Chemistry of Natural Compounds, Vol. 41, No. 3, 2005

LIPIDS FROM THE MARINE ALGA *Gracilaria verrucosa*

S. V. Khotimchenko

UDC 577.115+582.273

The composition of lipids and fatty acids from the red alga Gracilaria verrucosa, *for which a high content of 20:4n-6 acid is typical, was studied. The principal lipids were digalactosyldiacylglycerides, phosphatidylcholines (PC), monogalactosyldiacylglyderides (MGDG), and sulfoquinovosyldiacylglycerides, the fraction of each was approximately the same. Sphingophospholipids, inositephosphoceramides, were identified among the polar lipids. Each lipid class differed in the ratio of fatty acids (FA). The FA of all glycerolipids contained 20:4n-6 acid but its concentration was greatest in MGDG and PC, 67.2% and 56.5% of the acid mass.*

Key words: *Gracilaria verrucosa*, alga, arachidonic acid, fatty acids, glycolipids, triacylglycerides, phospholipids.

Marine red algae are interesting not only as sources of agar and carragheenan but also as rich concentrators of arachidonic 20:4 (*cis*-5,8,11,14) or 20:4n-6 and eicosapentaenoic 20:5 (*cis*-5,8,11,14,17) or 20:5n-3 acids, which are important for human and animal health. These acids are interesting because they are precursors in the biosynthesis of prostaglandins, thromboxans, and other eicosanoids, which are viewed as important bioregulators of many cellular processes [1]. Furthermore, 20:4n-6 is an essential fatty acid (FA) and should absolutely be present in food, especially for infants [2]. The principal polyunsaturated acid in most red algae is 20:5n-3. The content of 20:4n-6 and 20:5n-3 acids is approximately the same in many species. The content of 20:4n-6 is high, varying from 45.9 to 62.0% of the total FA depending on the season, only in several species of the genus *Gracilaria*, primarily *G. verrucosa* [3-5]. A study of lipids from this species was focused mainly on the FA composition [3, 4, 6]. However, FA are rarely found in the free state in algae but are incorporated into complex lipids as structural components. Therefore, we characterized acyl lipids from *G. verrucosa* and determined that the lipids have a high concentration of 20:4n-6 acid.

The lipid content of *G. verrucosa* is low, averaging 2.7 ± 0.7 mg/g fresh mass or 15.2 ± 2.7 mg/g dry mass. The lipid content varies due to habitat, age, or growth stage [7, 8]. Lipids from this species represent several groups: glycosyldiacylglycerides or glycolipids, phospholipids, and neutral lipids, which differ in structure and fulfill different functions. The glycolipids dominate, making up more than half of all lipids (Table 1). Glycolipids dominate also the lipids from other species of red alga. Their content reaches 50.3-75.1% of the lipid mass [3, 9]. We identified in *G. verrucosa* monogalactosyldiacylglycerides (MGDG), digalactosyldiacylglycerides (DGDG), and sulfoquinovosyldiacylglycerides (SQDG), among which DGDG dominated (25.0 ± 3.1% of total lipids). MGDG were second in abundance among the glycolipids whereas the SQDG content was much less. MGDG dominate in most marine algae. DGDG dominate the glycolipids of only a few species of red algae [10-12]. This group includes this species of *Gracilaria*.

The phospholipid composition of *G. verrucosa* is simple: phosphatidylcholines (PC), phosphatidylglycerines (PG), phosphatidylinosites (PI), and phosphatidylethanolamines (PE). In addition to these glycerophospholipids, we identified sphingophospholipids, inositephosphoceramides (IPC), the structure of which was recently proved [13]. Among the phospholipids, PC dominated (22.9 ± 3.1% of total lipids). Their content was significantly greater than that of each of the other phospholipids (Table 1). The PI, and especially PE, were among the minor components. This same composition of phospholipids was observed in other species of red algae. The IPC were previously designated as unknown lipids [14, 15].

Institute of Marine Biology, Far-East Division, Russian Academy of Sciences, Vladivostok, 690041, fax (4232)310900, e-mail: skhotim@hotmail.com. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 230-232, May-June, 2005. Original article submitted January 20, 2005.

0009-3130/05/4103-0285

TABLE 1. Composition of Lipids from Red Alga *Gracilaria verrucosa*

Lipids	% of total lipids
Neutral lipids	
Triacylglycerides	5.5±1.2
Glycolipids	
Monogalactosyldiacylglycerides	20.5±2.7
Digalactosyldiacylglycerides	25.0±3.1
Sulfoquinovosyldiacylglycerides	17.8±0.8
Phospholipids	
Phosphatidylcholines	22.9±3.1
Phosphatidylglycerines	4.3±1.1
Phosphatidylinosites	0.9±0.2
Phosphatidylethanolamines	0.5±0.2
Inositephosphoceramides	2.6±1.0

TABLE 2. Composition of FA in Individual Lipid Classes from Red Alga *Gracilaria verrucosa*, % of Total FA

Fatty acids	Triacylglycerides	Glycolipids			Phospholipids			Total lipids
		MGDG	DGDG	SQDG	PC	PG	IPC	
14:0	6.2	-	2.2	13.7	1.4	2.8	24.8	4.6
16:0	25.6	21.5	46.0	61.2	24.2	30.7	54.0	32.8
16:1n-7	18.6	1.0	0.6	0.8	0.7	3.1	6.5	2.2
16:1-*trans*	-	-	-	-	-	12.5	-	0.4
18:0	10.0	0.9	1.1	1.3	1.5	3.3	11.2	2.0
18:1n-9	10.5	5.9	18.1	2.7	3.7	11.0	3.4	8.4
18:2n-6	1.5	0.8	0.8	0.5	1.5	2.5	-	1.3
20:3n-6	1.6	0.8	0.6	-	7.6	3.8	-	2.8
20:4n-6	24.2	67.2	29.5	17.9	56.5	29.4	-	41.2
20:5n-3	-	0.9	-	-	0.7	-	-	0.5
Others	1.8	1.0	1.1	1.9	2.0	0.9	0.1	3.8
$\Sigma_{sat.}$ FA	41.8	22.4	49.3	76.2	27.1	36.8	90.0	39.4
$\Sigma_{monoen.}$ FA	29.1	6.9	18.7	3.5	4.4	26.6	9.9	11.0
$\Sigma_{PUFAn-6}$	27.3	68.8	30.9	18.4	65.6	35.7	-	45.3

According to TLC, the main components of the neutral lipids from *G. verrucosa* were triacylglycerides (TG). An insignificant amount of sterols and their esters was also found.

FA are important structural components of polar and neutral lipids. The qualitative FA composition of individual lipid classes from *G. verrucosa* was the same as for the total lipids. However, each lipid class differed in FA ratio. Thus, MGDG, the most unsaturated glycolipids owing to the high concentration in them of 20:4n-6 acid, made up 67.2% of the total FA (Table 2). The content of arachidonic acid in DGDG is 2.2 times less whereas that of 16:0 is significantly greater than in MGDG. If MGDG, DGDG, and SQDG are viewed as a series of glycolipids, the mass of 20:4n-6 acid in them decreased sharply and that of saturated acids 14:0 and 16:0 increased simultaneously, reaching 74.9% in SQDG. Similar trends for FA localization in glycolipids were noted for three other previously studid species of red algae. However, since these algae contained 20:5n-3 acid in addition to 20:4n-6, the MGDG unsaturation was due to the high concentration of 20:5n-3 acid [9, 12].

We observed the highest level of 20:4n-6 acid among the phospholipids in PC (56.5%). The FA compositions of PC and MGDG turned out to be rather similar. However, the PC had a significantly greater content of 20:3n-6 acid. Our results agree with those of Japanese researchers, who found a high concentration of 20:4n-6 in PC from *G. verrucosa* [3] and

demonstrated that the total PC contained from 56.2 to 64.2% diarachidonylphosphatidylcholine [16]. Saturated and polyunsaturated FA (PUFA) were localized in the PG of this species to approximately the same level, 36.8 and 35.7% of the total FA. The content of 20:3n-6 acid in these lipids was rather high. However, the main point is that the *trans*-isomer of 16:1 was concentrated exclusively in the PG whereas this is typical for terrestrial plants and other marine algae [17]. Sphingophospholipids and IPC had a limited set of FA that included saturated and monoenoic components, among which 16:0 acid dominated. The FA composition of PI and PE could not be determined reliably due to the exceedingly low concentration of them in *G. verrucosa*.

The main FA of TG in this alga species consisted of saturated and monoenoic FA (14:0, 16:0, 18:0, 16:1, 18:1) whereas the PUFA made up 27.3% of the total FA. *G. verrucosa* differs from other species of red algae in having a low PUFA content in the TG. Thus, these acids in *Polysiphoniua lanosa* and *Chondrus crispus* make up more that 50% of the total acids [9] whereas they are slightly greater than 25% in *G. verrucosa*.

Thus, the principal lipids from *G. verrucosa* were DGDG, PC, MGDG, and SQDG, the fractions of which were approximately the same. The TG, PG, and IPC formed a group of lipids of lesser content. PI and PE were present in insignificant quantities. Each lipid class differed in FA ratio. Arachidonic acid was present in FA of all glycerolipids. However, its concentration was greatest in MGDG and PC, which makes these lipids very unsaturated.

EXPERIMENTAL

Methyl esters of FA were analyzed by GC in a chromatograph with a Shimadzu-16A (Shimadzu, Kyoto, Japan) flame-ionization detector. The GC conditions were: capillary column (30 m × 0.32 mm) with Supelcowax 10M, column temperature 210°C, detector 240°C, He carrier gas, flow rate 40 mL/min.

Alga was collected in Peter the Great Bay in the Sea of Japan in August-September. It was thoroughly cleaned of other algae, fine invertebrates, and solids and washed with freshwater. The plants were immersed for 2-3 min in boiling water to inactivate enzymes and ground in a mortar with sand. Lipids were extracted using $CHCl_3$:CH_3OH (1:2) by a modified Bligh and Dyer method [20].

Lipids were separated using two-dimensional TLC on plates (6 × 6 cm) coated with a layer of silica gel. Polar lipids were separated using the solvent systems $CHCl_3$:CH_3COCH_3:CH_3OH:CH_3COOH:H_2O (100:40:20:20:8 v/v) in the first direction and CH_3COCH_3:C_6H_6:CH_3COOH:H_2O (200:30:3:10) in the second. Neutral lipids were separated by one-dimensional TLC using C_6H_{14}:$C_2H_5COC_2H_5$:CH_3COOH (70:30:1). Lipids were detected nonspecifically by spraying with H_2SO_4 (10%) in CH_3OH followed by heating to 100°C. Glyclipids were detected by anthrone (0.2%) in benzene [19]; phospholipids, molybdate reagent [20]. Lipids were also identified by comparing R_f values with those of standards. Lipids were determined quantitatively after separation using two-dimensional chromatography by collecting bands of silica gel containing the pure lipids from several plates and eluting the silica gel with $CHCl_3$:CH_3OH (1:1). The solvent was evaporated. The solid was treated with 17:0 acid (10 μg) and methylated by the Carreau and Dubacq method [21] to form the FA methyl esters, which were analyzed by GC. More forcing methylation conditions were used to prepare the FA methyl esters of IPC: HCl (5%) in CH_3OH, 80°C, 4 h [18]. The content of each lipid class was determined by multiplying the mass of isolated FA methyl esters by the apropriate coefficients: for MGDG, 1.45; DGDG, 1.72; SQDG, 1.57; phospholipids, 1.45; TG, 1.2.

FA were identified by comparing retention times with standards and the indices for the equivalent chain length [22].

REFERENCES

1. H. Tapiero, G. N. Ba, P. Couvreur, and K. D. Tew, *Biomed. Pharmacother.*, **56**, 215 (2002).
2. B. Koletzko and M. Braun, *Ann. Nutr. Metabol.*, **35**, 128 (1991).
3. S. Araki, T. Sakurai, T. Oohusa, M. Kayama, and K. Nisizawa, *Hydrobiologica*, **204/205**, 513 (1990).
4. C. J. Dawes, C. Kovach, and M. Friedlander, *Bot. Mar.*, **36**, 289 (1993).
5. S. V. Khotimchenko and I. S. Gusarova, *Biol. Morya (Vladivostok)*, **30**, 215 (2004).
6. S. V. Khotimchenko, V. E. Vaskovsky, and V. F. Przhemenetskaya, *Phytochemistry*, **30**, 207 (1991).
7. S. V. Khotimchenko and E. V. Levchenko, *Bot. Mar.*, **40**, 367 (1997).

8. E. V. Levchenko, I. R. Fomina, S. V. Khotimchenko, and K. Ya. Bill′, *Fiziol. Rast.*, **47**, 1 (2000).
9. T. R. Pettitt, A. L. Jones, and J. L. Harwood, *Phytochemistry*, **28**, 399 (1989).
10. S. V. Khotimchenko, *Khim. Prir. Soedin.*, 186 (2002).
11. V. M. Dembitsky, E. E. Pechenkina-Shubina, and O. A. Rozentsvet, *Phytochemistry*, **30**, 2279 (1991).
12. N. M. Sanina, S. N. Goncharova, and E. Y. Kostetsky, *Phytochemistry*, **65**, 721 (2004).
13. S. V. Khotimchenko and V. E. Vas′kovskii, *Bioorg. Khim.*, **30**, 190 (2004).
14. S. V. Khotimchenko, N. G. Klochkova, and V. E. Vaskovsky, *Biochem. Syst. Ecol.*, **18**, 93 (1990).
15. V. M. Dembitsky and O. A. Rozentsvet, *Phytochemistry*, **29**, 3149 (1990).
16. K. Kinoshita and K. Takahashi, *Bull. Jpn. Soc. Sci. Fish.*, **52**, 757 (1986).
17. S. V. Khotimchenko, *Lipids of Marine Algae-Macrophytes and Grasses* [in Russian], Dal′nauka, Vladivostok (2003).
18. M. Kates, *Techniques of Lipidology: Isolation, Analysis, and Identification of Lipids*, Elsevier, New York (1973).
19. C. M. van Gent, O. J. Roseuleur, and P. van der Bijl, *J. Chromatogr.*, **85**, 174 (1973).
20. V. E. Vaskovsky, E. Y. Kostetsky, and I. M. Vasendin, *J. Chromatogr.*, **114**, 129 (1975).
21. J. P. Carreau and J. P Dubacq, *J. Chromatogr.*, **151**, 384 (1978).
22. W. W. Christie, *J. Chromatogr.*, **447**, 305 (1988).