

Structure of New Carotenoids from Corbicula Clam *Corbicula japonica*

Takashi Maoka,^{*,†} Yasuhiro Fujiwara,[‡] Keiji Hashimoto,[§] and Naoshige Akimoto[⊥]

Research Institute for Production Development, 15 Shimogamo-morimoto-cho, Sakyo-ku, Kyoto 606-0805, Japan, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan, Nagahama Institute of Bio-sciences and Technology, Tamura-cho, Nagahama, Shiga 526-0829, Japan, and Graduate School of Pharmaceutical Sciences, Kyoto University, Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan

Received April 11, 2005

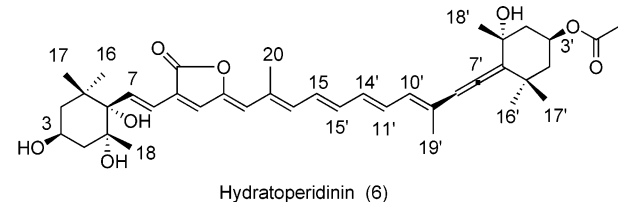
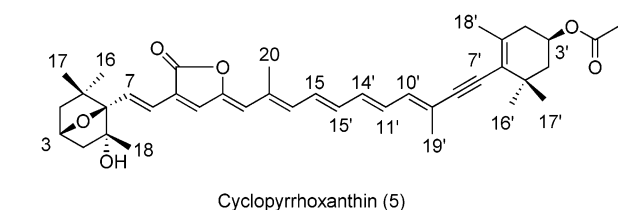
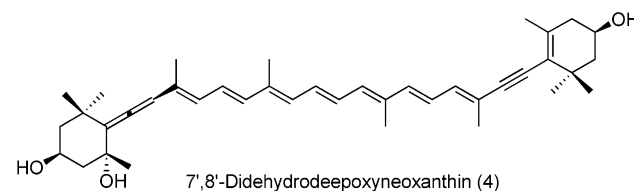
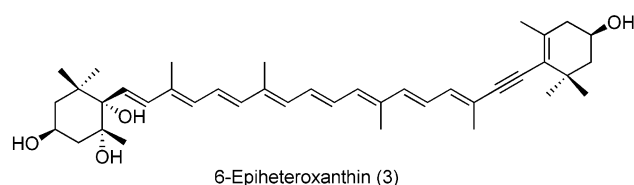
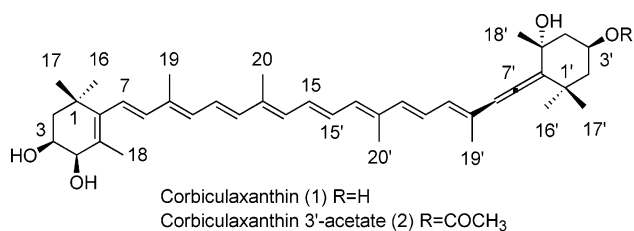
Six new carotenoids, named corbiculaxanthin (**1**), corbiculaxanthin 3'-acetate (**2**), 6-epiheteroxanthin (**3**), 7',8'-didehydrodepoxyneoxanthin (**4**), cyclopyrrhoxanthin (**5**), and hydratoperidin (**6**), were isolated from the corbicula clam (Shijimi in Japanese), *Corbicula japonica*. Their structures were determined by chemical and spectral data.

Corbicula clam (Shijimi in Japanese), *Corbicula japonica*, which inhabits brackish water, is an important edible shellfish in Japan. There have been several reports on carotenoids in marine shellfish,¹ but few studies on carotenoids in brackish and freshwater shellfish were carried out.¹ In the course of studies on the carotenoids in shellfish,² six new carotenoids were isolated from *C. japonica* as minor components along with β -carotene, alloxanthin, diadinoxanthin, diadinochrome, peridinin, pyrrhoxanthin, and fucoxanthin. This paper reports the isolation and structural elucidation of these new carotenoids.³

Results and Discussion

The acetone (Me₂CO) extract of the edible part of *C. japonica* (450 g) was chromatographed on silica gel using an increasing percentage of acetone (Me₂CO) in hexane. The fraction eluted with Me₂CO–hexane (6:4) was subjected to HPLC on silica gel with Me₂CO–hexane (6:4) and then on ODS with CHCl₃–MeCN (1:9) to yield **1** (0.2 mg), **2** (0.5 mg), **3** (1 mg), **4** (0.1 mg), **5** (1 mg), and **6** (0.03 mg).

Compound **1**, named corbiculaxanthin, showed UV–vis absorption maxima at 420, 443, and 472 nm. The molecular formula of **1** was determined to be C₄₀H₅₆O₄ by HRFABMS data. The presence of three secondary hydroxy groups and one tertiary hydroxy group in **1** was consistent with the formation of a triacetate and a tetratrimethylsilyl ether of **1**. The ¹H NMR data of **1** indicated the presence of a 3,4-(*cis*)-dihydroxy- β -end group, a 3,5-dihydroxy- β -end group, and a polyene chain containing an allenic group (δ 6.03, s, H-8').⁴ These partial structures were confirmed by ¹H–¹H COSY and NOESY (Figure 1) experiments. The *cis* configuration of the 3,4-dihydroxy group was revealed by a coupling constant between H-3 and H-4 of 3.5 Hz⁴ and NOE between H-3 and H-4. Furthermore, NOESY correlations H-3/H-16, H-3/H-4, H-4/H-18, H-3'/H-16', H-3'/H_{eq}-2'(α), and H-3'/H_{eq}-4'(α) revealed the relative stereochemistry of both end groups (Figure 1). The overall *E* geometry of the polyene chain was also confirmed by the NOESY data. Therefore, the structure of **1** was determined to be 6',7'-didehydro-5',6'-dihydro- β , β -carotene-3,4,3',5'-tetrol. The CD



spectrum of **1** showed a weak negative cotton effect, which was similar to that of deepoxyneoxanthin.⁵ On the basis of CD data, biosynthetic considerations, and the relative stereochemistry of 3,4-dihydroxy group, a (3*S*,4*R*,3'*S*,5'*R*,6'*R*) configuration was postulated for **1**.

Compound **2** showed the same absorption maxima as **1**. The molecular formula of **2** was determined to be C₄₂H₅₈O₅

* To whom correspondence should be addressed. Research Institute for Production Development, 15 Shimogamo-Morimoto-Cho, Sakyo-ku, Kyoto 607-0805, Japan. Tel: +81-75-781-1107. Fax: +81-75-722-8694. E-mail: maoka@mbox.kyoto-inet.or.jp.

† Research Institute for Production Development.

‡ Kyoto Pharmaceutical University.

§ Nagahama Institute of Bio-sciences and Technology.

⊥ Kyoto University.

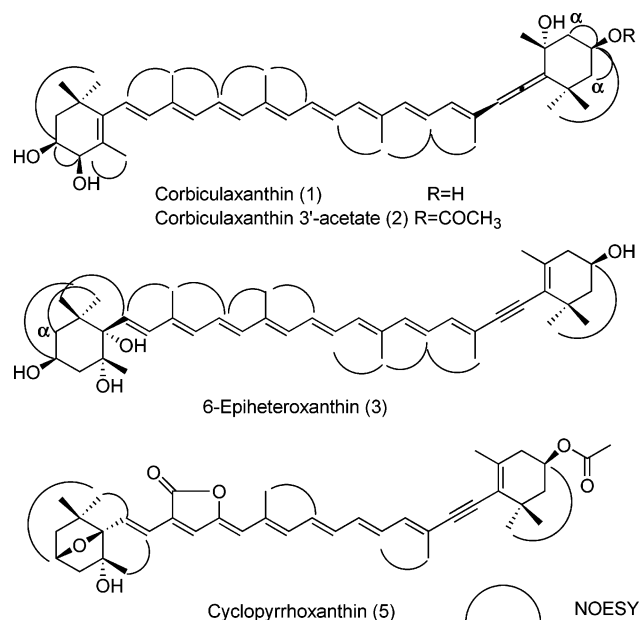


Figure 1. Key NOESY correlations of **1**, **2**, **3**, and **5**.

by HRFABMS data. Compound **2** showed almost the same ¹H NMR signals as **1** except for the presence of an acetyl group at δ 2.04 and an acetylated oxymethine at δ 5.38 instead of a free oxymethine at δ 4.31 (H-3') for **1**. These spectral data suggested that **2** was the 3'-acetate of **1**. This structure was confirmed by ¹H–¹H COSY and NOESY (Figure 1) experiments. Compound **2** showed also the same CD spectrum as that of **1**. Therefore, the structure of **2** was determined to be (3*S*,4*R*,3'*S*,5'*R*,6'*R*)-3'-ethanoyloxy-6',7'-didehydro-5',6'-dihydro- β , β -carotene-3,4,5'-triol, and the compound was named corbiculaxanthin 3'-acetate.

Compound **3** showed UV–vis absorption maxima at 420, 443, and 472 nm. The molecular formula of **3** was determined to be C₄₀H₅₆O₄ by HRFABMS data. The presence of two secondary hydroxy groups and two tertiary hydroxy groups in **3** was revealed by acetylation, HRFABMS, ¹H, and ¹³C NMR data. ¹H and ¹³C NMR of **3** showed the presence of an alloxanthin moiety (C-1' to C-20').⁴ The partial structure of the other end group (3,5,6-trihydroxy-5,6-dihydro- β -end group) was elucidated by ¹H–¹H COSY, HSQC, HMBC (Figure 2), and NOESY (Figure 1) experiments. Therefore, the structure of **3** was determined to be 7',8'-didehydro-5,6-dihydro- β , β -carotene-3,5,6,3'-tetrol. Concerning the naturally occurring carotenoids with the 3,5,6-trihydroxy-5,6-dihydro- β -end group, four kinds of stereoisomers [i.e., (3*S*,5*R*,6*R*), (3*S*,5*S*,6*S*), (3*S*,5*S*,6*R*), and (3*S*,5*R*,6*S*)] have been reported.⁶ The ¹H and ¹³C NMR spectral data of the 3,5,6-trihydroxy-5,6-dihydro- β -end group in **3** do not coincide with those of the (3*S*,5*R*,6*R*), (3*S*,5*S*,6*S*), and (3*S*,5*S*,6*R*) configurations but coincide with the (3*S*,5*R*,6*S*) configuration. Furthermore, NOESY correlations of H16/H_{eq}-2(α), H-16/H-3, and H17/H7 also supported this stereochemistry (Figure 1). Moreover, **3** showed a negative weak CD spectrum similar to that of heteroxanthin.^{6a} Therefore, the structure of **3** was determined to be (3*S*,5*R*,6*S*,3'*R*)-7',8'-didehydro-5,6-dihydro- β , β -carotene-3,5,6,3'-tetrol. This structure corresponds to the 6-epimer of heteroxanthin. Therefore, compound **3** was named 6-epiheteroxanthin.

Compound **4** showed UV–vis absorption maxima at 420, 443, and 472 nm. The molecular formula of **4** was determined to be C₄₀H₅₄O₃ by HRFABMS data. ¹H NMR of **4** showed the presence of neoxanthin (H-2 to H-20') and alloxanthin (H-2' to H-20') moieties.⁴ This was confirmed

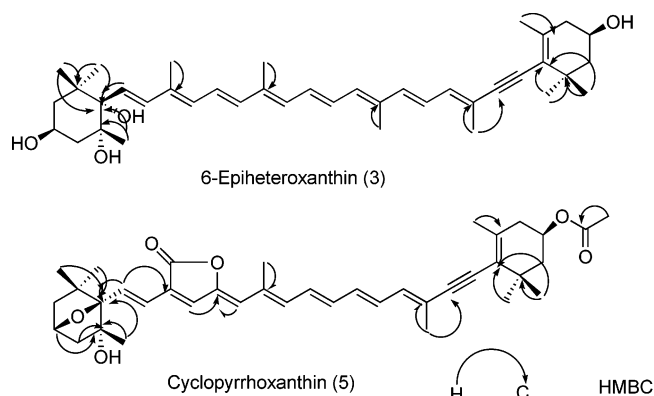


Figure 2. Key HMBC correlations of **3** and **5**.

by the ¹H–¹H COSY experiment. Therefore, the structure of **4** was determined to be 6,7,7',8'-tetrahydro-5,6-dihydro- β , β -carotene-3,5,3'-triol, and the compound was named 7',8'-didehydrodepoxyneoxanthin. The CD spectrum of **4** was almost the same as that of alloxanthin.^{2a} Therefore, (3*S*,5*R*,6*S*,3'*R*) chirality was postulated for **4**.

Compound **5** showed UV–vis absorption maxima at 455 and 475 nm. The molecular formula of **5** was determined to be C₃₉H₄₈O₆ by HRFABMS data. The ¹H and ¹³C NMR data of **5** were almost identical with those of pyrrhoxanthin⁴ except for the signals of the end group (C1 to C6 including C16, C17, and C18). The ¹H and ¹³C NMR signals of the remaining end group were similar to those of cycloviolaxanthin, which has a (3*S*,5*R*,6*R*)-3,6-epoxy-5,6-dihydro-5-hydroxy- β -end group.⁷ Thus, the structure 3,6-epoxy-3'-ethanoyloxy-3-hydroxy-7',8'-didehydro-5,6-dihydro-12',13',20'-trienor- β , β -caroten-19,11-olide was postulated for **5**. This structure was confirmed by ¹H–¹H COSY, TOCSY, NOESY (Figure 1), HSQC, and HMBC (Figure 2) experiments. The stereochemistry of **5** was elucidated from NOESY and CD data by comparing it with those of pyrrhoxanthin and cycloviolaxanthin.⁷ The CD spectrum of **5** was similar to that of pyrrhoxanthin. Therefore, (3*S*,5*R*,6*R*,3'*R*) chirality was postulated for **5**. Compound **5** was named cyclopyrrhoxanthin after the presence of a 3,6-epoxy end group, such as cycloviolaxanthin.

Compound **6** showed UV–vis absorption maxima at 455 and 475 nm. The molecular formula of **6** was determined to be C₃₉H₅₂O₈ by HRFABMS data. Acetylation of **6** gave a monoacetate. Because of the small amount of sample and contamination of lipids, two-dimensional NMR experiments could not be performed, so methylene signals in the end groups could not be completely assigned. The ¹H NMR data for the methyl groups of H-20, 16' to 19', methine signal of H-3', and olefinic protons H-10 to H-8' were almost the same as those of peridinin.⁸ Positive ion FAB MS/MS showed the characteristic product ions at m/z 630 [$M - H_2O$]⁺, 612 [$M - 2H_2O$]⁺, 588 [$M - AcOH$]⁺, and 570 [$M - AcOH - H_2O$]⁺, which were 18 mass units above the corresponding ions of peridinin. These data clearly indicated that **6** is a hydrate product of peridinin. The presence of a 3,5,6-trihydroxy-5,6-dihydro- β -end group in **6** was deduced from the HRFABMS, FAB MS/MS, and ¹H NMR data and the result of acetylation. Therefore, the planar structure of **6** was deduced to be 3'-ethanoyloxy-3,5,6,5'-tetrahydroxy-6',7'-didehydro-5,6,5',6'-tetrahydro-12',13',20'-trienor- β , β -caroten-19,11-olide, and the compound was named hydratoperidinin. Concerning the peridinin derivatives with a 3,5,6-trihydroxy-5,6-dihydro- β -end group, hydratopyrrhoxanthinol, having (3*S*,5*R*,6*R*,3'*R*) chirality, was isolated from marine shellfish, *Mytilus edulis*.⁹ The chemical shift values of methyl signals at H-16 and H-17 in **6**

were slightly different from those of hydratopyrroxanthinol. This fact indicated that the chirality of the 3,5,6-trihydroxy-5,6-dihydro- β -end group in **6** was different from that of hydratopyrroxanthinol. The CD spectrum of **6** was similar to that of peridinin, having (3*S*,5*R*,6*S*,3'*S*,5'*R*,6'*R*) chirality. On the basis of the CD data and biosynthetic considerations of epoxide hydrolysis of peridinin, (3*S*,5*R*,6*S*,3'*S*,5'*R*,6'*R*) chirality was proposed for **6**.

Experimental Section

General Experimental Procedures. The CD spectra were recorded in Et₂O at room temperature with a JASCO J-500 spectropolarimeter. The UV-vis spectra were recorded with a Shimadzu U-2001 spectrophotometer in diethyl ether (Et₂O). The ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) spectra were measured with a Varian UNITY INOVA 500 spectrometer in CDCl₃ with TMS as an internal standard. Gradient (g) ¹H-¹H COSY, NOESY (mixing time 1.3 s), gHSQC (¹J_{CH} = 142 Hz), gHMBC (ⁿJ_{CH} optimized for 8 Hz), and TOCSY (mixing time 0.08 s) spectra were acquired using the standard Varian pulse programs with the Varian software, version 6.1A. All two-dimensional NMR and ¹³C NMR spectra were measured in 0.2 mL of CDCl₃ solution using a SHIGEMI tube (Shigemi Co., Ltd, Tokyo, Japan). The positive ion FAB-MS and CID-MS/MS spectra¹⁰ were recorded using a JEOL JMS-HX/HX 110A four-sector tandem mass spectrometer with *m*-nitrobenzyl alcohol (*m*-NBA) as a matrix. The CID-MS/MS was performed with a FAB gun operated at 6 kV. A few micrograms of sample dissolved in CHCl₃ was placed on a stainless steel probe tip, and 1–2 μ L of *m*-NBA was added as a matrix. The sample was bombarded with xenon atoms, and the ions produced were accelerated through 10 keV. The radical cation M^{•+} selected as a precursor by MS1 was subjected to collisions with argon gas in the collision cell, floated at a potential of 8 kV, between MS1 and MS2. The amount of argon gas was adjusted to attenuate the intensity of the precursor ion by 30%. The resulting product ions were acquired with a JEOL ADS-11 array detector on MS2. HPLC was performed on a Shimadzu LC-6AD with a Shimadzu SPD-6AV spectrophotometer set at 450 nm. Acetylation and trimethyl silylations were carried out using standard procedures.¹¹

Animal Material. *Corbicula japonica* (grown in Lake Shinji, Shimane Prefecture), was purchased at a local fish market in January and February. Voucher specimens have been deposited at the Research Institute for Production Development.

Extraction and Isolation of Carotenoids. The Me₂CO extract of the edible parts of *C. japonica* (450 g) was partitioned between diethyl ether (Et₂O) and aqueous NaCl. The organic layer was dried over Na₂SO₄ and then concentrated to dryness. The residue was subjected to silica gel column chromatography using an increasing percentage of Me₂CO in *n*-hexane. The fraction eluted with Me₂CO–hexane (6:4) was subjected to a series of HPLCs on silica gel with Me₂CO–hexane (6:4) and then on ODS with CHCl₃–MeCN (1:9) to yield **1** (0.2 mg), **2** (0.5 mg), **3** (1 mg), **4** (0.1 mg), **5** (1 mg), and **6** (0.03 mg).

In the present investigation, the following known carotenoids were also isolated and identified: β -carotene (0.5 mg), alloxanthin (2.0 mg), diadinoxanthin (1.0 mg), diadinochrome (3.5 mg), peridinin (9.0 mg), pyrroxanthin (1.0 mg), and fucoxanthin (0.5 mg).

Corbiculaxanthin (1): CD (20 μ g/mL Et₂O) λ ($\Delta\epsilon$) nm 220 (0), 235 (–5.0), 280 (–3.0), 330 (–2.8), 350 (0); UV-vis (Et₂O) λ_{\max} 420, 443, 472 nm, %III/II = 50; ¹H NMR (CDCl₃, 500 MHz) δ 1.07 (6H, s, H-17, 17'), 1.09 (3H, s, H-16), 1.34 (1H, overlapped, H_{ax}-2'(β)), 1.34 (3H, s, H-16'), 1.35 (3H, s, H-18'), 1.41 (1H, dd, *J* = 12.5, 12.5 Hz, H_{ax}-4'(β)), 1.57 (1H, ddd, *J* = 12.5, 4, 1 Hz, H_{eq}-2(α)), 1.68 (1H, dd, *J* = 12.5, 12.5 Hz, H_{ax}-2(β)), 1.80 (3H, s, H-19'), 1.90 (3H, s, H-18), 1.95 (1H, overlapped, H_{eq}-2'(α)), 1.97 (9H, s, H-20, 19', 20'), 2.27 (1H, ddd, *J* = 12.5, 4.5, 2 Hz, H_{eq}-4'(α)), 3.87 (1H, m, H-3), 3.96 (1H, d, *J* = 3.5 Hz, H-4), 4.31 (1H, m, H-3'), 6.03 (1H, s, H-8'),

6.07 (1H, d, *J* = 16 Hz, H-7), 6.10 (1H, d, *J* = 11.5 Hz, H-10), 6.12 (1H, d, *J* = 11.5 Hz, H-10'), 6.17 (1H, d, *J* = 16 Hz, H-8'), 6.26 (1H, m, H-14'), 6.27 (1H, m, H-14), 6.34 (1H, d, *J* = 15.5 Hz, H-12), 6.38 (1H, d, *J* = 15.5 Hz, H-12'), 6.55 (1H, dd, *J* = 15.5, 11.5 Hz, H-11), 6.63 (1H, dd, *J* = 15.5, 11.5 Hz, H-11'), 6.64 (2H, m, H-15, 15'); HRFABMS *m/z* 600.4171 (C₄₀H₅₆O₄, calcd for 600.4178).

Acetylation of **1** in dry pyridine with Ac₂O at room temperature produced a triacetate which showed molecular ion at *m/z* 726 by FABMS, and trimethyl silylation of **1** provided tetra-trimethylsilyl ether of **1** which showed molecular ion at *m/z* 888 by FABMS.

Corbiculaxanthin 3'-acetate (2): CD (20 μ g/mL Et₂O) λ ($\Delta\epsilon$) nm 220 (0), 235 (–5.0), 280 (–3.0), 330 (–2.8), 350 (0); UV-vis (Et₂O) λ_{\max} 420, 443, 472 nm, %III/II = 50; ¹H NMR (CDCl₃, 500 MHz) δ 1.07 (6H, s, H-17, 17'), 1.09 (3H, s, H-16), 1.35 (3H, s, H-18'), 1.38 (3H, s, H-16'), 1.41 (1H, dd, *J* = 12.5, 12.5 Hz, H_{ax}-2'(β)), 1.51 (1H, dd, *J* = 12.5, 12.5 Hz, H_{ax}-4'(β)), 1.57 (1H, ddd, *J* = 12.5, 4, 1 Hz, H_{eq}-2(α)), 1.68 (1H, dd, *J* = 12.5, 12.5 Hz, H_{ax}-2(β)), 1.80 (3H, s, H-19'), 1.90 (3H, s, H-18), 1.97 (9H, s, H-20, 19', 20'), 1.99 (1H, overlapped, H_{eq}-2'(α)), 2.04 (3H, s, COCH₃), 2.28 (1H, ddd, *J* = 12.5, 4.5, 2 Hz, H_{eq}-4'(α)), 3.87 (1H, m, H-3), 3.96 (1H, d, *J* = 3.5 Hz, H-4), 5.38 (1H, m, H-3'), 6.05 (1H, s, H-8'), 6.07 (1H, d, *J* = 16 Hz, H-7), 6.10 (1H, d, *J* = 11.5 Hz, H-10), 6.15 (1H, d, *J* = 11.5 Hz, H-10'), 6.17 (1H, d, *J* = 16 Hz, H-8'), 6.26 (1H, m, H-14'), 6.27 (1H, m, H-14), 6.34 (1H, d, *J* = 15.5 Hz, H-12), 6.38 (1H, d, *J* = 15.5 Hz, H-12'), 6.55 (1H, dd, *J* = 15.5, 11.5 Hz, H-11), 6.63 (1H, dd, *J* = 15.5, 11.5 Hz, H-11'), 6.64 (2H, m, H-15, 15'); HRFABMS *m/z* 642.4290 (C₄₂H₅₈O₅, calcd for 642.4285).

6-Epithetoxanthin (3): CD (20 μ g/mL Et₂O) λ ($\Delta\epsilon$) nm 230 (–1.0), 240 (–2.0), 250 (–3.0), 320 (–1.2), 370 (0); UV-vis (Et₂O) λ_{\max} 420, 443, 472 nm, %III/II = 50; ¹H NMR (CDCl₃, 500 MHz) δ 0.82 (3H, s, H-17), 1.01 (3H, s, H-18), 1.14 (3H, s, H-16'), 1.20 (3H, s, H-17'), 1.25 (3H, s, H-16), 1.45 (1H, dd, *J* = 12.5, 12.5 Hz, H_{ax}-2'(β)), 1.54 (1H, dd, *J* = 12.5, 12.5 Hz, H_{ax}-2(β)), 1.61 (1H, dd, *J* = 13.5, 11 Hz, H_{ax}-4'(β)), 1.77 (1H, ddd, *J* = 12.5, 4, 2.5 Hz, H_{eq}-2(α)), 1.84 (1H, ddd, *J* = 12.5, 4, 1.5 Hz, H_{eq}-2'(α)), 1.92 (6H, s, H-19, 18'), 1.96 (3H, s, H-20'), 1.97 (3H, s, H-20), 2.01 (3H, s, H-19'), 2.07 (1H, dd, *J* = 18, 10 Hz, H_{ax}-4'(β)), 2.11 (1H, ddd, *J* = 13.5, 4, 2.5 Hz, H_{eq}-4'(α)), 2.43 (1H, ddd, *J* = 18, 5, 1.5 Hz, H_{eq}-4'(α)), 3.99 (1H, m, H-3'), 4.28 (1H, m, H-3), 5.87 (1H, d, *J* = 16 Hz, H-7), 6.22 (1H, d, *J* = 11.5 Hz, H-10), 6.27 (2H, m, H-14, H-14'), 6.36 (1H, d, *J* = 15.5 Hz, H-12'), 6.38 (1H, d, *J* = 15.5 Hz, H-12), 6.42 (1H, d, *J* = 16 Hz, H-8), 6.46 (1H, d, *J* = 11.5 Hz, H-10'), 6.51 (1H, dd, *J* = 15.5, 11.5 Hz, H-11'), 6.62 (1H, dd, *J* = 15.5, 11.5 Hz, H-11), 6.65 (2H, m, H-15, 15'); ¹³C NMR (CDCl₃, 125 MHz) δ 12.8 (q, C-20, 20'), 13.2 (q, C-19), 18.1 (q, C-19'), 22.5 (q, C-18'), 27.8 (q, C-18), 28.6 (q, C-17), 28.8 (q, C-16'), 29.7 (q, C-16), 30.5 (q, C-17'), 36.6 (s, C-1'), 39.7 (s, C-1), 41.5 (t, C-4'), 45.7 (t, C-4), 46.7 (t, C-2'), 46.9 (t, C-2), 64.4 (d, C-3), 64.9 (d, C-3'), 76.5 (s, C-5), 79.3 (s, C-6), 89.0 (s, C-7), 98.6 (s, C-8'), 120.0 (s, C-9'), 124.2 (d, C-11'), 124.8 (d, C-11), 124.9 (s, C-6'), 129.4 (d, C-7), 130.1 (d, C-15 or 15'), 130.4 (d, C-15' or 15), 132.0 (d, C-10), 132.8 (d, C-8), 133.1 (d, C-14 or 14'), 133.5 (d, C-14' or 14), 134.5 (s, C-9), 135.2 (d, C-10'), 136.3 (s, C-13' or 13), 136.7 (s, C-13 or C-13'), 137.3 (s, C-5'), 138.0 (d, C-12), 138.1 (d, C-12'); HRFABMS *m/z* 600.4174 (C₄₀H₅₆O₄, calcd for 600.4178).

Acetylation of **3** in dry pyridine with Ac₂O at room temperature produced a diacetate which showed molecular ion at *m/z* 684 by FABMS.

7,8-Didehydrodeepoxyneoxanthin (4): CD (20 μ g/mL Et₂O) λ ($\Delta\epsilon$) nm 240 (–1.5), 255 (–0.5), 280 (–2.0), 350 (0); UV-vis (Et₂O) λ_{\max} 420, 443, 472 nm, %III/II = 50; ¹H NMR (CDCl₃, 500 MHz) δ 1.07 (3H, s, H-17), 1.15 (3H, s, H-16'), 1.20 (3H, s, H-17'), 1.34 (1H, overlapped, H_{ax}-2(β)), 1.34 (3H, s, H-16), 1.35 (3H, s, H-18), 1.41 (1H, dd, *J* = 12.5, 12.5 Hz, H_{ax}-4'(β)), 1.45 (1H, dd, *J* = 12.5, 12.5 Hz, H_{ax}-2'(β)), 1.80 (3H, s, H-19), 1.84 (1H, ddd, *J* = 12.5, 4, 1.5 Hz, H_{eq}-2'(α)), 1.92 (3H, s, H-19'), 1.95 (1H, overlapped, H_{eq}-2(α)), 1.96 (6H, s, H-20, 20'), 2.01 (3H, s, H-19'), 2.07 (1H, dd, *J* = 18, 10 Hz, H_{ax}-4'(β)), 2.27 (1H, ddd, *J* = 12.5, 4, 2 Hz, H_{eq}-4'(α)), 2.43 (1H, ddd, *J* = 18, 5, 1.5 Hz, H_{eq}-4'(α)), 3.99 (1H, m, H-3'), 4.28 (1H, m, H-3), 6.03 (1H, s, H-8), 6.12 (1H, d, *J* = 11.5 Hz, H-10),

6.26 (1H, m, H-14), 6.27 (1H, m, H-14'), 6.36 (1H, d, $J = 15.5$ Hz, H-12'), 6.38 (1H, d, $J = 15.5$ Hz, H-12), 6.46 (1H, d, $J = 11.5$ Hz, H-10'), 6.55 (1H, dd, $J = 15.5, 11.5$ Hz, H-11), 6.63 (1H, dd, $J = 15.5, 11.5$ Hz, H-11), 6.64 (2H, m, H-15, 15'); HRFABMS m/z 582.4078 ($C_{40}H_{54}O_3$, calcd for 582.4073).

Cyclopyrroxanthin (5): CD (20 μ g/mL Et₂O) λ ($\Delta\epsilon$) nm 230 (−1.5), 250 (−2.5), 275 (−1.0), 350 (0); UV-vis (Et₂O) λ_{max} 455 and 475 nm, %III/II = 9; ¹H NMR (CDCl₃, 500 MHz) δ 0.89 (3H, s, H-17), 1.18 (3H, s, H-16'), 1.20 (3H, s, H-17'), 1.22 (3H, s, H-18), 1.47 (3H, s, H-16), 1.57 (1H, dd, $J = 12.5, 12.5$ Hz, H_{ax}-2'(β)), 1.63 (1H, d, $J = 12$ Hz, H_{eq}-2'(β)), 1.71 (1H, d, $J = 12$ Hz, H_{eq}-4'(β)), 1.84 (2H, overlapped, H_{ax}-2(α) and H_{eq}-2'(α)), 1.91 (3H, s, H-18'), 2.00 (3H, s, H-20), 2.04 (1H, ddd, $J = 12, 6, 2$ Hz, H_{ax}-4(α)), 2.05 (3H, s, CH₃CO), 2.13 (1H, dd, $J = 18, 9$ Hz, H_{ax}-4'(β)), 2.24 (3H, s, H-20), 2.50 (1H, ddd, $J = 18, 5.5, 2$ Hz, H_{eq}-4'(α)), 4.41 (1H, t, $J = 6$ Hz, H-3), 5.40 (1H, m, H-3'), 5.72 (1H, s, H-12), 6.41 (1H, dd, $J = 14.5, 11.5$ Hz, H-14'), 6.44 (1H, d, $J = 16$ Hz, H-8), 6.44 (2H, d, $J = 11.5$ Hz, H-10', H-14), 6.50 (1H, dd, $J = 14.5, 11.5$ Hz, H-15), 6.57 (1H, dd, $J = 14.5, 11.5$ Hz, H-11'), 6.64 (1H, dd, $J = 14.5, 11.5$ Hz, H-15), 6.96 (1H, d, $J = 16$ Hz, H-7), 7.01 (1H, s, H-10'); ¹³C NMR (CDCl₃, 125 MHz) δ 15.5 (q, C-20), 18.1 (q, C-19'), 21.4 (q, COCH₃), 22.4 (q, C-18'), 25.5 (q, C-16), 28.7 (q, C-16'), 30.2 (q, C-17'), 31.5 (q, C-18), 32.0 (q, C-17), 36.1 (s, C-1'), 37.5 (t, C-4'), 42.3 (t, C-2'), 44.0 (s, C-1), 47.5 (t, C-4), 48.4 (t, C-2), 67.9 (d, C-3'), 75.5 (d, C-3), 82.3 (s, C-5), 90.0 (s, C-7'), 91.9 (s, C-6), 98.5 (s, C-8'), 118.8 (d, C-12), 119.6 (d, C-8), 120.9 (s, C-9'), 124.3 (s, C-6'), 125.2 (s, C-9), 129.9 (d, C-15'), 130.5 (d, C-11'), 132.3 (d, C-7), 133.8 (d, C-14'), 134.6 (s, C-13), 135.7 (d, C-10'), 136.7 (d, C-10 and C-15), 137.2 (d, C-5'), 137.5 (d, C-14), 147.0 (s, C-11), 168.8 (s, C-19), 170.7 (s, COCH₃); HRFABMS m/z 612.3444 ($C_{39}H_{48}O_6$, calcd for 612.3451).

Hydratoperidin (6): CD (40 μ g/mL Et₂O) λ ($\Delta\epsilon$) nm 240 (−2.5), 253 (−4.0), 325 (−2.0), 350 (0); UV-vis (Et₂O) λ_{max} 455 and 475 nm, %III/II = 9; ¹H NMR (CDCl₃, 500 MHz) δ 0.83 (3H, s, H-16 or 17), 1.07 (3H, s, H-17'), 1.12 (3H, s, H-16 or 17), 1.35 (3H, s, H-18'), 1.39 (3H, s, H-16'), 1.43 (3H, s, H-18), 1.80 (3H, s, H-19'), 2.04 (3H, s, CH₃CO), 2.23 (3H, s, H-20), 4.24 (1H, m, H-3), 5.38 (1H, m, H-3'), 5.73 (1H, s, H-12), 6.06 (1H, s, H-8'), 6.12 (1H, d, $J = 11.5$ Hz, H-10'), 6.39 (1H, dd, $J = 14.5, 11.5$ Hz, H-14'), 6.45 (1H, d, $J = 11.5$ Hz, H-14), 6.51

(1H, dd, $J = 14.5, 11.5$ Hz, H-15'), 6.56 (1H, d, $J = 16$ Hz, H-8), 6.61 (2H, dd, $J = 14.5, 11.5$ Hz H-15, 11'), 7.03 (1H, s, H-10), 7.09 (1H, d, $J = 16$ Hz, H-7); HRFABMS m/z 648.3671 ($C_{39}H_{52}O_8$, calcd for 648.3662); FAB MS/MS m/z 630 [M − H₂O]⁺, 612 [M − 2H₂O]⁺, 588 [M − AcOH]⁺, 570 [M − AcOH − H₂O]⁺, and 552 [M − AcOH − 2H₂O]⁺.

Acetylation of **6** in dry pyridine with Ac₂O at room temperature produced a monoacetate which showed molecular ion at m/z 690.3767 ($C_{41}H_{54}O_9$, calcd for 690.3768) by HRFABMS.

Supporting Information Available: FAB MS/MS of M⁺ of hydratoperidin (6) and peridin. This material is available free charge via the Internet at <http://pubs.acs.org>.

References and Notes

- (1) Matsuno, T.; Hirao, S. In *Marine Biogenic Lipids, Fats, and Oils*; Ackman, R. G., Ed.; CRC Press: Boca Raton, FL, 1989; Vol. 1, pp 251–388.
- (2) (a) Maoka, T. *J. Nat. Prod.* **1997**, *60*, 616–617. (b) Maoka, T.; Hashimoto, K.; Akimoto, N.; Fujiwara, Y. *J. Nat. Prod.* **2001**, *64*, 578–581.
- (3) In the present paper, the IUPAC carotenoid nomenclature was based on the new rules described in the "Carotenoid Handbook". Britton, G.; Liaaen-Jensen, S.; Pfander, H. *Carotenoids Handbook*; Birkhauser Verlag: Basel, 2004.
- (4) Englert, G. NMR Spectroscopy. In *Carotenoids*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhauser Verlag: Basel, 1995; Vol. 1B, pp 147–160.
- (5) Buchecker, R.; Eugster, C. H. *Helv. Chim. Acta* **1980**, *63*, 2531–2537.
- (6) (a) Marki-Fischer, E.; Buchecker, R.; Eugster, C. H. *Helv. Chim. Acta* **1984**, *67*, 2143–2154. (b) Marki-Fischer, E.; Eugster, C. H. *Helv. Chim. Acta* **1985**, *68*, 1704–1707. (c) Marki-Fischer, E.; Eugster, C. H. *Helv. Chim. Acta* **1990**, *73*, 1637–1643. (d) Deli, J.; Molnar, P.; Matus, Z.; Toth, G.; Steck, A.; Pfander, H. *Helv. Chim. Acta* **1998**, *81*, 1233–1241.
- (7) Deli, J.; Molnar, P.; Toth, G.; Baumeler, A.; Eugster, H. *Helv. Chim. Acta* **1991**, *74*, 819–824.
- (8) Haugan, J. A.; Englert, G.; Aakermann, T.; Glinz, E.; Liaaen-Jensen, S. *Acta Chem. Scand.* **1994**, *48*, 769–779.
- (9) Hertzberg, S.; Partail, S.; Liaaen-Jensen, S. *Acta Chem. Scand.* **1988**, *B42*, 495–503.
- (10) Maoka, T.; Fujiwara, Y.; Hashimoto, K.; Akimoto, N. *Lipids* **2004**, *39*, 179–183.
- (11) Matsuno, T.; Tani, Y.; Maoka, T.; Matsuno, K.; Komori, T. *Phytochemistry* **1986**, *25*, 2837–2840.

NP058053N