Structures of Minor Carotenoids from the Japanese Common Catfish, *Silurus asotus*

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Three new carotenoids: 7′,8′,9′,10′-tetrahydro-β-cryptoxanthin, 7′,8′-dihydrodiatoxanthin, and (3*S*,6*S*,6′*S*)-ε-cryptoxanthin were isolated from the skin, fins, and gonads of the Japanese common catfish, *Silurus asotus*, as minor carotenoids. Their structures were determined based on chemical and spectroscopic data. Furthermore, 9*Z* and/or 9′*Z* geometrical isomers of parasiloxanthin, 7′,8′-dihydroparasiloxanthin, and 7′,8′-dihydro-β-cryptoxanthin were characterized by ^{1}H-NMR.

Key words catfish; *Silurus asotus*; carotenoid; tetrahydro-β-cryptoxanthin

The Japanese common catfish, *Silurus* (*Parasilurus*) *asotus*, belonging to the family Siluridae, contains unique structural carotenoids with 7,8-dihydro- or 7,8,7′,8′-tetrahydro-polyene chains.[1—4] In 1976, Matsuno *et al.* first isolated parasiloxanthin (**1**) and 7′,8′-dihydroparasiloxanthin (**2**) from the skin and fins of *S. asotus*.[1] Subsequently, a feeding experiment revealed that zeaxanthin was metabolized to 7′,8′-dihydroparasiloxanthin (**2**) *via* parasiloxanthin (**1**) in *S. asotus*.[5] In 2002, Tsushima *et al.* further isolated a series of carotenoids with 7,8-dihydro- or 7,8,7′,8′-tetrahydro-polyene chains: 7,8-dihydro-β-carotene (**3**), 7,8,7′,8′-tetrahydro-β-carotene (**4**), 7′,8′-dihydro-β-cryptoxanthin (**5**), and 7,8-didehydrolutein, from *S. asotus*.[4] Furthermore, Tsushima *et al.* proposed the stereochemistry of these compounds based on biosynthetic considerations.[4—6]

Following the further study of carotenoids in *S. asotus*, 7,8-dihydro-β-cryptoxanthin (**6**) and another three new carotenoids; 7′,8′,9′,10′-tetrahydro-β-cryptoxanthin (**7**), 7′,8′-dihydrodiatoxanthin (**8**), and (3*S*,6*S*,6′*S*)-ε-cryptoxanthin (**9**) were isolated from *S. asotus* as minor carotenoids (Fig. 1). Also, new geometrical isomers of parasiloxanthin, 7′,8′-dihydroparasiloxanthin, and 7′,8′-dihydro-β-cryptoxanthin, *i.e.*, 9*Z*-parasiloxanthin (**1b**), 9′*Z*-parasiloxanthin (**1c**), 9*Z*-7′,8′-dihydroparasiloxanthin (**2b**), and 9′*Z*-7′,8′-dihydro-

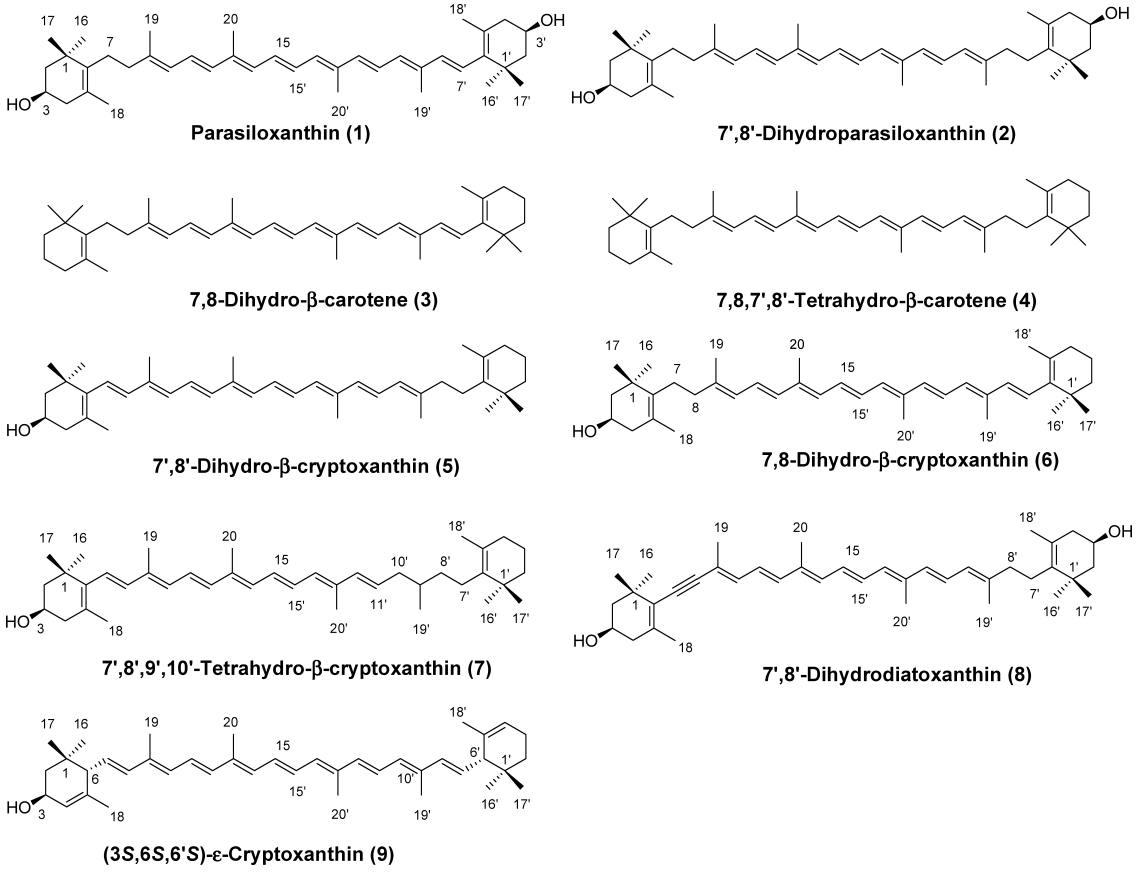

Fig. 1. Structure of Carotenoids Isolated from Japanese Common Catfish, *S. asotus*

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β-cryptoxanthin (**5b**), were isolated (Fig. 2). The present paper reports the isolation and structural elucidation of these new carotenoids.

Results and Discussion

An acetone (Me_2CO) extract of the skin, fins, and gonads of *S. asotus* was saponified with 5% KOH–MeOH at room temperature. The unsaponifiable matter was subjected to column chromatography on a silica gel followed by preparative HPLC on octadecyl silica (ODS) to obtain twenty-two carotenoids including three new compounds and four new geometrical isomers. Parasiloxanthin (**1**), 7′,8′-dihydroparasiloxanthin (**2**) 7,8-dihydro-β-carotene (**3**), 7,8,7′,8′-tetrahydro-β-carotene (**4**), 7′,8′-dihydro-β-cryptoxanthin (**5**), 7,8-didehydro-β-cryptoxanthin (**6**), β-carotene, β-cryptoxanthin, tunaxanthin A, tunaxanthin B, tunaxanthin C, lutein, zeaxanthin, diatoxanthin, and alloxanthin were identified by UV–vis, FAB-MS and ^{1}H-NMR in the case of **1**, **2**, and **5**.

There have been no reports providing complete ^{1}H-NMR data for parasiloxanthin (**1**), 7′,8′-dihydroparasiloxanthin (**2**), and 7′,8′-dihydro-β-cryptoxanthin (**5**).[1,4,5] Thus, complete ^{1}H-NMR assignments of **1**, **2**, and **5** (Table 1) were achieved by conducting correlation spectroscopy (COSY) and rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments.

Compound **6** showed the same UV–vis spectra as **5**. The molecular formula of **6** was also determined to be $C_{40}H_{58}O$ by HR-FAB-MS. These spectral data suggested that **6** was also a dihydro derivative of β-cryptoxanthin. The ^{1}H-NMR (Table 2) of **6** showed the presence of a 3-hydroxy-7,8-dihydro-β-end group[1,4,7] and a β-end group.[8] This clearly indicated that the double bond at 7, 8 positions in β-cryptoxanthin was saturated. This was confirmed by COSY and ROESY experiments. Thus, the compound **6** was identified to be 7,8-dihydro-β,β-caroten-3-ol (7,8-dihydro-β-cryptoxanthin), which was first isolated from the plumage of a bird by Stradi *et al.*[9] This structure was postulated only by UV–vis and MS spectral data.[9] In the present study, structure of **6** was completely characterized by ^{1}H-NMR spectral data. The chirality of this compound could not be determined from circular dichroism (CD) spectroscopic data because it showed a very weak CD spectrum. However, β-cryptoxanthin, exhibiting 3*R* chirality, is assumed to be the precursor of this compound.[2,3] Therefore, the 3*R* configuration is tentatively proposed for **6**.

Compound **7** showed absorption maxima at 384 (shoulder), 402, and 423 nm, indicating the presence of an octa-ene conjugate double bond system (seven conjugated double bonds in the polyene-chain and one conjugated double bond at 5, 6 positions in the end group)[10,11] in its molecule. The molecular formula of **7** was determined as $C_{40}H_{60}O$, which was four mass units higher than β-cryptoxanthin. In ^{13}C-NMR (Table 3), ten methyl carbons, eight methylene carbons, thirteen methin carbons, and nine quaternary carbons were observed. The presence of a secondary hydroxyl group in **7** is compatible with the formation of monoacetate. These data suggested that **7** was a tetra hydrogenated derivative of β-cryptoxanthin. The hydrogenated positions were determined by ^{1}H-NMR. The ^{1}H-NMR (Table 3), which was assigned by COSY, ROESY, and ^{1}H–^{1}H decoupling experiments, showed the presence of a 3-hydroxy-β-end group, a β-end group, and a partially saturated polyene chain in **7**. Due to the lacking of conjugated double bonds in the polyene chain, ^{1}H-NMR signals of the β-end group in **7** showed a slightly higher field shift than the ordinal β-end group.[8] The characteristic olefinic proton signal at δ 5.73 (1H, ddd, J=15, 7, 7 Hz) and doublet methyl signal at δ 0.93 (3H, d, J=6.5 Hz) were assigned as a terminal olefinic proton in the polyene chian at H-11′ and methyl group at H-19′, respectively. The methylene signals H-7′, 8′, 10′ and methin signal at H-9′ were assigned as shown in Table 3. These data clearly indicated that 7′, 8′, 9′, and 10′ positions in β-cryptoxanthin were hydrogenated. ^{13}C-NMR data also supported this structure. Furthermore, characteristic product ions in FAB-MS/MS, *m/z* 419 [M−137] (attributed to cleavage between C-7′ and C-8′), *m/z* 405 [M−151] (attributed to cleavage be-

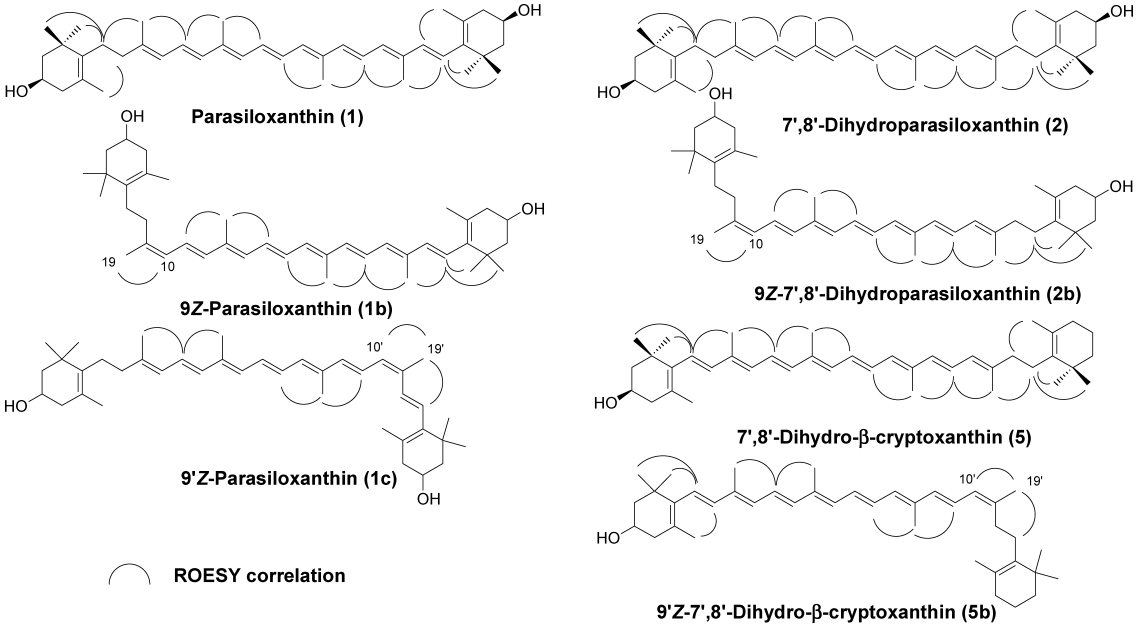

Fig. 2. Geometrical Isomers of Parasiloxanthin, 7′,8′-Dihydroparasiloxanthin, and 7′,8′-Dihydro-β-cryptoxanthin and Their ROESY Correlation

Table 1. ^{1}H-NMR (500 MHz) Spectral Data of Parasiloxanthin (**1**), 9*Z*-Parasiloxanthin (**1b**), 9′*Z*-Parasiloxanthin (**1c**), 7′,8′-Dihydroparasiloxanthin (**2**), 9*Z*-7′,8′-Dihydroparasiloxanthin (**2b**), 7′,8′-Dihydro-*β*-cryptoxanthin (**5**), and 9′*Z*-7′,8′-Dihydro-*β*-cryptoxanthin (**5b**) (in $CDCl_3$)

Position	**1**		**1b**		**1c**		Position	**2**		**2b**		**5**		**5b**	
	δ ^{1}H	mult. *J* (Hz)	δ ^{1}H	mult. *J* (Hz)	δ ^{1}H	mult. *J* (Hz)		δ ^{1}H	mult. *J* (Hz)	δ ^{1}H	mult. *J* (Hz)	δ ^{1}H	mult. *J* (Hz)	δ ^{1}H	mult. *J* (Hz)
H-2	1.44	dd (12, 12)	n.a.		1.44	dd (12, 12)	H-2	1.44	dd (12, 12)	n.a.		1.48	dd (12, 12)	1.48	dd (12, 12)
H-2	1.72	ddd (12, 4, 2)	n.a.		1.72	ddd (12, 4, 2)	H-2	1.72	ddd (12, 4, 2)	n.a.	ddd (12, 4, 2)	1.78	ddd (12, 5, 2)	1.78	ddd (12, 5, 2)
H-3	3.96	m	3.96	m	3.96	m	H-3	3.96	m	3.96	m	4.00	m	4.00	m
H-4	2.00	dd (16, 10)	2.02	dd (16, 10)	2.00	dd (16, 10)	H-4	2.00	dd (16, 10)	2.02	dd (16, 10)	2.04	dd (16, 10)	2.04	dd (16, 10)
H-4	2.26	ddd (16, 6, 2)	2.28	ddd (16, 6, 2)	2.26	ddd (16, 6, 2)	H-4	2.26	ddd (16, 6, 2)	2.28	ddd (16, 6, 2)	2.39	dd (16, 6, 2)	2.39	dd (16, 6, 2)
H-7	*ca.* 2.14	m	*ca.* 2.25	m	*ca.* 2.14	m	H-7	*ca.* 2.14	m	*ca.* 2.25	m	6.10	d (16)	6.10	d (16)
H-8	*ca.* 2.14	m	*ca.* 2.25	m	*ca.* 2.14	m	H-8	*ca.* 2.14	m	*ca.* 2.25	m	6.16	d (16)	6.16	d (16)
H-10	5.97	d (11)	5.93	d (11)	5.97	d (11)	H-10	5.97	d (11)	5.93	d (11)	6.14	d (11)	6.14	d (11)
H-11	6.49	dd (15, 11)	6.54	dd (15, 11)	6.49	dd (15, 11)	H-11	6.49	dd (15, 11)	6.54	dd (15, 11)	6.62	dd (15, 11)	6.62	dd (15, 11)
H-12	6.26	d (15)	6.24	d (15)	6.26	d (15)	H-12	6.26	d (15)	6.24	d (15)	6.36	d (15)	6.36	d (15)
H-14	6.20	d (12)	6.20	d (12)	6.20	d (12)	H-14	6.20	d (12)	6.20	d (12)	6.23	d (12)	6.23	d (12)
H-15	6.60	m	6.60	m	6.60	m	H-15	6.60	m	6.60	m	6.60	m	6.60	m
H-16	1.04	s	1.06	s	1.04	s	H-16	1.04	s	1.06	s	1.08	s	1.08	s
H-17	1.08	s	1.13	s	1.08	s	H-17	1.08	s	1.13	s	1.08	s	1.08	s
H-18	1.66	s	1.72	s	1.66	s	H-18	1.66	s	1.72	s	1.74	s	1.74	s
H-19	1.86	s	1.87	s	1.86	s	H-19	1.86	s	1.87	s	1.96	s	1.96	s
H-20	1.95	s	1.92	s	1.95	s	H-20	1.95	s	1.92	s	1.96	s	1.96	s
H-2′	1.48	dd (12, 12)	n.a.		1.50	dd (12, 12)	H-2′	1.44	dd (12, 12)	1.44	dd (12, 12)	1.43	t (7.5)	n.a.	
H-2′	1.78	ddd (12, 5, 2)	1.78	ddd (12, 5, 2)	1.78	ddd (12, 5, 2)	H-2′	1.72	ddd (12, 4, 2)	1.72	ddd (12, 4, 2)				
H-3′	4.00	m	4.00	m	4.03	m	H-3′	3.96	m	3.96	m	1.58	m	n.a.	
H-4′	2.04	dd (16, 10)	2.04	dd (16, 10)	2.07	dd (16, 10)	H-4′	2.00	dd (16, 10)	2.00	dd (16, 10)	1.92	t (7.5)	n.a.	
H-4′	2.39	dd (16, 6, 2)	2.39	dd (16, 6, 2)	2.42	dd (16, 6, 2)	H-4′	2.26	ddd (16, 6, 2)	2.26	ddd (16, 6, 2)				
H-7′	6.10	d (16)	6.10	d (16)	6.12	d (16)	H-7′	*ca.* 2.14	m	*ca.* 2.14	m	*ca.* 2.12	m	*ca.* 2.12	d (16)
H-8′	6.16	d (16)	6.16	d (16)	6.67	d (16)	H-8′	*ca.* 2.14	m	*ca.* 2.14	m	*ca.* 2.12	m	*ca.* 2.12	d (16)
H-10′	6.14	d (11)	6.14	d (11)	6.07	d (11)	H-10′	5.97	d (11)	5.97	d (11)	5.97	d (11)	5.93	d (11)
H-11′	6.62	dd (15, 11)	6.62	dd (15, 11)	6.74	dd (15, 11)	H-11′	6.49	dd (15, 11)	6.49	dd (15, 11)	6.49	dd (15, 11)	6.57	dd (15, 11)
H-12′	6.36	d (15)	6.36	d (15)	6.30	d (15)	H-12′	6.26	d (15)	6.26	d (15)	6.26	d (15)	6.24	d (15)
H-14′	6.23	d (12)	6.23	d (12)	6.23	d (12)	H-14′	6.20	d (12)	6.20	d (12)	6.20	d (12)	6.20	d (12)
H-15′	6.60	m	6.60	m	6.60	m	H-15′	6.60	m	6.60	m	6.60	m	6.60	m
H-16′	1.08	s	1.08	s	1.08	s	H-16′	1.04	s	1.04	s	1.01	s	1.04	s
H-17′	1.08	s	1.08	s	1.09	s	H-17′	1.08	s	1.08	s	1.01	s	1.04	s
H-18′	1.74	s	1.74	s	1.78	s	H-18′	1.66	s	1.66	s	1.63	s	1.69	s
H-19′	1.96	s	1.96	s	1.97	s	H-19′	1.86	s	1.86	s	1.86	s	1.87	s
H-20′	1.96	s	1.96	s	1.96	s	H-20′	1.95	s	1.95	s	1.97	s	1.97	s

Overlapping signals were assigned by decoupling and NOE difference spectra.

Table 2. ^{1}H-NMR (500 MHz) Spectral Data of 7,8-Dihydro-*β*-cryptoxanthin (**6**), 7′,8′-Dihydrodiatoxanthin (**8**), and (3*S*,6*S*,6′*S*)-*ε*-Cryptoxanthin (**9**) (in $CDCl_3$)

Position	**6**		**8**		**9**	
	δ ^{1}H	mult. *J* (Hz)	δ ^{1}H	mult. *J* (Hz)	δ ^{1}H	mult. *J* (Hz)
H-2	ax 1.44	dd (12, 12)	ax 1.45	dd (12, 12)	1.65	dd (14, 6)
	eq 1.72	ddd (12, 4, 2)	eq 1.84	ddd (12, 4, 2)	1.39	dd (14, 7)
H-3	3.96	m	3.99	m	4.24	m
H-4	ax 2.00	dd (16, 10)	ax 2.07	dd (16, 10)	5.49	br s
	eq 2.26	ddd (16, 6, 2)	eq 2.43	ddd (16, 6, 2)		
H-6					2.16	d (10)
H-7	*ca.* 2.14	m			5.53	dd (15, 10)
H-8	*ca.* 2.14	m			6.13	d (15)
H-10	5.97	d (11)	6.45	d (11)	6.14	d (11)
H-11	6.49	dd (15, 11)	6.54	d (15, 11)	6.62	dd (15, 11)
H-12	6.26	d (15)	6.36	d (15)	6.35	d (15)
H-14	6.20	d (12)	6.27	d (12)	6.25	d (12)
H-15	6.60	m	6.64	m	6.62	m
H_3-16	1.04	s	1.15	s	0.85	s
H_3-17	1.08	s	1.20	s	0.94	s
H_3-18	1.65	s	1.92	s	1.64	s
H_3-19	1.86	s	2.01	s	1.91	s
H_3-20	1.97	s	1.98	s	1.97	s
H-2′	H_2 1.46	t (7.5)	ax 1.44	dd (12, 12)	1.43	m
			eq 1.72	ddd (12, 4, 2)	1.18	m
H-3′	H_2 1.62	m	3.96	m	n.a.	
H-4′	H_2 2.02	t (7.5)	ax 2.00	dd (16, 10)	5.41	br s
			eq 2.26	ddd (16, 6, 2)		
H-6′					2.18	d (10)
H-7′	6.16	d (16)	*ca.* 2.14	m	5.53	dd (15, 10)
H-8′	6.14	d (16)	*ca.* 2.14	m	6.11	d (15)
H-10′	6.16	d (11)	5.97	d (11)	6.12	d (11)
H-11′	6.63	d (15, 11)	6.49	dd (15, 11)	6.63	dd (15, 11)
H-12′	6.35	d (15)	6.26	d (15)	6.35	d (15)
H-14′	6.24	d (12)	6.20	d (12)	6.25	d (12)
H-15′	6.60	m	6.60	m	6.62	m
H_3-16′	1.03	s	1.04	s	0.82	s
H_3-17′	1.03	s	1.08	s	0.90	s
H_3-18′	1.72	s	1.65	s	1.59	s
H_3-19′	1.97	s	1.86	s	1.91	s
H_3-20′	1.97	s	1.97	s	1.97	s

Overlapping signals were assigned by decoupling and NOE difference spectra.

Table 3. ^{1}H (500 MHz)- and ^{13}C (125 MHz)-NMR Spectral Data of 7′,8′,9′,10′-Tetrahydro-β-cryptoxanthin (**7**) (in $CDCl_3$)

Position	δ ^{13}C	δ ^{1}H	mult. J (Hz)
1	37.1		
2	48.4	ax 1.48	dd (12, 12)
		eq 1.78	ddd (12, 5, 2)
3	65.1	4	m
4	42.6	ax 2.04	dd (16, 10)
		eq 2.39	dd (16, 6, 2)
5	126.1		
6	137.7		
7	125.4	6.10	d (16)
8	138.6	6.16	d (16)
9	135.7		
10	131.3	6.14	d (11)
11	124.6	6.62	dd (15, 11)
12	137.7	6.36	d (15)
13	136.0		
14	132.5	6.23	d (12)
15	130.1	6.60	m
16	28.6	1.08	s
17	30.3	1.08	s
18	21.6	1.74	s
19	12.8	1.96	s
20	12.8	1.96	s
1′	34.7		
2′	39.9	1.40	m
3′	19.6	1.59	m
4′	32.7	1.89	t (7)
5′	126.1		
6′	137.6		
7′	27.2[a)]	*ca.* 2.01	m
8′	37.1[a)]	*ca.* 1.5	m
9′	34.9[a)]	*ca.* 1.56	m
10′	40.5	2.04	m
		2.18	m
11′	129.3	5.73	ddd (15, 7, 7)
12′	126.7[b)]	6.14	d (15)
13′	136.0		
14′	129.1[b)]	6.13	d (12)
15′	130.1	6.60	m
16′	28.6	0.98	s
17′	28.6	0.98	s
18′	19.8	1.57	s
19′	19.6	0.93	d (6.5)
20′	12.8	1.89	s

a) Assignments may be changeable. *b*) Assignments may be changeable. Overlapping signals were assigned by decoupling and NOE difference spectra. ^{13}C-NMR was assigned by heteronuclear singlet quantum coherence (HSQC) and heteronuclear multiplet bond coherence (HMBC) data and ^{13}C-NMR data of related compound.[8)]

tween C-8′ and C-9′), *m*/*z* 377 [M−179] (attributed to cleavage between C-9′ and C-10′), and *m*/*z* 363 [M−193] (attributed to cleavage between C10′ and C11′) were in agreement with this structure as shown in Fig. 3. Therefore, the structure of **7** was determined to be 7′,8′,9′,10′-tetrahydro-β,β-caroten-3-ol, and it was named 7′,8′,9′,10′-tetrahydro-β-cryptoxanthin (Fig. 1). The CD spectrum of **7** showed a similar curve to that of (3*R*)-galloxanthin,[10,11)] having the same chromophore system. Therefore, the chirality at C-3 of **7** was determined to be *R*. Concerning carotenoids with the 7′,8′,9′,10′-tetrahydro-polyene chain, Tsushima *et al.* reported 7′,8′,9′,10′-tetrahydro-β-carotene in *S. asotus*.[4)] However, its structure was tentatively proposed based on only UV–vis and MS spectral data. A detailed structural analysis using NMR spectroscopy was not carried out. Therefore, this is the first report of the structural determination of carotenoids with a 7′,8′,9′,10′-tetrahydro-polyene chain. 7′,8′,9′,10′-Tetrahydro-β-cryptoxanthin was assumed to be a reductive metabolite of β-cryptoxanthin *via* 7′,8′-dihydro-β-cryptoxanthin (**5**) in *S. asotus*.[2,3)]

Compound **8** showed absorption maxima at 407 (shoulder), 430, and 457 nm, resembling to those of parasiloxanthin.[1)] The molecular formula of **8** was determined to be $C_{40}H_{56}O_2$, which was two mass units less than parasiloxanthin. The presence of two secondary hydroxyl groups in **8** is compatible with the formation of diacetate. The ^{1}H-NMR (Table 2) of **8** showed the presence of the alloxanthin moiety (H-2 to H-20)[8)] and 7′,8′-dihydroparasiloxanthin moiety (H-2′ to H-20′).[1,4,7)] Thus, the structure of **8** was determined to be 7,8-didehydro-7′,8′-dihydro-β,β-carotene-3,3-diol, and it was named 7′,8′-dihydrodiatoxanthin (**8**). Previously, 7′,8′-dihydrodiatoxanthin (**8**) was reported as a reductive metabolite of diatoxanthin in *S. asotus*.[2,3)] However, detailed spectral data on **8** have yet to be reported. In the present investigation, the structure of 7′,8′-dihydrodiatoxanthin (**8**) was completely characterized based on the spectral data.

Compound **9** showed absorption maxima at 418, 439, and 468 nm. The molecular formula of **9** was determined to be $C_{40}H_{56}O$ by HR-FAB-MS. The ^{1}H-NMR (Table 2) showed the presence of a 3,6-*trans*-ε-end group,[8)] an ε-end group,[8)] and an all *trans* polyene chain. This was confirmed by COSY and ROESY experiments. Therefore, the structure of **9** was determined to be ε,ε-caroten-3-ol. The CD spectra of **9** showed a negative Cotton effect at 265 nm, indicating 6*S*,6′*S* chirality.[12)] Therefore, the structure of **9** was determined to be (3*S*,6*S*,6′*S*)-ε,ε-caroten-3-ol and it was named (3*S*,6*S*,6′*S*)-ε-cryptoxanthin (**9**). The structure of ε,ε-caroten-3-ol corresponded to the proposed structure of neothxanthin, isolated from marine fish.[13,14)] However, the structure of neothxanthin was postulated by only visible spectral data and partition coefficient. So, its structure was remained uncertain. Thus, this is the first report of structural determination of ε-carotene mono-ol.[6)] Furthermore, 9*Z*-parasiloxanthin (**1b**), 9′*Z*-parasiloxanthin (**1c**), 9*Z*-7′,8′-dihydroparasiloxanthin (**2b**), and 9′*Z*-7′,8′-dihydro-β-cryptoxanthin (**5b**) were isolated and characterized by ^{1}H-NMR (Table 1), including a ROESY experiment (Fig. 2).

In 9*Z*-parasiloxanthin (**1b**), down-field shifts of ^{1}H signals at H-8, H-11, and H-19 and a high-field shift at H-10 from the corresponding signals of all *E* parasiloxanthin (**1**) were observed. A ROESY correlation between H-19 and H-10, which was not noted in the case of **1**, was observed. These ^{1}H-NMR spectral data clearly indicated that **1b** exhibits 9*Z* geometry. In the case of 9′*Z*-parasiloxanthin (**1c**), low-field shifts of ^{1}H signals at H-8′, H-11′, and H-19′ and a high-field shift at H-10′ from the corresponding signals of **1** were observed. The ROESY correlation between H-19′ and H-10′ indicated 9′*Z* geometry. In a manner similar to that described above, the geometries of 9*Z*-7′,8′-dihydroparasiloxanthin (**2b**) and 9′*Z*-7′,8′-dihydro-β-cryptoxanthin (**5b**) were also characterized, as shown in Fig. 2.

Carotenoid content and composition in catfish were shown in Experimental. Catfish accumulates both in the integuments (fin and skin) and gonad. Carotenoid compositions of these organs are almost similar to each other. In general, animals do not synthesize carotenoids *de novo*, and those found in animals are either directly accumulated from food or partly

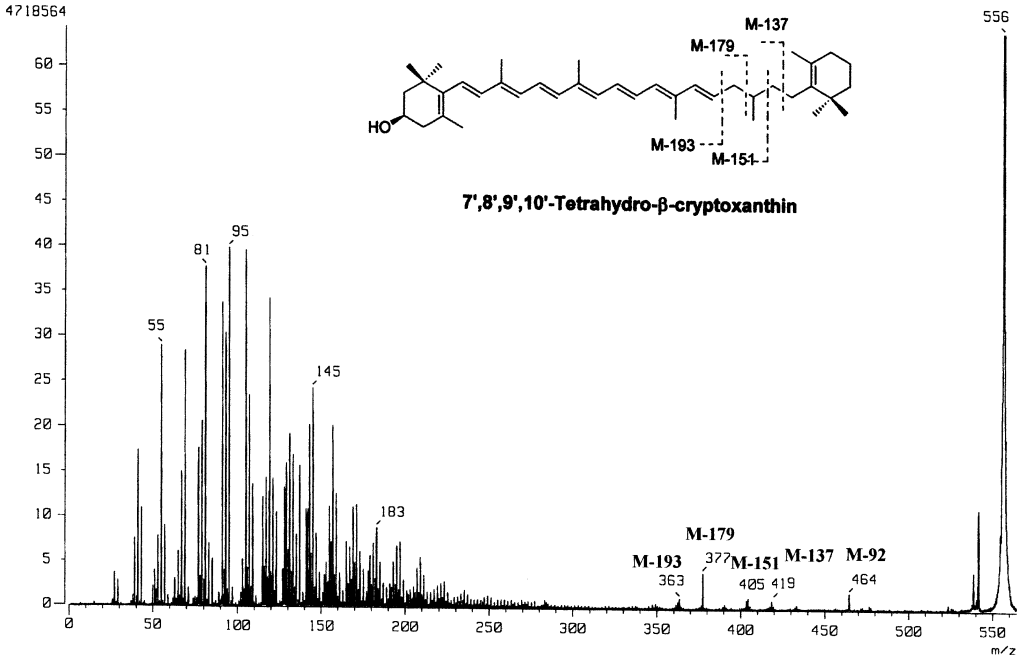

Fig. 3. FAB-MS/MS Spectrum of 7′,8′,9′,10′-Tetrahydro-β-cryptoxanthin (**7**)

modified through metabolic reactions.[15] Catfish is carnivores fish and feeds on small fishes and crustaceans. Catfish accumulates zeaxanthin, diatoxanthin, β-cryptoxanthin, and β-carotene from dietal small animals. These ingested carotenoids were converted to corresponding 7,8- and/or 7′,8′-dihydroderivatives in catfish.[3,4] Tunaxanthin and alloxanthin were also assumed to originate from dietal small fish such as goby.[16]

In conclusion, the new carotenoids, 7′,8′,9′,10′-tetrahydro-β-cryptoxanthin (**7**), 7′,8′-dihydrodiatoxanthin (**8**), and (3*S*,6*S*,6′*S*)-ε-cryptoxanthin (**9**) were isolated from the skin, fins, and gonads of *S. asotus* as minor components. Furthermore, 9*Z* and/or 9′*Z* geometrical isomers of parasiloxanthin, 7′,8′-dihydroparasiloxanthin, and 7′,8′-dihydro-β-cryptoxanthin were characterized by ^{1}H-NMR. There were the first reports of characterization of *cis* carotenoids with 7,8-dihydro- or 7,8,7′,8′-tetrahydro-polyene chains.

Experimental

General Experimental Procedures The UV–vis spectra were recorded with a Hitachi U-2001 spectrophotometer in Et_2O. The positive ion FAB-MS and MS/MS spectra were recorded using a JEOL JMS-HX/HX 110A mass spectrometer with *m*-nitrobenzyl alcohol as a matrix. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were measured with a Varian UNITY INOVA 500 spectrometer in $CDCl_3$ with tetramethyl silane (TMS) as an internal standard. Preparative HPLC was performed on a Shimadzu LC-6AD with a Shimadzu SPD-6AV spectrophotometer set at 400 nm. The column used was a 250×10 mm i.d., 10 μm Cosnosil 5C18-II (Nacalai Tesque, Japan).

Animal Material Catfish, *S. asotus* was purchased at a local fish market in Shiga Prefecture, June 2010.

Extraction and Isolation of Carotenoids Skin and fins (1 kg) and gonads (1.5 kg) were striped from the 25 fishes (5.6 kg) and were extracted with Me_2CO. The Me_2CO extract was partitioned between hexane/Et_2O (1 : 1, v/v) and water. The organic layer was evaporated to dryness, and the residue was saponified with 5% KOH/MeOH at room temperature for 2 h. After the time, unsaponifiable matter was extracted with hexane/Et_2O (1 : 1, v/v) from the reaction mixture by addition of water. The organic layer was dried over Na_2SO_4 and was evaporated. The residual was subjected to silica gel column chromatography The fraction eluted with Et_2O/hexane (20 : 80, v/v) gave β-carotene, 7,8-dihydro-β-carotene (**3**), and 7,8,7′,8′-tetrahydro-β-carotene (**4**). The fraction eluted with Et_2O/hexane (50 : 50, v/v) was subjected to HPLC on ODS with $CHCl_3$/CH_3CN (10 : 90, v/v) to afforded (3*S*,6*S*,6′*S*)-ε,ε-caroten-3-ol (**9**), β-cryptoxanthin, 7′,8′-didehydro-β-cryptoxanthin (**5**), 9′-*Z*-7′,8′-didehydro-β-cryptoxanthin (**5b**), 7,8-didehydro-β-cryptoxanthin (**6**), and 7′,8′,9′,10′-tetrahydro-β-cryptoxanthin (**7**). The fraction eluted with Et_2O was subjected to HPLC on ODS with $CHCl_3$/CH_3CN (10 : 90, v/v) to afforded tunaxanthin A, tunaxanthin B, tunaxanthin C, lutein, zeaxanthin, parasiloxanthin (**1**), 9*Z*-parasiloxanthin (**1b**), 9′*Z*-parasiloxanthin (**1b**), 7′,8′-dihydroparasiloxanrhin (**2**), 9*Z*-7′,8′-dihydroparasiloxanrhin (**2b**), diatoxanthin, 7,8-dihydrodiatoxanthin (**8**), and alloxanthin.

Quantification of Carotenoids The amount of carotenoid was calculated using extinction coefficient $E_{cm}^{1\%}$=2400 at λ_{max}.[17]

Parasiloxanthin (**1**): Yellow solid. Yield 2.5 mg. UV–vis (Et_2O) λ_{max} 405 (shoulder), 428, and 455 nm. CD λ ($\Delta\varepsilon$) 250 (0), 310 (−6.0), 328 (0), 352 (+3.0), and 385 (0) nm. ^{1}H-NMR Table 1. Key ROESY correlation Fig. 2. FAB-MS: *m/z* 570 $[M]^+$.

9*Z*-Parasiloxanthin (**1b**): Yellow solid. Yield 0.2 mg. UV–vis (Et_2O) λ_{max} 403 (shoulder), 426, and 453 nm. ^{1}H-NMR Table 1. Key ROESY correlation Fig. 2. FAB-MS: *m/z* 570 $[M]^+$.

9′*Z*-Parasiloxanthin (**1c**): Yellow solid. Yield 0.2 mg. UV–vis (Et_2O) λ_{max} 403 (shoulder), 426, and 453 nm. ^{1}H-NMR Table 1. Key ROESY correlation Fig. 2. FAB-MS: *m/z* 570 $[M]^+$.

7′,8′-Dihydroparasiloxanthin (**2**): Yellow solid. Yield 0.5 mg. UV–vis (Et_2O) λ_{max} 378, 400, and 425 nm. CD very weak Cotton effect almost overlapped baseline. ^{1}H-NMR Table 1. Key ROESY correlation Fig. 2. FAB-MS: *m/z* 572 $[M]^+$.

9*Z*-7′,8′-Dihydroparasiloxanthin (**2b**): Yellow solid; Yield 0.08 mg; UV–vis (Et_2O) λ_{max} 378, 400, and 425 nm. ^{1}H-NMR Table 1. Key ROESY correlation Fig. 2. FAB-MS: *m/z* 572 $[M]^+$.

7′,8′-Dihydro-β-cryptoxanthin (**5**): Yellow solid. Yield 0.2 mg; UV–vis (Et_2O) λ_{max} 405 (shoulder), 428, and 455 nm. CD λ ($\Delta\varepsilon$) 250 (0), 310 (−6.0), 328 (0), 352 (+3.0), and 385 (0) nm. ^{1}H-NMR Table 1. Key ROESY correlation Fig. 2. HR-FAB-MS: *m/z* 554.4494 $[M]^+$ (Calcd for $C_{40}H_{58}O$ 554.4488).

9′*Z*-7′,8′-Dihydro-β-cryptoxanthin (**5b**): Yellow solid. Yield 0.08 mg. UV–vis (Et_2O) λ_{max} 405 (shoulder), 428, and 455 nm. ^{1}H-NMR Table 1. FAB-MS: *m/z* 554 $[M]^+$.

7,8-Dihydro-β-cryptoxanthin (**6**): Yellow solid. Yield 0.2 mg. UV–vis (Et_2O) λ_{max}: 405 (shoulder), 428, and 455 nm. CD very weak Cotton effect almost overlapped baseline. ^{1}H-NMR Table 2. Key ROESY correlation H-16/H-3, H-16 and H-17/H-7, H18/H-8, H-19/H-7 and H-11, H-20/H-11 and

H-15, H-16′ and H-17′/H-2′ and H-7′, H-18′/H-4′ and H-8′, H-19′/H-7′ and H-H-11′, H-20′/H-11′ and H-15′. HR-FAB-MS: *m*/*z* 554.4494 $[M]^+$ (Calcd for $C_{40}H_{58}O$, 554.4488).

7′,8′,9′,10′-Tetrahydro-*β*-cryptoxanthin (**7**): Yellow solid. Yield 0.8 mg. UV–vis (Et_2O) λ_{max} 384 (shoulder), 402, and 423 nm. CD λ ($\Delta\varepsilon$) 220 (0), 240 (+4.0), 2650 (0), 294 (−6.4), 310 (0), 340 (+0.8), and 380 (0) nm. ^{1}H- and ^{13}C-NMR Table 3. Key ROESY correlation H-16/H-3, H-16 and H-17/H-7, H18/H-8, H-19/H-7 and H-11, H-20/H-11 and H-15, H-16′ and H-17′/H-2′, H-18′/H-4′, H-20′/H-11′ and H-15′. HR-FAB-MS: *m*/*z* 556.4641 $[M]^+$ (Calcd for $C_{40}H_{60}O$, 556.4644). Product ions of FAB-MS/MS: *m*/*z* 538 [M−18], 464 [M−92], 419 [M−137], 405 [M−151], 377 [M−179], 363 [M−193]. Acetylation of **7** with aceticanhydride in pyridine at room temperature for 1 h gave monoacetate: FAB-MS *m*/*z* 598 $[M]^+$.

7′,8′-Dihydrodiatoxanthin (**8**): Yellow solid. Yield 0.1 mg. UV–vis (Et_2O) λ_{max} 407 (shoulder), 430, and 457 nm. CD very weak Cotton effect almost overlapped baseline. ^{1}H-NMR Table 2. HR-FAB-MS: *m*/*z* 568.4280 $[M]^+$ (Calcd for $C_{40}H_{56}O_2$, 568.4283). Acetylation of **8** with aceticanhydride in pyridine at room temperature for 1 h gave diacetate: FAB-MS *m*/*z* 652 $[M]^+$.

(3*S*,6*S*,6′*S*)-*ε*-Cryptoxanthin (**9**): Yellow solid. Yield 0.1 mg. UV–vis (Et_2O) λ_{max} 418, 439, and 468 nm. CD λ ($\Delta\varepsilon$) 245 (0), 265 (−4.0), and 300 (0) nm. ^{1}H-NMR Table 2. Key ROESY correlation H-16/H-3, H-16/H-7, H18/H-4 and H-8, H-19/H-7 and H-11, H-20/H-11 and H-15, H-16′/H-3′, H-16′/H-7′, H-18′/H-4′ and H-8′, H-19′/H-7′ and H-11′, H-20′/H-11′ and H-15′. HR-FAB-MS: *m*/*z* 552.4340 $[M]^+$ (Calcd for $C_{40}H_{56}O$, 552.4331).

Carotenoid Content and Composition Carotenoid content; 0.5 mg/100 g (fin and skin), 0.8 mg/100 g (gonad). Carotenoid composition in fin and skin; *β*-carotene (1.4% of total carotenoid), 7,8-dihydro-*β*-carotene (0.8), 7,8,7′,8′-tetrahydro-*β*-carotene (1.2), (3*S*,6*S*,6′*S*)-*ε*-cryptoxanthin (1.5), *β*-cryptoxanthin (4.8), 7,8-dihydro-*β*-cryptoxanthin (2.1), 7′,8′-dihydro-*β*-cryptoxanthin (2.2), 9′*Z*-7′,8′-dihydro-*β*-cryptoxanthin (0.8), 7′,8′,9′,10′-tetrahydro-*β*-cryptoxanthin (5.1), tunaxanthin A (1.2), tunaxanthin B (1.3), tunaxanthin C (2.0), lutein (2.5), zeaxanthin (10.2), parasiloxanthin (24.0), 9*Z*-parasiloxanthin (2.5), 9′*Z*-parasiloxanthin (2.5), 7′,8′-dihydroparasiloxanthin (8.3), 9*Z*-7′,8′-dihydroparasiloxanthin (1.2), diatoxanthin (3.4), 7,8-dihydrodiatoxanthin (1.0), alloxanthin (17.0), unidentified carotenoids (3.0). Carotenoid composition in gonad was almost similar to that of fin and skin.

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