NOTE

Identification of Carotenoids in the Freshwater Shellfish *Unio douglasiae nipponensis*, *Anodonta lauta*, *Cipangopaludina chinensis laeta*, and *Semisulcospira libertina*

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Abstract: The biochemical properties of carotenoids from 2 species of freshwater bivalve, namely, *Unio douglasiae nipponensis* and *Anodonta lauta*, and 2 species of freshwater snail, namely, *Cipangopaludina chinensis laeta* and *Semisulcospira libertina*, were investigated. Diatoxanthin and fucoxanthin were identified as major carotenoids in both bivalves. In contrast, lutein and zeaxanthin were found to be the major carotenoids in *C. chinensis laeta*. In addition, a series of keto carotenoids was also identified in *S. libertina*.

Key words: carotenoids, freshwater shellfish, *Unio douglasiae nipponensis*, *Anodonta lauta*, *Semisulcospira libertina*, *Cipangopaludina chinensis laeta*

1 INTRODUCTION

Shellfish contain various carotenoids that possess structural diversity¹⁻⁴. The principal carotenoids found in marine shellfish include mytiloxanthin and isomytiloxanthin from *Mytilus edulis*⁵⁻⁷; diatoxanthin, alloxanthin, and pectenols A and B from *Mytilus coruscus*⁸; crassostreaxanthins A and B from *Crassostrea gigas*^{9, 10}; fucoxanthin and fucoxanthinol esters from *Macra chinensis*¹¹, *Ruditapes philippinarum*, and *Meretrix petechia*¹²; amarouciaxanthin A esters from *Paphia amabilis*¹³; and a series of carotenoids with a 5,6-dihydro- β -end group from *Fushinus perplexus*¹⁴. However, there are few reports on the content of carotenoids in freshwater shellfish, and only the carotenoids of the corbicula clams (*Corbicula sandai* and the Chinese corbicula clam) have been investigated by modern spectroscopic methods¹⁵.

A number of studies have compared the biochemical properties of carotenoids in shellfish⁸⁻¹⁵. In particular, the carotenoids of 4 species of edible freshwater shellfish have been investigated, comprising 2 species of bivalve, *Unio douglasiae nipponensis* (Ishigai in Japanese; Unionidae) and *Anodonta lauta* (Numagai in Japanese; Unionidae), and 2 species of snail, *Cipangopaludina chinensis laeta* (Marutanishi in Japanese; Pleuroceridae) and *Semisulcospira libertina* (Kawanina in Japanese; Viviparidae).

In the present study, we describe the content, composition, and identity of carotenoids found in these 4 species of freshwater shellfish. Furthermore, the origin and metabolism of carotenoids in the freshwater shellfish are discussed.

2 EXPERIMENTAL PROCEDURES

2.1 Apparatus

The UV-visible (UV-VIS) spectra were recorded with a Hitachi U-2001 spectrophotometer in diethyl ether (Et₂O). The positive-ion FAB-MS spectra were recorded using a JEOL JMS-HX 110A mass spectrometer with *m*-nitrobenzyl alcohol as a matrix. The ¹H-NMR (500 MHz) spectra were measured with a Varian UNITY INOVA 500 spectrometer in CDCl₃ using TMS as an internal standard. The CD spectra were recorded in Et₂O at room temperature with a Jasco J-500C spectropolarimeter. Preparative HPLC was performed using a Shimadzu LC-6AD with a Shimadzu SPD-6AV spectrophotometer set at 450 nm. A 10- μ m Cosmosil 5C18-II column (250 $\times$ 10 mm I.D.; Nacalai Tesque, Kyoto, Japan) and a 10- μ m Cosmosil 5SL-II column (250 $\times$ 10 mm I.D.; Nacalai Tesque) were used.

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Accepted September 13, 2011 (received for review September 1, 2011)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/> <http://mc.manuscriptcentral.com/jjocs>

2.2 Animal materials

U. douglasiae nipponensis (2000 g; 230 specimens) grown in Lake Biwa were purchased from a fish market in Otsu City, Shiga prefecture, Japan, in July 2010. *A. lauta* (1,200 g; 8 specimens), *S. libertina* (45 g; 50 specimens), and *C. chinensis laeta* (60 g; 20 specimens) were collected from a pond and river in Nara City, Nara prefecture, Japan, during May–July 2010 and 2011.

2.3 Extraction and isolation of carotenoids

The extraction and isolation of carotenoids was carried out according to our previously published methods^{8–15}. Carotenoids were extracted with acetone from the edible parts of *U. douglasiae nipponensis* (540 g), *A. lauta* (590 g), *S. libertina* (30 g), and *C. chinensis laeta* (30 g). Each acetone extract was partitioned between Et₂O and aqueous NaCl. The organic layer was dried over Na₂SO₄ and subsequently concentrated to dryness and subjected to silica gel column chromatography (300 × 10 mm) and preparative HPLC. The procedures for isolation and identification of carotenoids were carried out according to our previously published methods^{8–15}.

Individual carotenoids were identified by UV-Vis and FAB MS data and compared with standard samples by HPLC. In addition, the structural features of carotenoids were further characterized by ¹H-NMR and CD spectral data^{8–15}.

2.4 Quantification of carotenoids

The total carotenoid content and the amount of carotenoids eluted by column chromatography were calculated using the extinction coefficient, E_{1cm}^{1%} = 2,500 at λ_{max} (450 nm) or E_{1cm}^{1%} = 1,600 in the case of fucoxanthin derivatives¹⁶. For HPLC analysis, the relative amounts of individual carotenoids were estimated from the peak area detected at 450 nm.

2.5 Identification of carotenoids

Carotenoids in *Unio douglasiae nipponensis* and *Anodonta lauta*

β-Carotene (1). FAB-MS: *m/z* 536 [M]⁺; UV-VIS: 425, 449, 475 nm.

Zeaxanthin (2). FAB-MS: *m/z* 568 [M]⁺; UV-VIS 450, 475 nm.

Diatoxanthin (3). FAB-MS: *m/z* 566 [M]⁺; UV-VIS 451, 478 nm.

Diatoxanthin 3,6-epoxide (4). FAB-MS: *m/z* 582 [M]⁺; UV-VIS: 433, 470 nm. ¹H NMR (CDCl₃), δ 0.89 (3H, s, H-17), 1.15 (3H, s, 16'), 1.20 (3H, s, 17'), 1.22 (3H, s, H-18), 1.44 (3H, s, H-16), 1.46 (1H, dd, *J* = 12.5, 12.5 Hz, H-2' ax), 1.61 (1H, d, *J* = 11.5, H-2ax), 1.67 (1H, d, *J* = 12 Hz, H-4ax), 1.84 (2H, over lapped, H-2eq and H-2' eq), 1.92 (3H, s, H-18'), 1.95 (3H, s, H-20'), 1.96 (3H, s, H-19), 1.97 (3H, s, H-20), 2.01 (3H, s, H-19'), 2.06 (1H, ddd, *J* = 12, 6, 2 Hz,

H-4eq), 2.09 (1H, dd, *J* = 18, 9 Hz, H-4' ax), 2.43 (1H, ddd, *J* = 18, 5.5, 2 Hz, H-4' eq), 4.00 (1H, m, H-3'), 4.39 (1H, t, *J* = 6 Hz, H-3), 5.75 (1H, d, *J* = 16 Hz, H-7), 6.20 (1H, d, *J* = 11.5 Hz, H-10), 6.26 (1H, d, *J* = 10 Hz, H-14), 6.27 (1H, d, *J* = 10 Hz, H-14'), 6.36 (1H, d, *J* = 15 Hz, H-12), 6.36 (1H, d, *J* = 14.5 Hz, H-12'), 6.38 (1H, d, *J* = 16 Hz, H-8), 6.46 (1H, d, *J* = 11 Hz, H-10'), 6.51 (1H, dd, *J* = 14.5, 11.5 Hz, H-11'), 6.63 (1H, dd, *J* = 15, 11.5 Hz, H-11), 6.63 (1H, dd, *J* = 15, 11.5 Hz, H-15'), 6.63 (2H, m, H-15 and 15'), 6.65 (1H, dd, *J* = 15, 11.5 Hz, H-15), ¹H-NMR data were in agreement with previously published data¹⁰.

Alloxanthin (5). FAB-MS: *m/z* 564 [M]⁺; UV-VIS 451, 478 nm.

Halocynthiaxanthin 3'-acetate (6). FAB-MS: *m/z* 640 [M]⁺; UV-VIS 450, 475 nm. ¹H NMR (CDCl₃), δ 0.96 (3H, s, H-17), 1.04 (3H, s, H-16), 1.18 (3H, s, H-16'), 1.20 (3H, s, H-17'), 1.22 (3H, s, H-18), 1.36 (1H, dd, *J* = 12.5, 12 Hz, H-2ax), 1.50 (1H, ddd, *J* = 12.5, 3.5, 1.5 Hz, H-2eq), 1.57 (1H, dd, *J* = 12.5, 12.5 Hz, H-2' ax), 1.78 (1H, dd, *J* = 14, 9 Hz, H-4ax), 1.83 (1H, ddd, *J* = 12.5, 4, 2 Hz, H-2' eq), 1.92 (3H, s, H-18'), 1.94 (3H, s, H-19), 1.95 (3H, s, H-20'), 2.00 (3H, s, H-20), 2.01 (3H, s, H-19'), 2.04 (3H, s, CH₃CO), 2.13 (1H, dd, *J* = 18, 9 Hz, H-4' ax), 2.33 (1H, ddd, *J* = 14, 5, 1.5 Hz, H-4eq), 2.49 (1H, ddd, *J* = 18, 5.5, 2 Hz, H-4' eq), 2.60 (1H, d, *J* = 18.5 Hz, H-7), 3.66 (1H, d, *J* = 18.5 Hz, H-7), 3.82 (1H, m, H-3), 5.04 (1H, m, H-3'), 6.27 (1H, d, *J* = 11 Hz, H-14'), 6.36 (1H, d, *J* = 14.5 Hz, H-12'), 6.42 (1H, d, *J* = 11.5 Hz, H-14), 6.45 (1H, d, *J* = 11.5 Hz, H-10'), 6.51 (1H, dd, *J* = 14.5, 11.5 Hz, H-11'), 6.58 (1H, dd, *J* = 15.5, 11.5 Hz, H-11), 6.63 (1H, dd, *J* = 15, 11.5 Hz, H-15'), 6.65 (1H, dd, *J* = 15, 11.5 Hz, H-15), 6.66 (1H, d, *J* = 15.5 Hz, H-12), 7.14 (1H, d, *J* = 11.5 Hz, H-10)¹⁷.

Fucoxanthin (7). FAB-MS: *m/z* 658 [M]⁺; UV-VIS 445, 470 nm. ¹H-NMR data were in agreement with previously published data [18].

Fucoxanthinol (8). FAB-MS: *m/z* 616 [M]⁺; UV-VIS 445 and 470 nm.

Pectenol A (9). FAB-MS: *m/z* 582 [M]⁺; UV-VIS 451 478 nm, ¹H NMR (CDCl₃), δ 1.07 (3H, s, H-17), 1.08 (3H, s, H-16), 1.15 (3H, s, 16'), 1.20 (3H, s, 17'), 1.46 (1H, dd, *J* = 12.5, 12.5 Hz, H-2' ax), 1.57 (1H, dd, *J* = 12.5, 3.5 H-2ax), 1.67 (1H, d, *J* = 12 Hz, H-4ax), 1.69 (1H, dd, *J* = 12.5, 12.5 Hz, H-2ax), 1.84 (2H, over lapped, H-2eq and H-2' eq), 1.90 (3H, s, H-18), 1.92 (3H, s, H-18'), 1.95 (3H, s, H-20'), 1.96 (3H, s, H-19), 1.97 (3H, s, H-20), 2.01 (3H, s, H-19'), 2.09 (1H, dd, *J* = 18, 9 Hz, H-4' ax), 2.43 (1H, ddd, *J* = 18, 5.5, 2 Hz, H-4' eq), 3.88 (1H, m, H-3), 3.94 (1H, d, *J* = 3.5 Hz, H-4), 4.00 (1H, m, H-3'), 6.07 (1H, d, *J* = 16 Hz, H-7), 6.17 (1H, d, *J* = 16 Hz, H-8), 6.18 (1H, d, *J* = 11.5 Hz, H-10), 6.26 (1H, d, *J* = 10 Hz, H-14), 6.27 (1H, d, *J* = 10 Hz, H-14'), 6.36 (1H, d, *J* = 15 Hz, H-12), 6.36 (1H, d, *J* = 14.5 Hz, H-12'), 6.46 (1H, d, *J* = 11 Hz, H-10'), 6.51 (1H, dd, *J* = 14.5, 11.5 Hz, H-11'), 6.63 (1H, dd, *J* = 15, 11.5 Hz, H-11), 6.63 (1H, dd, *J* = 15, 11.5 Hz, H-15'), 6.63 (2H, m, H-15 and

15'), 6.65 (1H, dd, $J = 15, 11.5$ Hz, H-15). $^1\text{H-NMR}$ data were in agreement with previously published data^{8,17}.

Carotenoids in *Semisulcospira libertina*

Identification of β -carotene, zeaxanthin, diatoxanthin, fucoxanthin, and fucoxanthinol were described above.

α -Carotene (**10**). FAB-MS: m/z 535 $[\text{M}]^+$; UV-VIS 422, 443, 472 nm

β -Cryptoxanthin (**11**). FAB-MS: m/z 552 $[\text{M}]^+$; UV-VIS 450, 475 nm.

Lutein (**12**). FAB-MS: m/z 568 $[\text{M}]^+$; UV-VIS 422, 443, 472 nm.

Echinenone (**13**). FAB-MS: m/z 556 $[\text{M}]^+$; UV-VIS: 460 nm.

Canthaxanthin (**14**). FAB-MS: m/z 564 $[\text{M}]^+$; UV-VIS 470 nm.

Carotenoids in *Cipangopaludina chinensis laeta*

Identification of β -carotene, α -carotene, lutein, echinenone, and canthaxanthin were described above.

(3*S*)-Adonirubin (**15**). FAB-MS: m/z 580 $[\text{M}]^+$; UV-VIS 465 nm; CD nm ($\Delta\epsilon$) 220 (+12.0), 230 (0), 234 (−12.0), 252 (0), 275 (+10.0), 283 (0), 310 (−17.0), 340 (0), 370 (+4.4).

(3*S*,3'*S*)-Astaxanthin (**16**). FAB-MS: m/z 596 $[\text{M}]^+$; UV-VIS 472 nm; CD nm ($\Delta\epsilon$) 240 (−20.0), 252 (0), 270 (+20.0), 284 (0), 314 (−34.0), 355 (0), 372 (+3.0).

Fritschellaxanthin (**17**). FAB-MS: m/z 582 $[\text{M}]^+$; UV-VIS 452, 470 nm; CD nm ($\Delta\epsilon$) 255 (+10), 275 (0), 290 (−2.6).

(3*S*,3'*R*)-Adonixanthin (**18**). FAB-MS: m/z 582 $[\text{M}]^+$; UV-VIS 460 nm; CD nm ($\Delta\epsilon$) 235 (−17.0), 245 (0), 260 (+17.5), 275 (0), 300 (−20.0), 322 (0), 350 (+3.0).

3 RESULTS AND DISCUSSION

3.1 Carotenoids in *U. douglasiae nipponensis* and *A. lauta*

In general, since animals cannot synthesize carotenoids *de novo*, those found in animals are derived either directly from food or from partial modification of ingested carotenoids via metabolic reactions. Thus, the carotenoids found in animals provide an insight into both the food chain and metabolic pathways^{1–4}. Bivalves are filter feeders that accumulate carotenoids obtained from their diet of microalgae and modify carotenoids through metabolic reactions^{1–4}. The carotenoid content and composition of 2 species of freshwater bivalve that belong to the Unionidae family, *U. douglasiae nipponensis* and *A. lauta*, are shown in Table 1. The carotenoid composition of these 2 species was similar, and diatoxanthin (**3**) and fucoxanthin (**7**) were identified as the major carotenoids in both bivalves. Furthermore, halocynthiaxanthin 3'-acetate (**6**), diatoxanthin 3,6-epoxide (**4**), alloxanthin (**5**), pectenol A (**9**), zeaxanthin (**2**), and β -carotene (**1**) were also identified (Fig. 1). In particular, diatoxanthin (**3**) and fucoxanthin (**7**) are major characteristic carotenoids found in diatoms. Therefore, the carotenoid composition of the 2 bivalves indicates that their major food source consists of diatoms. Fucoxanthin was converted to halocynthiaxanthin 3'-acetate by these 2 bivalves. However, other fucoxanthin metabolites found in marine bivalves (sea mussels, oysters, and clams) such as mytiloxanthin, crassostreaxanthin A, and amarouciacanthin A were not identified in the freshwater bivalves belonging to the Unionidae family. Diatoxanthin 3,6-epoxide and pectenol A were assumed to be metabolites of diatoxanthin^{1–4}.

The carotenoid composition of the freshwater corbicula clam that inhabits Lake Biwa, *Corbicula sandai*, was more

Table 1 Carotenoid content and composition of *Unio douglasiae nipponensis* and *Anodonta lauta*.

Species	<i>U. douglasiae nipponensis</i>	<i>A. lauta</i>
Content (mg/g)	0.11	0.01
(mg/specimen)	0.03	0.73
Composition (%)		
β -Carotene (1)	10.6	16.6
Zeaxanthin (2)	6.6	5.5
Diatoxanthin (3)	13.7	22.6
Diatoxanthin 3,6-epoxide (4)	6.8	1.2
Alloxanthin (5)	15.1	2.8
Halocynthiaxanthin 3'-acetate (6)	11.0	9.3
Fucoxanthin (7)	19.0	33.0
Fucoxanthinol (8)	3.2	4.5
Pectenol A (9)	10.9	1.0
Others	3.1	3.5

Fig. 1 Carotenoids identified in *Unio douglasiae nipponensis* and *Anodonta lauta*.

Table 2 Carotenoid content and composition of *Cipangopaludina chinensis laeta* and *Semisulcospira libertina*.

Species	<i>C. chinensis laeta</i>	<i>S. libertina</i>
Content (mg/g)	0.022	0.032
(mg/specimen)	0.033	0.018
Composition (%)		
β -Carotene (1)	32.2	45.0
α -Carotene (10)	3.2	2.0
β -Cryptoxanthin (11)	3.2	
Lutein (12)	15.5	13.0
Zeaxanthin (2)	18.2	12.0
Diatoxanthin (3)	2.2	+
Fucoxanthin (7)	12.6	+
Fucoxanthinol (8)	8.1	+
Echinenone (13)	2.2	3.0
Canthaxanthin (14)	1.1	6.5
(3 <i>S</i>)-Adonirubin (15)		6.0
(3 <i>S</i> ,3' <i>S</i>)-Astaxanthin (16)		6.5
Fritschiellaxanthin (17)		0.5
(3 <i>S</i> ,3' <i>R</i>)-Adonixanthin (18)		1.0
Others	1.5	4.5

complex than those of the Unionidae bivalves. The major carotenoid of *C. sandai* was lutein, which originates from green algae. Loroaxanthin—a characteristic carotenoid found in green algae¹⁸⁾—as well as fucoxanthin, diatoxanthin, and their derivatives that originate from diatoms were also found in *C. sandai*¹⁵⁾. These differences mainly reflect variations in dietary algae between Unionidae and Corbicula clams.

3.2 Carotenoids in *C. chinensis laeta* and *S. libertina*

Phytoplanktons and detritus represent the major food sources of the freshwater snails *C. chinensis laeta* and *S. libertina*. The major carotenoids identified in *C. chinensis laeta* were β -carotene, lutein, zeaxanthin, and fucoxanthin (Table 2 and Fig. 2), which comprise the major characteristic carotenoids of green algae (β -carotene and lutein), cyanobacteria (β -carotene and zeaxanthin), and diatoms (fucoxanthin). In addition, echinenone and canthaxanthin were identified as minor carotenoids in *S. libertina*, which

Fig. 2 Carotenoids identified in *Bellamya chinensis laeta* and *Semisulcospira libertina*. The structures of β -carotene, diatoxanthin, fucoxanthin, and fucoxanthinol are shown in Fig. 1.

are also likely to originate from dietary cyanobacteria. Thus, the carotenoid composition of *C. chinensis laeta* indicates that this species directly absorbs carotenoids from dietary algae and that carotenoids are accumulated without further metabolic modification.

In *S. libertina*, β -carotene, lutein, and zeaxanthin were also found as major carotenoids. Furthermore, a series of carotenoids with a 3-hydroxy-4-keto- β end group, including fritschiellaxanthin, (3S)-adonirubin, (3S,3'S)-astaxanthin, and (3S,3'R)-adonixanthin, were also identified (Table 2 and Fig. 2). It was assumed that (3S,3'S)-astaxanthin is an oxidative metabolite of β -carotene through echinenone, canthaxanthin, and (3S)-adonirubin. Similarly, fritschiellaxanthin and (3S,3'R)-adonixanthin were assumed to be oxidative metabolites of lutein and zeaxanthin, respectively. These oxidative metabolic pathways have also been demonstrated in the marine and terrestrial snails *Fushinus perplexus*¹⁹⁾ and *Pomacea canaliculata*²⁰⁾, respectively.

4 CONCLUSION

Diatoxanthin, fucoxanthin, and their metabolites were identified as major carotenoids in the freshwater bivalves *U. douglasiae nipponensis* and *A. laeta* and were assumed to originate from dietary diatoms. In contrast, β -carotene, lutein, zeaxanthin, and fucoxanthin were the major carotenoids found in the freshwater snail *C. chinensis laeta*. Thus, *C. chinensis laeta* may directly absorb carotenoids from dietary green algae, cyanobacteria, and diatoms, and accumulate carotenoids without metabolic modification. A series of keto carotenoids comprising (3S,3'S)-astaxanthin, fritschiellaxanthin, and (3S,3'R)-adonixanthin were identified in the freshwater snail *S. libertina* and were assumed to represent oxidative metabolites of β -carotene, lutein, and zeaxanthin.

References

- 1) Liaaen-Jensen, S. Carotenoids in chemosystematics. in *Carotenoids*. Britton, G.; Liaaen-Jensen, S.; Pfander, H., ed. Birkhäuser. Basel. Vol. 3, pp. 217-247 (1998).
- 2) Matsuno, T. Aquatic animal carotenoids. *Fisheries Sci.* **67**, 771-789 (2001).
- 3) Maoka, T. Recent progress in structural studies of carotenoids in animals and plants. *Arch. Biochem. Biophys.* **483**, 191-195 (2009).
- 4) Maoka, T. Carotenoids in marine animals. *Mar. Drugs* **9**, 278-293 (2011).
- 5) Khare, A.; Moss, G. P.; Weedon, B. C. L. Mytiloxanthin and isomytiloxanthin, two novel acetylenic carotenoids. *Tetrahedron Lett.* 3921-3924 (1970).
- 6) Hertberg, S.; Partali, V.; Liaaen-Jensen, S. Animal carotenoids 32. Carotenoids of *Mytilus edulis* (Edible Mussel). *Acta Chem. Scand.* **B42**, 495-503 (1998).
- 7) Partali, V.; Tangen, K.; Liaaen-Jensen, S. Carotenoids in food chain studies-III. Resorption and metabolic transformation of carotenoids in *Mytilus edulis* (Edible Mussel). *Comp. Biochem. Physiol.* **92B**, 239-264 (1989).
- 8) Maoka, T.; Matsuno, T. Isolation and structural elucidation of three new acetylenic carotenoids from the Japanese sea mussel *Mytilus coruscus*. *Nippon Suisan Gakkaishi* **54**, 1443-1447 (1988).
- 9) Fujiwara, Y.; Maoka, T.; Ookubo, M.; Matsuno, T. Crassostreaxanthin A and B, Novel marine carotenoids from the oyster *Crassostrea gigas*. *Tetrahedron Lett.* **33**, 4941-4944 (1992).
- 10) Maoka, T.; Hashimoto, K.; Akimoto, N.; Fujiwara, Y. Structures of five new carotenoids from the oyster *Crassostrea gigas*. *J. Nat. Prod.* **64**, 578-581 (2001).
- 11) Maoka, T.; Fujiwara, Y.; Hashimoto, K.; Akimoto, N. Characterization of fucoxanthin and fucoxanthinol esters in the Chinese surf clam, *Macoma chinensis*. *J. Agric. Food Chem.* **55**, 1563-1567 (2007).
- 12) Maoka, T.; Akimoto, N.; Murakoshi, M.; Sugiyama, K.; Nishino, H. Carotenoids in clams, *Ruditapes philippinarum*.

- pinarum* and *Meretrix petechialis*. *J. Agric. Food Chem.* **58**, 5784-5788 (2010).
- 13) Maoka, T.; Akimoto, N.; Yim, M.-J.; Hosokawa, M.; Miyashita, K. A new C₃₇-skeletal carotenoid from the clam, *Paphia amabilis*. *J. Agric. Food Chem.* **56**, 12069-12072 (2008).
 - 14) Tsushima, M.; Maoka, T.; Matsuno, T. Structure of carotenoids with 5,6-dihydro- β -end groups from the spindle shell *Fushinus perplexus*. *J. Nat. Prod.* **64**, 1139-1142 (2001).
 - 15) Maoka, T.; Fujiwara, Y.; Hashimoto, K.; Akimoto, N. Carotenoids in three species of corbicula clams, *Corbicula japonica*, *Corbicula sandai*, and *Corbicula* sp. (Chinese freshwater corbicula clam). *J. Agric. Food. Chem.* **53**, 8357-8364 (2005).
 - 16) Schiedt, K.; Liaaen-Jensen, S. Isolation and analyses in *Carotenoids*. Britton, G.; Liaaen-Jensen, S.; Pfander, H. ed. Birkhäuser. Basel. Vol. 1A, pp. 81-108 (1995).
 - 17) Englert, G. NMR Spectroscopy. in *Carotenoids*. Britton, G.; Liaaen-Jensen, S.; Pfander, H. ed. Birkhäuser Verlag. Basel. Vol. 1B, pp. 147-160 (1995).
 - 18) Britton, G.; Liaaen-Jensen, S.; Pfander, H. ed. *Carotenoids Hand Book*. Birkhäuser. Basel. Switzerland (2004).
 - 19) Matsuno, T.; Katagiri, K.; Maoka, T.; Komori, T. Carotenoids of the spindle shell *Fushinus perplexus*. *Bull. Jpn. Soc. Sci. Fish.* **50**, 1583-1588 (1984).
 - 20) Tsushima, M.; Katsuyama, M.; Matsuno, T. Metabolism of carotenoids in the apple snail *Pomacea canaliculata*. *Comp. Biochem. Physiol.* **118B**, 431-436 (1997).
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