淡水魚ブラックバスより新カロテノイド2種の単離

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Isolation and Structural Elucidation of Two New Carotenoids from the Black Bass *Micropterus salmoides*[*1]

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Two new carotenoids, (3′*R*, 6′*S*)-β, ε-caroten-3′-ol and (3′*S*, 6′*S*)-β, ε-caroten-3′-ol have been isolated from the integuments of the black bass *Micropterus salmoides*.

β-Carotene, canthaxanthin, tunaxanthin, lutein, zeaxanthin, diatoxanthin, alloxanthin, α-doradexanthin, β-doradexanthin, astaxanthin, rhodoxanthin, parasiloxanthin and salmoxanthin have so far been known as principal carotenoids in fish.[1] As far as carotenoids of the black bass *Micropterus salmoides* are concerned, Sarphie and Crozier[2] reported the presence of lutein and Czeczuga[3] reported the following carotenoids: β-carotene, α-cryptoxanthin, β-crypotoxanthin, echinenone, canthaxanthin, lutein, zeaxanthin, neothxanthin, α-doradexanthin, β-doradexanthin, idoxanthin, astaxanthin, mutatochrome and mutatoxanthin.

In the course of our comparative biochemical studies of carotenoids in fish, we have isolated two new carotenoids, (3′*R*, 6′*S*)-β, ε-caroten-3′-ol and (3′*S*, 6′*S*)-β, ε-caroten-3′-ol along with α-carotene, β-carotene, β-cryptoxanthin, tunaxanthin A, B, C, D, G, H, lutein G, (3*R*, 3′*R*)-, (3*R*, 3′*S*: *meso*)-, (3*S*, 3′*S*)-zeaxanthin, diatoxantin and alloxanthin from the integuments of the black bass. We report in this paper the isolation and structural elucidation of these two new carotenoids.

Materials and Methods

Extraction and Isolation of Carotenoids

Black bass (300 specimens, 128 kg) were collected at the Lake of Biwa, Shiga, Japan. According to the method described previously,[4] carotenoids were extracted from the integuments (10 kg) of the black bass with acetone. They were transferred to ether/*n*-hexane (1 : 1) by addition of water. The extracted solution was concentrated under reduced pressure in N_2 below 40°C. The crude oil was saponified by 10% KOH/MeOH at 30°C for 12 h. Unsaponifiable matters were extracted with ether/*n*-hexane (1 : 1) by addition of water. The epiphase was dried over Na_2SO_4 and concentrated under reduced pressure in N_2 below 40°C. The crude carotenoids were separated and purified by column chromatography on MgO-Celite 545 (1 : 1) and high performance liquid chromatography (HPLC) on silica gel and chiral columns.

Identification and Structural Elucidation of Carotenoids

Identification and structural elucidation of each carotenoid were carried out by means of visible light absorption spectra (VIS), mass spectra (MS), circular dichroism spectra (CD), ^{1}H-NMR spectra, acetylation, benzoylation, allylic OH test, co-thin-layer chromatography (co-TLC) and co-high performance liquid chromatography (co-HPLC) with authentic samples. Chemical reactions were carried out by our routine procedures.[4,5] The authentic samples used were from our carotenoid collections.

Instruments

VIS were measured with a Shimadzu UV 240 spectrometer in ether. Concentrations were calculated using $E^{1\%}_{1cm}=2500$ at λ_{max}. ^{1}H-NMR spectra were measured in $CDCl_3$ on a Varian XL-300 spectrometer with a tetramethylsilane as an internal standard. MS spectra were recorded with a Hitachi M-80 mass spectrometer with an ionization energy of 25 eV. CD were measured on a Jasco J-500 C spectropolarimeter in ether/isopentane/EtOH (5 : 5 : 2) (EPA) solution at 20°C. HPLC was carried out on a Waters Model 510 instrument with a Waters Lambda-Max Model 481 LC spectrophotometer set at 445 nm.

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Isolation and Benzoylation of a New Carotenoid Fraction

A new carotenoid fraction was eluted with acetone/*n*-hexane (1: 9) from a MgO-Celite 545 (1: 1) column, and it was purified by HPCL on a silica gel column Shim-pack PREP-SIL (28×3 cm) (Shimadzu) with a mobile phase of acetone/*n*-hexane (1: 9) at a flow rate of 9.9 ml/min.

Benzoylation of a new carotenoid fraction was carried out by our routine procedure.[6] The reaction product was submitted to HPLC on a chiral column Sumipax OA-2000 (300×8 mm) (Sumitomo Chemical Co., Ltd.) with a mobile phase of *n*-hexane/CH_2Cl_2/EtOH (100: 2.5: 0.05) at a flow rate of 2.0 ml/min.

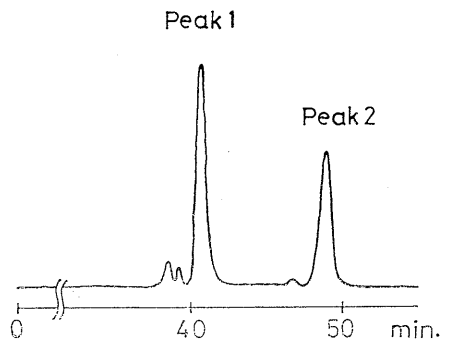

Fig. 1. HPLC separation of the product after benzoylation of a new carotenoid fraction obtained from the integuments of the black bass.
Column: Sumipax OA-2000, Mobile phase: *n*-hexane/CH_2Cl_2/EtOH (100: 2.5; 0.05), Flow rate: 2.0 ml/min., Detection: 445 nm.

Results and Discussion

A chromatogram of the product after benzoylation of a new carotenoid fraction obtained from the integuments of the black bass is shown in Fig. 1.

A New Carotenoid (1) (yield 0.12 mg)

A new carotenoid (1) was obtained by usual saponification of the carotenoid from peak 1 with 10% KOH/MeOH for 2 h at 20°C, and (1) was purified by HPLC on a chiral column Sumipax OA-2000 with a mobile phase of *n*-hexane/CH_2Cl_2/EtOH (48: 16: 0.05) at a flow rate of 2.0 ml/min. (1) showed VIS λ_{max} 420, 445 and 472 nm (in ether) indicating the presence of β, ε-carotene type chromophore and MS *m/z* 552 (M^+, $C_{40}H_{56}O$). The presence of a hydroxy group was consistent with the formation of a monoacetate, and the hydroxy group was proved to be in an allylic position by the allylic OH test. The characteristic ^{1}H-NMR signals (Fig. 2) at δ 1.029 (6H, *s*) and 1.719 (3H, *s*) indicated the presence of β-end-group.[7] On the other hand, ^{1}H-NMR signals (Fig. 2) at δ 0.853 (3H, *s*), 0.943 (3H, *s*), 1.645 (3H, *s*), 2.16 (1H, *d*, *J*=9.7 Hz), 4.23 (1H, *m*) and 5.487 (1H, *m*) were compatible with 3′, 6′-(*cis*)-3′-hydroxy-ε-end-group.[7] On the basis of the evidences described above, we assigned 3′, 6′-(*cis*)-β, ε-caroten-3′-ol for the structure of (1). The CD spectrum of (1) showed the negative Cotton effect at 243 nm. Contrary to the CD spectrum of (1), that of (3′*R*, 6′*R*)-β, ε-caroten-3′-ol showed the positive Cotton effect at 241 nm (Fig. 3).[8] It is well known that an additional hydroxy substituent in the allylic 3-position in ε-

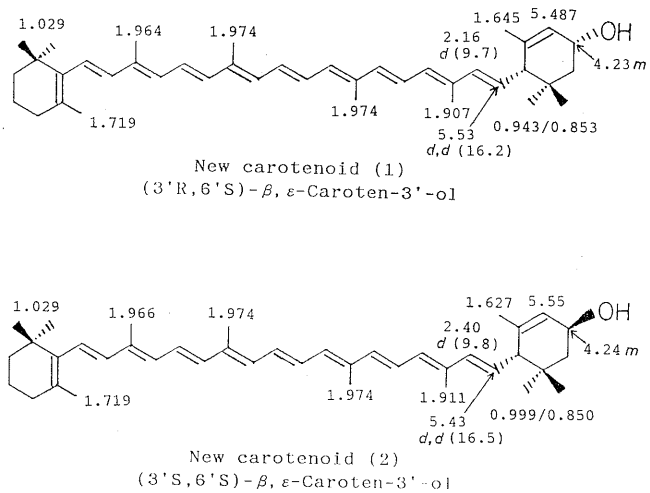

Fig. 2. ^{1}H-NMR assignments of the new carotenoids (1) and (2) at 300 MHz (in $CDCl_3$).

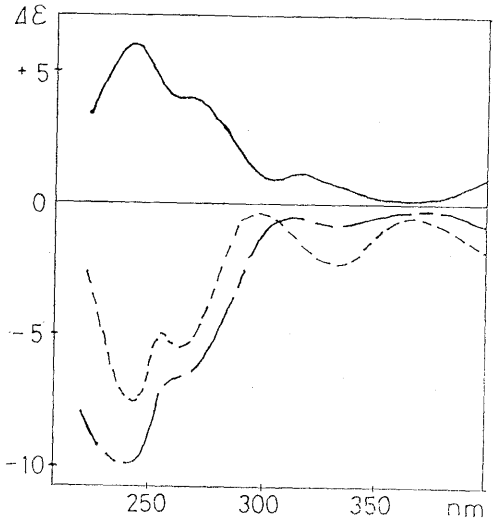

Fig. 3. CD spectra of (3′*R*, 6′*R*)-β, ε-caroten-3′-ol (from the red algae *Ceramium rubrum*) ——, new carotenoid (1) ------ and new carotenoid (2) ------- in EPA at 20°C.

end-group has no marked influence on the CD spectrum.[8-10] Therefore the CD spectrum of (1) indicated 6′*S*-chirality. Accordingly it was revealed that (1) had 3′*R*, 6′*S*-configulation by ^{1}H-NMR and CD spectral data. Consequently the structure of (1) has been determined to be (3′*R*, 6′*S*)-β, ε-caroten-3′-ol.

A New Carotenoid (2) (yield 0.09 mg)

A new carotenoid (2) was obtained by usual saponification of the carotenoid from peak 2 (Fig. 1) and submitted to purification by HPLC on a chiral colunm in the same manner as (1). (2) showed VIS λ_{max} 420, 445 and 472 nm (in ether) indicating the presence of β, ε-carotene type chromophore and MS *m/z* 552 (M^+, $C_{40}H_{56}O$). (2) gave a monoacetate and was positive for allylic OH test. 3′, 6′-(*trans*)-β, ε-caroten-3′-ol for the structure of (2) has been proposed by the ^{1}H-NMR spectral data (Fig. 2).[6] The CD spectrum (2) also showed the same negative Cotton effect at 240 nm as (1) (Fig. 3). Therefore it was revealed that (2) had 3′*S*, 6′*S*-configuration. Consequently the structure of (2) has been determined to be (3′*S*, 6′*S*)-β, ε-caroten-3′-ol.

Three kinds of β, ε-carotene-monol have so far been isolated from natural sources; zeinoxanthin [(3*R*, 6′*R*)-β, ε-caroten-3-ol] (from the maize) (3),[11] (2*R*, 6′*R*)-β, ε-caroten-2-ol (from the green alga *Trentephlia iolithus*) (4)[12] and (3′*R*, 6′*R*)-β, ε-caroten-3′-ol (from the red alga *Ceranium rubrum*) (5).[10] These three β, ε-carotene-monol, (3), (4) and (5) possessing 6′*R* chirality have been isolated from plants, while the new carotenoids, (1) and (2) having 6′*S*-chirality have been isolated from the fish, black bass. It is interesting from the comparative biochemical point of view.

Table 1. The content and percentage composition of individual carotenoids in the integuments of the black bass

Content	0.64 mg/100 g
Percentage composition	
α-Carotene	trace
β-Carotene	0.1
(3′*R*, 6′*S*)-β, ε-caroten-3′-ol	0.4
(3′*S*, 6′*S*)-β, ε-caroten-3′-ol	0.3
β-Cryptoxanthin	0.1
Tunaxanthin A	9.2
〃 B	13.4
〃 C	16.1
〃 D	6.5
〃 G	6.1
〃 H	15.7
Lutein G	5.0
(3*R*, 3′*R*)-Zeaxanthin	1.3
(3*R*, 3′*S*: *meso*)- 〃	1.3
(3*S*, 3′*S*)- 〃	0.5
Diatoxanthin	5.3
Alloxanthin	3.1
Unidentified carotenoids	15.5

The Other Known Carotenoids

The following carotenoids were identified: α-carotene, β-carotene β-cryptoxanthin, tunaxanthin A, B, C, D, G, H, lutein G, (3*R*, 3′*R*)-, (3*R*, 3′*S*: *meso*)-, (3*S*, 3′*S*)-zeaxanthin, diatoxanthin and alloxanthin.

The identification of each stereoisomer of tunaxanthin, lutein and zeaxanthin was performed by the method described previously.[6,13,14]

The content and percentage composition of individual carotenoids in the integuments of the black bass are shown in Table 1.

In conclusion, we have isolated the two new carotenoids from the integuments of the black bass *Micropterus salmoides* and elucidated the structures to be (3′*R*, 6′*S*)-β-, ε-caroten-3′-ol and (3′*S*, 6′*S*)-β, ε-caroten-3′-ol, respectively, by chemical and spectral data.

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