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Carotenoids in Three Species of Corbicula Clams, *Corbicula japonica*, *Corbicula sandai*, and *Corbicula* sp. (Chinese Freshwater Corbicula Clam)

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Carotenoids were investigated in three species of corbicula clams, *Corbicula japonica*, *Corbicula sandai*, and *Corbicula* sp. (Chinese freshwater corbicula clam). Forty-three carotenoids were isolated. Among them, 7,8-didehydro-β-cryptoxanthin (**12**), peridininol 5,8-furanoxide (**38**), pyrrhoxanthin 5,8-furanoxide (**40**), and pyrrhoxanthinol 5,8-furanoxide (**43**) are newly reported as naturally occurring carotenoids. Their structures were characterized on the basis of UV−vis, FAB-MS including MS/MS experiments, and ^{1}H NMR spectroscopic data. The total carotenoid contents in *C. japonica*, *C. sandai*, and Chinese freshwater corbicula clam were found to be 5.3, 2.6, and 0.3 mg/100 g in the edible part (wet weight), respectively. Peridinin (**34**) and its derivatives were found to be major carotenoids in *C. japonica*, which inhabits brackish water. On the other hand, lutein (**13**) was found to be the major carotenoid in *C. sandai* and Chinese corbicula clams, which inhabit freshwater. These patterns well reflected the carotenoids in their dietary algae. 7′,8′-Didehydrodeepoxyneoxanthin (**19**), corbiculaxanthin (**21**), corbiculaxanthin 3′-acetate (**22**), and 6-epiheteroxanthin (**24**) were found in all three species of corbicula clams and have not previously been found in other shellfishes. They were assumed to be peculiar carotenoids in corbicula clams.

KEYWORDS: Carotenoids; shellfish; *Corbicula japonica*; *Corbicula sandai*; FAB MS/MS; NMR

INTRODUCTION

Shellfish, which contain various carotenoids, are an important seafood in Japan. There have been several reports on carotenoids in marine shellfish (*1*). Mytiloxanthin and isomytiloxanthin from *Mytilus edulis* (*2*, *3*), diatoxanthin, alloxanthin, and pectinolone from *Pectene maximus* (*4*) and *Patinopectene yessoensis* (*5*), pectinols A and B from *Mytilus coruscus* (*6*), mactraxanthin from *Mactra chinensis* (*7*), crassostreaxanthins A and B from *Crassostrea gigas* (*8*), and a series of carotenoids with a 5,6-dihydro-β-end group from *Fushinus perplexus* (*9*) have been reported as principal carotenoids in marine shellfish.

However, there are few reports on carotenoids in brackish water and freshwater shellfish. Corbicula clams (shijimi in Japanese), which inhabit brackish or freshwater, are one of the most important edible shellfish in Japan. In the course of studies of the carotenoids in shellfish (*10*, *11*), carotenoids in three species of corbicula clams, *Corbicula japonica*, *Corbicula sandai*, and *Corbicula* sp. (Chinese freshwater corbicula clam) were investigated. Forty-three carotenoids were isolated from these clams and their identities established by spectroscopic methods. In the present paper, we describe the carotenoid contents, compositions, and identifications in three species of corbicula clams. Furthermore, the origin of carotenoids in corbicula clams is discussed.

MATERIALS AND METHODS

Apparatus. The UV−vis spectra were recorded with a Shimadzu U-240 spectrophotometer in diethyl ether (Et_2O). The positive ion FAB-MS and MS/MS spectra (*12*, *13*) were recorded using a JEOL JMS-HX/HX 110A four-sector tandem mass spectrometer with *m*-nitrobenzyl alcohol as a matrix. The 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*-nitrobenzyl alcohol 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 collision with argon gas in the collision cell, floated at a potential of 3 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 by linked scanning on MS2. The ^{1}H NMR (500 MHz) spectra were measured with a Varian Unity INOVA 500

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spectrometer in $CDCl_3$ with TMS as an internal standard. The CD spectra were recorded in Et_2O at room temperature with a JASCO J-500 spectropolarimeter. HPLC was performed on a Shimadzu LC-6AD with a Shimadzu SPD-6AV spectrophotometer set at 450 nm.

Animal Materials. Corbicula clams (belonging to Corbiculidae), *C. japonica* (yamatoshijimi in Japanese, grown in Lake Shinji, Shimane Prefecture, average shell length of 4 cm), *C. sandai* (setashijimi in Japanese, grown in Lake Biwa, Shiga Prefecture, average shell length of 3 cm), and *Corbicula* sp. (Chinese freshwater corbicula clam, imported from China as shucked and frozen matter), were purchased at a local fish market in January, February, and March.

Extraction and Isolation of Carotenoids. The edible part of *C. japonica* (450 g, about 700 specimens), *C. sandai* (120 g, about 250 specimens), and Chinese freshwater corbicula clam (2000 g) were extracted with Me_2CO at room temperature, respectively. The Me_2CO extract was partitioned between 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 (300 × 20 mm) using an increasing percentage of Me_2CO in *n*-hexane.

In the case of *C. sandai* and Chinese freshwater corbicula clam, the fraction eluted with 200 mL of hexane (fraction 1) was subjected to preparative HPLC on ODS, LiChrospher RP-18 (e) (particle size of 10 μm) 250 × 10 mm (Cica-Merck) with a CH_3CN flow rate of 2.0 mL/min to yield α-carotene [**1**, retention time (t_R) of 63.0 min] and *β*-carotene (**2**, t_R of 64.0 min). The fraction eluted with 200 mL of Me_2CO/hexane (3:7) (fraction 2) was subjected to preparative HPLC on ODS with $CHCl_3/CH_3CN$ (1:9) at a flow rate of 2.0 mL/min to yield canthaxanthin (**5**, t_R of 21.7 min), semi-α-carotenone (**6**, t_R of 25.8 min), semi-*β*-carotenone (**7**, t_R of 27.8 min), anhydrolutein I (**10**, t_R of 30.7 min), crocoxanthin (**11**, t_R of 36.8 min), 7,8-didehydro-*β*-cryptoxanthin (**12**, t_R of 41.2 min), isocryptoxanthin (**9**, t_R of 43.0 min), α-echinenone (**3**, t_R of 44.4 min), and *β*-echinenone (**4**, t_R of 46.9 min). The fraction eluted with 300 mL of Me_2CO/hexane (5:5) (fraction 3) was subjected to preparative HPLC on ODS with $CHCl_3/CH_3CN$ (1:9) at a flow rate of 2.0 mL/min to yield *β*-carotenone (**8**, t_R of 9.0 min), cycloviolaxanthin (**25**, t_R of 13.0 min), diatoxanthin 3,6-epoxide (**26**, t_R of 13.5 min), diadinochrome (**28**, t_R of 14.0 min), mutatoxanthin (**29**, t_R of 14.5 min), diadinoxanthin (**27**, t_R of 15.0 min), alloxanthin (**16**, t_R of 16.3 min), 9-*Z*-alloxanthin (**16b**, t_R of 16.3 min), diatoxanthin (**15**, t_R of 18.5 min), lutein (**13**, t_R of 20.0 min), and zeaxanthin (**14**, t_R of 20.8 min). The fraction eluted with 300 mL of Me_2CO/hexane (6:4) (fraction 4) was subjected to preparative HPLC on ODS with $CHCl_3/CH_3CN$ (1:9) at a flow rate of 2.0 mL/min to yield fucoxanthin (**30**, t_R of 9.2 min), corbiculaxanthin acetate (**22**, t_R of 10.0 min), pectinol A (**17**, t_R of 11.0 min), loroxanthin (**18**, t_R of 12.0 min), halocynthiaxanthin (**32**, t_R of 12.6 min), and crassostreaxanthin A (**33**, t_R of 13.5 min). The fraction eluted with 300 mL of Me_2CO/hexane (7:3) (fraction 5) was subjected to preparative HPLC on ODS with $CHCl_3/CH_3CN$ (1:9) at a flow rate of 2.0 mL/min to yield fucoxanthinol (**31**, t_R of 9.0 min), heteroxanthin (**23**, t_R of 10.0 min), 6-epiheteroxanthin (**24**, t_R of 11.0 min), corbiculaxanthin (**21**, t_R of 12.0 min), 7′,8′-didehydrodeepoxyneoxanthin (**19**, t_R of 12.6 min), and (3*S*,4*R*,3′*S*,4′*R*)-crustaxanthin (**20**, t_R of 15.5 min).

In the case of *C. japonica*, in addition to 33 carotenoids described above, the following 10 carotenoids were isolated. The fraction eluted with 300 mL of Me_2CO/hexane (5:5) (fraction 3) was subjected to preparative HPLC on ODS with $CHCl_3/CH_3CN$ (1:9) at a flow rate of 2.0 mL/min to additionally yield pyrrhoxanthin (**39**, t_R of 10.0 min), pyrrhoxanthin 5,8-furanoxide (**40**, t_R of 11.5 min), and cyclopyrrhoxanthin (**41**, t_R of 12.5 min). The fraction eluted with Me_2CO/hexane (6:4) (fraction 4) was subjected to preparative HPLC on ODS with $CHCl_3/CH_3CN$ (1:9) at a flow rate of 2.0 mL/min to additionally yield hydratoperidinin (**36**, t_R of 7.1 min), peridinin (**34**, t_R of 8.2 min), peridinin 5,8-furanoxide (**35**, t_R of 9.0 min), pyrrhoxanthinol (**42**, t_R of 10.6 min), and pyrrhoxnthinol 5,8-furanoxide (**43**, t_R of 11.5 min). The fraction eluted with Me_2CO/hexane (7:3) (fraction 5) was subjected to preparative HPLC on ODS with $CHCl_3/CH_3CN$ (1:9) at a flow rate of 2.0 mL/min to additionally yield peridininol (**37**, t_R of 8.0 min) and peridininol 5,8-furanoxide (**38**, t_R of 8.5 min).

Identification and Characterization of Carotenoids. α-*Carotene (1)*: UV−vis λ_{max} (Et_2O) 425, 443, and 472 nm; FAB MS/MS, *m*/*z* 536 $[M]^+$, 480 $[M-56]^+$, 444 $[M-92]^+$, 413 $[M-123]^+$; ^1H NMR, chemical shift, and spin coupling values of **1** were in agreement with previously published values (*14*).

β-Carotene (2): UV−vis λ_{max} (Et_2O) 425, 449, and 475 nm; FAB MS/MS, *m*/*z* 536 $[M]^+$, 444 $[M-92]^+$, 399 $[M-137]^+$; ^1H NMR, chemical shift, and spin coupling values of **2** were in agreement with previously published values (*14*).

α-*Echinenone (3):* UV−vis λ_{max} (Et_2O) 448 and 473 nm; FAB MS/MS, *m*/*z* 550 $[M]^+$, 494 $[M-56]^+$, 458 $[M-92]^+$, 427 $[M-123]^+$; ^1H NMR and CD spectroscopic data were in agreement with previously published values (*15*).

β-Echinenone (4): UV−vis λ_{max} (Et_2O) 455 nm; FAB MS/MS, *m*/*z* *m*/*z* 550 $[M]^+$, 456 $[M-92]^+$, 413 $[M-137]^+$; ^1H NMR, chemical shift, and spin coupling values of **2** were in agreement with previously published values (*14*).

Semi-α-carotenone (6): UV−vis λ_{max} (Et_2O) 463 and 487 nm; FAB MS/MS, *m*/*z* 568 $[M]^+$, 553 $[M-15]^+$, 525 $[M-43]^+$, 512 $[M-56]^+$, 476 $[M-92]^+$, 445 $[M-123]^+$, 441, 413 $[M-155]^+$; ^1H NMR δ ($CDCl_3$) 0.82 (3H, s, H-17′), 0.90 (3H, s, H-16′), 1.17 (6H, s, H-16, 17), ∼1.18 (1H, overlapped, H-2′), ∼1.44 (5H, overlapped, H-2, 3, 2′α), 1.60 (3H, s, H-18′), 1.92 (3H, s, H-19′), 1.98 (12H, s, H-19, 20, 19′, 20′), ∼2.01 (2H, m, H-3′), 2.11 (3H, s, H-18), 2.18 (1H, d, *J* = 9.5 Hz, H-6′), 2.40 (2H, t, *J* = 7 Hz, H-3), 5.41 (1H, br s, H-5′), 5.53 (1H, dd, *J* = 15.5, 9.5 Hz, H-7′), 6.11 (1H, d, *J* = 15.5 Hz, H-8′), 6.13 (1H, d, *J* = 11.5 Hz, H-10′), 6.25 (1H, d, *J* = 11 Hz, H-14′), 6.36 (1H, d, *J* = 15.5 Hz, H-12′), 6.36 (1H, d, *J* = 11.5 Hz, H-14), 6.52 (2H, d, *J* = 15.5 Hz, H-7, 12), 6.56 (1H, d, *J* = 11.5 Hz, H-10), 6.62 (1H, dd, *J* = 15.5, 11.5 Hz, H-11), 6.65 (1H, dd, *J* = 15.5, 11.5 Hz, H-11′), 6.68 (1H, dd, *J* = 15.5, 11.5 Hz, H-15 or 15′), 6.71 (1H, dd, *J* = 15.5, 11.5 Hz, H-15 or 15′), 7.40 (1H, d, *J* = 15.5, H-7).

Semi-β-carotenone (7): UV−vis λ_{max} (Et_2O) 467 and 492 nm; FAB MS/MS, *m*/*z* 568 $[M]^+$, 553 $[M-15]^+$, 525 $[M-43]^+$, 476 $[M-92]^+$, 455, 441 $[M-127]^+$, 431 $[M-137]^+$, 413 $[M-155]^+$; ^1H NMR δ ($CDCl_3$) 1.03 (6H, s, H-16′, 17′), 1.17 (6H, s, H-16, 17), ∼1.45 (5H, overlapped, H-2, 3, 2′), ∼1.62 (2H, m, H-3′), 1.72 (3H, s, H-18′), 1.98 (12H, s, H-19, 20, 19′, 20′), 2.02 (2H, t, *J* = 7 Hz, H-4′), 2.11 (3H, s, H-18), 2.40 (2H, t, *J* = 7 Hz, H-3), 6.14 (1H, d, *J* = 15.5 Hz, H-8′), 6.16 (1H, d, *J* = 11.5 Hz, H-10′), 6.17 (1H, d, *J* = 15.5 Hz, H-7′), 6.26 (2H, d, *J* = 11.5 Hz, H-14′), 6.36 (1H, d, *J* = 15.5 Hz, H-12′), 6.36 (1H, d, *J* = 11.5 Hz, H-14), 6.52 (2H, d, *J* = 15.5 Hz, H-7, 12), 6.56 (1H, d, *J* = 11.5 Hz, H-10), 6.62 (1H, dd, *J* = 15.5, 11.5 Hz, H-11), 6.65 (1H, dd, *J* = 15.5, 11.5 Hz, H-11′), 6.68 (1H, dd, *J* = 15.5, 11.5 Hz, H-15 or 15′), 6.71 (1H, dd, *J* = 15.5, 11.5 Hz, H-15 or 15′), 7.40 (1H, d, *J* = 15.5, H-7).

β-Carotenone (8): UV−vis λ_{max} (Et_2O) 440, 468, and 500 nm; FAB MS/MS, *m*/*z* 600 $[M]^+$, 585 $[M-15]^+$, 582, 571, 557 $[M-43]^+$, 508 $[M-92]^+$, 487, 473 $[M-127]^+$, 445 $[M-155]^+$; ^1H NMR δ ($CDCl_3$) 1.17 (12H, s, H-16, 17, 16′, 17′), ∼1.45 (8H, overlapped, H-2, 3, 2′, 3′), 1.98 (12H, s, H-19, 20, 19′, 20′), 2.11 (6H, s, H-18, 18′), 2.40 (4H, t, *J* = 7 Hz, H-3, 3′), 6.36 (2H, m, H-14, 14′), 6.53 (2H, d, *J* = 15.5 Hz, H-7, 7′), 6.54 (2H, d, *J* = 15.5 Hz, H-12, 12′), 6.56 (2H, d, *J* = 11.5 Hz, H-10, 10′), 6.65 (2H, dd, *J* = 15.5, 11.5 Hz, H-11, 11′), 6.70 (2H, m, H-15, 15′), 7.39 (2H, d, *J* = 15.5, H-7, 7′).

Anhydrolutein I (10): UV−vis λ_{max} (Et_2O) 420, 443, and 472 nm; FAB MS/MS, *m*/*z* 550 $[M]^+$, 535 $[M-15]^+$, 532 $[M-H_2O]^+$, 458 $[M-92]^+$, 428 $[M-122]^+$, 397 $[M-153]^+$; ^1H NMR, chemical shift, and spin coupling values of **10** were in agreement with previously published values (*16*).

Crocoxanthin (11): UV−vis λ_{max} (Et_2O) 420, 443, and 472 nm; FAB MS/MS, *m*/*z* 550 $[M]^+$, 535 $[M-15]^+$, 532 $[M-H_2O]^+$, 494 $[M-56]^+$, 458 $[M-92]^+$, 427 $[M-123]^+$; ^1H NMR δ ($CDCl_3$) 0.82 (3H, s, H-17′), 0.90 (3H, s, H-16′), 1.15 (3H, s, H-16), ∼1.18 (1H, overlapped, H-2′*β*), 1.20 (3H, s, H-17), ∼1.44 (2H, overlapped, H-2*β*, H-2′α), 1.60 (3H, s, H-18′), 1.84 (1H, ddd, *J* = 12.5, 4, 2 Hz, H-2α), 1.92 (3H, s, H-19′), 1.93 (3H, s, H-18), 1.95 (3H, s, H-20 or 20′), 1.96 (3H, s, H-20′ or 20), 2.01 (3H, s, H-19), ∼2.01 (2H, m, H-3′), 2.07 (1H, dd, *J* = 18, 10 Hz, H-4*β*), 2.18 (1H, d, *J* = 9.5 Hz, H-6′), 2.43 (1H, ddd, *J* = 18, 5, 1.5 Hz, H-4α), 3.99 (1H, m H-3), 5.41 (1H, br s, H-5′), 5.53 (1H, dd, *J* = 15.5, 9.5 Hz, H-7′), 6.11 (1H, d, *J* = 15.5 Hz, H-8′), 6.13 (1H, d, *J* = 11.5 Hz, H-10′), 6.25 (1H, d, *J* = 11 Hz, H-14′), 6.27 (1H, d, *J* = 11 Hz, H-14), 6.34 (1H, d, *J* = 15.5 Hz, H-12), 6.36

(1H, d, J = 15.5 Hz, H-12′), 6.46 (1H, d, J = 11.5 Hz, H-10), 6.51 (1H, dd, J = 15.5, 11.5 Hz, H-11), 6.61 (1H, dd, J = 15.5, 11.5 Hz, H-11′), 6.63 (2H, m, H-15, 15′).

*7,8-Didehydro-β-cryptoxanthin (**12**):* UV−vis λ_{max} (Et_2O) 425, 451, and 479 nm; HRMS-FAB (*m*/*z*), $[M]^+$ calcd for $C_{40}H_{54}O$ 550.4175, found 5550.4171; FAB MS/MS, *m*/*z* 550 $[M]^+$, 535 $[M - 15]^+$, 532 $[M - H_2O]^+$, 517 $[M - 33]^+$, 479, 458 $[M - 92]^+$, 427 $[M - 123]^+$, 413 $[M - 137]^+$; ^{1}H NMR, chemical shift, and spin coupling values of **12** were in agreement with previously published values (*17*).

*7′,8′-Didehydrodeepoxyneoxanthin (**19**):* UV−vis λ_{max} (Et_2O) 420, 443, and 472 nm; HRMS-FAB (*m*/*z*), $[M]^+$ calcd for $C_{40}H_{54}O_3$ 582.4073, found 582.4078; FAB MS/MS, *m*/*z* 582 $[M]^+$, 567 $[M - 15]^+$, 564 $[M - H_2O]^+$, 549 $[M - 33]^+$; ^{1}H NMR data were described elsewhere (*18*).

*(3S,4R,3′S,4′R)-Crustaxanthin (**20**):* UV−vis λ_{max} (Et_2O) 425, 449, and 475 nm; FAB MS/MS, *m*/*z* 600 $[M]^+$, 582 $[M - 18]^+$, 508 $[M - 92]^+$, 473, 447; ^{1}H NMR δ ($CDCl_3$) 1.07 (6H, s, H-17, 17′), 1.09 (6H, s, H-16, 16′), 1.57 [2H, ddd, J = 12.5, 4, 1 Hz, H-2, 2′ eq (α)], 1.68 [2H, dd, J = 12.5, 12.5 Hz, H-4, 4′ ax (β)], 1.90 (6H, s, H-18, 18′), 1.97 (6H, s, H-19, 19′), 1.98 (6H, s, H-20, 20′), 3.86 (2H, ddd, J = 12.5, 8, 3.5 Hz, H-3, 3′), 3.96 (2H, d, J = 3.5 Hz, H-4, 4′), 6.07 (2H, d, J = 16 Hz, H-7, 7′), 6.10 (2H, d, J = 11.5 Hz, H-10, 10′), 6.18 (2H, d, J = 16 Hz, H-8, 8′), 6.27 (2H, m, H-14, 14′), 6.34 (2H, d, J = 15.5 Hz, H-12, 12′), 6.55 (2H, dd, J = 15.5, 11.5 Hz, H-11, 11′), 6.65 (2H, m, H-15, 15′); CD (Et_2O) λ (Δε) 213 (0), 224 (−20.0), 236 (0), 245 (+20.0), 262 (0), 284 (−25.0), 332 (0), 350 (+4.0).

*Corbiculaxanthin (**21**):* UV−vis λ_{max} (Et_2O) 420, 443, and 472 nm; HRMS-FAB (*m*/*z*), $[M]^+$ calcd for $C_{40}H_{56}O_4$ 600.4178, found 600.4171; FAB MS/MS, *m*/*z* 600 $[M]^+$, 582 $[M - H_2O]^+$, 564 $[M - 2H_2O]^+$, 508 $[M - 92]^+$, 447 $[M - 153]^+$; ^{1}H NMR data were described elsewhere (*18*).

*Corbiculaxanthin acetate (**22**):* UV−vis λ_{max} (Et_2O) 420, 443, and 472 nm; HRMS-FAB (*m*/*z*), $[M]^+$ calcd for $C_{42}H_{58}O_5$ 642.4285, found 642.4290; FAB MS/MS, *m*/*z* 642 $[M]^+$, 624 $[M - H_2O]^+$, 606 $[M - 2H_2O]^+$, 599, 583, 582 $[M - AcOH]^+$, 550 $[M - 92]^+$, 489 $[M - 153]^+$; ^{1}H NMR data were described elsewhere (*18*).

*6-Epiheteroxanthin (**24**):* UV−vis λ_{max} (Et_2O) 420, 443, and 472 nm; HRMS-FAB (*m*/*z*), $[M]^+$ calcd for $C_{40}H_{56}O_4$ 600.4148, found 600.4174; FAB MS/MS, *m*/*z* 600 $[M]^+$, 585 $[M - 15]^+$, 582 $[M - H_2O]^+$, 567 $[M - 33]^+$, 508 $[M - 92]^+$, 447 $[M - 153]^+$; ^{1}H NMR data were described elsewhere (*18*).

*Peridinin 5,8-furanoxide (**35**):* UV−vis λ_{max} (Et_2O) 445 and 475 nm; HRMS-FAB (*m*/*z*), $[M]^+$ calcd for $C_{37}H_{48}O_6$ 588.3450, found, 588.3456; FAB MS/MS, *m*/*z* 588 $[M]^+$, 570 $[M - H_2O]^+$, 552 $[M - 2H_2O]^+$, 469, 435; ^{1}H NMR, chemical shift, and spin coupling values of **35** were in agreement with previously published values (*19*).

*Hydratoperidinin (**36**):* UV−vis λ_{max} (Et_2O) 455 and 475 nm; HRMS-FAB (*m*/*z*), $[M]^+$ calcd for $C_{39}H_{52}O_8$ 648.3662, found 648.3671; FAB MS/MS, *m*/*z* 648 $[M]^+$, 630 $[M - H_2O]^{\bullet+}$, 612 $[M - 2H_2O]^{\bullet+}$, 588 $[M - AcOH]^{\bullet+}$, 570 $[M - AcOH - H_2O]^{\bullet+}$, and 552 $[M - AcOH - 2H_2O]^{\bullet+}$; ^{1}H NMR data were described elsewhere (*18*).

*Peridininol 5,8-furanoxide (**38**):* UV−vis λ_{max} (Et_2O) 445 and 475 nm; HRMS-FAB (*m*/*z*), $[M]^+$ calcd for $C_{37}H_{48}O_6$ 588.3450, found 588.3456; FAB MS/MS, *m*/*z* 588 $[M]^+$, 570 $[M - H_2O]^+$, 552 $[M - 2H_2O]^+$, 469, 435; ^{1}H NMR δ ($CDCl_3$) 1.07 (6H, s, H-17′), 1.16 [3H, s, H-17 (8*R*)], 1.20 [3H, s H-17 (8*S*)], 1.32 [3H, s, H-16 (8*S*)], 1.34 [9H, s, H-16 (8*R*), 16′], 1.34 (2H, overlapped, H-2′ β), 1.35 (6H, s, H-17′), 1.41 (2H, dd, J = 12.2, 12.5 Hz, H-2′β), ∼1.49 [2H, overlapped, H-2α (8*R* and *S*)], 1.67 [3H, s, H-19 (8*R*)], 1.69 [3H, s, H-19 (8*S*)], 1.76 [2H, overlapped, H-2β (8*R* and *S*)], 1.80 (6H, s, H-18′), ∼1.94 [4H, overlapped, H-4α (8*R* and *S*), H-2′α], 2.18 [2H, overlapped, H-4β (8*R* and *S*)], 2.22 (6H, s, H-20), 2.27 (2H, ddd, J = 12.5, 4.5, 2 Hz, H-4α), 4.24 [1H, m, H-3 (8*R*)], 4.28 [1H, m, H-3 (8S)], 4.32 (2H, m, H-3′), 5.51 [1H, br s, H-8 (8*S*)], 5.53 [1H, br s, H-7 (8*R*)], 5.62 [1H, br s, H-8 (8*R*)], 5.64 [1H, d, J = 2 Hz, H-7 (8*S*)], 5.71 [1H, s, H-12 (8*R*)], 5.73 [1H, s H-12 (8*S*)], 6.04 (2H, s, H-8′), 6.10 (2H, d, J = 11.5 HZ, H-10′), 6.35−6.55 (10H, overlapped, olefinic H), 7.17 [1H, s, H-10 (8*R*)], 7.20 [1H, s H-10 (8*S*)].

*Pyrrhoxanthin 5,8-furanoxide (**40**):* UV−vis λ_{max} (Et_2O) 447 and 475 nm; HRMS-FAB (*m*/*z*), $[M]^+$ calcd for $C_{39}H_{48}O_6$ 612.3451, found 612.3461; FAB MS/MS, *m*/*z* 612 $[M]^+$, 597 $[M - 15]^+$, 594 $[M - H_2O]^+$, 579 $[M - 33]^+$, 552 $[M - AcOH]^+$, 537, 400; ^{1}H NMR δ ($CDCl_3$) 1.15 (6H, s, H-16′), 1.16 [3H, s, H-17 (8*R*)], 1.20 [9H, s H-17 (8*S*), H-17′], 1.32 [3H, s, H-16 (8*S*)], 1.34 [3H, s, H-16 (8*R*)], ∼1.49 [2H, overlapped, H-2α (8*R* and *S*)], ∼1.57 (2H, dd, J = 12.5, 12.5 Hz, H-2′β), 1.67 [3H, s, H-19 (8*R*)], 1.69 [3H, s, H-19 (8*S*)], 1.76 [2H, overlapped, H-2β (8*R* and *S*)], 1.83 (2H, ddd, J = 12.5, 4, 1.5 Hz, H-2′α), 1.92 (6H, s, H-19′), ∼1.94 [2H, overlapped, H-4 (8*R* and *S*)], 2.01 (6H, s, H-19′), 2.04 (6H, s, Ac), ∼2.18 [4H, overlapped, H-4β (8*R* and *S*), H-4′β], 2.22 (6H, s, H-20), 2.49 (2H, ddd, J = 18, 5.5, 1.5 Hz, H-4′α), 4.24 [1H, m, H-3 (8*R*)], 4.28 [1H, m, H-3 (8*S*)], 5.04 (2H, m, H-3′), 5.51 [1H, br s, H-8 (8*S*)], 5.53 [1H, br s, H-7 (8*R*)], 5.62 [1H, br s, H-8 (8*R*)], 5.64 [1H, d, J = 2 Hz, H-7 (8*S*)], 5.71 [1H, s, H-12 (8*R*)], 5.73 [1H, s H-12 (8*S*)], 6.35−6.55 (12H, overlapped, olefinic H), 7.17 [1H, s, H-10 (8*R*)], 7.20 [1H, s H-10 (8*S*)].

*Cyclopyrrohoxanthin (**41**):* UV−vis λ_{max} (Et_2O) 455 and 475 nm; HR-MS FAB (*m*/*z*), $[M]^+$ calcd for $C_{39}H_{48}O_6$ 612.3451, found 612.3444; FAB MS/MS, *m*/*z* 612 $[M]^+$, 597 $[M - 15]^+$, 594 $[M - H_2O]^{\bullet+}$, 579 $[M - 33]^+$, 552 $[M - AcOH]^{\bullet+}$, 537 $[M - 75]^{\bullet+}$, 517, 509; ^{1}H NMR data were described elsewhere (*18*).

*Pyrrhoxanthinol 5,8-furanoxide (**43**):* UV−vis λ_{max} (Et_2O) 447 and 475 nm; HRMS-FAB (*m*/*z*), $[M]^+$ calcd for $C_{37}H_{48}O_6$ 570.3345, found 570.3336; FAB MS/MS, *m*/*z* 570 $[M]^+$, 555 $[M - 15]^+$, 552 $[M - H_2O]^+$, 537 $[M - 33]^+$, 496; ^{1}H NMR δ ($CDCl_3$) 1.15 (6H, s, H-16′), 1.16 [3H, s, H-17 (8*R*)], 1.20 [9H, s H-17 (8*S*), H-17′], 1.32 [3H, s, H-16 (8*S*)], 1.34 [9H, s, H-16 (8*R*), 16′], ∼1.48 [4H, overlapped, H-2α (8*R* and 8*S*), H-2′β], 1.67 [3H, s, H-19 (8*R*)], 1.69 [3H, s, H-19 (8*S*)], 1.76 [2H, overlapped, H-2β (8*R* and *S*)], 1.84 (2H, ddd, J = 12.5, 4, 1.5 Hz, H-2′α), 1.92 (6H, s, H-19′), ∼1.94 [2H, overlapped, H-4α (8*R* and *S*)], 2.01 (6H, s, H-19′), 2.07 (2H, dd, J = 18, 10 Hz, H-4′β), 2.18 [4H, overlapped, H-4β (8*R* and *S*), H-4′β], 2.22 (6H, s, H-20), 2.43 (2H, ddd, J = 18, 5.5, 1.5 Hz, H-4′α), 3.99 (2H, m, H-3′), 4.24 [1H, m, H-3 (8*R*)], 4.28 [1H, m, H-3 (8*S*)], 5.51 [1H, br s, H-8 (8*S*)], 5.53 [1H, br s, H-7 (8*R*)], 5.62 [1H, br s, H-8 (8*R*)], 5.64 [1H, d, J = 2 Hz, H-7 (8*S*)], 5.71 [1H, s, H-12 (8*R*)], 5.73 [1H, s H-12 (8*S*)], 6.35−6.55 (12H, overlapped, olefinic H), 7.17 [1H, s, H-10 (*8R*)], 7.20 [1H, s. H-10 (8*S*)].

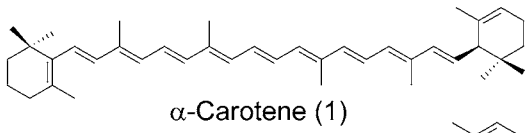
Identification of canthaxanthin (**5**), isocryptoxanthin (**9**), lutein (**13**), zeaxanthin (**14**), diatoxanthin (**15**), alloxanthin (**16**), pectinol A (**17**), loroxanthin (**18**), heteroxanthin (**23**), cycloviolaxanthin (**25**), diatoxanthin 3,6-epoxide (**26**), diadinoxanthin (**27**), diadinochrome (**28**), mutatoxanthin (**29**), fucoxanthin (**30**), fucoxanthinol (**31**), halocynthiaxanthin (**32**), crassostreaxanthin A (**33**), peridinin (**34**), peridininol (**37**), pyrrhoxanthin (**39**), and pyrrhoxanthinol (**42**) was based on comparison of spectroscopic data and chromatographic behavior with our authentic samples.

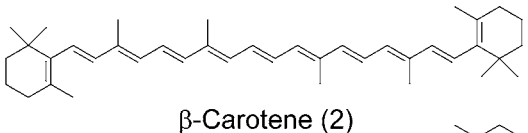
Quantification of Carotenoids. The total carotenoid content and the amount of carotenoids eluted from column chromatography were calculated using the extinction coefficient of $E_{cm}^{1\%} = 2500$ at λ_{max} (*20*). In the HPLC analysis, the relative amounts of individual carotenoids were calculated from peak area detected at 450 nm.

RESULTS AND DISCUSSION

Identification and Characterization of Carotenoids. Forty-three carotenoids (**Figure 1**) were isolated and identified on the basis of UV−vis, FAB-MS, and ^{1}H NMR spectroscopic data. Among them, 7,8-didehydro-β-cryptoxanthin (**12**), peridininol 5,8-furanoxide (**38**), pyrrhoxanthin 5,8-furanoxide (**40**), and pyrrhoxanthinol 5,8-furanoxide (**43**) were reported for the first time as naturally occurring carotenoids. Very recently, compounds **19**, **21**, **22**, **24**, **36**, and **41** were isolated from *C. japonica* by us, and their structures were elucidated on the basis of chemical and NMR including 2D experiments, HR FAB MS, FAB MS/MS, and CD spectroscopic data (*18*). In the present investigation, compounds **19**, **21**, **22**, and **24** were also isolated from *C. sandai* and Chinese freshwater corbicula clam. On the other hand, compounds **36** and **41** were not found in either of these species.

Peridinin 5,8-furanoxide (**35**), peridininol 5,8-furanoxide (**38**), pyrrhoxanthin 5,8-furanoxide (**40**), and pyrrhoxnthinol 5,8-


α-Carotene (1)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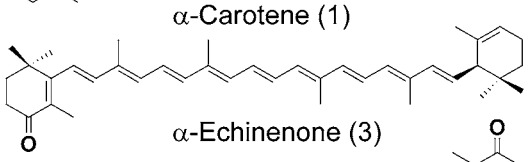
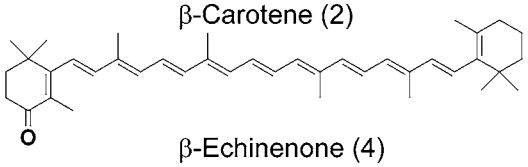
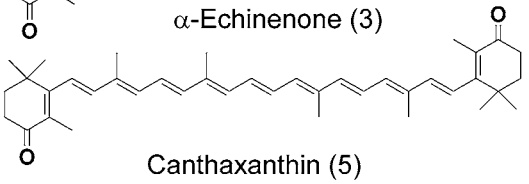
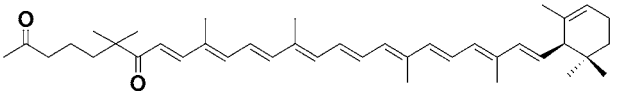
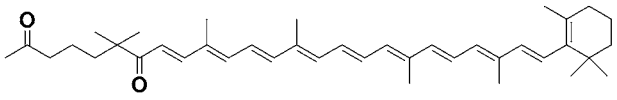
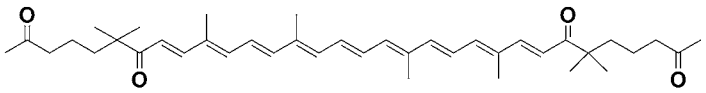

α-Echinenone (3)

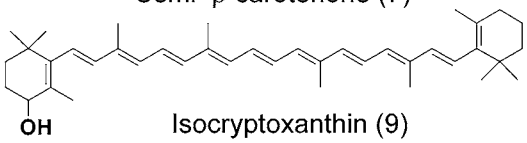

β-Echinenone (4)

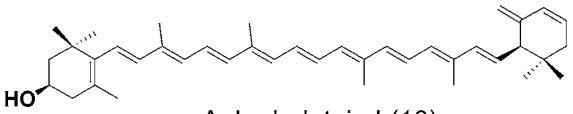

Canthaxanthin (5)

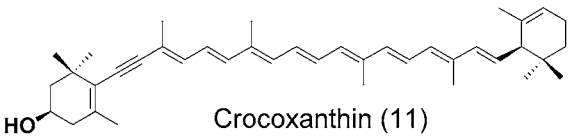

Semi–α-carotenone (6)

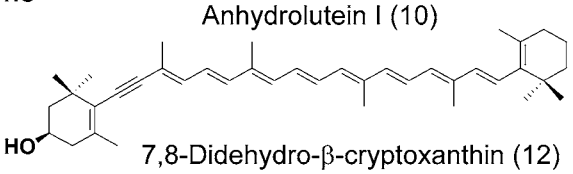

Semi–β-carotenone (7)

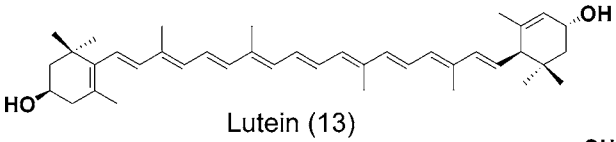

β-Carotenone (8)

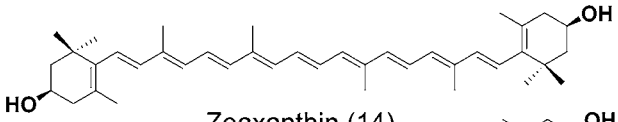

Isocryptoxanthin (9)

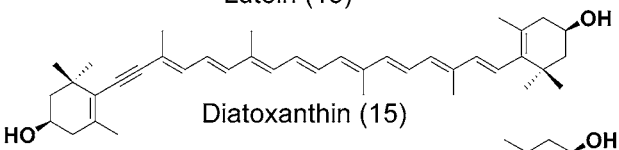

Anhydrolutein I (10)

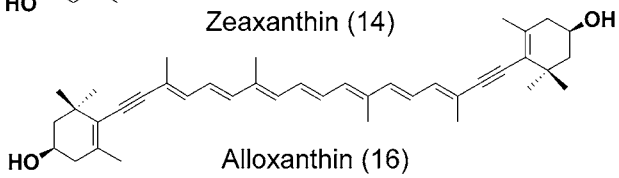

Crocoxanthin (11)

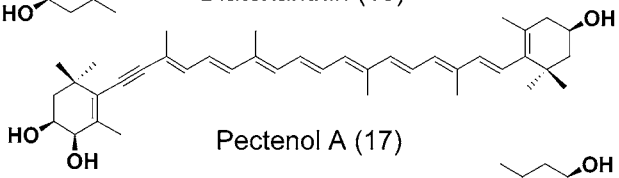

7,8-Didehydro-β-cryptoxanthin (12)

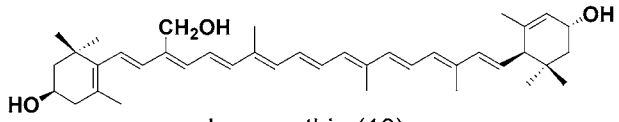

Lutein (13)

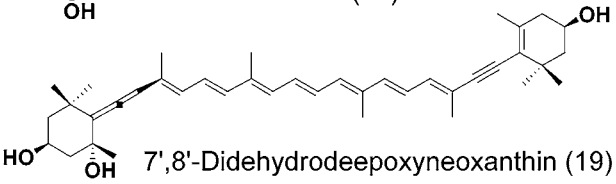

Zeaxanthin (14)

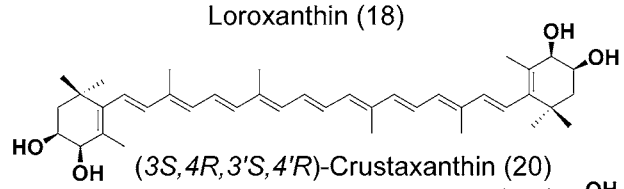

Diatoxanthin (15)

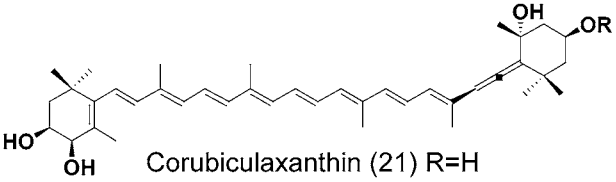

Alloxanthin (16)

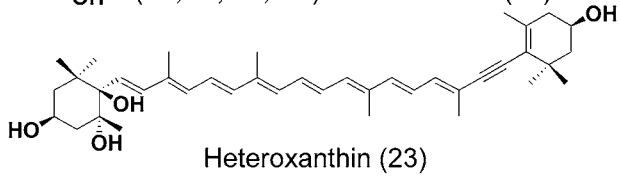

Pectenol A (17)

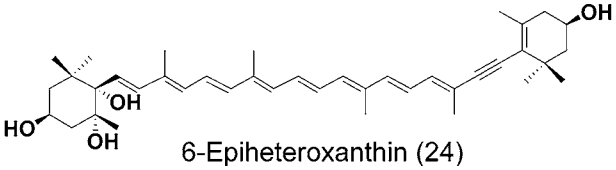

Loroxanthin (18)

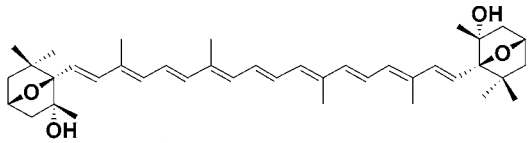

7',8'-Didehydrodeepoxyneoxanthin (19)


(3*S*,4*R*,3′*S*,4′*R*)-Crustaxanthin (20)


Corubiculaxanthin (21) R=H
Corbiculaxanthin acetate (22) R=Ac


Heteroxanthin (23)


6-Epiheteroxanthin (24)


Cycloviolaxanthin (25)

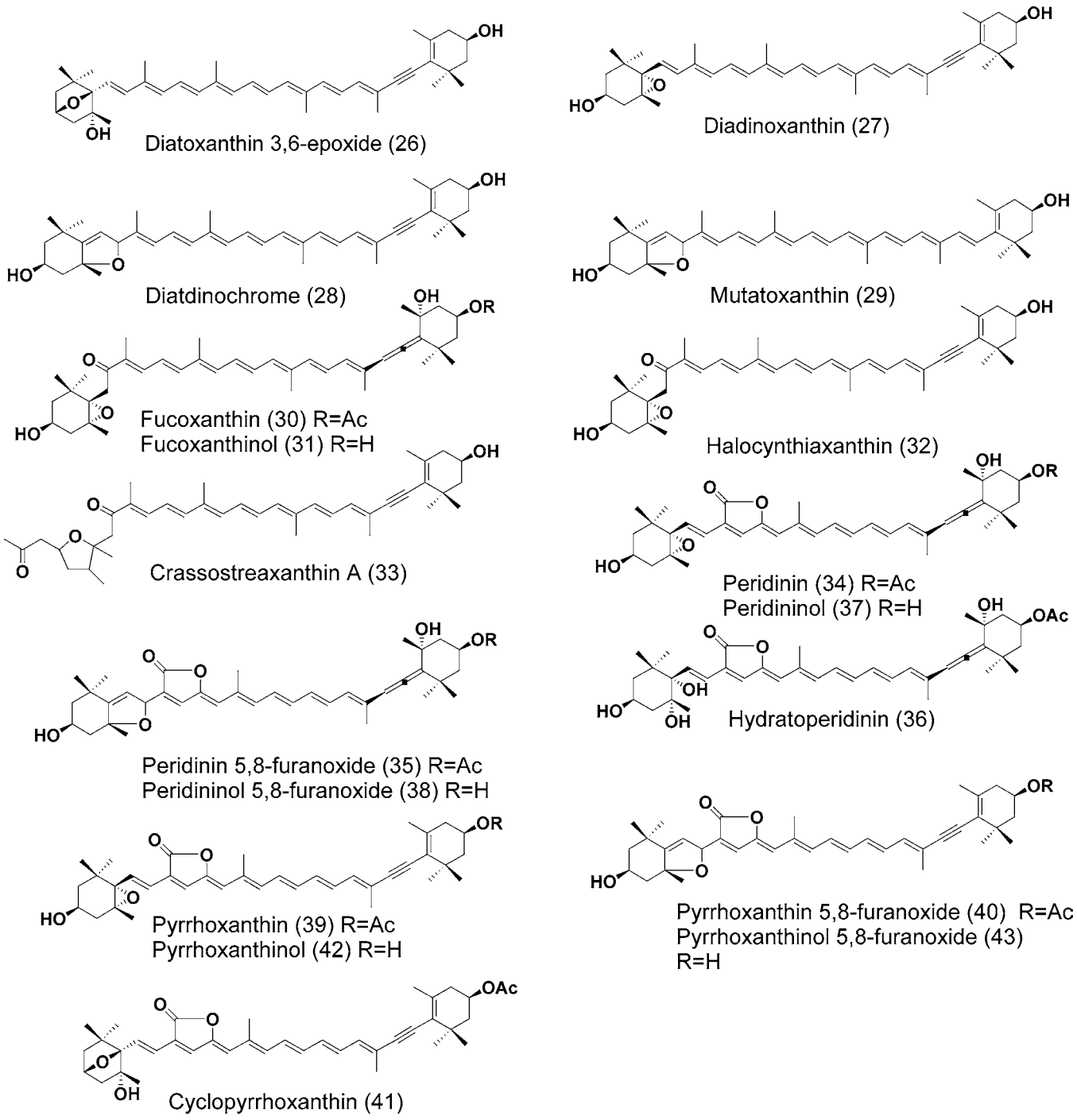


Figure 1. Structures of carotenoids isolated from corbicula clams.

furanoxide (**43**), which correspond to furanoid rearranged products of **34**, **37**, **39**, and **42**, respectively, were isolated from *C. japonica* as a mixture of (8*R*) and (8*S*) isomers. The ratio of the (8*R*) to (8*S*) isomer was estimated to be 1:1 from the intensity of the corresponding ^{1}H NMR signals. To determine whether these 5,8-furanoxide compounds (**35**, **38**, **40**, and **43**) are natural products or artificial products derived from the corresponding 5,6-epoxides through the isolation process, the Me_2CO solution of peridinin (**34**) was treated with the same procedure used for the isolation of carotenoids from corbicula clams. The formation of **35** from **34** was not observed during the isolation procedure. Therefore, these 5,8-furanoxide compounds were not artifacts formed by the isolation process but natural products. Peridinin 5,8-furanoxide (**35**) was first isolated from the dinoflagellate of *Symbiodinium* sp., a symbiont of the Okinawan soft coral *Clavularis virdis* (*19*). Pyrrhoxnthinol 5,8-furanoxide (**43**), which was previously reported as an artificial product derived from **42** (*3*), was first isolated as a natural product from *C. japonica*. Peridininol 5,8-furanoxide (**38**) and pyrrhoxanthin 5,8-furanoxide (**40**) have not previously been reported, and their structures were fully characterized by ^{1}H NMR including ^{1}H−^{1}H COSY and decoupling experiments and FAB MS/MS.

Previously, we reported the advantages of using FAB MS/MS to characterize natural carotenoids. Product ions resulting from molecular ion by MS/MS provided structural information on the end groups and polyene chains in carotenoids (*11*−*13*). Therefore, we attempted the characterization of carotenoids obtained from corbicula clams by FAB MS/MS. We could distinguish α-carotene (**1**) from *β*-carotene (**2**) on the basis of

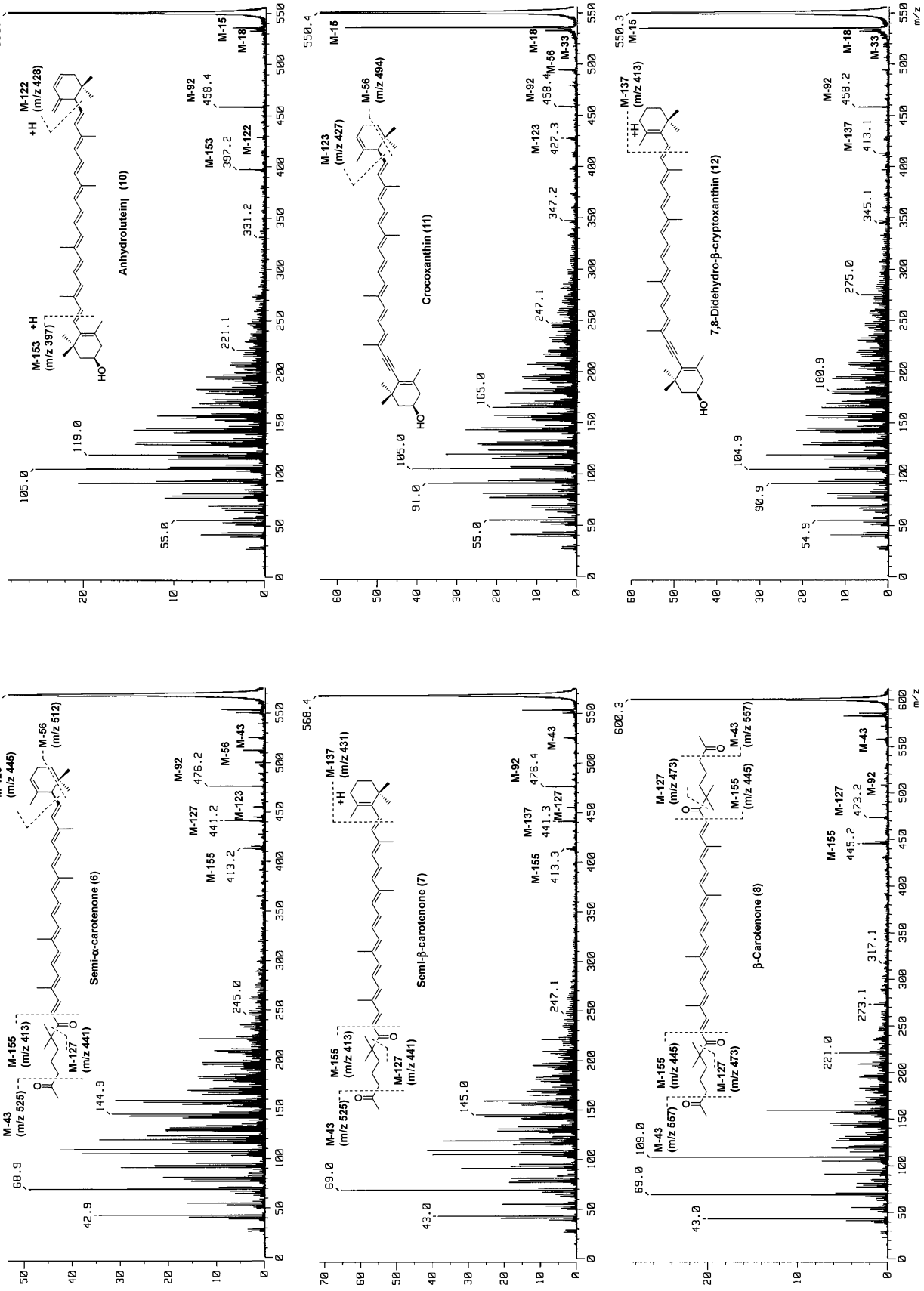


Figure 2. Positive ion FAB MS/MS of semi-α-carotenone (**6**), semi-β-carotenone (**7**), β-carotenone (**8**), anhydrolutein I (**10**), crocoxanthin (**11**), and 7,8-didehydro-β-cryptoxanthin (**12**).

$[M - 56]^+$ (cleavage between C-1 and C-6 and between C-2 and C-3 in the ϵ-end group) and $[M - 123]^+$ (elimination of the ϵ-end group), which were diagnostic product ions of a carotenoid possessing an ϵ-end group (*21*, *22*). Similarly, α-echinenone (**3**) was distinguished from β-echinenone (**4**) by the product ions $[M - 56]^+$ at *m*/*z* 494 and $[M - 123]^+$ at *m*/*z* 427. A symmetrical seco-carotenoid, β-carotenone (**8**), showed the characteristic product ions $[M - 43]^+$ [attributed to cleavage between C-4 and C-5 (C-4′ and C-5′)], $[M - 127]^+$ [attributed to cleavage between C-1 and C-6 (C-1′ and C-6′)], and $[M - 155]^+$ [attributed to cleavage between C-6 and C-7 (C-6′ and C-7′)]. Semi-β-carotenone (**7**) also showed $[M - 43]^+$ at *m*/*z* 525, $[M - 92]^+$ at *m*/*z* 476, $[M - 127]^+$ at *m*/*z* 441, and $[M - 155]^+$ at *m*/*z* 413. In the case of semi-α-carotenone (**6**), in addition to these product ions, $[M - 56]^+$ at *m*/*z* 512 and $[M - 123]^+$ at *m*/*z* 445 were observed as shown in **Figure 2**.

Three monohydroxy carotenoids, anhydrolutein I (**10**), crocoxanthin (**11**), and 7,8-didehydro-β-cryptoxanthin (**12**), which have the same molecular formula, $C_{40}H_{54}O$, could be characterized by FAB MS/MS as shown in **Figure 2**. All of these monohydroxy carotenoids showed $[M - H_2O]^+$ at *m*/*z* 532 and $[M - 92]^+$ at *m*/*z* 458. Both acetylenic carotenoids, **11** and **12**, showed $[M - 33]^+$ at *m*/*z* 517. Crocoxanthin (**11**), having an ϵ-end group, showed $[M - 56]^+$ at *m*/*z* 494 and $[M - 123]^+$ at *m*/*z* 427, which were absent from **10** and **12**. Anhydrolutein I (**10**) showed the characteristic product ions $[M - 122]^+$ at *m*/*z* 428 (elimination of the 3,4-didehydro-γ-end group) and $[M - 153]^+$ at *m*/*z* 397 (attributed to cleavage between C-7 and C-8 and transfer of hydrogen to the C-7 site) (*21*, *22*), which was absent from **12** having a 7,8-didehydro-3-hydroxy-β-end group. On the other hand, **12** showed the product ion $[M - 137]^+$ at *m*/*z* 413, indicating the presence of a β-end group (*21*, *22*).

There have been no reports on the complete ^{1}H NMR assignments of semi-α-carotenone (**6**), semi-β-carotenone (**7**), β-carotenone (**8**), and crocoxanthin (**11**) (*23*). Therefore, in the present study, complete ^{1}H NMR assignments of these compounds were made by conducting ^{1}H−^{1}H COSY or decoupling experiments.

Carotenoid Content and Composition of *C. japonica*, *C. sandai*, and *Corbicula* sp. (Chinese Freshwater Corbicula Clam). The amount and percentage composition of individual carotenoids in the three species of corbicula clams are shown in **Table 1**. The total carotenoid contents in *C. japonica*, *C. sandai*, and Chinese freshwater corbicula clam were found to be 5.3, 2.6, and 0.3 mg/100 g in the edible part (wet weight), respectively. The carotenoid contents of *C. japonica* and *C. sandai* were relatively higher than those of other clams (*1*). On the other hand, the carotenoid content of Chinese freshwater corbicula clam was very low, compared with another two corbicula clams. This clam was imported from China as shucked and frozen matter (not raw). It was assumed that carotenoids might be decreased during these procedures.

In general, animals do not synthesize carotenoids de novo, and those found in animals are either directly accumulated from food or partly modified through metabolic reactions. Thus, the carotenoid patterns in animals provide a key to the food chain as well as metabolic pathways (*24*, *25*).

It was reported that the major food sources of brackish water corbicula clam are phytoplankton such as dinoflagellates and detritus; on the other hand, those of freshwater corbicula clam are green algae and detritus (*26*). Peridinin (**34**), a characteristic carotenoid in dinoflagellates (*27*), was found to be the major carotenoid in *C. japonica*, which inhabits brackish water.

Table 1. Amount and Parcentage Composition of Carotenoids in Three Species of *Corbicula* Clams

	content[a] (mg/100 g)		
	C. japonica	*C. sandai*	Chinese freshwater corbicula clam
	5.3	2.6	0.3
α-carotene (**1**)	0.5	6.6	5.5
β-carotene (**2**)	1.0	9.1	11.5
α-echinenone (**3**)	0.5	0.5	2.0
β-echinenone (**4**)	1.0	2.0	3.5
canthaxanthin (**5**)	0.5	0.5	0.5
semi-α-carotenone (**6**)	0.5	1.5	1.1
semi-β-carotenone (**7**)	1.5	2.5	4.5
β-carotenon (**8**)	1.0	0.5	0.5
isocryptoxanthin (**9**)	0.5	1.0	0.5
anhydrolutein I (**10**)	0.5	1.5	2.0
crocoxanthin (**11**)	0.5	2.0	2.0
7,8-didehydro-β-cryptoxanthin (**12**)	0.5	2.0	2.5
lutein (**13**)	0.5	26.0	24.5
zeaxanthin (**14**)	0.5	2.0	4.5
diatoxanthin (**15**)	0.2	1.5	2.0
alloxanthin (**16**)	4.5	16.4	15.5
pectenol A (**17**)	2.3	2.5	1.0
7′,8′-didehydrodeepoxyneoxanthin (**18**)	1.0	1.0	0.5
loroxanthin (**19**)	nd[b]	2.0	1.5
(*3S*,*4R*,*3′S*,*4′R*)-crustaxanthin (**20**)	0.5	1.5	2.0
corbiculaxanthin (**21**)	1.0	1.0	2.0
corbiculaxanthin acetate (**22**)	2.0	2.0	0.5
heteroxanthin (**23**)	2.4	2.0	0.5
6-epiheteroxanthin (**24**)	3.0	1.0	1.0
cycloviolaxanthin (**25**)	1.5	1.0	1.5
diatoxanthin 3,6-epoxide (**26**)	1.0	2.0	1.5
diadioxanthin (**27**)	1.0	0.5	0.5
diadiochrome (**28**)	5.0	0.5	0.5
mutatoxanthin (**29**)	1.0	0.5	1.1
fucoxanthin (**30**)	1.5	2.3	1.5
fucoxanthinol (**31**)	1.0	1.0	0.5
halocynthiaxanthinol (**32**)	1.0	0.5	0.5
crassostreaxanthin A (**33**)	0.5	0.5	0.5
peridinin (**34**)	20.8	nd	nd
peridinin 5,8-furanoxide (**35**)	4.6	nd	nd
hydratoperidinin (**36**)	1.0	nd	nd
peridininol (**37**)	10.5	nd	nd
peridininol 5,8-furanoxide (**38**)	4.4	nd	nd
pyrrhoxanthin (**39**)	4.5	nd	nd
pyrrhoxanthin 5,8-furanoxide (**40**)	3.0	nd	nd
cyclopyrrhoxanthin (**41**)	3.5	nd	nd
pyrrhoxanthinol (**42**)	1.5	nd	nd
pyrrhoxanthinol 5,8-furanoxide (**43**)	1.0	nd	nd

[a] Content: mg/100 g in edible part (wet weight). [b] Not detected.

Furthermore, butenolide carotenoids (**35**−**43**), which were assumed to be derived from peridinin (**34**), were detected only in *C. japonica*. On the other hand, lutein (**13**), a characteristic carotenoid in green algae (*27*), was found to be the major carotenoid in *C. sandai* and Chinese corbicula clam, which inhabit freshwater. These carotenoid profiles well reflected the carotenoids in their dietary algae. α-Carotene (**1**), β-carotene (**2**), crocoxanthin (**11**), diatoxanthin (**15**), diadinoxanthin (**27**), heteroxanthin (**23**), and fucoxanthin (**30**), which were commonly found in microalgae such as diatom (*27*), were isolated from three species of corbicula clmas.

Ketocarotenoids such as α-echinenone (**3**), β-echinenone (**4**), canthaxanthin (**5**), α-carotenone (**6**), semi-β-carotenone (**7**), and β-carotenone (**8**) were found in the three species of corbicula

clmas and were also assumed to be oxidative metabolites of α-carotene (**1**) or β-carotene (**2**) in corbicula clams. Anhydrolutein I (**10**) might be formed from lutein (**13**) by dehydration of the hydroxy group at C-3′ (*16*). Pectenol A (**17**) and (3*S*,4*R*,3′*R*,4′*R*)-crastaxanthin (**20**), having a (3*S*,4*R*)-3,4-dihydroxy-β-end group, might be oxidative metabolites of diatoxanthin (**15**) and zeaxanthin (**14**), respectively.

7′,8′-Didehydrodeepoxyneoxanthin (**19**) has an interesting structure with both allenic and acetylenic groups. It was assumed that dehydration of the hydroxy group at C-5 of **19** produces the diacetylenic carotenoid alloxanthin (**16**), which is widely distributed in shellfish (*1*, *24*). The origin of alloxanthin in shellfish is still unclear (*24*). Therefore, **19** might be one of the possible precursors of alloxanthin in shellfish. Corbiculaxanthin (**21**) and its acetate (**22**) are allenic carotenoids having a (3*S*,4*R*)-3,4-dihydroxy-β-end group. It seemed likely that neoxanthin and/or dinoxanthin might be the precursor of these carotenoids. 6-Epiheteroxanthin (**24**), having (3*S*,5*R*,6*R*,3′*R*) chirality, is a 6-epimer of heteroxanthin (**23**) and might be formed from diadinoxanthin (**27**) by hydrolytic cleavage of the epoxy group. These four carotenoids (**19**, **21**, **22**, and **24)** were found in the three species of corbicula clams and have not previously been found in other shellfish (*1*). They were assumed to be peculiar carotenoids in corbicula clams. Two butenolide carotenoids with a C_{37} skeleton, hydratoperidinin (**36**), having (3*S*,5*R*,6*R*,3′*S*,-5′*R*,6′*R*) chirality, and cyclopyrrhoxanthin (**41**), having (3*S*,-5*R*,6*R*,3′*R*) chirality, were isolated from only *C. japonica* and assumed to have formed from peridinin (**34**) and pyrroxanthin (**39**), respectively, accumulated from dietary dinoflagellates (*27*).

Recently, peridinin (**34**) and related compounds were found to exhibit antitumor and anticarcinogenic activities (*19*, *28*). However, peridinin and related compounds do not exist in ordinary human food except for oysters (*1*, *11*). Therefore, *C. japonica* is a good dietary source for peridinin and related compounds for humans.

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