Triacylglycerols, Fucosterol, Diacylglyceroglycolipids and Fucoxanthin from *Fucus virsoides* J. AG.

G. Falsone*, F. Cateni, E. Vrech
Department of Pharmaceutical Sciences, University of Trieste, P.zle Europa 1, I-34127 Trieste
L. Birkofer
Institut für Organische Chemie I der Heinrich-Heine Universität Düsseldorf,
D-40225 Düsseldorf, F.R.G.
V. Lucchini
Department of Environmental Sciences, University of Venezia, Calle Larga 2137,
I-30123 Venezia
H. Wagner, O. Seligmann
Institut für Pharmazeutische Biologie der Universität München, Karlstraße 29,
D-80330 München
L. Coassini Lokar
Department of Biology, University of Trieste, Via Valerio 30, I-34127 Trieste

Z. Naturforsch. **49b,** 1297–1304 (1994); received March 3, 1994

Fucus virsoides J. AG., Triacylglycerols, Fucosterol, Diacylglyceroglycolipids, Fucoxanthin

From the marine brown macroalga *Fucus virsoides* J. AG. *(Fucales, Phaeophyceae)*, a mixture of triacylglycerols **1–3,** fucosterol **4,** a mixture of galactosyldiacylglycerols **5–8** and fucoxanthin **9** have been isolated. Normal phase column flash chromatography was effective for the isolation of the marine compounds. FAB-MS, CI-MS spectrometry, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY experiments were useful in providing informations for their structure elucidation.

Introduction

During the course of our investigation on marine natural products, we have analyzed the brown macroalga *Fucus virsoides* J. AG. *(Fucales, Phaeophyceae),* present in the North Adriatic sea.

The species *Fucus* was investigated with particular respect to basic physiological features, such as photosynthesis, dark carbon fixation and respiration [1]. In order to evaluate the nutritive value of some common seaweeds in the northern Adriatic as additional food and animal fodder, *Fucus virsoides* J. AG., has been analyzed to determine the content of ascorbic acid. In cold water areas *Fucus* species are a rich source of ascorbic acid [2]. Concerning the biological activity, in a preliminary screening for antitumour activity, the chloroform extract of *Fucus virsoides,* inhibited the growth of the tumour cells [3].

In this paper we report on the isolation and structure elucidation of triacylglycerols **1–3,** fucosterol **4,** monogalactosyl diacylglycerols **5–8** and fucoxanthin **9.**

Results and Discussion

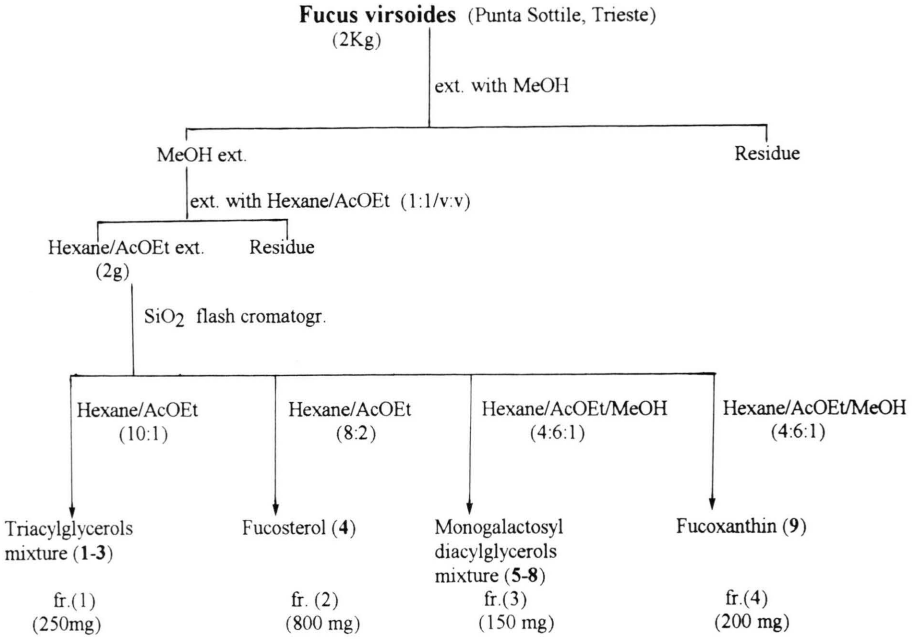
The alga *Fucus virsoides* J. AG., was treated as shown in Scheme 1. The *n*-hexane/ethyl acetate soluble portion, obtained from the methanolic extract of *Fucus virsoides,* was chromatographed over silica gel in order of polarity, and four fractions A–D, apparently pure, have been obtained. Chromatography (silica gel) of the fraction A using *n*-hexane/ethyl acetate (10:1) as eluent afforded the compounds **1–3** as mixture.

The ^{1}H NMR and ^{13}C NMR spectra of the mixture **1–3,** showed signals which were characteristically attributable to triacylglycerols. In the ^{1}H

---

* Reprint requests to Prof. Dr. G. Falsone.

0932–0776/94/0900–1297 $06.00



Scheme 1.

NMR spectrum of the mixture, namely, a triplet at $\delta = 0.87$ ppm (terminal methyl group) and a strong broad signal at $\delta = 1.27$ ppm (methylene chain in fatty acid moiety) were observed. The ^1H NMR spectrum also showed a signal due to two methylene and a methine group bearing ester oxygens at $\delta = 4.12$ (dd; $J = 12.0$, 5.9 Hz, 2H), 4.28 (dd; $J = 12.0$, 5.9 Hz, 2H) and 5.26 (m; 1H) respectively, and a signal at 5.35 ppm due to the olefinic protons. Furthermore, the signals of the methylene protons attached to α-position of esters-carbonyl and to α,β positions of the double bonds in fatty acid moiety of the compounds **1–3** were observed at $\delta = 2.00$, 2.30 and 1.60 ppm, respectively. The ^{13}C NMR spectrum of the mixture **1–3** exhibited signals of the carbonyl carbons at $\delta = 173.1$, 172.7 ppm; olefinic carbons at 128.0–130.0 and methylene groups at 29.1–34.2 ppm (Table I). In the CI-

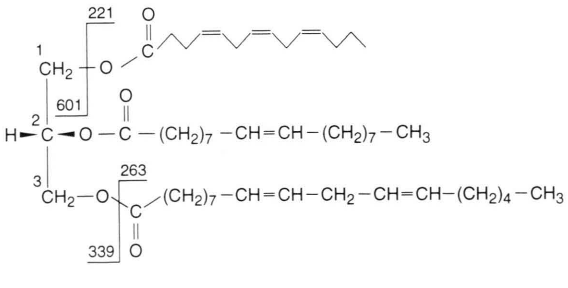


Fig. 1. CI-MS fragmentation of **1.**

Table I. ^1H NMR and ^{13}C NMR data of the mixture of compounds **1–3** in $CDCl_3$ (δ values in ppm from TMS, splitting in Hertz in parentheses).

	H	Carbon
H_2C-1	4.12 (dd, $J = 12.0$; 5.9 Hz, 2H)	62.5
H_2C-3	4.28 (dd, $J = 12.0$; 5.9 Hz, 2H)	62.3
HC-2	5.26 (m)	70.5
HC=	5.35 (m)	128.0–130.0
$=-CH_2$	2.00 (m)	27.2
$-OCOCH_2$	2.30 (t-like)	34.2–34.4
$=CH_2CH_2$	1.60 (m)	24.9
$=-CH_2-=$	2.80 (m)	25.7
CH_2 –alif.	1.27 (m)	29.5–29.8
CH_3 –term.	0.87 (t-like)	14.3
COO	–	2×173.1–172.7

MS spectrum of the mixture, a common molecular ion at m/z = 823 $(M+1)^+$ ($C_{53}H_{90}O_6$) and fragments, due to the elimination of an acyloxy residue, at m/z = 601 $(M-C_{14}H_{21}O_2)^+$ (**1**) (Fig. 1), 575 $(M-C_{16}H_{28}O_2)^+$ (**2**) and at m/z = 547 $(M-C_{18}H_{27}O_2)^+$ (**3**) respectively, indicating the loss of tetradecatrienic-, hexadecatetraenic- and octadecatetraenic- acyloxy residue from the molecular ion of **1–3,** have been observed. Other fragments at m/z = 339, 313 and at m/z = 285, due to $[RCO+C_3H_6O_2]^+$ ion [4], suggested the presence of a linoleoyl residue $(C_{18}H_{31}O)^+$, probably located on the carbon C-3 in **1–3,** oleoyl (339–74) in **1,** palmitoyl (313–74) in **2** and myristoyl residues (285–74) in **3** respectively, located on the carbon C-2 of the glycerol moiety. This fragmentation permits to determine not only the kind of acyl residue but also its position on the glycerol moiety, avoiding so the hydrolysis of the natural products [4]. On the basis of the above data the compounds **1–3** are: a mixture of 1–O–(4, 7, 10-tetradecatrienoyl)-2-O-(9-octadecenoyl)-3-O-(9, 12-octadecadienoyl) glycerol (**1**), 1-O-(4, 7, 10, 13-hexadecatetraenoyl)-2-O-(hexadecanoyl)-3-O-(9, 12-octadecadienoyl) glycerol (**2**) and 1-O-(5, 8, 11, 14-octadecatetraenoyl)-2-O-(tetradecanoyl)-3-O- (9, 12-octadecadienoyl) glycerol (**3**), (Fig. 2).

Concerning the biological activity, it has been shown that triglycerides and diacylglyceryl esters, isolated from the eggs of the sea hare *Aplysia Kurodai,* exhibit vomit and diarrhea [5, 6].

Chromatography (silica gel) of the fraction B using *n*-hexane/ethyl acetate (8:2) as eluent afforded the compound **4,** fucosterol. The ^{1}H NMR spectrum of **4** showed signals at δ = 5.30 ppm (d; J = 4.7 Hz) (H-6) and at δ = 5.20 ppm (q; J = 6.0 Hz) due to the olefinic proton H-28, and at δ = 3.51 ppm (m) due to the proton H-3. The multiplet at δ = 2.25 ppm, due to the proton H-25, is typical for the E-28 isomer, fucosterol, while in the Z-28 isomer, isofucosterol, it is at δ = 2.80 ppm [6, 7]. The molecular ion at m/z = 413 $(M+1)^+$, the fragment at m/z = 395 (M-18, 100%)$^+$ in the CI-MS spectrum, the fragment at m/z = 314 $(M-C_7H_{14}, 100\%)^+$ in the EI-MS spectrum and the ^{13}C NMR data at δ = 146.9 ppm (C-24), 140.7 (C-5), 121.6 (C-6), 115.3 (C-28) confirm the structure of fucosterol (Fig. 3). The presence of the Z-28 isomer, isofucosterol, and of other sterols hasn't been yet observed in *Fucus virsoides* J. AG.

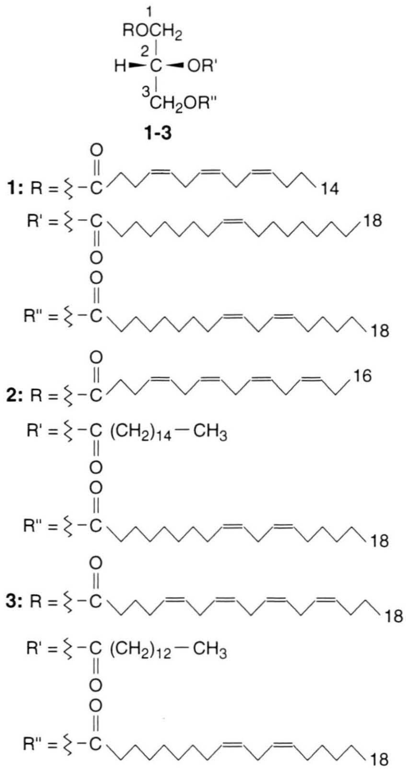


Fig. 2. Structures of the triacylglycerols **1–3.**

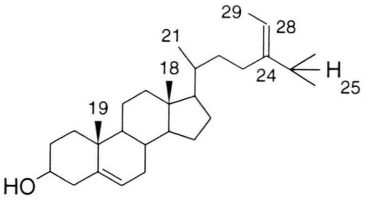


Fig. 3. Structure of fucosterol (**4**).

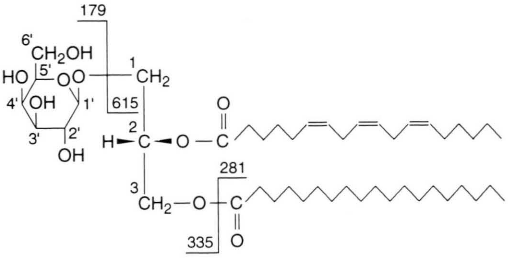


Fig. 4. CI-MS fragmentation of monogalactosyl diacylglycerol (**6**).

Table II. ^1H NMR and ^{13}C NMR data of the mixture of compounds **5–8** in $CDCl_3$ (δ values in ppm from TMS, splitting in Hertz in parentheses).

	H	Carbon		H	Carbon
1	3.91 (dd; J = 11.1, 5.4) 3.73 (dd; J = 11.2, 6.6)	68.3	$-COO$	–	173.8 173.5
2	5.30 (m)	70.2	$-CH{=}CH-$	5.34–5.40 (m)	127.9–130.3
3	4.40 (dd; J = 11.8, 3.3) 4.20 (dd; J = 12.2, 6.7)	62.7	$=-CH_2-=$	2.56 (m)	25.6
1′	4.27 (d; J = 7.5)	104.8	$-OCOCH_2$	2.33 (t-like)	34.2, 34.3
2′	3.66 (dd; J = 9.5, 7.8)	73.4	$=-CH_2CH_2-$	2.02 (m)	27.2
3′	3.59 (dd; J = 9.6, 3.2)	71.6	$-COCH_2CH_2$	1.60 (broad s)	24.9
4′	4.02 (d; J = 2.9)	69.4	CH_2-alif.	1.30 (m)	29.1–31.1
5′	3.54 (dd; 5.2, 4.6)	74.5	CH_3-term.	0.86 (t; J = 7.5)	14.2
6′	3.95 (dd; J = 12.1, 5.9) 3.85 (dd; J = 12.2, 4.6)	62.6			

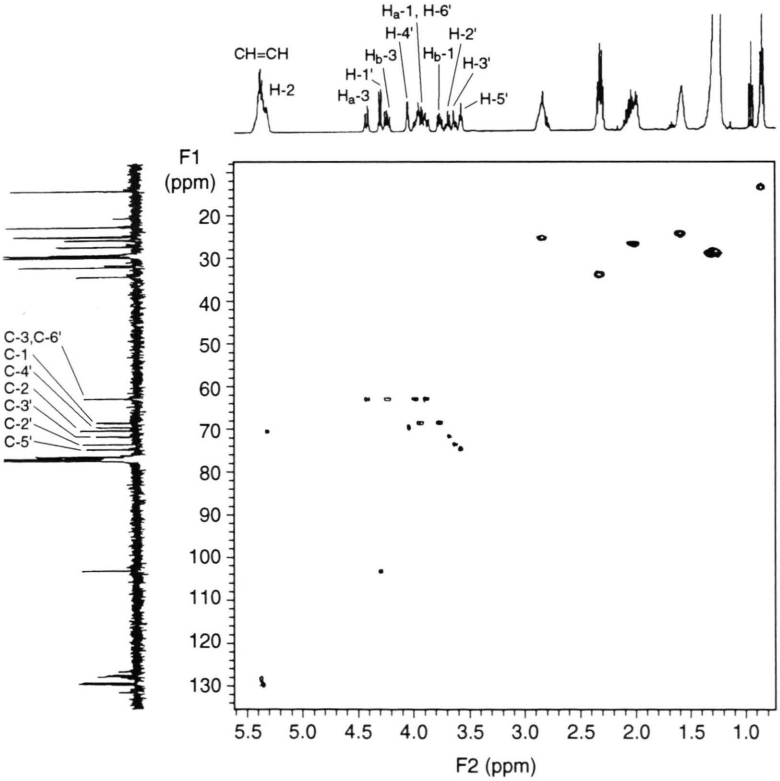


Fig. 5. ^1H-^{13}C Long-Range COSY of the mixture **5–8.**

The fraction C has been chromatographed on silica gel using *n*-hexane/ethyl acetate/methanol (4:6:1) as eluent to give the compounds **5–8** as mixture. The CI-MS spectrum of the mixture exhibited molecular ions at m/z = 773 in **5,** 795 in **6,** and at m/z = 805 $(M+1)^+$ in **7** and fragments at m/z = 593 in **5**, 615 in **6** and at m/z = 625 in **7** $(M-179)^+$ respectively, suggesting the loss of a sugar residue located on the carbon C-1 of the glycerol moiety (Fig. 4). Other fragments at m/z = 313, 335 and at m/z = 339 $(M-179-FA)^+$ in **5–7,** corresponding to the $(RCO+C_3H_6O_2)^+$ ion, due to the elimination of an acyl residue located probably on the carbon C-3 of the glycerol moiety have been characterized as nonadecanoyl- $(C_{19}H_{37}O)^+$ in **5, 6** and eicosatetraenoyl- $(C_{20}H_{31}O)^+$ in **7,** acyl residues respectively. The compound **8**, known as heterosigma-glycolipid I, isolated from the Raphidophycean Dinoflagellate *Heterosigma akashiwo* [8], showed in the CI-MS a molecular ion at m/z = 771 $(M+1)^+$, fragments at m/z = 591 $(M-179)^+$ and at m/z = 333 $(M-179-259+1)^+$. In the ^1H NMR spectrum of the mixture of **5–8,** the characteristic signals at δ = 4.40 (dd; J = 11.8, 3.37, 1H), 4.20 ppm (dd; J = 12.2, 6.7, 1H) and 5.28 (m; 1H) due to the methylen and methyne group bearing ester oxygens on the glycerol moiety respectively, and the signal at 4.27 (d; J = 7.5 Hz), due to the anomeric proton of the sugar moiety have been observed. The chemical shifts in the ^1H NMR and the signals at δ = 104.8 ppm and at δ = 74.5–62.7 ppm in the ^{13}C NMR spectrum of the mixture **5–8** (Table II) are in good aggreement with those reported in literature [9, 10] and this suggests that the sugar residue located on the carbon C-1 of the glycerol moiety is β-D-galactosyl. In the CI-MS spectrum of the mixture **5–8,** the fragments at m/z = 313, 333, 335 and 339, due to $(RCO+74)^+$ ion, were useful in providing informations for the determination of the fatty acid residues located on the carbon C-2, characterized as hexadecanoyl, octadecatetraenoyl, linolenoyl, oleoyl residues in **5–8** respectively. Further structural confirmations were obtained by TOCSY 1D, ^1H-^1H COSY, DQF-COSY and ^1H-^{13}C COSY long-range COSY spectral analysis, which displayed many correlations as shown in Fig. 5. In the TOCSY 1D, ^1H-^1H COSY, DQF-COSY spectra a strong correlation between the H-2 signal and the two methylene signals located at the C-1 and C-3 of the glycerol moiety has been observed, while the proton H-5′ in the galactosyl moiety correlated strongly with H-6′, but weakly with H-4′. In the proton-carbon long-range shift correlation (^1H-^{13}C COSY long-range COSY) spectrum, the CH_2-1 (δ = 3.91, 3.73 ppm) and the H-2′ were found to be correlated with the anomeric proton in the galactosyl moiety, while the H_a-3 correlates with the signal at δ = 173.8 ppm. Based on the findings mentioned above, the structures of the compounds **5–8** of the fraction C are: 2-O-(hexadecanoyl)-3-O-(nonadecanoyl) glyceryl β-D-galactopyranoside (**5**), 2-O-(6, 9, 12-octadecatrienoyl)-3-O-(nonadecanoyl)-glyceryl β-galactopyranoside (**6**) and 2-O-(9-octadecenoyl)-3-O-(6, 9, 12, 15-eicosatetraenoyl) glyceryl β-D-galactopyranoside (**7**) and 2, 3-di-O-(6, 9, 12, 15-octadecatetraenoyl) glyceryl β-D-galactopyranoside (**8**), respectively (Fig. 6). Concerning the biological activity of glycolipids, a galactolipids, which exhibits anti-inflammatory activity has been isolated from the Okinawan marine sponge *Phyllos-*

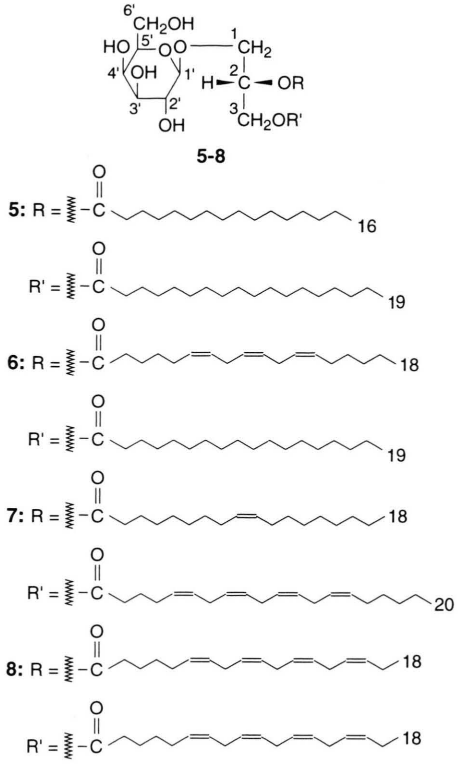


Fig. 6. Structures of monogalactosyl diacylglycerols (**5–8**).

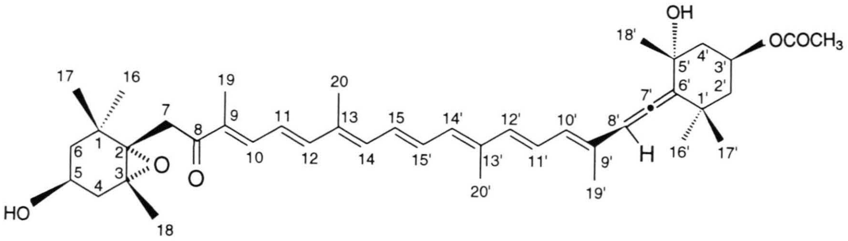


Fig. 7. Structure of fucoxanthin.

pongia foliascens (Pallas) [10]. Recently, an examination of the antialgal activities of the isolated glyceroglycolipids for *Phormidium tenue* revealed that the monogalactosyl diacylglycerols, which are major membrane lipids, induced the lysis of this cyanobacterium and showed anti-tumor activity [11, 12].

From the fraction D, throught repeated column chromatography with silica gel as absorbent, a yellow, crystallized compound **9** has been obtained. The FAB-MS spectrum of the compound **9** shows only a molecular ion at $m/z = 665$ $(M+Li)^+$, and in the ^1H NMR spectrum a number of characteristic signals of fucoxanthin in the field at $\delta = 0.96-3.66$ ppm have been observed and these are in good aggreement with those reported in literature [13]. The ^{13}C NMR spectrum of **9** and DEPT experiments confirmed the structure of fucoxanthin [1, 13]. Fucoxanthin (Fig. 7) occurs in brown algae and is probably the most abundant carotenoid in nature [13].

Experimental

^1H NMR spectra: Varian Unity 400 spectrometer and Varian Gemini 200 spectrometer. ^{13}C NMR: 100.4 MHz. NMR spectra were obtained by using $CDCl_3$ as solvent. FAB-MS, EI-MS, DCI-MS: Kratos MS 80 RFA. FAB-MS (8 Kv, Xe; methanol as solvent and glycerol matrix + LiCl), EI-MS: (4 Kv, 70 eV), DCI-MS: (4 Kv, 120 eV; NH_3). Flash-chromatography: Kieselgel 60 (230–400 Mesh, 60 Å, Merck). All solvents were distilled before use. TLC: Kieselgel 60 F_{254} (20×20 cm; 0.2 mm, Merck).

Fucus virsoides J. AG. (2 Kg) was collected in Punta Sottile (Trieste, Italy) in October 1991.

Isolation of the compounds **1–9**

The alga *Fucus virsoides* J. AG. was harvested in October 1991 from Punta Sottile (North Adriatic Sea, Trieste; Italy). It was first washed and freed from extraneous matter by hand sorting, then was refrigerated. The frozen alga was minced and lyophilized to give a green powder (200 g) which was then treated according to Scheme 1.

The powder was extracted with MeOH in 5-liter glass bottles for 15 days with occasional shaking. The methanol extract, concentrated *in vacuo,* (10 g) was extracted successively with a mixture *n*-hexane/ethyl acetate (1:1). The *n*-hexane/ethyl acetate extract (2 g) was chromatographed over silica gel with *n*-hexane/ethyl acetate (10:1) to yield fraction A (250 mg), then *n*-hexane/ethyl acetate (8:2) to yield fraction B (800 mg) and finally with *n*-hexane/ethyl acetate/methanol (4:6:1) to yield fraction C (150 mg) and fraction D (200 mg). The fraction A was purified by flash chromatography using *n*-hexane/ethyl acetate (10:1) and the compounds **1–3** were obtained as mixture.

1-O-(4,7,10-tetradecatrienoyl)-2-O-(9-octadecenoyl)-3-O-(9,12,octadecadienoyl)glycerol (**1**)

^1H NMR and ^{13}C NMR data, in $CDCl_3$, of **1–3** are reported in Table I. CI-MS: $m/z = 823$ $(M+1)^+$, 601 $(M-221)^+$, 339 $(M-221-263+1)^+$, 339 $(C_{18}H_{31}O+C_3H_6O_2)^+$. Calcd. for $C_{53}H_{90}O_6$: 822.7.

1-O-(4,7,10,13-hexadecatetraenoyl)-2-O-(hexadecanoyl)-3-O-(-9,12-octadecadienoyl)glycerol (**2**)

CI-MS: $m/z = 823$ $(M+1)^+$, 575 $(M-247)^+$, 313 $(M-247-263+1)^+$, 313 $(C_{16}H_{31}O+C_3H_6O_2)^+$. Calcd. for $C_{53}H_{90}O_6$: 822.7.

1-O-(5,8,11,14-octadecatetraenoyl)-2-O-(tetradecanoyl)-3-O-(9,12-octadecadienoyl)glycerol (**3**)

CI-MS: $m/z = 823$ $(M+1)^+$, 547 $(M-275)^+$, 285 $(M-275-263+1)^+$, 285 $(C_{14}H_{27}O+C_3H_6O_2)^+$. Calcd. for $C_{53}H_{90}O_6$: 822.7.

The fraction B was purified by flash chromatography using *n*-hexane/ethyl acetate (8:2) and the compound **4** was obtained in pure form.

Fucosterol (**4**)

^{1}H NMR ($CDCl_3$): δ = 5.30 ppm (d; 1 H, H-6), 5.20 (q; 1 H, J = 6 Hz, H-28), 3.51 (m; 1 H, H-3), 2.25 (m; 1 H, H-25), 2.00–1.85 (m; 2 H, H-7; 2 H, H-22), 1.57 (d; 3 H, J = 6 Hz, CH_3-29), 1.01 (s; 3 H, CH_3-19), 0.99 (3 H, CH_3-26; 3 H, CH_3-27), 0.94 (d; 3 H, J = 6 Hz, CH_3-21). ^{13}C NMR ($CDCl_3$): δ = 146.9 ppm (C-24), 140.7 (C-5), 121.6 (C-6), 115.3 (C-28), 71.8 (C-3), 56.7 (C-14), 55.7 (C-17), 50.1 (C-9), 36.4 (C-20), 34.2 (C-25), 31.6 (C-8), 42.3 (C-4), 39.2 (C-16), 36.5 (C-1), 35.2 (C-22), 31.1 (C-2), 31.6 (C-7), 29.2 (C-12), 25.6 (C-23), 24.3 (C-15), 22.2 (C-27), 22.1 (C-26), 21.0 (C-11), 19.4 (C-19), 18.7 (C-21), 13.2 (C-18), 11.0 (C-29). CI-MS: *m/z* = 413 $(M+1)^+$, 395 $(M\text{-}18+1, 100\%)^+$. EI-MS: *m/z* = 412 $(M, 23\%)^+$, 314 $(M\text{-}C_7H_{14}, 100\%)^+$. Calcd. for $C_{29}H_{48}O_1$: 412.4.

The fraction C was purified by flash chromatography using *n*-hexane/ethyl acetate/methanol (4:6:1) and the compounds **5–8** were obtained as mixture.

2-O-(hexadecanoyl)-3-O-(nonadecanoyl) glyceryl β-D-galactopyranoside (**5**)

^{1}H NMR and ^{13}C NMR data of **5–8** ($CDCl_3$) are reported in Table II. CI-MS: *m/z* = 773 $(M+1)^+$, 593 $(M\text{-}179)^+$, 313 $(M\text{-}179\text{-}281+1)^+$, 313 $(C_{16}H_{31}O+C_3H_6O_2)^+$. Calcd. for $C_{44}H_{84}O_{10}$: 772.6.

2-O-(6,9,12-octadecatrienoyl)-3-O-(nonadecanoyl)glyceryl β-galactopyranoside (**6**)

CI-MS: *m/z* = 795 $(M+1)^+$, 615 $(M\text{-}179)^+$, 335 $(M\text{-}179\text{-}281+1)^+$, 335 $(C_{18}H_{29}O+C_3H_6O_2)^+$. Calcd. for $C_{46}H_{82}O_{10}$: 794.6.

2-O-(9-octadecenoyl)-3-O-(5,8,11,14-eicosatetraenoyl) glyceryl β-D-galactopyranoside (**7**)

CI-MS: *m/z* = 805 $(M+1)^+$, 625 $(M\text{-}179)^+$, 339 $(M\text{-}179\text{-}287+1)^+$, 339 $(C_{18}H_{33}O+C_3H_6O_2)^+$. Calcd. for $C_{47}H_{80}O_{10}$: 804.6.

2,3-di-O-(6,9,12,15-octadecatetraenoyl) glyceryl β-D-galactopyranoside (**8**)

CI-MS: *m/z* = 771 $(M+1)^+$, 591 $(M\text{-}179)^+$, 333 $(M\text{-}179\text{-}259+1)^+$. Calcd. for $C_{45}H_{70}O_{10}$: 770.5.

The fraction D was purified by flash chromatography using *n*-hexane/ethyl acetate/methanol (4:6:1) and the compound **9** was obtained in the pure form.

Fucoxanthin (**9**)

^{1}H NMR ($CDCl_3$): δ = 7.14 ppm (d; 2 H, J = 10.5 Hz, H-10), 6.90–6.12 (m; CH=CH), 6.05 (s; 1 H, H-8′), 5.37 (m; 2 H, H-3′, OH-5′), 3.81 (m; 1 H, H-3), 3.66–2.59 (ABq; 2 H, J = 18.3 Hz, H-7), 2.04 (s; 3 H, CH_3CO), 1.99 (s; 6 H, CH_3-20, 20′), 1.94 (s; 3 H, CH_3-19), 1.81 (s; 3 H, CH_3-19′), 1.38 (s; 3 H, CH_3-18′), 1.35 (s; 3 H, CH_3-17′), 1.22 (s; 3 H, CH_3-18), 1.07 (s; 3 H, CH_3-16′), 1.03 (s; 3 H, CH_3-17), 0.96 (s; 3 H, CH_3-16). ^{13}C NMR ($CDCl_3$): 202.3 (C=C=C), 197.3 (C=O), 170.4 (COO), 125.3–129.6/132.4–145.0 (HC=CH), 103.3 (C-8′), 67.0 (C-6), 66.1 (C-5), 68.0 (C-3), 64.3 (C-3′), 47.0 (C-7), 45.4 (C-2′), 45.2 (C-2), 41.6 (C-4′), 40.2 (C-4), 32.0 (C-17′), 31.2 (C-17), 29.1 (C-16′), 28.1 (C-16), 25.0 (C-18), 21.4 (C-18′), 21.1 (CH_3–C=O), 13.9 (C-19′), 12.8 (C-20′), 12.7 (C-20), 11.8 (C-19). FAB-MS: *m/z* = 665 $[M+Li]^+$. Calcd. for $C_{42}H_{58}Li_1O_6$: 665.4.

Acknowledgement

This work is part of the research program "Sistema Lagunare Veneziano". We thank the Regione Veneto, Department for Industry and Energy, for financing the purchase of the Varian Unity 400 spectrometer.

[1] B. P. Kremer, I. H. Munda, Marine Ecology **3,** 75 (1982).

[2] I. M. Munda, Hydrobiologia **151/152,** 477 (1987).

[3] A. Avanzini, V. Kosovel, V. Scarcia, A. Furlani, L. Ravalico, Fitoterapia **4,** 391 (1987).

[4] K. Satouchi, M. Oda, K. Yasunaga, K. Saito, Biochem. Biophys. Res. Commun. **128,** 1409 (1985). (By CI-MS analysis of trioleoyl glycerol sample, the fragments at *m/z* = 885 (M+1), 603 (M-281), 339 (M-281-265) and $[265+C_3H_6O_2]^+$ were observed and they supported the fragmentation of compounds (**1**–**3**).

[5] T. Miyamoto, R. Higuchi, M. Funatsu, H. Seike, T. Nohara, T. Komori, Liebigs Ann. Chem. **1988,** 585.

[6] S. S. Papov, N. L. Marekov, M. I. Konaklieva, M. I. Panayotova, S. Dimitrova-Konaklieva, Phytochemistry **24,** 1987 (1985).

[7] J. Frost, J. Ward, Tetrahedr. Lett. **1968,** 3779.

[8] M. Kobayashi, K. Hayashi, K. Kawazoe, I. Kitagawa, Chem. Pharm. Bull. **40** (6), 1404–1410 (1992).

[9] B. W. Son, Phytochemistry **29,** 307 (1990).

[10] H. Kikuchi, Y. Tsukitani, T. Manda, T. Fujh, H. Nakanischi, M. Kobayashi, I. Kitagawa, Chem. Pharm. Bull. **30,** 3544 (1982).

[11] N. Murakami, T. Morimoto, H. Imamura, T. Ueda, S. Nagai, J. Sakakibara, N. Yamada, Chem. Pharm. Bull. **39** (9), 2277–2281 (1991).

[12] H. Shirahashi, N. Murakami, M. Watanabe, A. Nagatsu, J. Sakakibara, H. Tokuda, H. Nishino, A. Iwashima, Chem. Pharm. Bull. **41,** 1664 (1993).

[13] R. Bonnet, A. K. Maliams, A. A. Spark, J. L. Tee, B. C. L. Weedon, A. McCormick, J. Chem. Soc. (C) **1969,** 429.