

NATURAL OCCURRENCE OF ENANTIOMERIC AND MESO ASTAXANTHIN 7*-CRUSTACEANS INCLUDING ZOOPLANKTON

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(Received 14 February 1986)

Abstract—1. The isomeric ratio of enantiomeric and *meso* astaxanthin (free and esterified) in the crustaceans *Calanus finmarchicus*, *Euphausia superba*, *Thysanoessa inermis*, *Acanthephyra purpurea* and *Cancer pagurus* is reported.

2. The ratios observed in *C. finmarchicus* and *T. inermis*, both considered as important feed ingredients for wild salmon, are compatible with those reported by others for wild salmon and with recent evidence demonstrating that salmonids obtain the three optical isomers of astaxanthin from the diet.

3. The origin of the three optical isomers of astaxanthin in zooplankton is discussed briefly.

INTRODUCTION

Previously we have reported the ratio of the natural occurrence of the three optical isomers 3*S*,3'*S* (1, Scheme 1), 3*R*,3'*S* (2, *meso*) and 3*R*,3'*R* (3) of astaxanthin and its esters in lobster (*Hommarus gammarus*; Rønneberg *et al.*, 1980; Renstrøm *et al.*, 1982), shrimp (*Pandalus borealis*; Renstrøm *et al.*, 1981a) and flowers of *Adonis annua* (Renstrøm and Liaaen-Jensen, 1981b).

Schiedt *et al.* (1981) studied this ratio for wild salmon, which was remarkably similar for different species and geographical localities. Subsequent feeding experiments with synthetic, individual optical isomers of astaxanthin (1, 2 and 3) to rainbow trout and to salmon have demonstrated that salmonids resorb each isomer to an equal extent, are not able to carry out an epimerization at C-3,3' in astaxanthin, and that the isomeric ratio observed merely reflects the configuration of the astaxanthin present in the diet (Foss *et al.*, 1984; Storebakken *et al.*, 1985). A similar conclusion was also reached from studies on the isomeric ratio (1:2:3) of astaxanthin in zooplankton and salmonids in two Norwegian subalpine lakes (Storebakken *et al.*, 1984). The ratio of the optical isomers of astaxanthin may be useful in food chain studies.

MATERIALS AND METHODS

Biological materials

Euphausia superba, caught December 1978 near the Bouvet island, *Thysanoessa inermis* caught February 1982 near Svartnes, Balsfjord, Norway, *Calanus finmarchicus*

caught in May 1984 near Sunndalsøra, Norway and *Acanthephyra purpurea* caught May 1981, were stored frozen. *Cancer pagurus* was purchased at the local fish market 1980.

Physical and chemical methods

Isolation of the carotenoids. The carotenoids were extracted with acetone at room temperature. Whole frozen animals were extracted, except for *C. pagurus* where only the shell was used. Chromatography was carried out by TLC (SiO₂, 30% acetone-hexane). An extinction coefficient of $E_{1\text{cm}}^{1\%} = 2100$ was used, calculating the esters as astaxanthin equivalents.

Astaxanthin diester. $R_F = 0.70$, inseparable from an authentic sample *ex Pandalus borealis*; VIS λ_{max} nm (acetone) 470.

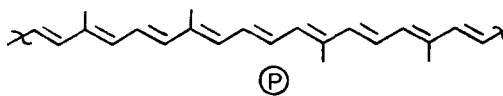
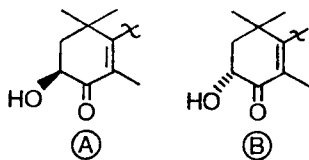
Astaxanthin monoester. $R_F = 0.46$, inseparable from an authentic standard *ex P. borealis*; VIS λ_{max} nm (acetone) 470.

Astaxanthin. $R_F = 0.30$, inseparable from synthetic astaxanthin; VIS λ_{max} nm (acetone) 470; MS (200°C) m/z 596 (M^+ , 10%), 594 ($M-2$, 14%), 504 ($M-92$, 4%), 490 ($M-106$, 8%), 91 (100%).

Astaxanthin dicamphanate. The mono- and diesters were submitted to anaerobic hydrolysis to astaxanthin as described elsewhere (Renstrøm *et al.*, 1981c). Astaxanthin (0.1 mg) was esterified with (-)-camphanoyl chloride (30-90 mg), providing the dicamphanate $R_F = 0.84$ (SiO₂, diethyl ether); VIS λ_{max} nm (acetone) 470 (Schiedt *et al.*, 1981; Foss *et al.*, 1984). The diastereomeric camphanates were submitted to HPLC by the standard procedure (Vecchi and Müller, 1979).

RESULTS AND DISCUSSION

We now report the isomeric ratio (1:2:3) of five species of crustaceans: *Thysanoessa inermis*, *Acan-*



1 (3 <i>S</i> ,3' <i>S</i>)	A-P-A
2 (3 <i>R</i> ,3' <i>S</i> ; <i>meso</i>)	B-P-A
3 (3 <i>R</i> ,3' <i>R</i>)	B-P-B

Table 1. Carotenoid composition in per cent of total carotenoids, and ratio of optical isomers [*S,S*:*R,S* (*meso*):*R,R*] of free and esterified astaxanthin in some Crustaceans

Carotenoid	<i>Euphausia superba</i>	<i>Thysanoessa inermis</i>	<i>Calanus finmarchicus</i>	<i>Acantheephyra purpurea</i>	<i>Cancer pagurus</i> shell
Astaxanthin diester	64	61	46	43	22
Astaxanthin monoester	31	35	43	37	13
Astaxanthin	5	4	11	20	58
	(9:21:70)	(50:24:26)	(84:5:11)	(20:44:15)	(20:24:56)
		(55:7:38)	(83:3:14)		

theephyra purpurea, *Cancer pagurus* and the zooplankton *Calanus finmarchicus*, all from the North Sea and *Euphausia superba* from Antarctic waters, Table 1.

Consistent with previous studies (Barbier *et al.*, 1966; Batham *et al.*, 1951; Fischer *et al.*, 1955; Herring, 1973; Lenel *et al.*, 1978; Wieser, 1965) free and esterified astaxanthin were the only carotenoids present. A mixture of the three astaxanthin isomers was demonstrated in each case. In *E. superba* and *C. pagurus* the *R*-configuration was dominant, whereas in *T. inermis* and *C. finmarchicus* the *S*-configuration dominated. The two species *T. inermis* and *C. finmarchicus* are recognized as important feed ingredients for wild salmon, and the ratios in these two crustaceans are compatible with the isomeric astaxanthin mixture encountered in wild salmon (Schiedt *et al.*, 1981).

We have recently demonstrated that in the freshwater zooplankton *Daphnia magna* optically pure (3*S*,3'*S*)-astaxanthin (1) was formed from optically pure (3*R*,3'*R*)-zeaxanthin (β , β -carotene-3,3'-diol) of algal origin (Partali *et al.*, 1986). However, the origin of the *R*-configured astaxanthin isomers (2 and 3) in zooplankton is still obscure. The formation of particularly 3 and 2 by zooplankton, presumably from phytoplankton carotenoid precursors will be studied.

Acknowledgements—We thank Dr P. J. Herring, Institute of Oceanographic Sciences, Surrey, England, for the sample of *Acantheephyra purpurea*, cand. agric. Trond Storebakken, Research Station for Salmonids, Sunndalsøra, Norway, for the sample of *Calanus finmarchicus* and Dr Trond Ellingsen and Siv.ing. Olav Sæter, Institute of Biotechnology, this University for samples of *Euphausia superba* and *Thysanoessa inermis*, respectively.

REFERENCES

- Barbier M., Charniaux-Cotton H. et Fried-Montaufer M. (1966) Presence dans les secondes antennes des mâles *Talitrus saltator* et *Orchestia gammarella* (Crustacea Amphipodes) d'un taux relativement élevé d'astaxanthine. *C. r. Acad. Sci. Paris*, **263**, 1508–1510.
- Batham E., Fisher L. R., Henry K. M., Kon S. K. and Thompson S. Y. (1951) Preformed vitamin A in marine crustacea. *Biochem. J.* **48**.
- Fisher L. R., Kon S. K. and Thompson S. Y. (1955) Vitamin A and carotenoids in certain invertebrates III. Euphausiacea. *J. mar. Biol. Assoc., UK* **34**, 81–100.
- Foss P. (1985) Applied carotenoid chemistry. Algal chemosystematics and food chain studies. Dr. ing. Thesis, Univ. Trondheim-NTH, 327 p.
- Foss P., Storebakken T., Schiedt K., Liaaen-Jensen S., Austreng E. and Streiff K. (1984) Carotenoids in diets for salmonids I. Pigmentation of rainbow trout with the individual optical isomers of astaxanthin in comparison with canthaxanthin. *Aquaculture* **41**, 213–226.
- Herring P. J. (1973) The distribution of carotenoid pigments and lipids of some oceanic animals. 2. Decapod crustaceans. *J. mar. Biol. Assoc., UK* **53**, 339–568.
- Lenel R., Negre-Sadargues G. et Castillo R. (1978) Les Pigments Carotenoides Chez Les Crustaces. *Arch. Zool. Exp.* **119**, 297–334.
- Partali V., Olsen Y., Foss P. and Liaaen-Jensen S. (1985) Carotenoids in food Chain Studies. 1. Zooplankton (*Daphnia magna*) response to a unialgal (*Scenedesmus acutus*) carotenoid diet, to spinach and to yeast diets supplemented with individual carotenoids. *Comp. Biochem. Physiol.* **82B**, 767–772.
- Renstrøm B., Borch G. and Liaaen-Jensen S. (1981a) Natural occurrence of enantiomeric and *meso*-astaxanthin. 4. *Ex* shrimps (*Pandalus borealis*). *Comp. Biochem. Physiol.* **69B**, 621–624.
- Renstrøm B. and Liaaen-Jensen S. (1981b) Natural occurrence of enantiomeric and *meso*-astaxanthin. 6. Esterified, optically pure (3*S*,3'*S*)-astaxanthin from flowers of *Adonis annua*. *Biochem. Syst. Ecol.* **9**, 249–250.
- Renstrøm B., Borch G., Skulberg O. and Liaaen-Jensen S. (1981c) Natural occurrence of enantiomeric and *meso*-astaxanthin. 3. Optical purity of (3*S*,3'*S*)-astaxanthin *ex Haematococcus pluvialis* (green algae). *Phytochemistry* **20**, 2561–2564.
- Renstrøm B., Rønneberg H., Borch G. and Liaaen-Jensen S. (1982) Animal carotenoid 27. Further studies on the carotenoproteins crustacyanin and overoverdin. *Comp. Biochem. Physiol.* **71B**, 249–252.
- Rønneberg H., Renstrøm B., Aareskjold K., Liaaen-Jensen S., Vecchi M., Leuenberger F. J., Müller R. K. and Mayer H. (1980) Natural occurrence of enantiomeric and *meso*-astaxanthin. 1. *Ex* lobster eggs (*Homarus gammarus*). *Helv. chim. Acta* **63**, 711–715.
- Schiedt K., Leuenberger F. J. and Vecchi M. (1981) Natural occurrence of enantiomeric and *meso*-astaxanthin. 5. *Ex* wild salmon (*Salmo salar* and *Oncorhynchus*). *Helv. chim. Acta* **64**, 415–424.
- Storebakken T., Foss P., Åsgård T., Austreng E. and Liaaen-Jensen S. (1984) Carotenoids in food chain studies—Optical isomer composition of astaxanthin in crustaceans and fish from two sub-alpine lakes. *Abstr. 7th Int. IUPAC Symp. on Carotenoids*, p. 31. München 1984.
- Storebakken T., Foss P., Austreng E. and Liaaen-Jensen S. (1985) Carotenoids in diets for salmonids. II. Epimerization studies with astaxanthin in Atlantic salmon. *Aquaculture* **44**, 259–269.
- Vecchi M. and Müller R. K. (1979) Separation of (3*S*,3'*S*)-, (3*R*,3'*R*)-astaxanthin and (3*S*,3'*R*)-astaxanthin via (–)-camphanic acid esters. *J. High Res. Chrom.* **2**, 195–196.
- Wieser W. (1965) Electrophoretic studies on blood proteins in an ecological series of isopod and amphipod species. *J. mar. Biol. Assoc., UK* **45**, 507–523.