73. Natural Occurrence of Enantiomeric and *meso*-Astaxanthin 1. *Ex* Lobster Eggs *(Homarus gammarus)*

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Summary

Astaxanthin (**1**; 3,3′-dihydroxy-β,β-carotene-4,4′-dione) isolated from lobster eggs *(Homarus gammarus)* was unexpectedly found to be a mixture of all three optical isomers as determined by HPLC. analysis of the corresponding diesters of (−)-camphanic acid. This is the first finding of *meso*-astaxanthin and a *meso*-carotenoid in general in nature.

The constitution of astaxanthin (**1**; 3,3′-dihydroxy-β,β-carotene-4,4′-dione), elucidated by *Kuhn & Sørensen* [1], was later confirmed by partial synthesis of the derivative astacene (**2**) from canthaxanthin (**3**) [2].

Assignment of the (3*S*,3′*S*)-configuration **1a** rests on the following evidence: Complex metal hydride reduction of the monoacetate of a natural astaxanthin monoester *ex Haematococcus pluvialis* (green alga) yielded a mixture of diastereomeric tetrols. Conformational analysis of these tetrols revealed that the helicity of the preferred half-chair of the cyclohexene end groups would be determined by the chirality at C(3) and C(3′). CD. correlation with (3*R*,3′*R*)-zeaxanthin (**4**) favoured the corresponding (3*S*,3′*S*)-configuration for the tetrol and for the astaxanthin from which it was derived [3]. Qualitative CD. comparison of astaxanthin *ex Haematococcus pluvialis* with astaxanthin *ex Homarus gammarus* and *Halocynthia papillosa* supported the same (3*S*,3′*S*)-configuration [3]. This was also the case for astaxanthin *ex* spider mite *(Schizonobia sycophanta)* [4].

Subsequently, the natural occurrence of (3*R*,3′*R*)-astaxanthin (**1b**) from the yeast *Phaffia rhodozyma* was reported [5]. The configurational assignment rested on the opposite *Cotton* effect to that of **1a** and comparative ^{1}H-NMR. studies of **1a** and **1b** in the presence of *d*-Eu(hfc)$_3$.

More recently, the total synthesis of optically pure (3*S*,3′*S*)-astaxanthin (**1a**) has been reported [6] and the quantitative CD. data have been recorded [7]. The

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total synthesis of (3*R*,3'*R*)-astaxanthin (**1b**) and of the *meso*-form **1c** has also been achieved [8].

Separation of enantiomers as diastereomeric diesters of (−)-camphanic acid [9] was successfully adopted to the carotenoid field during the total synthesis of (3*S*,3'*S*)-actinioerythrin [10]. Analytical methods for investigations of the enantiomeric purity of carotenols were subsequently studied independently in our laboratories. The procedure for the separation of enantiomeric and *meso*-astaxanthin as diesters of (−)-camphanic acid has recently been published [11].

The evidence hitherto taken in favour of the (3*S*,3'*S*)-configuration for astaxanthin from different sources does not rule out a co-occurrence with smaller relative amounts of the (3*R*,3'*R*)-enantiomer and the presence of the optically inactive *meso*-form. With the synthetic reference compounds and the methodology now available, investigations on the natural occurrence of enantiomeric and *meso*-astaxanthin may be carried out. We now present the first communication in a series dealing with the investigation of astaxanthin from a variety of sources.

Stored crystallized astaxanthin from lobster eggs, previously found to contain 4.4% of the acetylenic 7,8-didehydro and 0.3% of the 7,8,7',8'-tetradehydro derivative [12] was converted to the diester of (−)-camphanic acid and analyzed by HPLC. It was unexpectedly found to consist of a mixture of (3*S*,3'*S*)-, (3*R*,3'*R*)- and (3*R*,3'*S*; *meso*)-isomers in the ratio of 5:3:2. The identification of the peaks was achieved by means of reference samples prepared from synthetic **1a**, **1b** and **1c**. In addition, VIS. and mass spectra were recorded for every eluted compound. The composition of the ester mixture on a column also allowing the separation of all-*trans*- and *cis*-isomers [11] is given in the *Figure* and the *Table* (sample 1). The

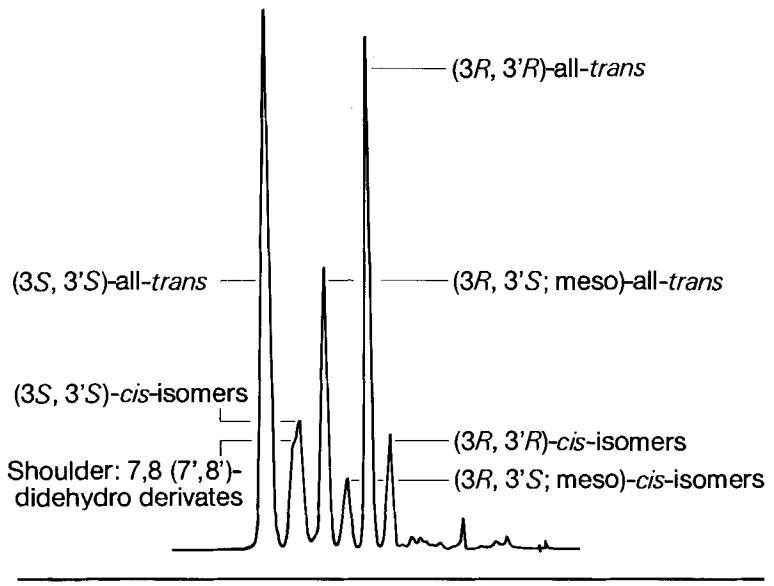

Figure. *Determination of configurational isomers of astaxanthin* ex *lobster eggs* (Homarus gammarus) *by HPLC. analysis of diesters of* (−)*-camphanic acid*

Table. *Composition of enantiomeric and* meso*-astaxanthin determined by HPLC. of diesters of (−)-camphanic acid*

Substrate	Diesters of (−)-camphanic acid as % of total of					
	1a		**1b**		**1c**	
	all-*trans*	*cis*	all-*trans*	*cis*	all-*trans*	*cis*
Synthetic samples[a])						
(3*S*,3′*S*)-all-*trans*	89	8			3	
(3*R*,3′*R*)-all-*trans*			86	5	8	1
(3*R*,3′*S*; *meso*)-all-*trans*	3		1		94	3
Biological samples						
1 Sample 1	35	13	24	6	16	5
1 Sample 2	6	18	30	9	26	11
1a (3*S*,3′*S*)-all-*trans*[b])	71	29				

[a]) Results consistent with optical purity of the appropriate intermediates in the synthesis of **1a**, **1b** and **1c** [16].

[b]) Treatment of **1a** in a simulated isolation like sample 2; s. exper. part.

quantitative CD. spectrum of the original astaxanthin sample, with $\Delta\varepsilon$ values lower than for optically pure **1a** was compatible with this result.

Further confirmation for the presence of a mixture of configurational isomers was obtained by careful extraction of fresh frozen lobster eggs, quick isolation of astaxanthin by TLC. and HPLC. analysis of its esters of (−)-camphanic acid (s. *Table*, sample 2).

We consequently conclude that lobster eggs contain both astaxanthin enantiomers and the *meso*-form. This is the first finding of *meso*-astaxanthin (**1c**) and a *meso*-carotenoid in general in nature.

Provided that lutein (**5**; (3*R*,3′*R*,6′*R*)-configuration) is a dietary precursor of astaxanthin in lobster eggs (*cf.* [13–15]), the existence of *meso*-astaxanthin can be explained. However, the occurrence of the (3*R*,3′*R*)-enantiomer suggests that lobster contains a C(3)-isomerase. Chemically, the isomerization of an α-hydroxyketone can, of course, be rationalized *via* the enol.

The formation of a quasi-equilibrium of **1a**, **1b** and **1c** from either pure **1a** or **1b** during extraction and laboratory manipulations is rejected by the following experiments: i) the preparation of pure diesters of (−)-camphanic acid of **1a**, **1b** and **1c** in individual runs, ii) an experiment with **1a** checking the influence of isolation conditions, and iii) separate studies on the configurational stability of α-hydroxycarbonyl compounds [16]. The conditions of the experiments i) and ii) caused only *trans/cis* isomerization of the polyene chain (see *Table*). The *cis*-isomers have not been identified, but are presumably mono- and di-*cis*-isomers with one or two of the $\Delta^{9,9',13,13'}$-bonds in *cis*-configuration [17].

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Experimental Part

General remarks. All operations were carried out in inert atmosphere, in subdued light and at temp. not exceeding room temp. Optimum HPLC. separations were under conditions reported elsewhere [11] with *Spherisorb* S-5CN as a stationary phase and hexane/isopropyl acetate/acetone 76:17:7 as mobile phase at low flow rate. For the Trondheim laboratory, the HPLC. equipment has been described before [18]. CD.: $\lambda_{max}(\Delta\varepsilon)$ in nm.

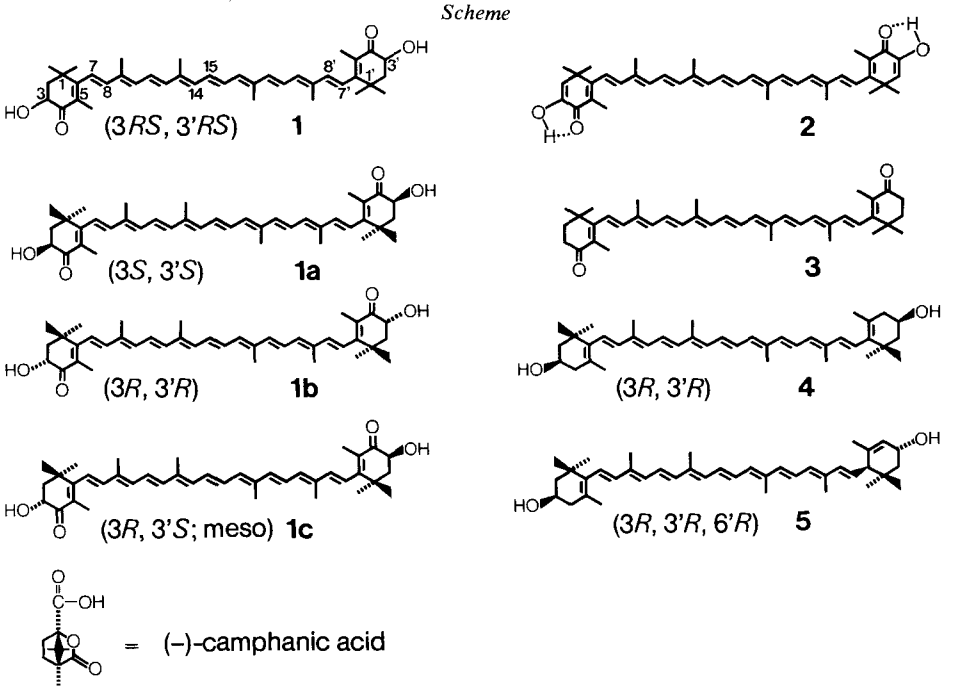
Natural astaxanthin from lobster eggs. i) Sample 1 was left from a previous study [12]. – CD. (CH_2Cl_2): 388 (+2.25), 323 (−7.45), 279 (+3.54), 248 (−4.22); *cf.* CD. (CH_2Cl_2) of optically pure **1a** [7]: 384 (+6.7), 323 (−23.1), 280 (+12.5), 249 (−14.4).

ii) Sample 2 was obtained from *Homarus gammarus,* collected near Bergen, Norway, frozen fresh. The eggs (37 g) were readily crushed in a mortar, transferred to an Erlenmeyer flask, and extracted with acetone (300 ml) by standing under N_2 at 5° (refrigerator) overnight. The extract was filtered through a glass-sinter, leaving decoloured cell debris behind (having washed the debris with acetone). Total carotenoid content 9.6 mg ($E^{1\%}_{1cm}=2500$ at λ_{max}). Colourless lipids were precipitated from the acetone concentrate at −10°, and the extract evaporated to dryness upon addition of benzene (rotatory evaporator, vacuum). TLC. (SiO_2, acetone/hexane 3:7) revealed 8 yellow-orange zones. The main carotenoid, astaxanthin (Rf 0.37) was identified from λ_{max} 475 nm in acetone and co-chromatography with synthetic **1b**.

Attempted racemization of **1a** *under extraction conditions.* Pure, synthetic **1a** (0.5 mg) dissolved in acetone (20 ml) containing H_2O (1 ml) was kept as a homogeneous solution at 5° overnight. The mixture was evaporated to dryness upon addition of benzene. The residue was esterified with (−)-camphanoyl chloride followed by HPLC. analysis of the ester mixture obtained (s. *Table*).

Preparation of the esters of (−)-camphanic acid. Carotenoid samples (about 0.5 mg) in dry pyridine (1 ml) and (−)-camphanoyl chloride (25 mg) were reacted at 0° for 40 min. Ether (10 ml) was added and the organic phase washed several times with water. The ether extract was evaporated to dryness upon addition of benzene and subsequently, by co-chromatography with authentic standards, submitted to HPLC. analysis. A typical separation is given in the *Figure.* Peaks were identified from their VIS. and

Scheme



mass spectra and by co-injection with authentic samples (diesters of (−)-camphanic acid of **1a**, **1b** and **1c**). Results are summarized in the *Table* with an approximate accuracy of about ±2% for the biological samples and for the simulated isolation experiment.

REFERENCES

[1] *R. Kuhn & N.A. Sørensen,* Ber. deutsch. chem. Ges. *71,* 1879 (1938).
[2] *J.B. Davis & B.C.L. Weedon,* Proc. chem. Soc. *1960,* 182.
[3] *A.G. Andrewes, G. Borch, S. Liaaen-Jensen & G. Snatzke,* Acta chem. Scand. *B28,* 730 (1974).
[4] *A. Veerman, G. Borch, R. Pedersen & S. Liaaen-Jensen,* Acta chem. Scand. *B29,* 525 (1975).
[5] *A.G. Andrewes & M.P. Starr,* Phytochemistry *15,* 1009 (1976).
[6] *F. Kienzle & H. Mayer,* Helv. *61,* 2609 (1978).
[7] *G. Englert, F. Kienzle & K. Noack, Helv. 60,* 1209 (1977).
[8] *R.K. Müller & H. Mayer,* Helv. to be published; *H. Mayer,* Pure appl. Chemistry *51,* 535 (1979); *R.K. Müller, K. Bernhard, F. Kienzle, H. Mayer & A. Rüttimann,* Food Chemistry *15,* 5 (1980).
[9] *H. Gerlach,* Helv. *51,* 1587 (1968); *H. Gerlach & W. Müller,* Helv. *55,* 2277 (1972); *H. Gerlach, K. Oertle & A. Thalmann,* Helv. *59,* 755 (1976).
[10] *R.K. Müller, H. Mayer, K. Noack, J.J. Daly, J.D. Tauber & S. Liaaen-Jensen,* Helv. *61,* 2881 (1978); *R.K. Müller, J.J. Daly, G. Englert, H. Mayer & K. Noack,* Helv. to be published.
[11] *M. Vecchi & R.K. Müller,* Journal of High Resolution Chromatography & Chromatography Communications *2,* 195 (1979).
[12] *H. Thommen, F. Leuenberger, R. Berger & S. Liaaen-Jensen,* Biochemical Systematics and Ecology *4,* 131 (1976).
[13] *T. Katayama, K. Hirata, H. Yokoyama & C.O. Chichester,* Bulletin of the Japanese Society of Scientific Fisheries *36,* 702 (1970).
[14] *T. Katayama, H. Yokoyama & C.O. Chichester,* Intern. J. Biochemistry *1,* 438 (1970).
[15] *W.-J. Hsu, D.B. Rodriguez & C.O. Chichester,* Intern. J. Biochemistry *3,* 333 (1972).
[16] *R.K. Müller, K. Bernhard, H. Mayer, A. Rüttimann & M. Vecchi,* to be published.
[17] *G. Englert & M. Vecchi,* unpublished results.
[18] *A. Fiksdahl, J.T. Mortensen & S. Liaaen-Jensen,* J. Chromatogr. *157,* 111 (1978).