217. Carotenoids of the Carotenoprotein Asteriarubin. Optical Purity of Asterinic Acid

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Summary

The carotenoid composition of the carotenoprotein asteriarubin *ex* the starfish *Asterias rubens*, determined by HPLC., comprised canthaxanthin (**6**, 3% of total), all-*trans*-astaxanthin (**1**, 14%), all-*trans*-7,8-didehydroastaxanthin (**2**, 24%), all-*trans*-7,8,7′,8′-tetradehydroastaxanthin (**3**, 43%) and 4-oxomytiloxanthin (**7**, 10%). The previously unknown 4-oxomytiloxanthin was tentatively identified by the UV./VIS., ^{1}H-NMR. spectra and MS. data.

The optical purity was determined by HPLC. of the di-(−)-camphanates, by comparison with those of synthetic standards: 7,8,7′,8′-tetradehydroastaxanthin (92% (3*S*,3′*S*), 2% *meso*), 7,8-didehydroastaxanthin (89% (3*S*,3′*S*), 2% *meso?*), and astaxanthin (78% (3*S*,3′*S*), 14% (3*R*,3′*S*), and 5% (3*R*,3′*R*)). It is concluded that the acetylenic derivatives of astaxanthin in contrast to astaxanthin from marine animal sources are essentially pure (3*S*,3′*S*)-isomers. This might reflect their probable metabolic formation by 4-oxo modification of acetylenic (3*R*,3′*R*)-carotenols *ex Mytilus edulis* in their diet.

1. Introduction. – Analyses of the carotenoids of the carotenoprotein asteriarubin *ex* the starfish *Asterias rubens* have established the presence of 7,8,7′,8′-tetradehydroastaxanthin (**3**, *Scheme 1*, 50–79% of total), 7,8-didehydroastaxanthin (**2**, 22–40%), and astaxanthin (**1**, 5–10%) [1] [2]. Previous studies on asterinic acid [3] (**2/3**) support a variation in the amounts of the acetylenic analogs present [4–6].

Total syntheses of the three optical isomers of astaxanthin (**1a** (3*S*,3′*S*), **1b** (3*R*,3′*S*; *meso*) and **1c** (3*R*,3′*R*)) have recently been achieved [7]. The natural occurrence of the pure (3*S*,3′*S*)-isomer in algae [8] and higher plants [9], of the pure (3*R*,3′*R*)-enantiomer in a yeast [10–11] and of mixtures of all three optical isomers in various marine animals [12–15] have been established.

The 7,8,7′,8′-tetradehydroastaxanthin (**3**) and 7,8-didehydroastaxanthin (**2**) have been assigned (3*S*,3′*S*)-configuration (**3a** resp. **2a**) from CD. correlations of

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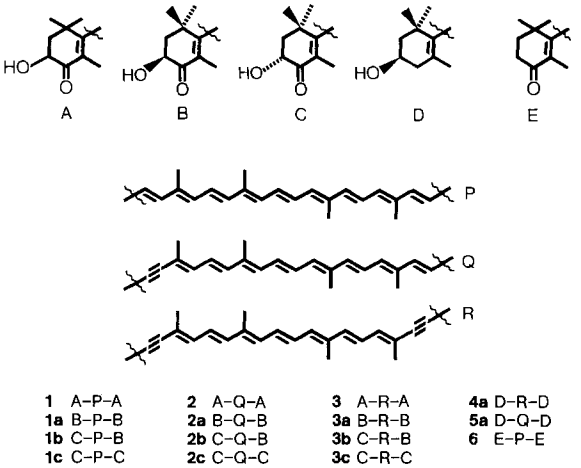
Scheme 1



the corresponding tetrols with (3*R*,3′*R*)-alloxanthin (**4a**) and (3*R*,3′*R*)-diatoxanthin (**5a**), respectively [6], subsequently confirmed by total syntheses of the pure all-*trans*-(3*S*,3′*S*)-isomers **2a** and **3a** [16]. Recently, also the 9,9′-di-*cis*-isomers of **3**, **3a** and **3c** have been prepared by total syntheses [17].

We now report the re-isolation of asteriarubin for a further analysis of the carotenoid components including a study of the optical purity of the acetylenic astaxanthin derivatives **2** and **3**.

2. Results. – The carotenoprotein asteriarubin was reisolated from the skin of *Asterias rubens* by a combination of previous procedures [1] [2], and the carotenoids were extracted from the lyophilized carotenoprotein immediately before HPLC. separation. The results are given in *Table 1*.

Table 1. *HPLC. analysis of the carotenoid complement in asteriarubin*

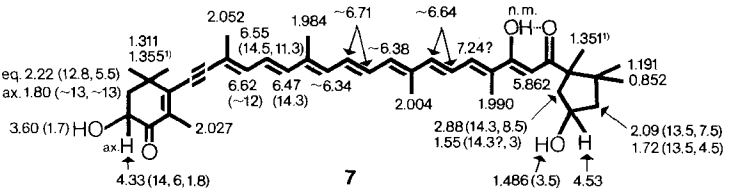
Retention time [sec]	Peak area [%]	Identification
610	2.4	Canthaxanthin (**6**)
690	0.7	9,9′-di-*cis*-**3**?
730	0.3	
798 (803)[a])	45.7	all-*trans*-7,8,7′,8′-Tetradehydroastaxanthin (**3**)
914 (918)[a])	24.3	all-*trans*-7,8-Didehydroastaxanthin (**2**)
986	1.3	
1042 (1047)[a])	13.6	all-*trans*-Astaxanthin (**1**)
1134	0.5	
1165	0.9	
1337	1.6	*cis*-**1**?
1712	8.8	4-Oxomytiloxanthin (**7**)
	100.1	

[a]) In parentheses the retention times of authentic synthetic standards.

The all-*trans* nature of astaxanthin (**1**) and its mono- and diacetylenic derivatives **2** and **3**, respectively, was demonstrated by comparison with HPLC. data obtained for the authentic compounds *(Table 1)*.

Canthaxanthin (**6**) and the unknown **7** were isolated by HPLC. on a semi-preparative column. Canthaxanthin (**6**) was identified by its retention time, UV./VIS. and ^{1}H-NMR. spectra. Based on its UV./VIS., ^{1}H-NMR. spectra and MS. data, the most polar component was tentatively identified as 4-oxomytiloxanthin (**7**; *cf.* assignments in *Scheme 2*, disregarding configuration) by comparison with known data for the acetylenic α-hydroxy ketones **2** and **3** and mytiloxanthin [18] [19].

Scheme 2. *400-MHz-^{1}H-NMR. of* **7** (chemical shifts in ppm (J in Hz))[a]



[a]) The assignments characterized by [1]) may be interchanged; n.m. = not measured.

The α-hydroxy ketones **1**–**3** were esterified with (−)-camphanoyl chloride by a standard procedure [14] and the camphanates submitted to HPLC. analysis, see *Table 2*. Allowing for a slight racemization during the esterification [11], the results support that the two acetylenic astaxanthin derivatives were the essentially optically pure (3 *S*,3′ *S*)-isomers **2a** and **3a**, whereas, for astaxanthin, the (3 *S*,3′ *S*)-isomer **1a** was dominant, but occurring together with the *meso*-form **1b** and the (3 *R*,3′ *R*)-enantiomer **1c**.

Available for HPLC. comparison were the (−)-camphanates of the three optical isomers of the synthetic diacetylenic all-*trans*-astaxanthin derivative (**3a** (*S*, *S*), **3b** (*R*, *S; meso*) and **3c** (*R*, *R*)) and of the three optical isomers of the mono-acetylenic all-*trans*-astaxanthin derivative (**2a** (*S*, *S*), **2b** (*R*, *S; meso*), and **2c** (*R*, *R*))

Table 2. *HPLC. analysis of the di-(−)-camphanates of 7,8,7′,8′-tetradehydroastaxanthin* (**3**), *7,8-didehydroastaxanthin* (**2**) *and astaxanthin* (**1**) ex *asteriarubin*

Di-(−)-camphanates of	Retention time [sec]	Peak area [%]	Identification of carotenoid
3	1071	1.7	all-*trans*-(3 *R*,3′ *S*)
	1289	91.8	all-*trans*-(3 *S*,3′ *S*)
2	1168	1.7	all-*trans*-(3 *R*,3′ *S*)
	1406	88.8	all-*trans*-(3 *S*,3′ *S*)
1	1060	5.3	all-*trans*-(3 *R*,3′ *R*)
	1268	14.0	all-*trans*-(3 *R*,3′ *S*)
	1410	7.6	*cis*-(3 *S*,3′ *S*)
	1542	70.1	all-*trans*-(3 *S*,3′ *S*)

[16], as well as the (−)-camphanates of the corresponding optical isomers of astaxanthin (**1a–c**) [7] [20]. The separation of the diastereomeric (−)-camphanates of the all-*trans*-compounds **3a–c**, and **2a–c** is illustrated in the *Figure.*

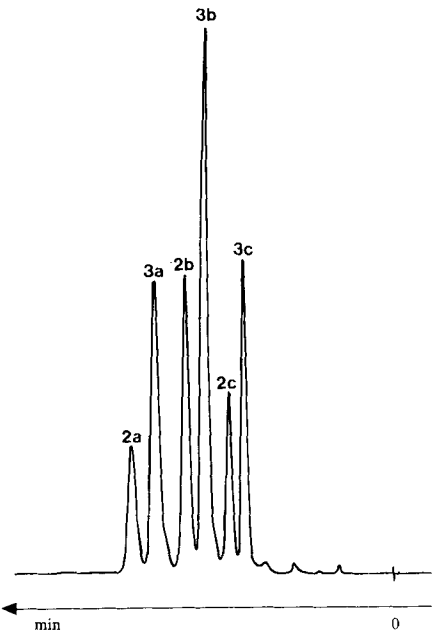


Figure. *HPLC. separation of the di-(−)-camphanates of the all-*trans*-isomers of 7,8,7′,8′-tetradehydroastaxanthins* (**3a–c**) *and 7,8-didehydroastaxanthins* (**2a–c**)

3. Discussion. – The present work confirms previous findings that (3*S*,3′*S*)-7,8,7′,8′-tetradehydroastaxanthin (**3a**), (3*S*,3′*S*)-7,8-didehydroastaxanthin (**2a**) and astaxanthin (**1**) are major components of the prosthetic group in the carotenoprotein asteriarubin. Furthermore, the optical purity of the acetylenic *α*-hydroxy ketones **2a** and **3a** and the partly racemic nature of astaxanthin (**1a/1b/1c**) have now been demonstrated. Canthaxanthin (**6**) and 4-oxomytiloxanthin (**7**) were identified as minor components. All carotenoid constituents of asteriarubin share the common 4-oxo moiety, 95% are *α*-hydroxy ketones mainly with the (3*S*)-end group B, consistent with the general capacity of such *α*-hydroxy ketones for protein binding [21] [22]. Carotenoids with triple bonds in 7,8(7′,8′)-positions are dominant [1], a phenomenon that has previously raised the question whether a 7,8(7′,8′)-dehydrogenation occurs metabolically in *A. rubens* [23]. However, the present isolation of 4-oxomytiloxanthin (**7**) and the previously established carotenoid pattern in the edible mussel *(Mytilus edulis)* [24], comprising mytiloxanthin [18] and alloxanthin (pectenoxanthin) (**4a**, chirality not proved from this source) [25], together with the known feeding habits of *A. rubens* including *M. edulis* [26], suggest that the acetylenic carotenoids are derived from the diet. The optical purity of the acetylenic astaxanthin derivatives suggests a metabolic 4-oxo modification from optically

pure (3*R*,3′*R*)-alloxanthin (**4a**) and diatoxanthin (**5a**) to be present in the diet. Such a capacity is indicated by the isolation of 4-oxomytiloxanthin (**7**). The biosynthetic origin of racemized astaxanthin (**1**) in marine animals remains to be answered.

The potential use of carotenoids and their detailed configuration as indicators for food chain relationships seems obvious.

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Experimental Part

General remarks. All operations were carried out under an Ar atmosphere. HPLC.: *Altex 100* pump. Sample injection was carried out by syringe *(Hamilton) via* septum injection port from *Perkin-Elmer*. The analytical column (500×3.1 mm) was home-made and filled with *Spherisorb S 5-CN*. The mixture hexane/isopropyl ethanoate/2-propanol/*N*-ethyldiisopropylamine 82.4:17:0.5:0.1 (*v/v*) served as mobile phase; flow rate 1 ml/min. The spectrophotometer *LC-55* (λ=450 nm) from *Perkin-Elmer* was used as detector. Retention times for authentic standards were determined in parallel experiments (s. *Table 1*). The semi-preparative isolation of the five main components **1**–**3**, **6**, and **7**, was carried out under the same conditions with the exception of the column having the dimensions 500×6 mm and the flow rate being 1.8 ml/min. The results from *Table 2* were achieved using the same conditions, but hexane/isopropyl ethanoate/acetone 76:17:7 (*v/v*) served as the mobile phase. The detector operated at 476 nm. For peak assignment, an aliquot of the solution to be analyzed was spiked with the di-(−)-camphanates of synthetic **3a**–c, **2a**–c, and **1a**–c. – ^{1}H-NMR. spectra were run at 400 MHz (solvent $CDCl_3$, 100% D-quality) on a *Bruker-WM-400*-FT. spectrometer equipped with an *ASPECT-2000* computer (32 K data) and disk unit; chemical shifts in ppm relative to TMS (=0 ppm), coupling constants *J* in Hz. – MS.: On *AEI MS 9;* using direct introduction, vaporizing the sample from the tip of a glass rod close to the electron beam. The ion source was held at 200°, electron energy 70 eV.

Asteriarubin [1] [2] [27]. Skins of *Asterias rubens* collected in the Trondheimsfjord, Norway, October 1980, were used. Asteriarubin was isolated by the procedure described in [1] involving extraction, concentration by ultrafiltration, $(NH_4)_2SO_4$-precipitation, chromatography on *Sephadex* and lyophilization followed by a final precipitation [2] with Na-acetate buffer; yield 65 mg of lyophilized asteriarubin. – UV./VIS. (phosphate buffer): 280, 570. D_{554}/D_{280}=4.7, containing *ca.* 1 mg carotenoid.

Carotenoid composition. The carotenoids were extracted from the carotenoprotein by acetone and quickly submitted to HPLC. The five major components were isolated by a semi-prep. column (conditions see general remarks).

Canthaxanthin (**6**). – UV./VIS. (mobile phase): 469. – ^{1}H-NMR. (*ca.* 2 µg in 0.2 ml): 1.195 (*s*, *ca.* 12 H, 2 H_3C–C(1), 2 H_3C–C(1′)); 1.853 (*t*, *J*=6.7, 4 H, H_2C(2), H_2C–C(2′)); 1.873 (*s*, 6 H, H_3C–C(5), H_3C–C(5′)); 1.983 (*s*, 6 H, H_3C–C(13), H_3C–C(13′)); 2.003 (*s*, 6 H, H_3C–C(9), H_3C–C(9′)); 2.508 (*t*, *J*=6.7, 4 H, H_2C(3), H_2C(3′)); 6.24 (*d*, *J*≈16, H–C(7), H–C(7′)); 6.28 (*d*, *J*≈12, H–C(10), H–C(10′)); *ca.* 6.30 (*m*, H–C(14), H–C(14′)); 6.35 (*d*, *J*=16, 2 H, H–C(8), H–C(8′)); 6.43 (*d*, *J*=14.8, 2 H, H–C(12), H–C(12′)); *ca.* 6.65 (*m*, 4 H, H–C(11), H–C(11′) and H–C(15), H–C(15′)).

4-Oxomytiloxanthin (**7**). – UV./VIS. (mobile phase): 447, 473, 502. – ^{1}H-NMR. (*ca.* 10 µg in 0.2 ml): see *Scheme 2*. – MS.: 612 (1, M^+), 610 (1), 594 (1), 592 (1), 590 (1), 576 (1), 413 (2), 383 (2), 359 (3), 328 (4), 315 (3), 303 (3), 197 (7), 179 (35), 127 (20), 109 (79), 95 (36), 91 (44), 83 (64), 69 (56), 55 (67), 43 (74), 41 (100). The MS. of **7** shows a very weak molecular ion at *m/z* 612. No detailed interpretation of the central part of the mass spectrum is possible. The lower part with *m/z* 197, 179, 127 and 109 shows the 8-hydroxycapsorubin pattern.

Optical purity of 7,8,7′,8′-tetradehydroastaxanthin (**3**), *7,8-didehydroastaxanthin* (**2**) *and astaxanthin* (**1**). Compounds **1**–**3**, separated by HPLC., were esterified individually with (−)-camphanoyl chloride by the previously described procedure [14] [22] and the esters submitted to HPLC. (s. *Table 2*).

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