Chemistry of Natural Compounds, Vol. 35, No. 1, 1999

LIPIDS OF TWO SPECIES OF BROWN ALGAE OF THE GENUS *Laminaria*

S.V. Khotimchenko and I. V. Kulikova

UDC 547.953:577.115:582.26

The compositions of the neutral lipids, glycolipids, phospholipids and fatty acids of the brown algae Laminaria japonica *and* Laminaria cichorioides *have been studied. It has been found that the glycolipids are the main lipid classes in these algae. Palmitic, oleic, and polyunsaturated acids with 18 and 20 carbon atoms form the group of main acids. The lipids of both species of the* Laminaria *genus have a complex composition but they differ by the ratios of their fatty acids.*

Brown algae dominate among the marine macrophytes in the seas of moderate latitudes and have been used by Man since antiquity. The most important algae for their industrial value and abundance are representatives of the genus *Laminaria.* These algae have found use as food and industrial raw material and also in pharmacology and medicine [1, 2]. In recent years, interest has increased in marine algae as sources of polyunsaturated fatty acids (PUFAs), which are essential components in nutrition and play an important role in the life of Man and animals [3, 4]. It has recently been found that algal glycolipids exhibit biological activity [5], which has increased interest in the lipids of these plants even further. The fatty acids (FAs) of algae of the genus *Laminaria* — *L. digitata, L. japonica,* and *L. saccharina* — have been investigated previously [6—11]. Extremely contradictory statements have been made about the FAs of *L. japonica.* Thus, some authors have found octadecatetraenoic acid as the main polyenic acid in this algal species, making up 39.9% of the total FAs [6]. Others have reported that the level of this acid is 4.6—5.3% [9, 10]. A third group of authors failed to detect any of the 18:4ω—3 acid whatever in *L. japonica.* We have been unable to find any information on the lipids of *L. cichorioides* in the literature. We have therefore determined the fatty acid compositions of the two species of *Laminaria* algae — *L. japonica* and *L. cichorioides* — that are most widespread in the Sea of Japan. In addition, we have identified the main lipids in these algae and have determined their ratios. The results obtained are of definite value, since algae of this genus are used most widely by Man in food.

The majority of the lipids that we found in *L. japonica* and *L. cichorioides* proved to be known compounds that had been found previously in higher plants and in other species of algae [12]. The lipids were representatives of several classes: neutral, glycosyldiacylglycerides or glycolipids, and phospholipids, which differ in structure and fulfill various functions in algae. As can be seen from Table 1, glycolipids were the main lipids in the two *Laminaria* species. They amounted to 65.9% of the total lipids in *L. japonica* and to 71.0% in *L. cichorioides.* Glycolipids also dominated among the lipids of other species of brown algae, amounting to 47.2—83.1% of all the lipids [13—15]. Among the glycolipids, monogalactosyldiacylglycerols predominated in both *L. japonica* and *L. cichorioides,* which is characteristic for the majority of other species of brown algae [13—15], apart from species belonging to the order Fucales. In representatives of this order, sulfoquinovosyldiacylglycerols predominated among the glycolipids [14, 16].

L. japonica and *L. cichorioides* had the same phospholipid composition. They contained phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), phosphatidylglycerols (PGs), phosphatidylinositols (PIs), and phosphatidic acids (PAs). The same phospholipids have been found previously in other species of brown algae [14, 17, 18]. However, in both *Laminaria* species we also found another phospholipid, which was identified on the basis of its chromatographic behavior and reactions with specific reagents as a phosphatidyl-O-[N-(2-hydroxyethyl)]glycine (PHEG). The structure of this lipid has been established

Institute of Marine Biology, Far-eastern Scientific Center, Russian Academy of Sciences, Vladivostok, fax (4232) 310900, e-mail: lipid@biom.marine.su. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 22—26, January-February, 1999. Original article submitted December 12, 1998.

0009-3130/99/3501-0017$22.00

TABLE 1. Composition of the Lipids of Algae of the Genus *Laminaria* (% of the total lipids)

Lipids	L. japonica	L. cichorioides
Neutral	19.7	15.1
Monogalactosyldiacylglycerols	25.0	27.0
Digalactosyldiacylglycerols	19.5	23.9
Sulfoquinovosyldiacylglycerols	21.4	20.1
Phosphatidylcholines	6.4	7.1
Phosphatidylethanolamines	2.9	2.6
Phosphatidylglycerols	3.5	3.3
Phosphatidylinositols	0.7	0.5
Phosphatidylhydroxyethylglycerols	0.6	0.3
Phosphatidic acids	0.3	0.1
Total lipids *	3.5	4.0

* In percentages of the dry weight of the algae.

Figures given as the mean values of three measurements.

TABLE 2. Fatty Acid Compositions of Algae of the *Laminaria* Genus

Fatty acids	L. japonica	L. cichrioides
14:0	5.3	5.4
trans-14:0	1.7	1.2
16:0	14.6	19.7
16:1n-7	3.9	3.7
trans-16:1	0.3	0.2
16:2n-6	0.3	0.3
18:0	1.0	2.9
18:1n-9	8.4	13.9
18:2n-6	8.4	7.2
18:3n-6	4.2	3.2
18:3n-3	6.1	3.9
18:4n-3	13.9	5.9
20:0	0.3	0.7
20:2n-6	0.3	0.2
20:3n-6	0.6	0.5
20:4n-3	0.7	0.4
20:4n-6	14.0	10.9
20:5n-3	14.0	13.0
Others*	2.0	6.8
PUFAs (n-3)	34.7	23.2
PUFAs (n-6)	27.5	22.0
C_{18} PUFAs	32.6	20.2
C_{20} PUFAs	29.6	25.0

*Others 12:0, 14:1, 15:0, 16:1-9, 17:0, 18:1n-7, 18:2n-4, 20:1, 20:3n-3.

The figures are given as the mean values of three determinations.

recently [18] and it was shown that PHEG was present in all the species of brown algae investigated [18—20]. Japanese researchers have shown that phosphatidylserines (PSs) are present in *L. japonica* [21]. We did not find PSs in the two *Laminaria* species, just as other authors have failed to find this type of phospholipid in other species of brown algae [14, 17, 18]. The reason for this disagreement in the results probably consists in the fact that PSs and PHEGs show some similarity,

since they both give a positive reaction with ninhydrin. However, these lipids differ slightly in chromatographic mobility in the separation of the phospholipids by TLC: PSs migrate more slowly than PIs, and PHEGs more quickly.

Phosphatidylcholines were the main phospholipids in both species of *Laminaria*, amounting to 6.4% of the total lipids in *L. japonica* and 7.1% in *L. cichorioides*. The PEs and PGs may be assigned to the main phospholipids, while the PIs, PHEGs, and PAs form a group of minor components (Table 1).

According to TLC, the main components of the neutral lipids were the triacylglycerols, and only insignificant amounts of sterols and their esters were present in the two *Laminaria* species.

L. japonica and *L. cichorioides* had fatty acid compositions that were similar and characteristic for brown algae [9, 14, 22]. Palmitic and oleic acids, and also PUFAs with 18 and 20 carbon atoms, formed the group of main fatty acids, amounting to 83.6% of the total FAs in *L. japonica* and to 77.7% in *L. cichorioides* (Table 2). However, these species of algae differed in their ratios of individual FAs. *L. japonica* proved to be richer in PUFAs than *L. cichorioides*, and in *L. japonica* among the polyenic fatty acids C_{18} PUFAs predominated, while in *L. cichorioides* the C_{20} PUFAs did so. The fatty acids of *L. japonica* have been studied more than once, and the results obtained on the ratios of the main fatty acids are rather contradictory. However, all the authors reported C_{18} PUFAs as the dominating polyenic fatty acids of *L. japonica* [6, 9, 10]. The two *Laminaria* species also differed by their ratio of the PUFAs of the (ω—3) and (ω—6) series. In *L. japonica* the content of the (ω—3) PUFAs exceeded the level of the (ω—6) polyenic FAs, these being 34.7% and 27.5% of the total FAs, respectively, while in *L. cichorioides* the PUFAs of the (ω—3) and (ω—6) series were present in comparable amounts: 23.2 and 22.0%, respectively. The levels of octadecatetraenoic, arachidonic, and eicosapentaenoic acids were higher, and the levels of palmitic and oleic acids lower, in *L. japonica* than in *L. cichorioides*.

It is known that polyunsaturated FAs linoleic, linolenic, and arachidonic are essential for marine organisms, animals, and Man [3, 23]. Arachidonic and eicosapentaenoic acids also serve as precursors in the biosynthesis of prostaglandins and other eicosanoids, which influence many cell processes and organ functions [24]. In view of the fact that both species of algae of the *Laminaria* genus contain fairly considerable amounts of such acids, these algae can be considered as excellent sources of important acids and a valuable addition to traditional foodstuffs.

EXPERIMENTAL

L. japonica and *L. cichorioides* were gathered in Peter the Great Bay, Sea of Japan, from June to August. For analysis we selected thalli not overgrown with epiphytes, and we carefully freed them from contaminants and washed them in fresh water. The algae were ground in a mortar with sand, and the lipids were extracted with $CHCl_3$—MeOH by Bligh and Dyer's method [25].

TLC of the Lipids. The lipids were separated by two-dimensional TLC on 6 × 6 cm plates coated with a layer of silica gel [26]. To separate the polar lipids we used the solvent systems chloroform—acetone—methanol—formic acid—water (100:40:20:20:8) in the first direction, and acetone—benzene—formic acid—water (200:30:3:10) in the second direction. The phospholipids were separated in the solvent systems chloroform—methanol—benzene—28% ammonia (65:30:10:6) in the first direction, and chloroform—methanol—benzene—acetic acid—water (70:30:10:3:4:1) in the second direction. Neutral lipids were separated by one-dimensional TLC in the hexane—diethyl ether—acetic acid (70:30:1) system. For the nonspecific detection of lipids, the plates were sprayed with 10% H_2SO_4 in MeOH and were then charred on a hotplate. Specific reagent were used to identify lipids: a molybdate reagent for phospholipids [27], anthrone for glycolipids [28], 0.5% ninhydrin in acetone for aminophospholipids, and the Dragendorff reagent for choline-containing lipids [29]. The lipids were also identified by comparing their R_f values with standards.

Quantitative Determination of Lipids. Total lipids, were determined gravimetrically, phospholipids from the phosphorus content [27], and glycolipids and neutral lipids by the GLC method from their fatty acid contents, using the 15:0 acid as an internal standard [30].

Fatty Acid Analysis. Fatty acid were analyzed in the form of methyl esters. To obtain them, the total lipid extract was treated with 1% Na in MeOH and heated at 55°C for 15 min; then a 5% solution of HCl in MeOH was added and heating was continued under the same conditions [31]. The FA methyl esters were analyzed by GLC on a chromatograph with a flame-ionization detector (Shimadzu GC-9A) and a Chromatopack C-R3A integrator (Shimadzu, Kyoto, Japan). GLC conditions: capillary columns (30 m × 0.32 mm) with Supelcowax 10 and SPB-5, column temperatures 210 and 230°C, respectively;

carrier gas helium at a rate of flow of 40 ml/min. The fatty acids were identified by comparing their retention times (R_f) with standards and from their carbon numbers [32]. For additional identification, the FA methyl esters were separated by $AgNO_3$—TLC in the hexane—diethyl ether—acetic acid (94:4:3) system according to their degrees of unsaturation [33]. The silica gel zones were collected, and the FA methyl esters were eluted with $CHCl_3$ and analyzed by GLC.

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