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

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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 (SiO_2, 30% acetone–hexane). An extinction coefficient of $E^{1\%}_{1cm} = 2100$ 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.

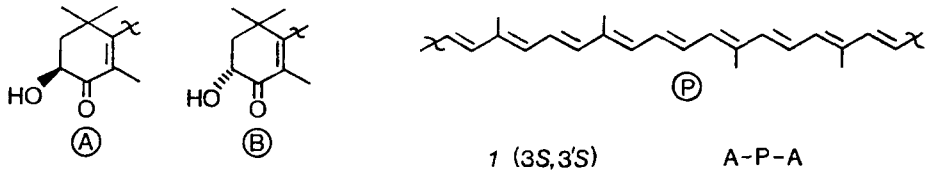
Astaxanthin monoester. $R_F = 0.46$, inseparable from an authentic standard *ex P. borealis*; VIS λ_{max} nm (acetone) 470.

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 $R_F = 0.84$ (SiO_2, 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*S*,3′*S*) A-P-A
2 (3*R*,3′*S*; meso) B-P-A
3 (3*R*,3′*R*) 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	*Euphausia superba*	*Thysanoessa inermis*	*Calanus finmarchicus*	*Acanthephyra purpurea*	*Cancer pagurus* shell
Astaxanthin diester	64 } (9:21:70)	61 } (50:24:26)	46 } (84:5:11)	43	22
Astaxanthin monoester	31	35	43	37	13
Astaxanthin	5	4 (55:7:38)	11 (83:3:14)	20 (20:44:15)	58 (20:24:56)

thephyra 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*-configurated 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.

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