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FATTY ACIDS AND PHOSPHOLIPIDS FROM LICHENS OF THE ORDER LECANORALES

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Abstract—Three lichen species from the order Lecanorales were examined for their fatty acids by capillary GC-MS. Among the 70 fatty acids identified, 22 were saturated, including six *iso*-acids, two *anteiso*-acids, one pristanic acid and one phytanic acid; their total amount varied from 10.6 to 26.8%. Twenty-four acids were monoenoic (14.9–24.4% five including 25:1, 26:1, 28:1 and 30:1), dienoic (8.0–12.0%), eleven trienoic (21.3–33.9%) and eight were polyenoic (8.6–41.1%). Lichen samples were also examined for total lipids and phospholipids. Total lipids varied from 13.4 to 30.7 mg g^{-1} dry wt. The major phospholipid was phosphatidylcholine.

INTRODUCTION

Our previous studies concerned phospholipids [1] and fatty acids [1–4] from lichen species collected in the Volga river basin. Lipid composition of lichens from the family Usneaceae have been reviewed in a limited number of publications [5, 6] where mass spectrometric methods were not used extensively [7]. Fatty acids and phospholipids, examined by GC-MS and TLC, respectively, from three lichen species belonging to the order Lecanorales, are reported in the present paper.

RESULTS AND DISCUSSION

Tree-growing epiphytic species from the family Usneaceae and rock-growing, epilytic species from the family Lecanoraceae, were chosen for a comparative examination of their fatty acid and phospholipid compositions. The GC-MS analysis of fatty acids in the three species, revealed a high polyenoic content in *Aspicilia transbaicalica* (41.1%, Table 1), but much lower amounts of such acids in two *Evernia* species, viz. 27.4% in *E. mesomorpha* and only 8.6% in *E. prunastri*. The amounts of trienoic acids, however, were higher in the samples from tree-growing species, 33.9% and 28.2%, compared to 21.3% found in the rock-growing species. All three species studied had almost identical dienoic acid contents. Unusually long chain fatty acids, 25:1, 26:1, 28:1 and 30:1 were present only in the *Evernia* species. *trans* 16:1 (n-13), pristanic and phytanic acids were detected and identified only in *E. prunastri*, where they constituted equal amounts (0.1%). According to Dertien *et al.* [5], in *E. prunastri*, collected in Holland, the respective amounts of saturated, dienoic and trienoic acids were 13.0, 14.3 and 43.9%; acids with more than 20 carbon atoms constituted as little as 7%. Our sample of *E. prunastri* growing in the Volga river basin, contained 26.8% saturated, 24.4% monoenoic, 12.0% dienoic, 28.2% trienoic acids and 17.1% of acids with chains longer than C_{20}. The major fatty acid reported by Beltman *et al.* [6] for *E. prunastri*, also collected in Holland but three years later, was 18:3 which comprised 57% of the total acids. Such a broad variation of acids in the same species collected at different sites and times, can probably be attributed to the differences between the substrates where the species had grown. Solberg [7] used mass spectrometric analyses for the identification of some fatty acids, particularly, 16:0, 20:1, 20:2 and 20:4 from four lichen species; he also identified a number of dicarboxylic acids, hydroxy acids (2-OH 16:0, 2-OH 18:0, 2-OH 22:0, 2-OH 24:0 and 2-OH 24:1), as well as keto acids (5-keto 18:0, 5-keto 21:0 and 5-keto 23:0). Quantification of these compounds was not attempted.

Lichens are symbiotic associations between cyanobacteria and fungi and it is of interest to know that contributions are made by phyco- and mycobiont. According to Yamamoto and Vatanabe [8], who studied the fatty acid composition of phycobionts in various species of lichens, *Trebouxia* in *Cladonia rangiferina* and *Usnea diffracta* contain mainly 16:0 (18.6 and 15%), 18:1 (40.5 and 29.1%) and 18:2 (27.3 and 27.7%) with small amounts of arachidonic acid (2.2 and 1.6%). For a number of cyanobacteria the above mentioned fatty acids are the main ones, but for primitive red eukaryotic algae that can be phycobionts of lichens polyunsaturated fatty acids such as arachidonic and eicosapentaenoic are found; their content in *Porphydium* sp. can reach 44% [9], and in *Porphyridium cruentum* 53% [10].

Of interest also is the occurrence of branched-chain fatty acids. Our recent studies of fatty acids of 25 species of fungi belonging to the Basidiomycetes [11] using GC-MS showed that this class of fungi is rich in *iso*- and *anteiso*-fatty acids, the content of the former varying from 7 to 10%, the latter from 3 to 5%. Among dienoic, trienoic and polyenoic acids in this class of fungi we have identified only three acids: 18:2 (n-9) from 5 to 8%, 18:3 (n-6) from 0.3 to 4% and 18:3 (n-9) from 1 to 10%. Among higher fungi polyunsaturated acids are rarely

Table 1. Fatty acid composition in lichens from the order Lecanorales

	Usneaceae		Lecanoraceae
	Evernia mesomorpha	*Evernia prunastri*	*Aspicilla transbaicalica*
iso-12:0	0.20	0.23	0.20
n-12:0	0.41	0.11	0.41
iso-13:0	0.20	—	0.20
iso-14:0	0.10	—	0.10
n-14:0	0.20	3.67	3.25
iso-15:0	0.10	1.49	0.51
anteiso-15:0	0.10	0.80	0.71
n-15:0	0.30	0.34	0.20
iso-16:0	0.30	0.80	0.30
Pristanic	—	0.11	—
n-16:0	3.25	4.82	6.50
iso-17:0	0.20	0.34	0.30
anteiso-17:0	0.30	0.57	0.71
Phytanic	—	0.11	—
n-17:0	0.20	0.34	0.20
n-18:0	2.95	11.24	0.81
n-19:0	—	0.23	0.10
n-20:0	0.20	0.23	0.10
n-22:0	0.20	0.23	0.10
n-23:0	—	0.11	—
n-24:0	1.22	0.92	0.10
n-26:0	0.20	0.11	—
Total saturated	**10.63**	**26.80**	**14.80**
12:1	0.10	0.11	0.10
13:1	0.30	—	0.30
14:1 (n-5)	0.10	2.06	1.42
15:1 (n-8)	0.41	0.23	0.81
15:1 (n-6)	0.71	0.46	0.41
16:1 (n-9)	4.18	2.06	0.81
16:1 (n-7)	3.76	3.55	2.34
trans			
16:1 (n-13)	—	0.11	—
17:1 (n-10)	0.10	0.57	0.10
17:1 (n-8)	0.71	0.80	0.41
18:1 (n-11)	2.85	2.63	0.71
18:1 (n-9)	3.15	6.65	5.69
18:1 (n-7)	0.81	1.49	0.91
19:1 (n-8)	0.10	0.11	—
20:1 (n-11)	0.10	0.23	—
20:1 (n-9)	0.51	0.80	0.51
21:1 (n-8)	0.10	0.11	0.10
22:1 (n-11)	0.20	0.11	—
22:1 (n-9)	0.10	1.49	0.20
24:1 (n-9)	0.91	0.69	0.10
25:1 (n-8)	0.30	0.11	—
26:1 (n-11)	0.30	—	—
28:1	0.10	—	—
30:1	0.10	—	—
Total monoenes	**20.00**	**24.37**	**14.92**

Table 1. (*Continued*)

	Usneaceae		Lecanoraceae
	Evernia mesomorpha	*Evernia prunastri*	*Aspicilla transbaicalica*
14:2 (n-5)	—	0.46	0.20
16:2 (n-4)	0.71	0.34	0.41
18:2 (n-6)	6.51	7.80	6.70
20:2 (n-6)	0.30	0.34	0.20
22:2 (n-6)	0.51	3.09	0.30
Total dienes	**8.03**	**12.03**	**7.81**
16:3 (n-6)	3.25	0.46	1.32
16:3 (n-3)	2.85	1.03	2.84
18:3 (n-6)	0.41	1.49	0.91
18:3 (n-3)	13.76	21.32	14.43
20:3 (n-9)	0.20	0.23	0.81
20:3 (n-6)	0.10	0.69	0.10
20:3 (n-3)	3.87	0.46	0.41
22:3 (n-6)	0.61	2.06	0.41
22:3 (n-3)	6.41	0.46	0.10
24:3 (n-3)	1.63	—	—
26:3 (n-3)	0.81	—	—
Total trienes	**33.89**	**28.20**	**21.33**
16:4 (n-3)	3.15	1.49	3.25
18:4 (n-3)	4.89	2.52	6.40
20:4 (n-6)	0.20	1.95	3.25
20:4 (n-3)	4.58	0.69	5.28
20:5 (n-3)	7.02	1.03	7.31
22:4 (n-3)	3.76	0.23	0.71
22:5 (n-3)	2.95	0.46	6.40
22:6 (n-3)	0.81	0.23	8.54
Total tetra-, penta- and hexaenes	**27.36**	**8.6**	**41.14**

found, but among fungi belonging to the Phycomecetes there have been found species where the content of arachidonic acid varies from 2 to 27% [12, 13]. Therefore, we can suppose that phycobionts contribute polyunsaturated acids and mycobionts branched-chain fatty acids.

Phospholipids of the lichen species presently studied, have not been previously reported. According to our results, total lipids, for the epiphytic species, constituted 16.5 mg g^{-1} dry wt, in *E. mesomorpha* and 13.4 mg g^{-1} dry wt in *E. prunastri*. The epilytic *A. transbaicalica* contained 30.7 mg g^{-1} dry wt of total lipids (Table 2). The studied species differed in their phospholipid composition. Phosphatidycholine in the *Evernia* species was present in amounts of 59.4% and 58.3% of total phospholipids, while in *A. transbaicalica* it constituted 33.3% (Table 2). The contents of phosphatidylglycerol were 32.3%, in *A. transbaicalica*, 8.6%, in *E. mesomorpha* and 5.3%, in *E. prunastri*. The amount of phosphatidylserine in the *Evernia* species was three times higher than that in *A. transbaicalica*.

Table 2. Phospholipid composition in lichens from the order Lecanorales

	Evernia mesomorpha	*Evernia prunastri*	*Aspicilia trasbaicalica*
PC	59.4±1.3	58.3±1.2	33.3±0.9
PG	6.7±0.3	7.2±0.4	10.2±0.4
PI	8.6±0.8	5.3±0.3	32.3±0.8
PE	13.3±0.5	14.7±0.4	20.2±0.9
PS	12.0±0.3	14.5±0.7	4.0±0.2
PL	22.8	19.9	37.4
TL	16.52	13.43	30.67

PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phospatidylinositol; PE, phosphatidylethanol amine; PS, phosphatidylserine; PL, phospholipids, as a per cent of total lipids (TL), mg g^{-1} dry wt.

During our studies of lichen composition we have not found unidentified polar lipids, but practically all lichens had an unidentified lipid not containing phosphorus with an R_f value very close to that of diacylglyceryl trimethylhomoserine (DGTS). Quantity determination and identification of this lipid were not performed. It should be noted that among fungi DGTS is reported [14–19]; further analysis is required to distinguish between DGTS and DGTA (diacylglyceryl oxymethyl trimethyl β-alanine).

Thus, the reported results clearly indicate differences in fatty acid and phospholipid compositions between epiphytic and epilytic lichens. Of major importance, in this context, is the presence of long-chain fatty acids (C_{25}–C_{30}) in the epiphytic species and their absence from the epilytic ones. This difference may imply metabolic particularities of fatty acids or ecological factors.

EXPERIMENTAL

Lichens were harvested in August 1990 in the Samarskaya Luka nature reserve near Togliatti. Fr. collected lichens were thoroughly cleansed of extraneous matter and only clean lichen tissues were used for homogenization in a high-speed unit. Lipids were extracted by the methods of refs [20, 21]; total lipids and phospholipids were determined as described in refs [1, 22].

Me esters of fatty acids were prepd by alkaline hydrolysis and reaction of free acids with BF_3–MeOH [23]. The oxazolines and/or their TMSi ethers were prepd by modifications of the methods of refs [24, 25]; 5 mg of dicyclohexylcarboiimide was added to a soln of 5 mg fatty acids in 1 ml CH_2Cl_2. After stirring (10 min) 5 mg 2-amino-2-methylpropanol 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 eluted through a column with dry Na_2SO_4 and silica gel. The eluate was evapd, dissolved in pyridine and heated with trimethylsilyl chloride (50°, 4 hr).

Me esters were identified using a fused silica capillary column (60 m × 0.32 mm i.d. SPB-1, Supelco) with splitless injection and He as carrier gas. The oven temp. was progd from 100° to 320° at 4° min^{-1}. The ionization energy was 70 eV, electron multiplier voltage 2.5 kV. TMSi ethers of oxazolines were identified under conditions similar to those used for Me ester identification, the only difference being that the column was progd from 150° to 330° at 5° min^{-1}.

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REFERENCES

1. Dembitsky, V. M., Bychek, I. A., Shustov, M. V. and Rozentsvet, O. A. (1991) *Phytochemistry* **30**, 837.
2. Dembitsky, V. M., Rezanka, T., Bychek, I. A. and Shustov, M. V. (1992) *Phytochemistry* (in press).
3. Dembitsky, V. M., Rezanka, T., Bychek, I. A. and Shustov, M. V. (1992) *Phytochemistry* (in press).
4. Dembitsky, V. M., Rezanka, T. and Bychek, I. A. (1992) *Phytochemistry* (in press).
5. Dertien, B. K., De Kok, L. J. and Kuiper, P. J. C. (1977) *Physiol. Plant* **40**, 175.
6. Beltman, I. H., De Kok, L. J., Kuiper, P. J. C. and van Hasselt, P. R. (1980) *OIKOS* **35**, 321.
7. Solberg, Y. (1987) *J. Hattori Bot. Lab.* **63**, 357.
8. Yamamoto, Y. and Watanabe, A. (1974) *J. Gen. Appl. Microbiol.* **20**, 83.
9. Ackman, R. G., Tocher, C. S. and McLachlan, J. (1968) *J. Fish. Res. Bd. Can.* **25**, 1603.
10. Nichols, B. W. and Appleby, R. S. (1969) *Phytochemistry* **8**, 1907.
11. Dembitsky, V. M., Rezanka, T., Shubina, E. E. and Rozenberg, G. S. (1992) *Biochem. Syst. Ecol.* (in press).
12. Haskins, R. H., Tulloch, A. P. and Micetich, R. G. (1964) *Can. J. Microbiol.* **10**, 187.
13. Tyrrell, D. (1967) *Can. J. Microbiol.* **13**, 755.
14. Wassef, M. K. (1977) *Adv. Lipid Res.* **15**, 159.
15. Brennan, P. J. and Losel, D. M. (1978) *Adv. Microbiol. Physiol.* **17**, 47.
16. Weete, J. D. (1980) *Lipid Biochemistry of Fungi and Other Organisms*. Plenum Press, New York.
17. Weete, J. D., Furter, R., Hanseler, E. and Rast, D. M. (1985) *Can. J. Microbiol.* **31**, 1120.
18. Sugai, A., Itoh, T., Kaneko, H., Kinjo, N. and Muramatsu, T. (1986) *Lipids* **21**, 666.
19. Vaskovsky, V. E., Khotimchenko, S. V. and Benson, A. A. (1991) *Lipids* **26**, 254.
20. Bligh, E. G. and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911.
21. Khotimchenko, S. V., Klochkova, N. G. and Vaskovsky, V. E. (1990) *Biochem. Syst. Ecol.* **18**, 93.
22. Dembitsky, V. M., Rozentsvet, O. A. and Pechenkina, E. E. (1990) *Phytochemistry* **29**, 3417.
23. Rezanka, T., Sokolov, M. Yu. and Viden, I. (1990) *FEMS Microbiol. Ecol.* **73**, 231.
24. Yu, Q. T., Liu, B. N., Zhang, J. Y. and Hung, Z. H. (1988) *Lipids* **23**, 804.
25. Tulloch, A. P. (1985) *Lipids* **20**, 652.