Structure of New Carotenoids from Corbicula Clam *Corbicula japonica*

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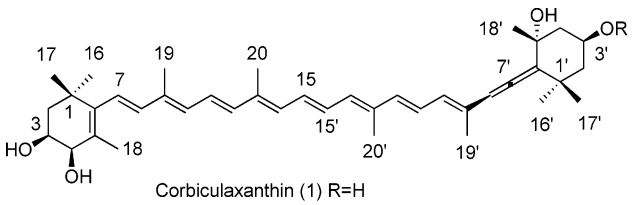
Six new carotenoids, named corbiculaxanthin (**1**), corbiculaxanthin 3′-acetate (**2**), 6-epiheteroxanthin (**3**), 7′,8′-didehydrodeepoxyneoxanthin (**4**), cyclopyrrhoxanthin (**5**), and hydratoperidinin (**6**), were isolated from the corbicula clam (Shijimi in Japanese), *Corbicula japonica*.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

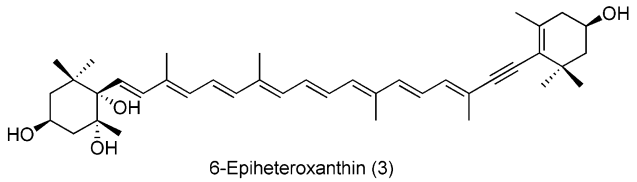
Corbicula clam (Shijimi in Japanese), *Corbicula japonica,* which inhabits brackish water, is an important edible shellfish in Japan. There have been several reports on carotenoids in marine shellfish,[1] but few studies on carotenoids in brackish and freshwater shellfish were carried out.[1] In the 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]

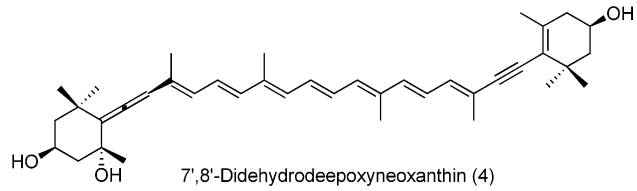
Results and Discussion

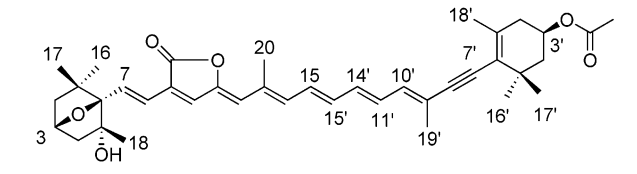
The acetone (Me_2CO) extract of the edible part of *C. japonica* (450 g) was chromatographed on silica gel using an increasing percentage of acetone (Me_2CO) in hexane. The fraction eluted with Me_2CO−hexane (6:4) was subjected to HPLC on silica gel with Me_2CO−hexane (6:4) and then on ODS with $CHCl_3$−MeCN (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).

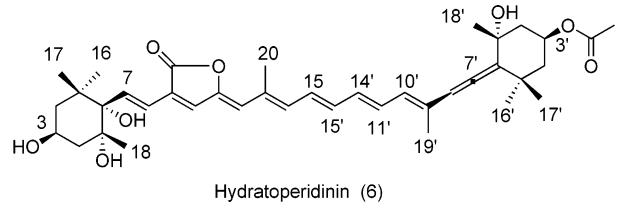
Compound **1**, named corbiculaxanthin, showed UV−vis absorption maxima at 420, 443, and 472 nm. The molecular formula of **1** was determined to be $C_{40}H_{56}O_4$ by HRFABMS data. The presence of three secondary hydroxy groups and one tertiary hydroxy group in **1** was consistent with the formation 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 Hz[4] 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 $C_{42}H_{58}O_5$

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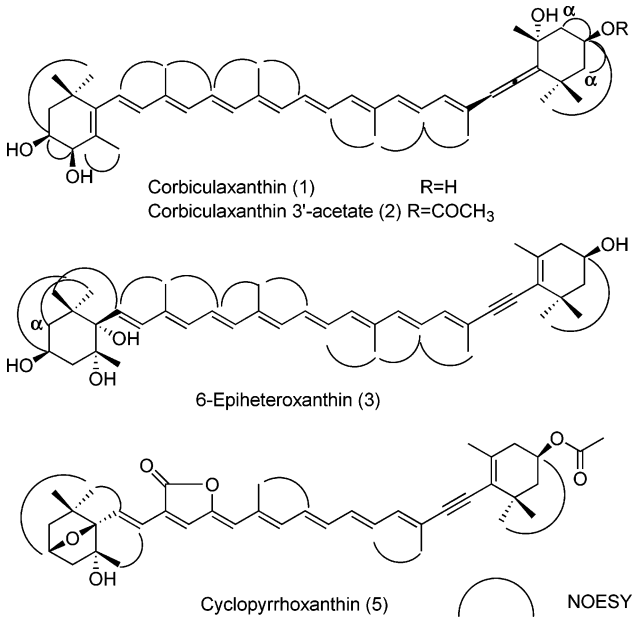

Figure 1. Key NOESY correlations of **1**, **2**, **3**, and **5**.

by HRFABMS data. Compound **2** showed almost the same ^{1}H 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 ^{1}H−^{1}H 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 $C_{40}H_{56}O_4$ by HRFABMS data. The presence of two secondary hydroxy groups and two tertiary hydroxy groups in **3** was revealed by acetylation, HRFABMS, ^{1}H, and ^{13}C NMR data. ^{1}H and ^{13}C 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 ^{1}H−^{1}H 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 ^{1}H and ^{13}C 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 $C_{40}H_{54}O_3$ by HRFABMS data. ^{1}H 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

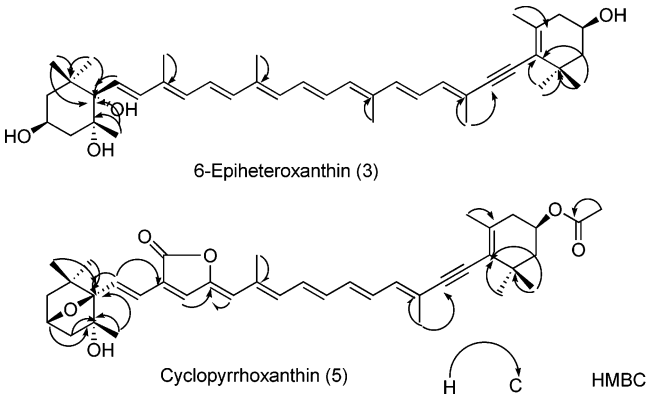

Figure 2. Key HMBC correlations of **3** and **5**.

by the ^{1}H−^{1}H COSY experiment. Therefore, the structure of **4** was determined to be 6,7,7′,8′-tetradehydro-5,6-dihydro-β,β-carotene-3,5,3′-triol, and the compound was named 7′,8′-didehydrodeepoxyneoxanthin. 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 $C_{39}H_{48}O_6$ by HRFABMS data. The ^{1}H and ^{13}C NMR data of **5** were almost identical with those of pyrrhoxanthin[4] except for the signals of the end group (C1 to C6 including C16, C17, and C18). The ^{1}H and ^{13}C 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′-trinor-β,β-caroten-19,11-olide was postulated for **5**. This structure was confirmed by ^{1}H−^{1}H 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 $C_{39}H_{52}O_8$ 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 ^{1}H 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]$^{+\bullet}$, 612 [M − 2H_2O]$^{+\bullet}$, 588 [M − AcOH]$^{+\bullet}$, and 570 [M − AcOH − H_2O]$^{+\bullet}$, 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 ^{1}H 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′- trinor-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 hydratopyrrhoxanthinol. 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 hydratopyrrhoxanthinol. 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 Et_2O at room temperature with a JASCO J-500 spectropolarimeter. The UV−vis spectra were recorded with a Shimadzu U-2001 spectrophotometer in diethyl ether (Et_2O). The ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured with a Varian UNITY INOVA 500 spectrometer in $CDCl_3$ 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 ^{13}C NMR spectra were measured in 0.2 mL of $CDCl_3$ solution using a SHIGEMI tube (Shigemi Co., Ltd, Tokyo, Japan). The positive ion FAB-MS and CID-MS/MS spectra[10] 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 $CHCl_3$ was placed on a stainless steel probe tip, and 1−2 μL 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 $M^{+\bullet}$ 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 clam, *C. 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 Me_2CO extract of the edible parts of *C. japonica* (450 g) was partitioned between diethyl ether (Et_2O) and aqueous NaCl. The organic layer was dried over Na_2SO_4 and then concentrated to dryness. The residue was subjected to silica gel column chromatography using an increasing percentage of Me_2CO in *n*-hexane. The fraction eluted with Me_2CO−hexane (6:4) was subjected to a series of HPLCs on silica gel with Me_2CO−hexane (6:4) and then on ODS with $CHCl_3$−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), pyrrhoxanthin (1.0 mg), and fucoxanthin (0.5 mg).

Corbiculaxanthin (1): CD (20 μg/mL Et_2O) λ ($\Delta\epsilon$) nm 220 (0), 235 (−5.0), 280 (−3.0), 330 (−2.8), 350 (0); UV−vis (Et_2O) λ_{max} 420, 443, 472 nm, %III/II = 50; ^1H NMR ($CDCl_3$, 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 ($C_{40}H_{56}O_4$, calcd for 600.4178).

Acetylation of **1** in dry pyridine with Ac_2O at room temperature produced a triacetate which showed molecular ion at *m/z* 726 by FABMS, and trimethyl silylation of **1** provided tetratrimethylsilyl ether of **1** which showed molecular ion at *m/z* 888 by FABMS.

Corbiculaxanthin 3′-acetate (2): CD (20 μg/mL Et_2O) λ ($\Delta\epsilon$) nm 220 (0), 235 (−5.0), 280 (−3.0), 330 (−2.8), 350 (0); UV−vis (Et_2O) λ_{max} 420, 443, 472 nm, %III/II = 50; ^1H NMR ($CDCl_3$, 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, 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, $COCH_3$), 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 ($C_{42}H_{58}O_5$, calcd for 642.4285).

6-Epiheteroxanthin (3): CD (20 μg/mL Et_2O) λ ($\Delta\epsilon$) nm 230 (−1.0), 240 (−2.0), 250 (−3.0), 320 (−1.2), 370 (0); UV−vis (Et_2O) λ_{max} 420, 443, 472 nm, %III/II = 50; ^1H NMR ($CDCl_3$, 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′); ^{13}C NMR ($CDCl_3$, 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 ($C_{40}H_{56}O_4$, calcd for 600.4178).

Acetylation of **3** in dry pyridine with Ac_2O at room temperature produced a diacetate which showed molecular ion at *m/z* 684 by FABMS.

7′,8′-Didehydrodeepoxyneoxanthin (4): CD (20 μg/mL Et_2O) λ ($\Delta\epsilon$) nm 240 (−1.5), 255 (−0.5), 280 (−2.0), 350 (0); UV−vis (Et_2O) λ_{max} 420, 443, 472 nm, %III/II = 50; ^1H NMR ($CDCl_3$, 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).

Cyclopyrrhoxanthin (5): CD (20 μg/mL Et_2O) λ (Δε) nm 230 (−1.5), 250 (−2.5), 275 (−1.0), 350 (0); UV−vis (Et_2O) λ_{max} 455 and 475 nm, %III/II = 9; ^{1}H NMR ($CDCl_3$, 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, CH_3CO), 2.13 (1H, dd, J = 18, 9 Hz, H_{aq}-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′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 15.5 (q, C-20), 18.1 (q, C-19′), 21.4 (q, CO*C*H_3), 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, *C*OCH_3); HRFABMS *m/z* 612.3444 ($C_{39}H_{48}O_6$, calcd for 612.3451).

Hydratoperidinin (6): CD (40 μg/mL Et_2O) λ (Δε) nm 240 (−2.5), 253 (−4.0), 325 (−2.0), 350 (0); UV−vis (Et_2O) λ_{max} 455 and 475 nm, %III/II = 9; ^{1}H NMR ($CDCl_3$, 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, CH_3CO), 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 $[M - H_2O]^{+\bullet}$, 612 $[M - 2H_2O]^{+\bullet}$, 588 $[M - AcOH]^{+\bullet}$, 570 $[M - AcOH - H_2O]^{+\bullet}$, and 552 $[M - AcOH - 2H_2O]^{+\bullet}$.

Acetylation of **6** in dry pyridine with Ac_2O 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 $M^{+\bullet}$ of hydratoperidinin (**6**) and peridinin. This material is available free charge via the Internet at http://pubs.acs.org.

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