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Carotenoids of Sea Squirts. I. New Marine Carotenoids, Halocynthiaxanthin and Mytiloxanthinone from *Halocynthia roretzi*

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New marine carotenoids, halocynthiaxanthin (**4**) and mytiloxanthinone (**5**), were isolated from the sea squirt, *Halocynthia roretzi* ("Maboya" in Japanese). The structures of **4** and **5** were established to be (3*S*,5*R*,6*S*,3′*R*)-5,6-epoxy-3,3′-dihydroxy-7′,8′-didehydro-5,6,7,8-tetrahydro-β,β-caroten-8-one and 3,8′-dihydroxy-7,8-didehydro-β,κ-carotene-3′,6′-dione, respectively.

Keywords——marine new carotenoid; halocynthiaxanthin; mytiloxanthinone; sea squirt; *Halocynthia roretzi*, halocynthiaxanthin synthesis; absolute configuration

The sea squirt *Halocynthia roretzi*, which belongs to the family Pyuridae in Prochordata, inhabits the northern Pacific Ocean and is cultured in Tohoku district in Japan. In earlier studies, only a few carotenoids, cynthiaxanthin (**1**),[1,2] astaxanthin (**2**),[1,2] β-carotene (**3**)[2] and an antheraxanthin-like product,[2] had been isolated from *H. roretzi*. On the other hand Campbell *et al.*[3] reported that both cynthiaxanthin and pectenoxanthin were identical with alloxanthin (**1**), diacetylenic diol obtained from *Cryptomonas* algae.[4,5] The authors now wish to report the isolation and structural elucidation of two new carotenoids, halocynthiaxanthin (**4**)[6] and mytiloxanthinone (**5**),[7] along with eleven known carotenoids from *H. roretzi*.

Halocynthiaxanthin (**4**) was obtained as reddish needles, $C_{40}H_{54}O_4$, mp 158—160 °C. The visible light absorption spectrum of **4** showed absorption maxima at 430, 452 and 470 nm. The infrared absorption (IR) spectrum of **4** showed distinctive absorption bands at 3350 (OH), 2165 (–C≡C–), 1645 (conj. C=O) and 960 (*trans* –CH=CH–) cm^{-1}. Compound **4** gave a diacetate and a ditrimethylsilyl ether. On reduction with $NaBH_4$, **4** gave a corresponding secondary alcohol (**6**). The proton nuclear magnetic resonance (^{1}H-NMR) signals of **4** are compiled in Table I, together with those for other carotenoids [mytiloxanthinone (**5**), alloxanthin (**1**), fucoxanthin (**7**)[8] and mytiloxanthin (**8**)[9]]. The assignments were made by comparison with the known chemical shifts of **1**, **7** and **8**. These data indicate that the halocynthiaxanthin molecule consisted of two parts (A and B), A being a part of **7**[8] and B being a part of **1**.[9]

In order to establish the absolute configuration of **4**, conversion of naturally occurring (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*R*)-fucoxanthin (**7**)[8] isolated from brown algae into semi-synthetic (3*S*,5*R*,6*S*,3′*R*)-5,6-epoxy-3,3′-dihydroxy-7′,8′-didehydro-5,6,7,8-tetrahydro-β,β-caroten-8-one (**4**) was performed. Acetylation of **7** gave fucoxanthin monoacetate (**9**). Dehydration[10] of **9** yielded semi-synthetic (3*S*,5*R*,6*S*,3′*R*)-5,6-epoxy-3,3′-dihydroxy-7′,8′-didehydro-5,6,7,8-tetrahydro-β,β-caroten-8-one-3,3′-diacetate (**10**), $C_{44}H_{58}O_6$, mp 163 °C. Enzymatic hydrolysis of **10** with lipase gave a corresponding alcohol, $C_{40}H_{54}O_4$, mp 158—160 °C, which was shown to be identical with naturally occurring halocynthiaxanthin. The IR, mass, ^{1}H-NMR and circular dichroism (CD) (Fig. 1) spectral data of these two compounds were in good agreement with each other. Thus, the structure of halocynthiaxanthin isolated

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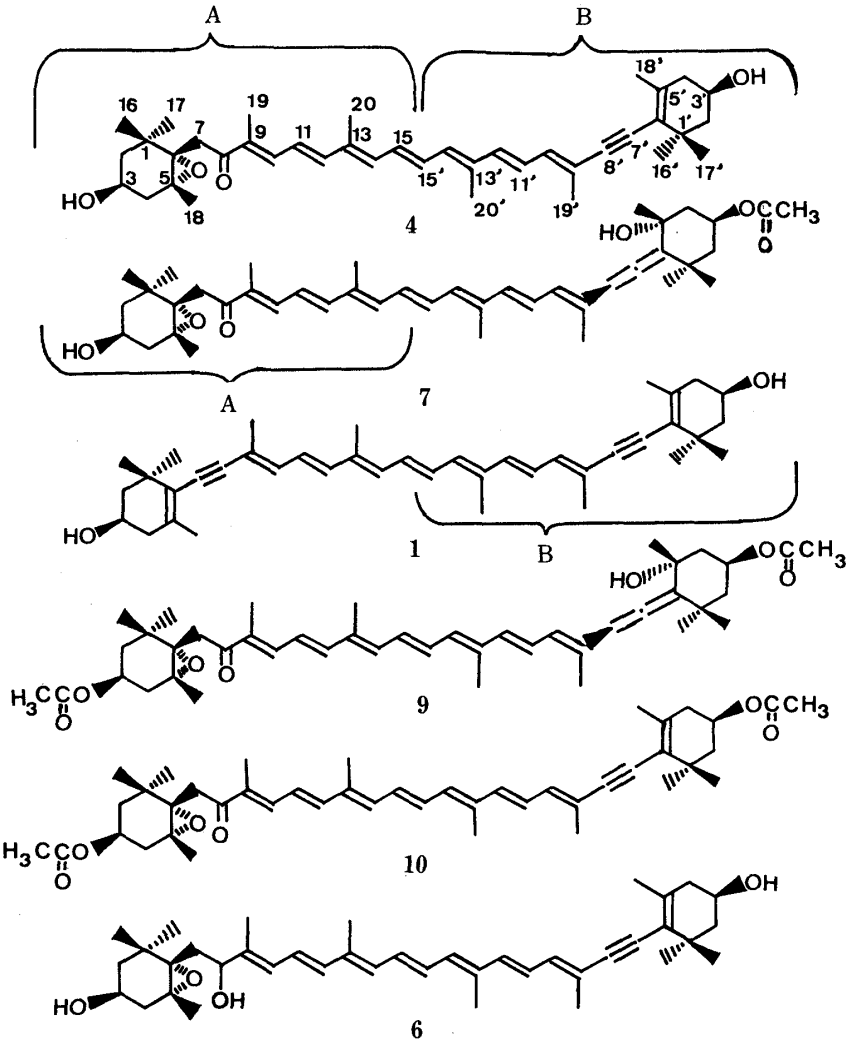

Chart 1

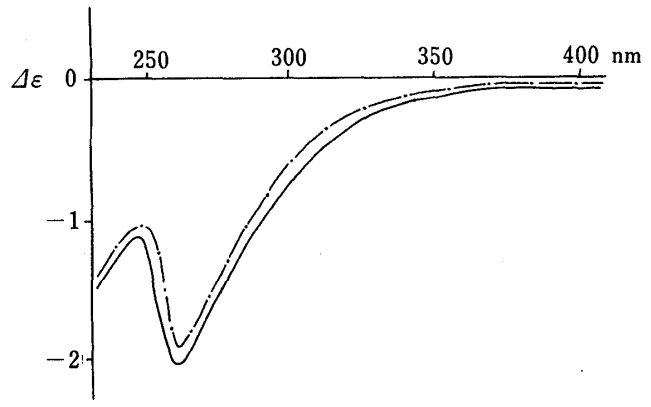

Fig. 1. CD Spectra of Naturally Occurring Halocynthiaxanthin (——) and Semi-synthetic (3*S*, 5*R*, 6*S*, 3′*R*)-5,6-Epoxy-3,3′-dihydroxy-7′,8′-didehydro-5,6,7,8-tetrahydro-β, β-caroten-8-one (**4**) (— - —) in EPA (Ether–Isopentane–Ethanol 5:5:2) at 20 °C

from *H. roretzi* was established to be (3*S*,5*R*,6*S*,3′*R*)-5,6-epoxy-3,3′-dihydroxy-7′,8′-didehydro-5,6,7,8-tetrahydro-β,β-caroten-8-one (**4**).

Mytiloxanthinone (**5**) was obtained as reddish needles, $C_{40}H_{52}O_4$ (high resolution MS *m/z*: M^+ 596.3850), mp 226—228 °C. The visible light absorption spectrum of **5** showed absorption maximum at 295, 475 (ether), 298, 475 (ethanol) and 270, 455 (ethanol–KOH) nm. Absorption bands in the IR spectrum of **5** were observed at 3390 (OH), 2165 (–C≡C–), 1738

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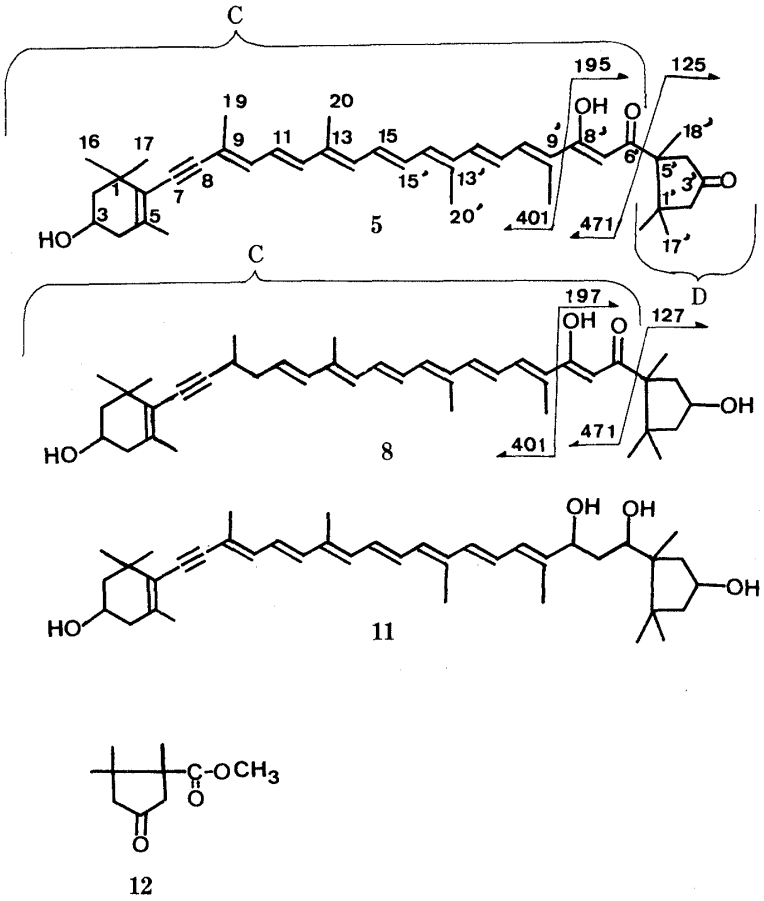

Chart 2

TABLE I. ^{1}H-NMR Chemical Shifts of Halocynthiaxanthin (**4**), Mytiloxanthinone (**5**) and Reference Carotenoids [Alloxanthin (**1**), Fucoxanthin (**7**) and Mytiloxanthin (**8**)] (δ ppm, $CDCl_3$)

Carotenoid	**4**	**5**	**1**	**7**	**8**
C-16 CH_3	0.96	1.14	1.14	0.96	1.14
C-17 CH_3	1.03	1.20	1.20	1.04	1.20
C-18 CH_3	1.22	1.92	1.90	1.22	1.92
C-19 CH_3	1.93	1.99	1.98	1.95	1.98
C-20 CH_3	1.99	1.99	1.98	1.98	1.98
C-20′ CH_3	1.99	1.99	1.98	1.98	1.98
C-19′ CH_3	1.99	1.99	1.98	1.82	1.98
C-18′ CH_3	1.93	1.34	1.90	1.35	1.34
C-17′ CH_3	1.20	1.22	1.20	1.35	1.19
C-16′ CH_3	1.14	1.03	1.14	1.08	0.85
C-7 CH_2	2.59 d			2.59 d	
	3.66 d			3.68 d	
C-7′=CH–		5.90			5.86
C-8′ –OH		16.25			16.28
Olefinic protons	*Ca.* 6.00—7.00	*Ca.* 6.00—7.00	*Ca.* 6.00—7.00	*Ca.* 6.00—7.00	*Ca.* 6.00—7.00

Abbreviation: d, doublet.

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Table II. The Amounts of Carotenoids[a] and Carotenoids Composition in Each Tissue of *Halocynthia roretzi*

	Tunic	Mantle	Liver
Total carotenoids[a] (mg/100 g wet tissue)	18.9	2.0	21.5
Composition (%)			
β-Carotene (**3**)	0.1	0.4	4.1
Lutein	0.1	—	0.1
Zeaxanthin	2.0	4.0	9.0
Diatoxanthin	13.2	16.7	6.0
Alloxanthin (**1**)	49.3	20.7	12.0
Diadinochrome	4.1	18.0	6.0
(3*S*, 3*S*′)-Astaxanthin (**2**)	5.0	10.0	2.5
Pectenolone	2.0	3.0	—
Mytiloxanthinone (**5**)	10.0	3.0	—
Mytiloxanthin (**8**)	4.7	8.0	2.5
Fucoxanthin (**7**)	—	—	3.0
Halocynthiaxanthin (**4**)	4.2	7.4	24.0
Fucoxanthinol	Trace	Trace	16.0
Unidentified carotenoids	5.3	8.8	14.8

a) The total carotenoids and carotenoids composition were determined by the method of McBeth.[22]

(C=O in a cyclopentane ring), 1610, 1595 and 1588 (enolic β-diketone) and 960 (*trans* –CH=CH–) cm^{-1}. Acetylation of **5** gave a monoacetate. On reduction with $NaBH_4$ **5** gave the corresponding secondary tetrol (**11**). The ^{1}H-NMR spectrum of **5** also indicated the presence of an enolic β-diketone group[11] [δ 5.90 and 16.25]. The presence of the fragment ions at *m/z* 401 and 195 was attributed to cleavage of the 8′, 9′ bond (Chart 2). Taking into consideration the absorption band at 1738 cm^{-1} (characteristic of a carbonyl group in a cyclopentane ring), the presence of a cyclopentanone end group in **5** is clear from observation of the fragment ions at *m/z* 471 and 125 in the mass spectrum of **5** (Chart 2). The ^{1}H-NMR data for **5** (Table I) showed signals due to methyl protons of group C and the cyclopentanone end group D in comparison with those of group C in mytiloxanthin (**8**) and of methyl 1,2,2-trimethyl-4-oxo-cyclopentanecarboxylate (**12**) (Chart 2).[12] On the basis of the above evidence, the structure of mytiloxanthinone was determined to be 3,8′-dihydroxy-7,8-didehydro-β,κ-carotene-3′,6′-dione (**5**).

The carotenoids composition in the sea squirt *H. roretzi* is shown in Table II.

Experimental

Melting points were measured on a Yanagimoto micro melting point apparatus. Visible light absorption and IR spectra were recorded on a Shimadzu UV-200 spectrophotometer and a Shimadzu IR-27G spectrophotometer, respectively. Mass spectra (MS) were recorded with a Nippon Denshi JEOL JMS-O1SG mass spectrometer at an ionization energy of 25 eV and with a Hitachi M-80 spectrometer at 70 eV. CD spectra were measured in ether–isopentane–ethanol (5:5:2) (EPA) with a Jasco J-20 instrument. ^{1}H-NMR spectra were obtained at 80 MHz on a Varian CFT-20 spectrometer. Chemical shifts are reported in ppm from internal tetramethylsilane for the compounds in deuterio-chloroform ($CDCl_3$) solution.

Preparative thin layer chromatography (PTLC) was carried out on glass plates coated with Silica gel 60 G (Merck) and column chromatography was carried out using MgO (Merck) and Celite 545 (Kanto Chemical).

Materials——*Halocynthia roretzi* (40 kg wet weight) was transported to our laboratory by air from Yamada Bay, Iwate Prefecture, October 1981. The carotenoids were extracted with acetone and transferred to petroleum ether; this solution was washed with distilled water and dried over anhydrous sodium sulfate. The resulting petroleum ether solution was concentrated under reduced pressure in nitrogen below 40 °C in the dark, and the residue afforded

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eight bands (band 1, *Rf* 0.95; band 2, *Rf* 0.55; band 3, *Rf* 0.50; band 4, *Rf* 0.35—0.45; band 5, *Rf* 0.30; band 6, *Rf* 0.25; band 7, *Rf* 0.20; band 8, *Rf* 0.10) on PTLC with petroleum ether–acetone (70 : 30) as the developing solvent.

β-Carotene (3)——β-Carotene (**3**) was isolated from band 1 of the first silica gel PTLC. Recrystallization from petroleum ether gave reddish needles, mp 184 °C, UV λ_{max}^{ether} nm: 448, 475, MS *m/z*: 536 (M^+, $C_{40}H_{56}$), which were proved to be identical with β-carotene (Merck) by mixed fusion, and MS and thin layer chromatography (TLC) comparisons.

Astaxanthin (2)——Astaxanthin (**2**) was isolated from band 2 by repeated PTLC. Recrystallization from petroleum ether gave reddish needles, mp 216 °C, UV λ_{max}^{ether} nm: 469. MS *m/z*: 596 (M^+, $C_{40}H_{52}O_4$). ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H × 2, s), 1.32 (3H × 2, s), 1.94 (3H × 2, s), 1.99 (3H × 4, s). CD (c = 0.02, EPA) $\Delta\varepsilon$: +8.8 (220), 0 (230), −14.7 (243), 0 (255), +5.9 (275), 0 (283), −20.0 (315), 0 (343). The CD spectrum of **2** suggested the 3*S*,3′*S* configuration in comparison with that of synthetic-(3*S*,3′*S*)-astaxanthin.[13]

Mytiloxanthinone (5)——Mytiloxanthinone (**5**) was isolated from band 3 by repeated PTLC. Recrystallization from petroleum ether gave reddish needles, mp 226—228 °C, UV λ_{max}^{ether} nm: 295, 475, UV $\lambda_{max}^{ethanol}$ nm: 298, 475, UV $\lambda_{max}^{ethanol-KOH}$ nm: 270, 455. MS *m/z*: 596 (M^+, $C_{40}H_{52}O_4$, Calcd: 596.3862: Found: 596.3850), 578 (M^+-18), 560 ($M^+-18-18$), 504 (M^+-92), 486 ($M^+-92-18$), 471 (M^+-125), 401 (M^+-195), 195 (M^+-401), 125 (M^+-471). IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 2165 (–C≡C–), 1738 (C=O in a cyclopentane ring), 1610, 1595, 1588 (enolic β-diketone), 960 (*trans* –CH=CH–). ^{1}H-NMR: Table II.

Acetylation of 5——**5** (1 mg) was dissolved in 1 ml each of dry pyridine and acetic anhydride, and the mixture was allowed to stand for 6 h at room temperature. The solution was diluted with water, and extracted with petroleum ether. After being washed with water, the petroleum ether layer was dried over anhydrous sodium sulfate. After evaporation, the residue was purified by PTLC [petroleum ether–acetone (80 : 20)]. Recrystallization of the product from petroleum ether gave 0.5 mg of needles, mp 142 °C, monoacetate of **5**. MS *m/z*: 638 (M^+, $C_{42}H_{54}O_5$), 578 (M^+-60).

Reduction of 5——Sodium borohydride (30 mg) was added to a solution of 1 mg of **5** in 10 ml of methanol with stirring at room temperature. The reaction mixture was allowed to stand for 1 h, then poured into 100 ml of water and extracted with petroleum ether. After being washed with water, the petroleum ether layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure in nitrogen below 40 °C in the dark, the residue was subjected to PTLC [petroleum ether–acetone (90 : 10)]. Recrystallization of the product from petroleum ether gave 0.4 mg of reddish needles, mp 102—104 °C, **11**. MS *m/z*: 602 (M^+, $C_{40}H_{58}O_4$).

Zeaxanthin——Zeaxanthin was isolated from band 4 of the first silica gel PTLC, followed by MgO column chromatography.[14] Recrystallization from petroleum ether gave reddish needles, mp 206 °C, UV λ_{max}^{ether} nm: 448, 475. MS *m/z*: 568 (M^+, $C_{40}H_{56}O_2$). This compound was identical with an authentic sample of zeaxanthin obtained from the cycad[15] on direct comparison (TLC, UV, MS and mixed fusion).

Lutein——Lutein was isolated from band 4 of the first silica gel PTLC, followed by MgO column chromatography. Recrystallization of the product from petroleum ether gave orange needles, mp 195—196 °C, UV λ_{max}^{ether} nm: 419, 443, 473. MS *m/z*: 568 (M^+, $C_{40}H_{56}O_2$). This compound was identical with authentic lutein obtained from the yolk[16] on direct comparison (TLC, UV, MS and mixed fusion).

Pectenolone——Pectenolone was isolated from band 4 by repeated PTLC. Recrystallization from petroleum ether gave reddish needles, mp 162—163 °C, UV λ_{max}^{ether} nm: 459—462. MS *m/z*: 580 (M^+, $C_{40}H_{52}O_3$), 562 (M^+-18), 544 ($M^+-18-18$), 488 (M^+-92). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 2165 (–C≡C–), 1660 (conj. C=O), 960 (*trans* –CH=CH–). ^{1}H-NMR ($CDCl_3$) δ: 1.14 (3H, s), 1.20 (3H × 2, s), 1.32 (3H, s), 1.94 (3H × 4, s), 1.99 (3H × 2, s). This compound was identical with an authentic sample of pectenolone isolated from *Patinopecten yessoensis*[17,18] on direct comparison (TLC, UV, MS, IR, ^{1}H-NMR and mixed fusion).

Diadinochrome——Diadinochrome was isolated from band 4 of the first silica gel PTLC, followed by MgO column chromatography. Recrystallization of the product from petroleum ether gave orange needles, mp 127—130 °C, UV λ_{max}^{ether} nm: 400, 428, 454. MS *m/z*: 582 (M^+, $C_{40}H_{54}O_3$), 568 (M^+-18), 502 (M^+-80). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 2165 (–C≡C–), 960 (*trans* –CH=CH–). This compound was assigned as diadinochrome by direct comparison (TLC, UV, IR, MS and mixed fusion) with authentic diadinochrome derived from diadinoxanthin[19] obtained from diatoms.

Diatoxanthin——Diatoxanthin was isolated from band 4 of the first silica gel PTLC, followed by MgO column chromatography. Recrystallization of the product from petroleum ether gave reddish needles, mp 201 °C, UV λ_{max}^{ether} nm: 425, 450, 478. MS *m/z*: 566 (M^+, $C_{40}H_{54}O_2$), 548 (M^+-18), 530 ($M^+-18-18$), 474 (M^+-92). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 2165 (–C≡C–), 960 (*trans* –CH=CH–). This compound was identical with authentic diatoxanthin obtained from striped mullet *Mugil cephalus*[20] on direct comparison (TLC, UV, MS, IR and mixed fusion).

Alloxanthin (1)——Alloxanthin (**1**) was isolated from band 5 of the first silica gel PTLC, followed by MgO column chromatography. Recrystallization of the product from petroleum ether gave reddish needles, mp 186—188 °C, UV λ_{max}^{ether} nm: 428, 452, 480. MS *m/z*: 564 (M^+, $C_{40}H_{52}O_2$), 546 (M^+-18), 528 ($M^+-18-18$), 472 (M^+-92). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 2165 (–C≡C–), 960 (*trans* –CH=CH–). ^{1}H-NMR ($CDCl_3$) δ: 1.14 (3H × 2, s), 1.20 (3H × 2, s), 1.92 (3H × 2, s), 1.95 (3H × 2, s), 1.99 (3H × 2, s), 6.0—7.0 (10H, m). CD (c = 0.02, EPA) $\Delta\varepsilon$: 0 (350),

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+0.2 (335), 0 (320), −3.0 (285), −0.8 (250). This compound was identical with authentic alloxanthin[5] isolated from *Cryptomonas* algae on direct comparison (TLC, UV, MS, IR, ^{1}H-NMR, CD and mixed fusion).

Mytiloxanthin (8)——Mytiloxanthin (**8**) was isolated from band 5 of the first silica gel PTLC, followed by MgO column chromatography. Recrystallization from petroleum ether gave reddish needles, mp 147 °C, UV λ_{max}^{ether} nm: 295, 470, UV $\lambda_{max}^{ethanol}$ nm: 298, 471, UV $\lambda_{max}^{ethanol+KOH}$ nm: 270, 455. MS *m/z*: 598 (M^+, $C_{40}H_{54}O_4$), 580 (M^+-18), 562 ($M^+-18-18$), 506 (M^+-92), 448 ($M^+-92-18$), 471 (M^+-127), 401 (M^+-197). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 2165 (–C≡C–), 1610, 1590, 1588 (enolic β-diketone), 960 (*trans* –CH=CH–). ^{1}H-NMR ($CDCl_3$) δ: 0.85 (3H, s), 1.14 (3H, s), 1.19, (3H×2, s), 1.34 (3H, s), 1.92 (3H, s), 1.98 (3H×4, s), 5.86 (1H, s), 16.25 (1H, s) (O=C–CH=C–OH). This compound was identical with authentic mytiloxanthin obtained from *Mytilus edulis*[9] on direct comparison (TLC, UV, MS, IR, ^{1}H-NMR and mixed fusion).

Fucoxanthin (7)——Fucoxanthin (**7**) was isolated from band 6 by repeated PTLC. Recrystallization from ethanol gave reddish needles, mp 168—169 °C, UV λ_{max}^{ether} nm: 410, 446, 470. MS *m/z*: 658 (M^+, $C_{42}H_{58}O_6$). This compound was identical with authentic fucoxanthin isolated from *Sargassum fulvellum*[8,21] on direct comparison (TLC, UV, IR, MS and mixed fusion).

Halocynthiaxanthin (4)——Halocynthiaxanthin (**4**) was isolated from band 7 by repeated PTLC. Recrystallization of the product from petroleum ether gave reddish needles, mp 158—160 °C, UV λ_{max}^{ether} nm: 430, 452, 470. High resolution MS: Found 598.4024. Calcd for $C_{40}H_{54}O_4$: 598.4022. MS *m/z*: 598 (M^+), 582 (M^+-16), 580 (M^+-18), 565 ($M^+-18-15$), 562 ($M^+-18-18$), 506 (M^+-92), 490 ($M^+-92-16$), 488 ($M^+-92-18$). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 2165 (–C≡C–), 1645 (conj. C=O), 960 (*trans* –CH=CH–). ^{1}H-NMR: Table I.

Fucoxanthinol——Fucoxanthinol was isolated from band 8. Recrystallization of the product from ethanol gave orange needles, mp 146—148 °C, UV λ_{max}^{ether} nm: 410, 446, 470. MS *m/z*: 616 (M^+, $C_{40}H_{56}O_5$). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1928 (>C=C=C<), 1660 (conj. C=O), 960 (*trans* –CH=CH–). This compound was identical with authentic fucoxanthinol obtained from brown algae, *Sargassum fulvellum*,[8,21] on direct comparison (TLC, UV, IR, MS and mixed fusion).

Preparation of (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*R*)-5,6-Epoxy-3,3′,5′-trihydroxy-6′,7′-didehydro-5,6,7,8,5′,6′-hexahydro-β,β-caroten-8-one-3′-acetate, (Fucoxanthin) (7)——Fucoxanthin (**7**) (500 mg, mp 168—169 °C, M^+, 658, $C_{42}H_{58}O_6$), was isolated from brown algae, *Sargassum fulvellum* (10 kg wet weight), by means of PTLC [petroleum ether–acetone (70 : 30)]. On the basis of the mp, MS, ^{1}H-NMR and CD spectral data, this compound was concluded to be identical with (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*R*)-fucoxanthin (**7**).[8,21]

Fucoxanthin Monoacetate [(3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*R*)-5,6-Epoxy-3,3′,5′-trihydroxy-6′,7′-didehydro-5,6,7,8,5′,6′-hexahydro-β,β-caroten-8-one-3,3′-diacetate] (9)——Fucoxanthin (**7**, 500 mg) was treated with acetic anhydride (3 ml) in dry pyridine (5 ml) to give **9** (450 mg, mp 148—150 °C, M^+, 700, $C_{44}H_{60}O_7$), in 90% yield. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1920 (>C=C=C<), 1730 (OAc), 1660 (conj. C=O), 1608 (>C=C<), 960 (*trans* –CH=CH–). ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, s), 1.05 (3H, s), 1.06 (3H, s), 1.21 (3H, s), 1.33 (3H, s), 1.37 (3H, s), 1.78 (3H, s), 1.92 (3H, s), 1.97 (3H×2, s), 2.00 (3H, s), 2.53 (1H, d, J=18 Hz), 3.60 (1H, d, J=18 Hz) and 6.0—7.0 (11H, m).

Preparation of Semi-synthetic (3*S*,5*R*,6*S*,3′*R*)-5,6-Epoxy-3,3′-dihydroxy-7′,8′-didehydro-5,6,7,8-tetrahydro-β,β-caroten-8-one-3,3′-diacetate (10)——**9** (450 mg) was treated with $POCl_3$ (50 drops) in dry pyridine (10 ml) for 5 h at 40 °C to give dehydrated fucoxanthin acetate. The reaction product was subjected to preparative paper chromatography [(Scheicher and Schull 287, acetone–petroleum ether (2 : 98)]. Recrystallization of the product from ethanol afforded semi-synthetic (3*S*,5*R*,6*S*,3′*R*)-**10** (20 mg, mp 163 °C) in 4.4% yield. MS *m/z*: 682 (M^+, $C_{44}H_{58}O_6$), 664 (M^+-18), 622 (M^+-60). IR ν_{max}^{KBr} cm^{-1}: 2165 (–C≡C–), 1735 (OAc), 1660 (conj. C=O), 1608 (>C=C<), 960 (*trans* –CH=CH–). ^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, s), 1.07 (3H, s), 1.18 (3H, s), 1.21 (3H×2, s), 1.91 (3H, s), 1.94 (3H, s), 1.98 (3H, s), 1.99 (3H×2, s), 2.01 (3H, s), 2.02 (3H, s), 2.06 (1H, d, J=18 Hz), 3.66 (1H, d, J=18 Hz), 6.0—7.0 (10H, m). CD (c=0.02, EPA)Δε: −0.3 (250), −1.2 (265), 0 (350).

Preparation of Semi-synthetic (3*S*,5*R*,6*S*,3′*R*)-5,6-Epoxy-3,3′-dihydroxy-7′,8′-didehydro-5,6,7,8-tetrahydro-β,β-caroten-8-one (4): Enzymatic Hydrolysis of Semi-synthetic (3*S*,5*R*,6*S*,3′*R*)-10——Commercially available lipase (Meito Sangyo Co., Ltd., Lipase-OF-360) (activity: 360000 units/g solid at 37 °C, pH 7.0) was used in this experiment. A solution of semi-synthetic (3*S*,5*R*,6*S*,3′*R*)-**10** (20 mg in 5 ml methanol) in 0.05 M Tris-HCl buffer (20 l) was treated with lipase (20 g) with stirring at 37 °C for 48 h. The reaction mixture was then extracted with ether and the ethereal solution was dried over anhydrous sodium sulfate, then concentrated under reduced pressure in nitrogen below 40 °C in the dark. The resulting residue was subjected to PTLC [(acetone–petroleum ether (30 : 70)]. Recrystallization from petroleum ether afforded semi-synthetic (3*S*,5*R*,6*S*,3′*R*)-**4** (3 mg), mp 158—160 °C. MS *m/z*: 598 (M^+, $C_{40}H_{54}O_4$), 582 (M^+-16), 580 (M^+-18), 565 ($M^+-18-15$), 562 ($M^+-18-18$), 506 (M^+-92), 490 ($M^+-92-16$), 488 ($M^+-92-18$). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 2165 (–C≡C–), 1645 (conj. C=O), 960 (*trans* –CH=CH–). ^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, s), 1.03 (3H, s), 1.14 (3H, s), 1.20 (3H, s), 1.22 (3H, s), 1.93 (3H×2, s), 1.99 (3H×3, s), 2.60 (1H, d, J=18 Hz), 3.66 (1H, d, J=18 Hz), 6.0—7.0 (10H, m). CD (c=0.02, EPA)Δε: −1.0 (250), −2.0 (260), 0 (330).

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