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Animal Carotenoids. 12.* Chirality of Asterinic Acid

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The chirality of monoacetylenic asterinic acid [(3*S*,3′*S*)-7,8-didehydroastaxanthin, *1a*] has been established by $NaBH_4$-reduction and hydrolysis of the corresponding diesters *1b* and *1c* providing the tetrol *9* and CD-correlation with diatoxanthin [(3*R*,3′*R*)-7,8-didehydro-β,β-carotene-3,3′-diol, *10*].

Diacetylenic asterinic acid [(3*S*,3′*S*)-7,8,7′,8′-tetradehydro-β,β-carotene-3,3′-diol, *2a*] was assigned the some absolute configuration by similar conversion to the diacetylenic tetrol *11* and CD-correlation with alloxanthin [(3*R*,3′*R*)-7,8,7′,8′-tetradehydro-β,β-carotene-3,3′-diol, *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 [1] from the starfish *Asterias rubens* (Linné), has also been encountered in the soft coral *Alcyonium digitatum* (Linné) [2] and recently in lobster roe.[3]

Asterinic acid occurs as a protein complex in *Asterias rubens*,[1,3] presumably also in the lobster eggs,[1,3] 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.[5,6]

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

RESULTS AND DISCUSSION

The chirality of astaxanthin (*3*) *ex* lobster was assigned in our laboratory by conversion to a diastereomeric mixture of tetrols (*4*) by $LiAlH_4$-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.[7] Since the CD of the tetrol mixture (*4*) was identical with that of zeaxanthin (*5*) of known 3*R*,3′*R* configuration,[8] the same chirality at C-3,3′ of the tetrols *4* and zeaxanthin (*5*) and consequently also in astaxanthin (*3*) was concluded. The chirality of astaxanthin (*3*) has since been confirmed by Kienzle [9] by total synthesis of (3*S*,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 diacetylenic derivatives (*1* and *2*) of astaxanthin (*3*) could be solved by the same approach.

A mixture of astaxanthin (*3*) and the monoacetylenic (*1*) and diacetylenic 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 monoacetylenic diacetate *1c* and the diacetylenic diacetate *2c* were characterized by electronic, IR and mass spectra.

To simplify the isolation the monoacetylenic diesters *1b* and *1c* were treated with $NaBH_4$, rather than $LiAlH_4$, resulting in reduction of the keto groups, followed by alkaline hydrolysis of the ester functions to provide the monoacetylenic 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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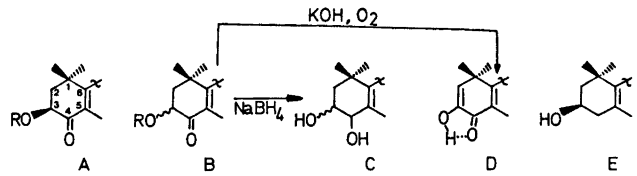

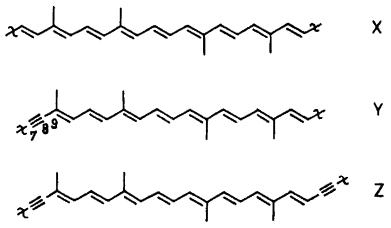

1	B-Y-B	R=H
1a	A-Y-A	R=H
1b	B-Y-B	R=Acyl
1c	B-Y-B	R=Acetyl
2	B-Z-B	R=H
2a	A-Z-A	R=H
2b	B-Z-B	R=Acyl
2c	B-Z-B	R=Acetyl

3	A-X-A	R=H
3b	A-X-A	R=Acyl
3c	A-X-A	R=Acetyl
4	C-X-C	
5	E-X-E	
6	D-X-D	
7	D-Y-D	
8	D-Z-D	
9	C-Y-C	
10	E-Y-E	
11	C-Z-C	
12	E-Z-E	

by electronic, mass and CD spectra. The CD spectrum (Fig. 1) showed agreement with that of authentic all-*trans* diatoxanthin (*10*)[10] of known 3*R*,3′*R*-configuration.[11] 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 (3*S*,3′*S*)-7,8-didehydroastaxanthin (*1a*).

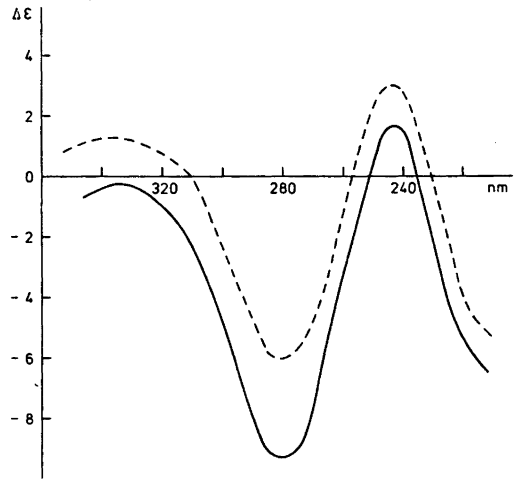

Fig. 1. CD spectra in EPA solution of ——— the monoacetylenic tetrol *9* and of – – – diatoxanthin (*10*).

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.[12] Moreover, a *cis* bond in the polyene chain reverses the sign of the Cotton effect.[10,11] However, the spectral characteristics (λ_{max} 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 $NaBH_4$ 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 3*R*,3′*R*-configuration,[11] Fig. 2. As references are used the CD spectrum of all-*trans* alloxanthin (*12*) calculated by a Koenig-Kramers transformation[13–15] of the published[11] ORD spectrum and the measured CD spectra

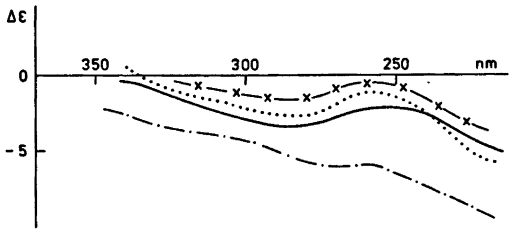

Fig. 2. CD spectra in EPA solution of ——— the diacetylenic tetrol *11*, — — alloxanthin (*12*) *ex Microciona prolifera*,[16] — × — alloxanthin (*12*) *ex Asterias rubens* and of · · · alloxanthin (*12*) in solution calculated from the published [11] ORD spectrum. *Δε*-values only valid for the three former curves.

of alloxanthin *ex Asterias rubens* and *ex* the sponge *Microciona prolifera*.[14] In all cases a negative Cotton effect is observed below 310 nm. It is concluded that the diacetylenic tetrol *11* has the same chirality at C-3,3′ as alloxanthin (*12*) and thus that the diacetylenic asterinic acid is (3*S*,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

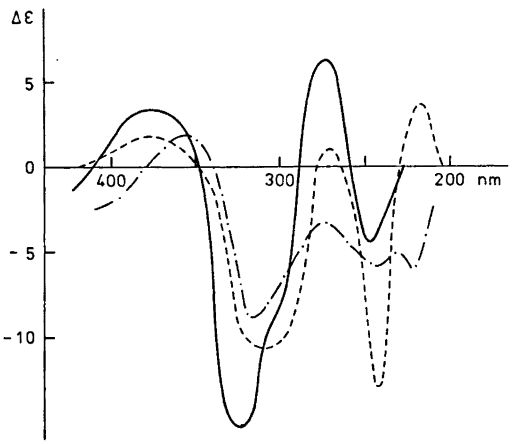

Fig. 3. CD spectra in EPA solution of — — — astaxanthin (*3*),[7] ——— 7,8-didehydroastaxanthin diacetate (*1c*) and —·— 7,8,7′,8′-tetradehydroastaxanthin diacetate (*2c*); *Δε*-values not valid for *3*.

precursor relationship to any of these 3,3′-diols. It should be mentioned that astaxanthin occurs with 3*S*,3′*S* chirality (*3*) in all sources studied hitherto,[11,17] except a red yeast [18] which produces the enantiomeric (3*R*,3′*R*)-astaxanthin.

ORD spectra of zeaxanthin (*5*), diatoxanthin (*10*) and alloxanthin (*12*) are published by Bartlett *et al.*[11] CD spectra of the same series are reported elsewhere [7] and in the present work. In Fig. 3 are compiled the CD spectra of (3*S*,3′*S*)-astaxanthin (*3*), (3*S*,3′*S*)-7,8-didehydroastaxanthin diacetate (*1c*) and (3*S*,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 "flattering" of the CD spectra ascribed to chromophoric changes.

EXPERIMENTAL

Materials and methods were those commonly employed in the Trondheim laboratory.[19–21] Chromatography was effected by TLC System A (kieselgel G; 0.75 or 1 mm layers) or System B [22] (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. 287 (System C), using mixtures of acetone in hexane (A-hex) for development.

CD spectra were recorded on a Roussel-Jouan Dicrographe. *Δε*-Values are based on calculated concentrations. The following $E(1\ \%,\ 1\ cm)$ values at λ_{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-Trøndelag, August 1974.

Pigment 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 blendor with water, and the suspension extracted with water (5 l + 3.5 l) at room temperature for 2 days,[1,5,6] followed by decantation and filtration.

Addition of aqueous, saturated NH_4Cl-solution to aliquots 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 $E(1\ \%,\ 1\ cm) = 2500$) after transfer to ether. Lipids were removed from the crude extract by repeated precipitation from acetone at low temperature.

Pigment 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.

Zone	R_F	Yield mg	% of total	Compound
1	0.96	0.12	0.5	β,β-carotene
2	0.86	0.57	2.5	alloxanthin (*12*) diester
3	0.70	0.23	1.0	u
4	0.57	10.0	46.0	diesters *1b*, *2b* and *3b*
5	0.50	0.12	0.5	u
6	0.42	0.91	4.0	u
7	0.25	0.10	0.5	u
8	0.16	6.6	29.0	α-ketols *1a*, *2a* and *3*
9	0.08	3.6	16.0	alloxanthin (*12*) and diosphenols *6*, *7* and *8*

β,β-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*); λ_{max} (acetone) (430), 454 and 483 nm; *m/e* 564 (100 %), M−15, M−92, M−106.

The mixed diesters (Zone 4) *1b*, *2b* and *3b* were identified from R_F-value, λ_{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_3; 18), 575 (M_1-17; 3), 573 (M_2-17; 2), 500 (M_1-92; 3), 498 (M_3-92; 2), 496 (M_3-92; 1), 486 (M_1-106; 3), 484 (M_2-106; 2) and 482 (M_3-106; 1), *cf.* Ref. 6.

The mixed diesters *1b*, *2b* and *3b* were separated in System B (35 % A-hex): R_F *3b* 0.67, *1b* 0.43 and *2b* 0.18.

The mixed α-ketols *1a*, *2a* and *3* (Zone 8) were crystallized from diethyl ether, yield 4 mg, and identified from R_F-value, λ_{max} (acetone 476 and 503 nm) and *m/e* 596 (M_1), 594 (M_2), 592 (M_3), M_n-92 and M_n-106 peaks.

Standard acetylation [18] of the mixed α-ketols gave the diacetates *1c*, *2c* and *3c* with unchanged λ_{max} and *m/e* (%): 680 (M_1; 9), 678 (M_2; 29), 676 (M_3; 36), 620 (M_1-60; 2), 618 (M_2-60; 4), 616 (M_3-60; 3), 588 (M_1-92; 2), 586 (M_2-92; 1), 584 (M_3-92; 1) and 574 (M_1-106; 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-Didehydroastaxanthin natural diester (*1b*) had λ_{max} (acetone) 478 and (CS_2) 503 (535) nm; ν_{max} (KBr) 2920, 2850, 2161 (−C≡C−), 1740 (ester), 1680 (conj. C=O), 1568, 1520, 1468, 1380, 1365, 1290, 1270, 1243, 1160, 1120, 1090, 1072, 1040, 964 (*trans*-CH=CH−), 929, 830 (>C=CH−), 742 and 720 cm^{-1}.

1b (0.2 mg) in chloroform (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); λ_{max}(tetrahydrofurane) (430), 456 and 484 nm; *m/e* 598 (M); CD (EPA) Fig. 1.

7,8-Didehydroastaxanthin diacetate (*1c*) had λ_{max} (CS_2) 500 nm; ν_{max} (KBr) 2958, 2922, 2855, 2161 (−C≡C−, medium), 1740 (ester) 1672 (conj. 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'-Tetradehydroastaxanthin natural diester (*2b*) had λ_{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 diacetylenic tetrol *11*, yield 0.1 mg (48 %); $R_F = 0.50$; λ_{max} (acetone) (428) 454 and 483 nm, % III/II [23] = 26, (tetrahydrofurane) (432), 457 and 485 nm; *m/e* 596 (M); CD (EPA) Fig. 2.

7,8,7',8'-Tetradehydroastaxanthin diacetate (*2c*) had λ_{max} (CS_2) 503, (535) nm; ν_{max} (KBr) 2958, 2922, 2161 (−C≡C−, relatively stronger than for *1c*), 1740 (ester), 1672 (conj. 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 diacetylenic tetrol *11* by the same procedure as for *2b*.

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