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Characterization of Fucoxanthin and Fucoxanthinol Esters in the Chinese Surf Clam, *Mactra chinensis*

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Fucoxanthin and fucoxanthinol fatty acid esters in the clam, *Mactra chinensis*, were characterized on the basis of ^{1}H NMR and FAB-MS spectra. ^{1}H NMR revealed that the hydroxy group at C-3 in fucoxanthin and fucoxanthinol was acylated. 3′-*O*-Acylated compounds such as fucoxanthinol 3′-ester or fucoxanthinol 3,3′-diester were not found in the clam. The fatty acids esterified with fucoxanthin and fucoxanthinol were identified as C24:6, C22:5, C22:6, C20:5, C20:0, C20:1, C18:0, C18:1, C16:0, C16:1, and C14:0 by FAB-MS data.

KEYWORDS: Carotenoids; fucoxanthin 3-ester; fucoxanthinol 3-ester; esterified fatty acid composition; clam; *Mactra chinensis*

INTRODUCTION

Fucoxanthin, a marine carotenoid possessing allenic, conjugated carbonyl, epoxide, and acetyl groups in its molecule, is widely distributed in marine algae such as Chrysophyceae, Prymnesiophyceae, Bacillariophyceae, Prasinophyceae, and Phaeophyceae (*1*, *2*). Fucoxanthin contributes >10% of the estimated total production of carotenoids in nature (*3*). Fucoxanthiol, a deacetyl product of fucoxanthin, is also found in these algae (*1*, *2*). Fucoxanthin has been found to have biological activities such as antioxidative (*4*, *5*), antitumor, anticarcinogenic (*6*−*13*), and antiobesity (*14*) activities.

Fucoxanthin and fucoxanthinol were also found in some marine invertebrates such as shellfish and tunicates as free and esterified forms (*15*−*18*). However, because of a large amount of lipid contaminants and instability for saponification, detailed structures including acylation position and fatty acid compositions of fucoxanthin and fucoxanthin esters in marine animals have not yet been determined. Recently, we isolated these esters from the Chinese surf clam, *Mactra chinensis*, which is an important edible shellfish in Japan and its muscle exhibits bright orange color, by using gel permeation chromatography (GPC) and reversed phase HPLC on ODS. The present study was undertaken to determine the structures of fucoxanthin and fucoxanthinol esters (**Figure 1**) by ^{1}H NMR and FAB-MS spectra.

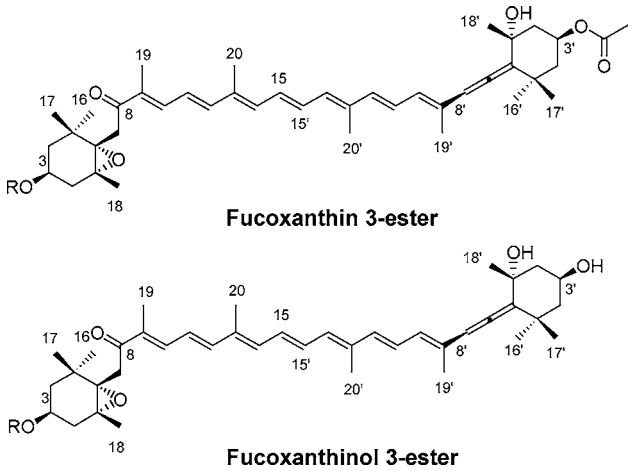

Figure 1. Structures of fucoxanthin and fucoxanthinol esters in clam *M. chinensis*.

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 spectra were recorded using a JEOL JMS-HX 110A mass spectrometer with *m*-nitrobenzyl alcohol as a matrix. The ^{1}H NMR (500 MHz) spectra were measured with a Varian UNITY INOVA 500 spectrometer in $CDCl_3$ with TMS as an internal standard. Preparative HPLC was performed on a Shimadzu LC-6AD with a Shimadzu SPD-6AV spectrophotometer set at 450 nm. The column used was a 250 × 10 mm i.d., 10 μm LiChrospher RP-18 (e) (Cica-Merck, Darmstadt, Germany). Preparative GPC was performed on an LC-908 HPLC system using a 600 × 20 mm i.d., 16 μm JAIGEL 2H column (Japan Analytical Industry Co., Ltd., Tokyo, Japan) with an IR-5 detector

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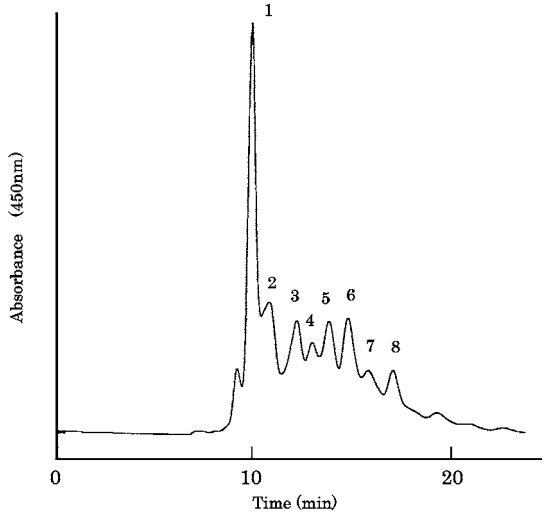

Figure 2. HPLC of fucoxanthin ester obtained from clam, *M. chinensis*. For peak assignment see **Table 1.**

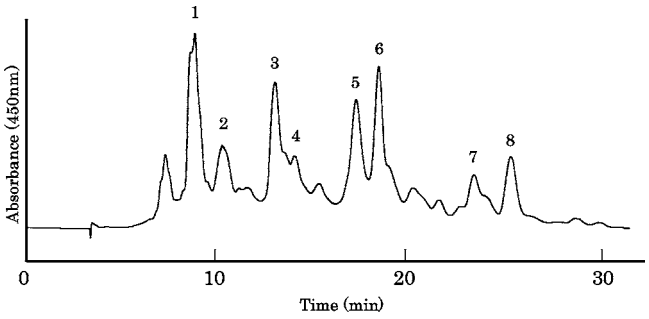

Figure 3. HPLC of fucoxanthinol ester obtained from clam, *M. chinensis*. For peak assignment see **Table 2.**

(Japan Analytical Industry Co., Ltd.) and $CHCl_3$ as an eluting solvent at the flow rate of 3.8 mL/min.

Animal Materials. The clam, *M. chinensis* (Mactridae), was purchased at a local fish market in February and March.

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} (450 nm) (*19*) and 1600 in the case of fucoxanthin derivatives (*20*). In the HPLC analysis, the relative amounts of individual carotenoids were calculated from the peak area detected at 450 nm.

Extraction and Isolation of Fucoxanthin and Fucoxanthinol Esters. The muscle of *M. chinensis* (140 g, about 50 specimens) was extracted with Me_2CO at room temperature. 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 on a 300 × 20 mm column using *n*-hexane, Et_2O, and Me_2CO as solvents. The fraction eluted with Et_2O from the silica gel column contained fucoxanthin esters and a large amount of steroids and lipids. To remove these contaminants from fucoxanthin esters, this fraction was subjected to GPC on JAIGEL 2H with $CHCl_3$ as an eluting solvent. The orange fraction eluted at 60 min contained fucoxanthin esters, and the colorless fraction eluted at 65−90 min contained steroids and lipids. The fucoxanthin ester fraction was further purified by preparative HPLC on ODS with $CHCl_3/CH_3CN$ (3:7) at a flow rate of 2.0 mL/min as shown in **Figure 2**. The fraction eluted with Me_2CO-Et_2O (4:6) from silica gel column chromatography contained fucoxanthinol esters and was further purified by GPC on JAIGEL 2H with $CHCl_3$ in order to remove lipid contaminants. The fucoxanthinol esters obtained by GPC (retention time 60 min) was further purified by preparative HPLC on ODS with $CHCl_3-CH_3CN$ (1:9) as shown in **Figure 3**.

Fucoxanthin 3-polyunsaturated fatty acid (PUFA) ester: UV−vis, λ_{max} (Et_2O) 445 and 475 nm; ^{1}H NMR ($CDCl_3$), δ 0.96 (3H, s, H-17), 0.98 (3H, t, $J = 7.5$ Hz, CH_3 in fatty acid moiety), 1.07 (6H, s, H-16, 17′), 1.22 (3H, s H-18), 1.35 (3H, s, H-18′), 1.38 (3H, s, H-16′), 1.39 (1H, dd, $J = 12.5$, 12 Hz, H-2β), 1.41 (1H, dd, $J = 12.5$, 7 Hz, H-2′β), 1.51 (1H, dd, $J = 13$, 12.5 Hz, H-4′β), 1.54 (1H, ddd, $J = 12.5$, 3.5, 1.5 Hz, H-2α), 1.69 (2H, quintet, $J = 7.5$ Hz, $-CH_2-CH_2-CH_2-COO$ in fatty acid moiety), 1.81 (3H, s, H-19′), 1.86 (1H, dd, $J = 14$, 9 Hz, H-4β), 1.94 (3H, s, H-19), 1.99 (1H, overlapped, H-2′α), 2.00 (6H, s, H-20, 20′), 2.04 (3H, s, CH_3CO), 2.08 (2H, quintet d, $J = 7.5$, 1 Hz, $CH_3-CH_2-CH=CH-$ in fatty acid moiety), 2.11 (2H, m, $J = 7.5$ Hz, $=CH-CH_2-CH_2-CH_2-COO$), 2.27 (1H, ddd, $J = 13$, 4.5, 1.5 Hz, H-4′α), 2.28 (2H, t, $J = 7.5$ Hz, $-CH_2-COOH$ in fatty acid moiety), 2.36 (1H, ddd, $J = 14$, 5, 1.5 Hz, H-4α), 2.61 (1H, d, $J = 18$ Hz, H-7), 2.80−2.85 (about 10H, m, $=CH-CH_2-CH=$ in fatty acid moiety), 3.66 (1H, d, $J = 18$ Hz, H-7), 4.88 (1H, m, H-3), 5.37 (1H, m, H-3′), 5.37 (about 12H, m, $-CH=CH-$ in fatty acid moiety), 6.06 (1H, s, H-8′), 6.13 (1H, d, $J = 11.5$ Hz, H-10′), 6.27 (1H, d, $J = 11.5$ Hz, H-14′), 6.35 (1H, d, $J = 15.5$ Hz, H-12′), 6.42 (1H, d, $J = 11.5$ Hz, H-14), 6.58 (1H, dd, $J = 15.5$, 11.5 Hz, H-11), 6.61 (1H, dd, $J = 15.5$, 11.5 Hz, H-11′), 6.65 (1H, dd, $J = 15$, 11.5 Hz, H-15), 6.66 (1H, d, $J = 15.5$ Hz, H-12), 6.75 (1H, dd, $J = 15$, 11.5 Hz, H-15′), 7.14 (1H, d, $J = 11.5$ Hz, H-10); FAB-MS (**Table 1**); CD (Et_2O), λ-($\Delta\epsilon$) 230 (−0.5), 250 (−1.5), 270 (−1.0), 300 (−0.5), 320 (−0.7), 380 (0) nm.

Fucoxanthin 3-ester (in the case of fucoxanthin 3-palmitate): UV−vis, λ_{max} (Et_2O) 445 and 475 nm; ^{1}H NMR ($CDCl_3$), δ, signals of the carotenoid moiety were identical with fucoxanthin 3-PUFA ester described above, and signals of the fatty acid moiety were as follows: 0.88 (3H, t, $J = 7.5$ Hz, CH_3 in fatty acid moiety), 1.25 (about 28H, CH_2 in fatty acid moiety) 2.07 (2H, t, $J = 7.5$ Hz, $-CH_2-COO$ in fatty acid moiety); FAB-MS (**Table 1**); CD of fucoxanthin 3-ester was almost the same as that of fucoxanthin 3-PUFA ester described above.

Fucoxanthinol 3-PUFA ester: UV−vis, λ_{max} (Et_2O) 445 and 475 nm; ^{1}H NMR ($CDCl_3$), δ 0.96 (3H, s, H-17), 0.98 (3H, t, $J = 7.5$ Hz, CH_3 in fatty acid moiety), 1.07 (6H, s, H-16, H-17′), 1.21 (3H, s H-18), 1.34 (3H, s, H-16′), 1.34 (1H, dd, $J = 12$, 7 Hz, H-2′β), 1.35 (3H, s, H-18′), 1.39 (1H, dd, $J = 12.5$, 12 Hz, H-2β), 1.41 (1H, dd, $J = 13$, 12.5 Hz, H-4′β), 1.54 (1H, ddd, $J = 12.5$, 3.5, 1.5 Hz, H-2α), 1.69 (2H, quintet, $J = 7.5$ Hz, $-CH_2-CH_2-CH_2-COO$ in fatty acid moiety), 1.81 (3H, s, H-19′), 1.86 (1H, dd, $J = 14$, 9 Hz, H-4β), 1.94 (3H, s, H-19), 1.95 (1H, overlapped, H-2′α), 2.00 (6H, s, H-20, 20′), 2.08 (2H, quintet d, $J = 7.5$, 1 Hz, $CH_3-CH_2-CH=CH-$ in fatty acid moiety), 2.11 (2H, m, $J = 7.5$ Hz, $=CH-CH_2-CH_2-CH_2-COO$), 2.27 (1H, ddd, $J = 13$, 4.5, 1.5 Hz, H-4′α), 2.28 (2H, t, $J = 7.5$ Hz, $-CH_2-COO$ in fatty acid moiety), 2.36 (1H, ddd, $J = 14$, 5, 1.5 Hz, H-4α), 2.61 (1H, d, $J = 18$ Hz, H-7), 2.80−2.85 (about 10H, m, $=CH-CH_2-CH=$ in fatty acid moiety), 3.66 (1H, d, $J = 18$ Hz, H-7), 4.32 (1H, m, H-3′), 4.88 (1H, m, H-3), 5.37 (about 12H, m, $-CH=CH-$ in fatty acid moiety), 6.04 (1H, s, H-8′), 6.12 (1H, d, $J = 11.5$ Hz, H-10′), 6.27 (1H, d, $J = 11.5$ Hz, H-14′), 6.35 (1H, d, $J = 15.5$ Hz, H-12′), 6.42 (1H, d, $J = 11.5$ Hz, H-14), 6.58 (1H, dd, $J = 15.5$, 11.5 Hz, H-11), 6.61 (1H, dd, $J = 15.5$, 11.5 Hz, H-11′), 6.65 (1H, dd,

Table 1. Identification of Fucoxanthin Esters in Chinese Surf Clam, *M. chinensis*

peak	MS[a] *m/z* (M^+)	HR MS[a] *m/z* (formula, Δ error[b])	identification[c]	composition[d]
1	996	not measured	F-C24:6	35.7[e]
	970	not measured	F-C22:5	
	968	968.6542 ($C_{64}H_{88}O_7$, Δ −0.6)	F-C22:6	
	942	942.6349 ($C_{62}H_{86}O_7$, Δ −2.4)	F-C20:5	
2	868	not measured	F-C14:1	2.5
3	894	not measured	F-C16:1	12.1
4	896	896.6495 ($C_{58}H_{88}O_7$, Δ +4.5)	F-C16:0	7.0
5	922	not measured	F-C18:1	10.7
6	924	924.6860 ($C_{60}H_{92}O_7$, Δ +1.7)	F-C18:0	11.7
7	950	not measured	F-C20:1	6.7
8	952	not measured	F-C20:0	7.7

[a] Positive ion FAB-MS. [b] Error indicated as milli mass unit. [c] F = fucoxanthin. [d] Expressed as percent of the fucoxanthin 3-ester fraction. [e] Sum of PUFA esters.

Table 2. Identification of Fucoxanthinol Esters in Chinese Surf Clam, *M. chinensis*

peak	MS[a] *m/z* (M^+)	HR MS[a] *m/z* (formula, Δ error[b])	identification[c]	composition[d]
1	954	954.6724 ($C_{64}H_{90}O_6$, Δ −1.3)	Fol-C24:6	17.0[e]
	928	928.6575 ($C_{62}H_{88}O_6$, Δ −0.6)	Fol-C22:5	
	926	926.6429 ($C_{62}H_{86}O_6$, Δ +0.4)	Fol-C22:6	
	900	900.6248 ($C_{60}H_{84}O_6$, Δ −2.0)	Fol-C20:5	
2	826	not measured	Fol-C14:0	6.4
3	852	not measured	Fol-C16:1	14.6
4	854	854.6460 ($C_{56}H_{86}O_6$, Δ+3.6)	Fol-C16:0	8.9
5	880	880.6760 ($C_{58}H_{88}O_6$, Δ +0.2)	Fol-C18:1	12.5
6	882	882.6760 ($C_{58}H_{92}O_6$, Δ +2.2)	Fol-C18:0	15.7
7	908	908.6915 ($C_{60}H_{92}O_6$, Δ +2.1)	Fol-C20:1	6.4
8	910	910.7015 ($C_{60}H_{94}O_6$, Δ −3.6)	Fol-C20:0	7.8

[a] Positive ion FAB-MS. [b] Error indicated as milli mass unit. [c] Fol = fucoxanthinol. [d] Expressed as percent of the fucoxanthinol 3-ester fraction. [e] Sum of PUFA esters.

J = 15, 11.5 Hz, H-15), 6.66 (1H, d, J = 15.5 Hz, H-12), 6.75 (1H, dd, J = 15, 11.5 Hz, H-15′), 7.14 (1H, d, J = 11.5 Hz, H-10); FAB-MS (**Table 2**); CD of fucoxanthinol 3-PUFA ester was almost the same as that of fucoxanthin 3-PUFA ester described above.

Fucoxanthinol 3-ester (in the case of fucoxanthinol 3-palmitate): UV−vis, λ_{max} (Et_2O) 445 and 475 nm; ^{1}H NMR ($CDCl_3$), δ, signals of the carotenoid moiety were identical with fucoxanthinol 3-PUFA ester described above, and signals of the fatty acid moiety were as follows: 0.88 (3H, t, J = 7.5 Hz, CH_3 in fatty acid moiety), 1.25 (about 28H, CH_2 in fatty acid moiety), 2.07 (2H, t, J = 7.5 Hz, CH_2−COO, in fatty acid moiety); FAB-MS (**Table 2**); CD of fucoxanthinol 3-etser was almost the same as that of fucoxanthin 3-PUFA ester.

Identification of Other Carotenoids in Clam. α-Carotene, β-carotene, crassostreaxanthin A acetate, crassostreaxanthin A, crassostreaxanthin B acetate, crassostreaxanthin B, halocynthiaxanthin 3′-acetate, halocynthiaxanthin, alloxanthin, diatoxanthin, diadinoxanthin, heteroxanthin, and mactraxanthin were identified on the basis of comparison of spectroscopic data and chromatographic behavior with our authentic samples obtained from the oyster, *Crassostrea gigas* (*21*). Heteroxanthin and mactraxanthin were isolated as esterified form and were identified as free form after saponification. Because of the small amount of samples, fatty acid moieties of those esterified carotenoids could not be determined.

Carotenoid content and composition in *M. chinensis* are shown in **Table 3**.

Table 3. Carotenoid Content and Composition of Chinese Surf Clam, *M. chinensis*

	muscle	digestive organ	gonad
total carotenoid content (mg/100 g)	0.62	0.53	7.3
carotenoid composition (%)			
α-carotene	0.5	0.5	0.5
β-carotene	1.0	2.5	2.0
fucoxanthin 3-ester	45.5	2.0	nd[a]
fucoxanthin	3.0	10.0	7.8
fucoxanthinol 3-ester	25.0	2.5	nd
fucoxanthinol	2.0	6.0	14.7
crassostreaxanthin A acetate	2.0	5.5	2.0
crassostreaxanthin A	2.5	5.5	5.3
crassostreaxanthin B acetate	1.0	3.5	3.3
crassostreaxanthin B	0.8	6.5	8.2
halocynthiaxanthin 3′-acetate	1.0	6.2	3.2
halocynthiaxanthin	1.0	11.0	10.5
alloxanthin	2.0	8.0	7.0
diatoxanthin	1.5	10.0	12.5
diadinoxanthin	1.5	5.0	9.0
heteroxanthin 3,3′-diester	3.0	2.0	nd
heteroxanthin	1.0	10.4	10.4
mactraxanthin 3,3′-diester	2.0	nd	nd
unidentifieds	3.7	2.9	3.6

[a] Not detected.

RESULTS AND DISCUSSION

Fucoxanthin Ester in Clam. The fraction eluted with Et_2O from silica gel column chromatography contained fucoxanthin esters accompanied with a large amount of steroids and lipids. Contaminant lipids were removed from fucoxanthin esters by GPC. Fucoxanthin esters were further separated by HPLC on ODS with $CHCl_3$/MeCN (3:7) as shown in **Figure 2**. The carotenoids in peak 1 were identified as fucoxanthin 3-polyunsaturated fatty acid (PUFA) esters by ^{1}H NMR, which was assigned by COSY and NOESY experiments, and FAB-MS. The ^{1}H NMR signal of H-3 (δ 4.88), which showed a 0.97 ppm downfield shift relative to the corresponding signal in fucoxanthin (*20*, *22*), indicated that the hydroxy group at C-3 was acylated. Furthermore, the following series of characteristic ^{1}H NMR signals, 0.98 (t), 1.69 (quintet), 2.08 (quartet), 2.11 (m), 2.28 (quartet), 2.80 (m), and 5.37 (m), indicated that the fatty acid moiety was consistent with a polyunsaturated fatty acid (*23*). The FAB-MS showed molecular ion peaks at *m/z* 996, 970, 968, and 942, which were assigned to tetracosahexaenoic acid (C24:6), docosapentaenoic acid (C22:5), docosahexaenoic acid (C22:6), and eicosapentaenoic acid (C20:5) esters of fucoxanthin, respectively (**Table 1**). The carotenoids in peaks 2−8 were identified as fucoxanthin 3-saturated fatty acid or monounsaturated fatty acid esters. The acylated position in fucoxanthin was also determined to be the hydroxy group at C-3 by ^{1}H NMR spectral data. Compounds exhibiting molecular ions at *m/z* 952, 950, 924, 922, 896, 894, and 868 were assigned as eicosanoic acid (C20:0), eicosenoic acid (C20:1), octadecanoic acid (C18:0), octadecenoic acid (C18:1), hexadecanoic acid (C16:0), hexadecenoic acid (C16:1), and tetradecanoic acid (C14:0) esters of fucoxanthin as shown in **Table 1**. Among them, the molecular formulas of C22:5, C20:5, C18:0, and C16:0 esters of fucoxanthin were confirmed by HR FAB-MS data. The composition of individual fucoxanthin esters expressed as percentage of the total of fucoxanthin 3-ester fraction is shown in **Table 1**.

Fucoxanthinol Ester in Clam. The fraction eluted with Me_2CO/Et_2O (4:6) from silica gel column chromatography contained fucoxanthinol esters accompanied by a large amount of steroids and lipids. This fraction was also purified by GPC to remove these lipid impurities. The fucoxanthinol esters obtained by GPC were further separated by HPLC on ODS with $CHCl_3$/MeCN (1:9) as shown in **Figure 3**. Peak 1 was identified as a mixture of fucoxanthinol 3-PUFA esters by FAB-MS and ^{1}H NMR. The fucoxanthinol molecule bears two secondary hydroxy groups located at C-3 and C-3′. Thus, two possible structures of fucoxanthinol monoesters, that is, fucoxanthinol 3-ester and fucoxanthinol 3′-ester, could be considered. In the ^{1}H NMR of fucoxanthinol ester, assigned by COSY and NOESY experiments, the H-3 signal (δ 4.88) showed a remarkable downfield shift of 0.97 ppm relative to the corresponding signal of fucoxanthinol (*20*, *22*). On the other hand, the H-3′ signal showed the same chemical shift (δ 4.32) as fucoxanthinol (*20*, *22*). This clearly indicated that the hydroxy group at C-3 was acylated and that the hydroxy group at C-3′ existed as the free form. The ^{1}H NMR signals of the fatty acid moiety were in agreement with the structure of the polyunsaturated fatty acid. The FAB-MS showed molecular ions at *m/z* 954, 928, 926, and 900, which were assigned as C24:6, C22:5, C22:6, and C20:5 esters of fucoxanthinol, respectively (**Table 2**); they were also confirmed by HR FAB-MS data.

The carotenoids in peaks 2−8 were identified as fucoxanthinol 3-saturated fatty acid or monounsaturated fatty acid esters. Compounds exhibiting molecular ions at *m/z* 910, 908, 882, 880, 854, 852, and 826 were assigned as C20:0, C20:1, C18:0, C18:1, C16:0, C16:1, and C14:0 esters of fucoxanthinol as shown in **Table 2**. The acylated position in those fucoxanthinol esters was also determined by ^{1}H NMR spectral data. Neither fucoxanthinol 3′-ester nor fucoxanthinol 3,3′-diester were found in the clam. Therefore, it was concluded that only the hydroxy group at C-3 in fucoxanthinol was esterified in clam. The composition of individual fucoxanthinol esters expressed as percentage of the total of fucoxanthinol 3-ester fraction is shown in **Table 2**.

Concerning the carotenoid ester in marine animals, astaxanthin esters in Crustaceae (shrimp, crab, and krill) have been investigated by several authors (*24*−*28*). On the other hand, there are only a few reports on the fucoxanthin and fucoxanthinol esters. Only 19′-hexanoyloxy fucoxanthin and 19′-hexanoyloxy fucoxanthinol from marine algae (*29*, *30*) and fucoxanthinol 3′-sulfate from fucoxanthin supplemented hens' egg yolks (*31*) have been reported. To our knowledge, fucoxanthin 3-ester and fucoxanthinol 3-ester have not yet been reported (*32*).

Carotenoid content and composition of muscle, digestive organ, and gonad in *M. chinensis* are shown in **Table 3**. Carotenoid content in gonad (7.3 mg/100 g) is higher than that of muscle (0.62 mg/100 g) and digestive organ (0.53 mg/100 g). In gonad and digestive organ, fucoxanthin, fucoxanthinol, halocynthiaxanthin, diatoxanthin, and heteroxanthin were found to be major carotenoids, and they are mainly present as the free form. On the other hand, in muscle, fucoxanthin 3-ester and fucoxanthinol 3-ester were found to be major carotenoids, and they consisted of >70% of the total carotenoids. The major food sources of the clam are microalgae such as diatom, which contains fucoxanthin as a major carotenoid (*1*, *2*). From the carotenoid composition in muscle, digestive organ, and gonad described above, the following metabolic pathways of fucoxanthin in clam might be proposed. Fucoxanthin accumulated from dietary microalgae was metabolized to fucoxanthinol and halocynthiaxanthin in digestive organ, and they were mainly deposited in gonad. On the other hand, some of the fucoxanthin and fucoxanthinol was esterified with fatty acid and deposited in muscle.

In conclusion, fucoxanthin 3-ester and fucoxanthinol 3-ester were isolated from the muscle of *M. chinensis*. The acylation position of these compounds was characterized by ^{1}H NMR data. The fatty acids esterified with fucoxanthin and fucoxanthinol were identified as C24:6, C22:5, C22:6, C20:5, C20:0, C20:1, C18:0, C18:1, C16:0, C16:1, and C14:0 by FAB-MS data.

LITERATURE CITED

(1) Goodwin, T. W. *The Biochemistry of Carotenoids, Vol. 1, Plants*; Chapman and Hall: London, U.K., 1980.

(2) Liaeen-Jensen, S. Carotenoids in chemosystematics. In *Carotenoids, Vol. 3, Biosynthesis and Metabolism*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, Switzerland, 1998; pp 217−247.

(3) Liaaen-Jensen, S. Marine carotenoids. In *Marine Natural Products, Chemical and Biochemical Perspective Vol. 2*; Scheuer, P. J., Ed.; Academic Press: New York, 1978; pp 1−73.

(4) Yan, X.; Chuda, Y.; Suzuki, M.; Nagata, T. Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible sea weed. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 605−607.

(5) Shimidzu, N.; Goto, M.; Miki, W. Carotenoid as singlet oxygen quenchers in marine organisms. *Fish. Sci.* **1996**, *62*, 134−137.

(6) Okuzumi, J. Antiproliferative and antitumor promoting activities of fucoxanthin, a natural carotenoid. *J. Kyoto Pref. Univ. Med.* **1991**, *100*, 551−566.

(7) Okuzumi, J.; Nishino, H.; Murakoshi, M.; Iwashima, A.; Tanaka, Y.; Yamane, T.; Fujita, Y.; Takahashi, T. Inhibitory effects of fucoxanthin, a natural carotenoid, on N-myc expression and cell cycle progression in human malignant tumor cells. *Cancer Lett.* **1990**, *55*, 75−81.

(8) Okuzumi, J.; Takahashi, T.; Yamane, T.; Kitao, T.; Inagaki, M.; Ohya, K.; Nishino, H.; Tanaka, Y. Inhibitory effects of fucoxanthin, a natural carotenoid, on *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine-induced mouse duodenal carcinogenesis. *Cancer Lett.* **1993**, *68*, 159−168.

(9) Nishino, Y. Cancer prevention by carotenoids. *Mutat. Res.* **1998**, *402*, 159−163.

(10) Tsushima, M.; Maoka, T.; Katuyama, M.; Kozuka, M.; Matsuno, T.; Tokuda, H.; Nishino, H.; Iwashima, A. Inhibitory effect of natural carotenoids on Epstein−Barr virus activation activity of a tumor promoter in Raji cells. A screening study for anti-tumor promoters. *Biol. Pharm. Bull.* **1995**, *18*, 227−233.

(11) Hosogawa, M.; Wanezaki, S.; Miyauchi, K.; Kurihara, H.; Kohono, H.; Kawabata, J.; Odashima, S.; Takahashi, K. Apotosis-inducing effect on fucoxanthin on human leukemia cell line HL-60. *Food Sci. Technol. Res.* **1999**, *5*, 243−246.

(12) Hosokawa, M.; Kudo, M.; Maeda, H.; Kohno, H.; Tanaka, T.; Miyashita, K. Fucoxanthin induces apotosis and enhances the antiproliferative effect on the PPARγ ligand, troglitazone, on colon cancer cells. *Biochim. Biophys. Acta* **2004**, *1675*, 113−119.

(13) Kotake-Nara, E.; Sugawara, T.; Nagao, A.; Antiproliferative effect on neoxnthin and fucoxanthin on cultured cells. *Fish. Sci.* **2005**, *71*, 459−461.

(14) Maeda, H.; Hosokawa, M.; Sashima, T.; Funayama, K.; Miyashita, K. Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem. Biophys. Res. Commum.* **2005**, *332*, 392−397.

(15) Matsuno, T.; Hirao, S. Marine carotenoids. In *Marine Biogenic Lipids, Fats, and Oils*; Ackman, R. G., Ed.; CRC Press: Boca Raton, FL, 1989; Vol. 1, pp 251−388.

(16) Liaeen-Jensen, S. Carotenoids in food chains. In *Carotenoids Vol. 3, Biosynthesis and Metabolism*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, Switzerland, 1998; pp 259−371.

(17) Matsuno, T.; Maoka, T.; Shiba, K.; Ookubo, M. Isolation of fucoxanthinol from short-necked clam *Tapes philippinarum* [Asari in Japanese]. *Nippon Suisan Gaku* **1986**, *52*, 167.

(18) Stancher, B.; Zonta, F.; Favretto, L. G.; High-performance, liquid chromatography of carotenoids from some marine shellfish. *J. Chromatogr.* **1988**, *440*, 37−46.

(19) Schiedt, K.; Liaaen-Jensen. S. Isolation and analysys. In *Carotenoids*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, Switzerland, 1995; Vol. 1A, pp 81−108.

(20) Haugan, J. A.; Aakermann, T.; Liaaen-Jensen, S. Isolation of fucoxanthin and peridinin. *Methods Enzymol.* **1992**, *213*, 231−246.

(21) Maoka, T.; Hashimoto, K.; Akimoto, N.; Fujiwara, Y. Structures of five new carotenoids from the oyster *Crassostrea gigas*. *J. Nat. Prod.* **2001**, *64*, 578−581.

(22) Englert, G. NMR spectroscopy. In *Carotenoids*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds; Birkhäuser: Basel, Switzerland, 1995; Vol. 1B, pp 147−260.

(23) Igarashi, T.; Watanabe, K.; Miyake, Y.; Shimidzu, N.; Oshima, Y.; Kushida, K.; Nonaka, M.; Wada, A. Determination of docosahexanoeic acid and n-3 fatty acids in fish oils by high resolution proton nuclear magnetic resonance spectroscopy: collaborative study by working group (DHA/NMR subcommittee), Japan Oil Chemists' Society. *J. Oleo Sci.* **2002**, *51*, 175−182.

(24) Renstrome, B.; Liaaen-Jensen, S. Fatty acid composition of some esterified carotenols. *Comp. Biochem. Physiol.* **1981**, *69B*, 625−627.

(25) Coral-Hinostroza, G. N.; Bjerkeng, B. Astaxanthin from the red crab langostilla (*Pleuroncodes planipes*): optical R/S isomers and fatty acid moieties of astaxanthin esters. *Comp. Biochem. Physiol.* **2002**, *133B*, 437−444.

(26) Takaichi, S.; Matsui, K.; Nakamura, M.; Muramatsu, M.; Hanada, S. Fatty acid of astaxanthin esters in krill determined by mild mass spectrometry. *Comp. Biochem. Physiol.* **2003**, *136B*, 317−322.

(27) Breithaupt, D. E. Identification and quantification of astaxanthin esters in shrimp (*Pandalus borealis*) and in a microalgae (*Haematococcus pluvialis*) by liquid chromatography−mass spectrometry using negative ion atomospheric pressure chemical ionization. *J. Agric. Food. Chem.* **2004**, *52*, 3870−3875.

(28) Lin, W.-C.; Chien, J.-J.; Chen, B.-H.; Determination of carotenoids in spear shrimp shells (*Parapenaeopsis hardwickii*) by liquid chromatography. *J. Agric. Food. Chem.* **2005**, *53*, 5144−5149.

(29) Aprin, N.; Srec, W. A.; Liaaen-Jensen, S. New fucoxanthin-related carotenoids from *Coccolithus huxleyi*. *Phytochemistry* **1976**, *15*, 529−532.

(30) Bjornland, T.; Throndsen, J.; Liaaen-Jensen, S. Carotenoids of the marine chrysophyte *Pelagococcus subviridis*. *Phytochemistry* **1989**, *28*, 3347−3353.

(31) Strand, A.; Herstad, O.; Liaaen-Jensen, S. Fucoxanthin metabolites in egg yolks of laying hens. *Comp. Biochem. Physiol. A* **1998**, *119*, 963−974.

(32) Britton, G.; Liaaen-Jensen, S.; Pfander, H. *Carotenoids Handbook*; Birkhäuser Verlag: Basel, Switzerland, 2004.

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