>$\beta$,-carotene</td>
</tr>
<tr>
<td>2</td>
<td>0.86</td>
<td>0.57</td>
<td>2.5</td>
<td>alloxanthin (12) diester</td>
</tr>
<tr>
<td>3</td>
<td>0.70</td>
<td>0.23</td>
<td>1.0</td>
<td>u</td>
</tr>
<tr>
<td>4</td>
<td>0.67</td>
<td>10.0</td>
<td>46.0</td>
<td>diesters Ib, 2b and 3b</td>
</tr>
<tr>
<td>5</td>
<td>0.50</td>
<td>0.12</td>
<td>0.5</td>
<td>u</td>
</tr>
<tr>
<td>6</td>
<td>0.42</td>
<td>0.91</td>
<td>4.0</td>
<td>u</td>
</tr>
<tr>
<td>7</td>
<td>0.25</td>
<td>0.10</td>
<td>0.5</td>
<td>u</td>
</tr>
<tr>
<td>8</td>
<td>0.16</td>
<td>6.2</td>
<td>29.0</td>
<td>$\alpha$-ketols 1a, 2a and 3</td>
</tr>
<tr>
<td>9</td>
<td>0.08</td>
<td>3.6</td>
<td>16.0</td>
<td>alloxanthin (12) and diosphenols 6, 7 and 8</td>
</tr>
</tbody>
</table>

$\beta$,-Carotene was identified from electronic and mass spectra and co-chromatography (System C, hexane) with an authentic sample.

Alloxanthin (12) diester had upon rechromatography (System A, 15 % A-hex) $R_F=0.71$. Saponification followed by chromatography (System A, 50 % A-hex) gave alloxanthin (12) by the criteria given below.

Free alloxanthin (12) isolated by rechromatography (System B, 60 % A-hex) had $R_F=0.26$, slightly more strongly adsorbed than authentic zeaxanthin (5); $\lambda_{max}$ (acetone) (430), 454 and 483 nm; m/e 564 (100 %), M -- 15, M -- 92, M -- 106.

The mixed diesters (Zone 4) Ib, 2b and 3b were identified from $R_F$-value, $\lambda_{max}$ (acetone) 475 and (502) nm and by alkali treatment providing the mixed diosphenols 6, 7 and 8; m/e (%): 592 (M+1; 27), 590 (M+2; 32), 588 (M+4; 48), 575 (M+1; 17; 3), 573 (M+1; 17; 2), 500 (M+2; 92; 3), 498 (M+2; 92; 2), 496 (M+4; 92; 1), 486 (M+1; 106; 3), 484 (M+3; 106; 2) and 482 (M+4; 106; 1), cf. Ref. 6.

The mixed diesters Ib, 2b and 3b were separated in System B (35 % A-hex): $R_F$ 2b 0.67, Ib 0.43 and 2b 0.18.

The mixed $\alpha$-ketols 1a, 2a and 3 (Zone 8) were crystallized from diethyl ether, yield 4 mg, and identified from $R_F$-value, $\lambda_{max}$ (acetone 476 and 503 nm) and m/e 596 (M+2), 594 (M+4), 592 (M+4), M+4 -- 92 and M+4 -- 106 peaks.

Standard acetylation 18 of the mixed $\alpha$-ketols gave the diacetates 1c, 2c and 3c with unchanged $\lambda_{max}$ and m/e (%): 680 (M+1; 9), 678 (M+2; 29), 676 (M+3; 36), 620 (M+6; 60; 2), 618 (M+6; 60; 4), 616 (M+6; 60; 3), 588 (M+1; 92; 2), 586 (M+2; 92; 1), 584 (M+3; 92; 1) and 574 (M+4; 106; 1), cf. Ref. 6.

The diacetates were separated in System B (60 % A-hex): $R_F$ 3c 0.87, 1c 0.51 and 2c 0.21.

$7,8$-Tetradehydrostaxanthin natural diester (15) had $\lambda_{max}$ (acetone) 478 and (CS$_2$) 503 (535 nm); $r_{max}$ (KBr) 2920, 2850, 2161 (\(-\mathrm{C\equiv\mathrm{C}}\)), 1740 (ester), 1680 (conjug. C=O), 1568, 1520, 1468, 1380, 1365, 1290, 1270, 1243, 1160, 1120, 1090, 1072, 1040, 964 (trans-CH=CH-) 929, 830 (\(\mathrm{>C}=\mathrm{C}=\mathrm{H}\)) 742 and 720 cm$^{-1}$.

$\lambda_{max}$ (referred (0.5 ml) and ethanol (5 ml) was treated with NaBH$_4$ (0.1 g). The reaction was monitored by TLC and after 1 h 10 % methanolic KOH (2 ml) was added. Extractive isolation, followed by TLC (System A, 60 % A-hex) gave tetrol 9 as a major product; $R_F=0.51$, 0.08 mg (39 % yield); $\lambda_{max}$ (tetralyufuran) (430), 456 and 484 nm; m/e 598 (M); CD (EPA) Fig. 1.

7,8-Didehydrostaxanthin diacetate (1c) had $\lambda_{max}$ (CS$_2$) 500 nm; $r_{max}$ (KBr) 2958, 2922, 2855, 2161 (\(-\mathrm{C\equiv\mathrm{C}}\), \(-\mathrm{\mathrm{H}}\)) medium), 1740 (ester) 1672 (conjug. C=O), 1600, 1571, 1551, 1460, 1375, 1345, 1304, 1288, 1270, 1240, 1232, 1180, 1150, 1122, 1070, 1045, 975, 952, 905, 834 and 760 cm$^{-1}$; CD (EPA) Fig. 3.

The diacetate 1c was reduced with NaBH$_4$ and hydrolyzed with KOH in the same manner as 1b above, providing the tetrol 9 with properties as reported above.

7,8,7',8'-Tetradehydrostaxanthin natural diester (2b) had $\lambda_{max}$ (acetone) 482 (510), (CS$_2$) 503, 535 nm.

2b (0.2 mg) was reduced with NaBH$_4$ and hydrolyzed with KOH in the same manner as 1b. Extractive isolation and chromatography in System A (60 % A-hex) gave the dicetylenic tetrol 11, yield 0.1 mg (46 %); $R_F=0.50$; $\lambda_{max}$ (acetone) (428) 450 and 483 nm, %III/II'=26, (tetralyufuran) (432), 457 and 485 nm; m/e 596 (M); CD (EPA) Fig. 2.

7,8,7',8'-Tetradehydrostaxanthin diacetate (2c) had $\lambda_{max}$ (CS$_2$) 503, 535 nm; $r_{max}$ (KBr) 2958, 2922, 2161 (\(-\mathrm{C\equiv\mathrm{C}}\), \(-\mathrm{\mathrm{H}}\)) medium, 1740 (ester) 1672 (conjug. C=O), 1600, 1571, 1551, 1460, 1378, 1348, 1304, 1288, 1270, 1240, 1232, 1180, 1150, 1122, 1070, 1045, 975, 952, 905, 834 and 740 cm$^{-1}$; CD (EPA) Fig. 3.

The diacetate 2c was converted to the dicetylenic tetrol 11 by the same procedure as for 2b.

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