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LIPID COMPOSITION OF SOME LICHENS

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Abstract—Five lichen species from the subclass Gymnocarpeae, or Discolichenes, growing in the Volga river basin were examined for their fatty acid compositions using capillary GC-MS. Seventy-three fatty acids were identified: 24 saturated, 25 monoene, five diene, 11 triene and eight tetra-, penta- and hexaene. Very long-chain fatty acids: 26:0, 26:1(n-11), 26:3(n-3), 27:1, 28:1 and 30:1 were also identified. The total lipid content varied from 18 to 34 mg g^{-1} dry wt. The phospholipid compositions of the same species were studied. Phospholipids were found to vary from 17 to 31% of the total lipid. Among the phospholipids, phosphatidylcholine had the highest concentration, varying from 37 to 61%.

INTRODUCTION

The presence of various chemical components in lichens has long been used as a chemotaxonomic marker, particularly with regard to aromatic acids [1], carotenoids [2] and fatty acids [3], providing useful information for chemotaxonomic investigations. There are numerous reports on the fatty acid composition of lichens [e.g. 4]. Our previous analyses of fatty acids from some lichens growing in the Volga river basin [5, 6] suggest that lichens are organisms of great diversity. Our recent report on the fatty acids found in the genus *Cladonia* [7] has indicated the need for further examination of various species from other families. The present work reports the results of a continued investigation on lichen fatty acids and phospholipids using GC-MS and TLC methods, respectively.

RESULTS AND DISCUSSION

The fatty acid composition of the lichen species examined by capillary GC-MS is shown in Tables 1–3. We identified 73 fatty acids, including 24 saturated acids. The proportion of saturated fatty acids in the examined species varied from 14.6 to 40.8%. The saturated fatty acids included: *iso*-acids 12:0, 13:0, 14:0, 15:0, 16:0 and 17:0, as well as two *anteiso*-acids, 15:0 and 17:0. For the first time lichen species were shown to contain prystanic acids which constituted 0.33% and 0.13% in *Peltigera spurta* and *Diploschistes* sp., respectively, and also phytanic acids which constituted 1.4%, 0.2% and 0.3% in *P. spuria*, *P. aphthosa* and *Anaptychia ciliaris*, respectively (Table 1).

The major saturated acids were 16:0 and 18:0. Of interest were the long-chain fatty acids 26:0 and 28:0, previously identified in lichens of the genus *Cladonia* [7]. According to Solberg [4], who studied the chemistry of four lichen species, the fatty acids he identified ranged from C_6 to C_{28}. In the qualitative analysis, the author showed some species to contain dicarboxylic, 2-hydroxy- and keto acids.

Most of the acids identified in the course of the present study were monoenes ranging from 12:1 to 30:1 (Table 2). C_{18} acids were represented by three isomers, 18:1(n-11), 18:1(n-9) and 18:1(n-7). 16:1 acids were also represented by three isomers, 16:1(n-9), 16:1(n-7) and *trans*-16:1(n-13). Among long-chain fatty acids, 25:1(n-8), 26:1(n-11), 27:1, 28:1 and 30:1 were identified. Similar acid types have recently been identified in the genus *Cladonia* [7]. 18:1 was one of the main fatty acids and showed a broad variation, due to variations of the lichen substrates [8, 9].

Monoene acids in different lichen species were shown to vary from 17.15 to 51.91%. Dienoic acids made up a small portion of the total fatty acids and varied from 2.1 to 9.9%, being represented by five acids only, namely, 14:2(n-5), 16:2(n-4), 18:2(n-6), 20:2(n-6) and 22:2(n-6) (Table 3). Eleven fatty acids were identified as trienes, among which were the long-chain fatty acids 24:3(n-3), found in just two lichen species, and 26:3(n-3), also found to be present only in two species. The total trienes varied widely, from 4.8% in *Peltigera spuria* to 33.2% in *Anaptychia ciliaris*.

Among other interesting acids, 16:4 (n-3), the presence of which is characteristic of green marine algae [10], and 18:4 (n-3), characteristic of brown marine algae [11], were detected. It seems likely that the lichen phycobionts synthesize these acids. In *Peltigera aphthosa*, the total level of isomers of 20:4 (n-6) and 20:4 (n-3) was considerably higher than that from the rest of the examined species, reaching 12.8%. These amounts of arachidonic acid isomers are the highest known in the lichen literature with regard to the total lipid extract, although still higher levels have been found in the individual lipid classes, for example, in mosses [3].

Small amounts of very long-chain fatty acids are reported to be present in certain photosynthetic microorganisms, particularly from the freshwater autotrophic *Euglena gracilis* which contains C_{24}–C_{26} [12]. The freshwater green alga, *Botryococcus braunii*, is known to produce 26:1 and 28:2 [13], and the algae *Spirulina*

Table 1. Saturated fatty acid composition of lichens (subclass Gymnocarpeae)

	Peltigeraceae		Umbilicariaceae	Diploschistaceae	Physciaceae
	Peltigera spuria	*Peltigera aphthosa*	*Umbilicaria deusta*	*Diploschistes* sp.	*Anaptychia ciliaris*
	1	2	3	4	5
iso-12:0	0.33	0.15	0.10	—	0.08
n-12:0	1.31	0.46	0.49	0.27	0.31
iso-13:0	0.98	—	0.10	—	0.08
iso-14:0	0.44	0.46	—	0.27	0.23
n-14:0	0.98	3.19	0.88	1.73	1.38
iso-15:0	0.87	1.06	0.39	—	0.31
anteiso-15:0	0.76	0.91	0.10	—	0.23
n-15:0	1.53	0.76	0.68	1.47	1.00
iso-16:0	0.76	0.76	0.39	3.07	0.15
Pristanic	0.33	—	—	0.13	—
n-16:0	9.15	6.22	6.04	7.47	4.82
iso-17:0	0.76	0.15	0.29	0.27	0.23
anteiso-17:0	0.44	1.06	0.49	—	0.08
Phytanic	1.42	0.15	—	—	0.31
n-17:0	0.98	0.15	0.10	—	0.08
n-18:0	7.85	1.97	4.09	0.53	6.19
n-19:0	1.09	—	—	—	0.08
n-20:0	1.96	0.91	0.29	0.13	0.92
n-21:0	1.96	0.15	—	—	0.54
n-22:0	1.96	—	—	—	1.07
n-23:0	0.98	—	—	—	0.23
n-24:0	1.53	0.15	0.19	0.13	0.61
n-26:0	0.98	—	—	—	0.69
n-28:0	1.42	—	—	—	0.15
Total saturated	40.77	18.66	14.62	15.47	19.77

Table 2. Monoenoic fatty acid composition of lichens (subclass Gymnocarpeae)

	1	2	3	4	5
n-12:1	1.20	0.30	0.10	0.53	0.23
n-13:1	0.76	—	0.19	—	0.38
14:1(n-5)	1.42	1.97	0.58	1.07	0.38
15:1(n-8)	1.42	0.61	0.39	0.93	0.31
15:1(n-6)	2.07	0.61	—	0.80	0.23
16:1(n-9)	4.80	1.06	1.27	1.07	5.44
16:1(n-7)	3.05	3.79	1.36	0.80	0.69
trans-16:1(n-13)	0.11	0.15	—	—	—
17:1(n-10)	1.53	—	0.10	0.27	0.15
17:1(n-8)	1.31	1.37	1.07	1.07	0.23
18:1(n-11)	4.69	1.21	3.12	1.20	1.30
18:1(n-9)	15.04	3.19	22.91	19.48	7.03
18:1(n-7)	2.29	1.21	1.07	0.80	0.69
19:1(n-8)	1.31	—	—	—	0.54
20:1(n-11)	1.53	0.46	—	—	0.46
20:1(n-9)	1.20	0.46	0.39	0.13	1.38
21:2(n-8)	0.76	0.15	—	—	0.08
22:1(n-11)	0.98	—	0.10	—	0.61
22:1(n-9)	1.42	0.46	0.10	0.27	0.23
24:1(n-9)	1.42	0.15	0.19	0.13	1.00
25:1(n-8)	0.76	—	0.29	0.27	0.08
26:1(n-11)	1.31	—	—	—	0.61
n-27:1	0.22	—	—	—	0.08
n-28:1	0.87	—	—	—	0.08
n-30:1	0.44	—	—	—	0.08
Total monoenes	51.91	17.15	33.23	28.82	22.29

Table 3. Dienoic and trienoic fatty acid composition of lichens (subclass Gymnocarpeae)

	1	2	3	4	5
14:2(n-5)	—	—	—	—	0.61
16:2(n-4)	—	1.67	0.88	0.13	0.61
18:2(n-6)	0.98	7.28	5.46	5.60	6.57
20:2(n-6)	0.98	0.61	0.19	0.40	0.54
22:2(n-6)	0.11	0.30	0.29	0.13	0.08
Total dienes	2.07	9.86	6.82	6.26	8.41
16:3(n-6)	—	1.21	3.02	3.07	0.31
16:3(n-3)	—	1.82	0.88	3.20	1.07
18:3(n-6)	1.42	1.97	1.07	0.93	10.25
18:3(n-3)	2.29	9.26	4.19	6.93	14.08
20:3(n-9)	—	0.15	0.58	0.40	0.31
20:3(n-6)	0.22	0.91	0.49	0.93	0.08
20:3(n-3)	0.22	0.61	—	0.40	2.45
22:3(n-6)	0.44	3.95	0.49	0.53	1.76
22:3(n-3)	0.22	0.76	0.88	0.67	1.30
24:3(n-3)	—	—	—	0.53	1.07
26:3(n-3)	—	0.15	—	—	0.54
Total trienes	4.81	20.79	11.60	17.59	33.22
16:4(n-3)	—	2.28	2.05	1.20	0.61
18:4(n-3)	0.22	0.61	5.17	9.07	2.83
20:4(n-6)	0.11	7.74	1.85	3.73	2.22
20:4(n-3)	—	5.01	6.14	0.93	3.68
20:5(n-3)	—	5.61	5.95	3.60	4.98
22:4(n-3)	—	1.82	0.68	0.53	0.46
22:5(n-3)	—	4.70	6.14	4.67	0.69
22:6(n-3)	0.11	5.77	5.75	8.13	0.84
Total tetra-, penta and hexaenes	0.44	33.54	33.73	31.86	16.31

Table 4. Phospholipid composition of lichens (subclass Gymnocarpeae)

Species	PC	PI	PG	PE	PS	X	PL	TL
Anaptychia ciliaris	61.1	—	7.4	9.3	13.9	8.3	17.6	18.6
Diploschistes sp.	58.2	—	19.3	22.5	—	—	26.4	18.3
Peltigera aphthosa	52.3	12.0	18.1	15.5	1.1	1.0	25.2	20.9
Peltigera spuria	44.6	14.3	16.8	17.6	0.9	5.8	20.9	28.4
Umbilicaria deusta	37.8	33.9	8.1	20.2	—	—	31.2	34.6

PC, phosphatidylcholine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PS, phosphatidylserine; X, unidentified phospholipid; PL, phospholipids; in per cent of total lipids (TL), mg g^{-1} dry wt.

platensis [14] and *Stauroneis amphioxys* are reported to contain small amounts of acids 24:0–30:0 [15]. *Chlorella kessleri*, grown under heterotrophic conditions, reportedly contains a number of monoenoic acids identified as 29:1–36:1 [14]. It is most likely that lichen phycobionts contribute greatly to the biosynthesis of very long fatty acids. The biosynthesis of long-chain polyunsaturated fatty acids is well studied in primitive multicellular animals, particularly in sponges [16]. The role of the elongases and desaturases in lichens is probably quite similar to that of the same enzymes in sponges, though the majority of the latter are known to contain from 20 to 79% of very long-chain fatty acids [17], while the variation of very long-chain fatty acids in lichens is only small. Lichen fatty acid biosynthesis has been little studied and our present day knowledge does not allow us to state the possible ways of lichen biosynthesis.

The total lipid content in the lichen species studied varied from 18.3 to 34.6 mg g^{-1} dry wt (Table 4). As lichens are complex organisms combining properties of fungi and algae, they were compared with each of the latter plant groups. Thus, the total lipids contained in fungi are known to constitute 3–48% of the dry wt in the tissue of *Pythium ultimum* [18], belonging to Phycomycetes, and to vary from 0.4% in *Ustilago maydis* [19] to 35% in *Tilletia controversa* [20], belonging to the Basidiomycetes. In red algae, the total lipid content is generally much lower and reportedly varies from 0.6 to

2.6 mg g^{-1} dry wt [21], while in green algae it amounts to 0.8–2.6 mg g^{-1} dry wt [22]. The data obtained in the course of the present study show that the total lipids contained in lichens are well within the total lipid range found for fungi.

Examination of the lichen phospholipid composition showed phosphatidylcholine (PC) to be the major phospholipid with concentrations varying from 37.8 to 61.1% of total phospholipids (Table 4). The PC content in various fungal species is known to vary from 20 to 55%, whereas PC contained in red algal species varies from 61.6 to 77.8% [23]. Phosphatidylserine was detected in three out of the five lichen species studied; its level was low in two species (*ca* 1%), but reached 13.9% in *Anaptychia ciliaris*. Phospatidylinositol was found in three out of the five lichen species studied; its level was highest in *Umbilicaria deusta* (33.9%). Three lichen species were shown to possess an unidentified phosphorus-containing lipid; its concentration was 8.3% in *Anaptychia ciliaris* and 5.8% in *Peltigera spuria*.

Thus a fairly broad diversity of fatty acids was revealed, many of which were added to the list of fatty acids previously identified in lichen species, for example, the long-chain acids with a number of carbons greater than 24. The results of the fatty acid and phospholipid examination demonstrated a marked difference between lipids from lichens, on the one hand, and lipids from fungi and/or algae, on the other.

EXPERIMENTAL

Lichens were harvested in August 1990 in the Samarskaya Luka nature reserve near Togliatti. Freshly collected lichens were thoroughly cleansed of extraneous matter, homogenized and extracted ×3 with $CHCl_3$ [24]. The lipid extracts were washed with 0.9% KCl and stored at −20°.

Me esters of fatty acids were prepd by alkaline hydrolysis and reaction of free acids with BF_3–MeOH [25]. Oxazolines and/or their TMSi ethers were prepd by modification of the methods of refs [26, 27]: 5 mg dicyclohexylcarbodiimide was added to a soln of 5 mg fatty acid in 1 ml CH_2Cl_2. After stirring (10 min) 2-amino-2-methylpropanol (5 mg) was added (20°, 4 hr). The evapd mixt. was dissolved in 1 ml Et_2O and treated with 0.5 ml $SOCl_2$ (20°, 1 hr), washed with ice-cold H_2O and filtered through a column of dry Na_2SO_4 and silica gel. The eluate was evapd, dissolved in pyridine and heated with trimethylsilylchloride (50°, 4 hr). Me esters were identified by GC using a fused silica capillary column (60 m × 0.32 mm i.d./SPB-1), using splitless inj. and He as carrier gas. Oven temp. was progd from 100 to 320° at 4° min^{-1}. Ionization energy was 70 eV and the electron multiplier voltage 2.5 kV. TMSi ethers of oxazolines were identified under conditions similar to those for Me esters, the only difference being the column temp. progd from 150 to 330° at 5° min^{-1}. The total lipids and phospholipid classes were identified and estimated as described previously [24].

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