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IDENTIFICATION OF FATTY ACIDS FROM *CLADONIA* LICHENS

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Key Word Index—*Cladonia* lichens; fatty acids; GC-MS.

Abstract—Fatty acids of eight lichen species belonging to the genus *Cladonia* were examined by GC-MS. Twenty-two saturated, 23 monoene, five diene, nine triene and eight tetra-, penta- and hexaene fatty acids were identified. Unusually for lichens, very long-chain fatty acids, 25:1, 26:0, 26:1, 26:3, 28:0, 28:1, and 30:1 were detected.

INTRODUCTION

From the lichen fatty acid analyses reported in the literature, it follows that practically all lichen lipids contain fatty acids with C_{14}–C_{18} chains with only a few exceptions when fatty acids having a greater number of carbons were detected [1–8]. Such results may be due to the conventional packed columns used for analyses. The present study is the first known report on fatty acids of lichens from the Volga river basin and deals with fatty acids from lichens belonging to the genus *Cladonia*.

RESULTS AND DISCUSSION

The lichen fatty acid data previously obtained by us using conventional packed columns revealed the difficulty of identifying minor fatty acid components contained in biological samples [7, 8]. Among fatty acids, we identified 22 saturated fatty acids, with *n*-16:0 and *n*-18:0 as major acids (1.3 to 9.3 and 0.3 to 5.1%, respectively). We also detected very long-chain fatty acids, *n*-24:0, *n*-26:0 and *n*-28:0 in small amounts (Table 1). Total saturation in various species varied from 3.2 to 25.4%. The most numerous group among the identified fatty acids were monoenes, 23 acids. In this group, 24:1 (n-9), 25:1 (n-8), 26 (n-11), 28:1 and 30:1 were detected in lichens for the first time. The content of monoene fatty acids in various species was found to vary from 5.5 to 29.3%. Small amounts of very long-chain fatty acids (those with 23 to 33 carbons in a chain) were found in autotrophic [9–14], and in heterotrophic [15–20] microorganisms. High concentrations of very long-chain fatty acids (VLCFAs) 26:2, 26:3, 28:1, 28:2 and of still longer-chain fatty acids were found in marine [21–24] and freshwater [25–27] sponges. Biosynthesis of VLCFAs was elucidated for various marine sponge species [28–32]. The mechanism of VLCFA biosynthesis in lichens may be similar to that in sponges, but to be sure, more experimental work is needed.

Among the identified triene fatty acids, the most interesting are 24:3 (n-3) and 26:3 (n-3), large amounts of which were detected in various marine sponges [21–24, 33–36]. The contents of polyene fatty acids: tetra-, penta-, and hexaenoic, in various lichen species showed fairly wide variation from 12.13 to 54.9%. Thus, the analyses of fatty acids from eight species of the genus *Cladonia* have shown the presence of unusual very long-chain fatty acids: *n*-24:0, *n*-26:0, *n*-28:0, 24:1 (n-9), 25:1 (n-8), 26:1 (n-11), 28:1, 30:1, 24:3 (n-3), and 26:3 (n-3). Small amounts of such acids were found in some fungal species [18–20, 37, 38]. As the mycobiont component may form the main part of lichens, the presence of the above acids is to be expected for lichens.

EXPERIMENTAL

Samples. The lichens were harvested in different regions of the Volga river basin during July–August 1989. Freshly collected lichens were carefully cleaned of impurities and only clean tissue was used for homogenization.

Lipid extraction. Macerated lichen tissue was treated with $CHCl_3$–MeOH prepn (1:1). Lipids were extracted in three stages, and the extracts combined. $CHCl_3$–MeOH–H_2O prepn (8:1:4) was added. Lipid extracts were then subjected to triple washing in cooled 0.9% KCl.

Methyl esters and pyrrolidines of fatty acids. The total lipid extract was evapd to dryness and saponified by boiling for 2 hr in 50 ml of 2 M NaOH in 50% EtOH. The non-saponified portion was extracted with Et_2O, the soln was acidified to pH 1 with HCl, and fatty acids were extracted with $CHCl_3$. Me esters of fatty acids were prepared by reaction with a 14% BF_3 soln in MeOH (Supelco) [39] and separated by TLC (petrol–Et_2O, 9:1) on silica gel G (Merck). Pyrrolidines of fatty acids were prepared by boiling 10 mg of fatty acid Me esters with 1 ml pyrrolidine and 0.1 ml HOAc for 1 hr [40]. The pyrrolidines were purified by TLC (petrol–Et_2O, 1:1, v/v) on silica gel G (Merck).

Gas chromatography-mass spectrometry. The mixtures of both fatty acid Me esters and pyrrolidines were analysed using a SCOT glass capillary column (77 m × 0.5 mm i.d.) (SGE) with SE-30 as the stationary phase. The operating conditions of the instrument were as follows: ionization energy, 70 eV; scan speed, 690 a.m.u. sec.; mass range, 4–600 a.m.u. For separation of Me esters, the capillary column oven temp. was programmed from 160° to 280° at 4° min^{-1}; for separation of pyrrolidines, from 240° to 280° at 2° min^{-1}, this latter operation then proceeding isothermally for 30 min. The carrier gas (He) flow-rate was 1.5 ml min^{-1} in the capillary column. The injector temp. was

Table 1. Fatty acid composition of *Cladonia* spp.

Fatty acids	Cladonia sp.1	C.sp.2	C.sp.3	C.sp.4	C.sp.5	C.sp.6	C.sp.7	C.sp.8
iso-12:0	0.2	—	0.1	—	0.2	—	—	—
n-12:0	1.3	1.4	0.9	0.3	0.6	0.2	0.2	0.2
iso-13:0	0.2	—	—	—	0.1	—	—	—
iso-14:0	—	—	0.1	—	—	0.2	—	—
n-14:0	1.8	1.9	2.9	2.1	2.7	0.5	1.7	0.6
iso-15:0	0.2	—	0.1	0.8	0.5	—	0.6	—
anteiso-15:0	0.3	—	0.3	0.1	0.2	—	0.8	—
n-15:0	1.3	0.8	0.5	0.1	1.0	0.2	1.3	0.2
iso-16:0	1.0	0.1	0.5	0.1	0.2	—	0.9	—
n-16:0	9.3	8.5	6.9	6.9	4.0	2.0	7.2	1.3
iso-17:0	0.4	—	0.3	—	0.1	—	0.7	—
anteiso-17:0	1.0	0.1	0.3	0.3	0.2	—	1.3	—
n-17:0	0.4	0.1	0.3	0.2	0.1	—	—	—
n-18:0	5.1	5.2	3.3	4.8	7.1	0.3	0.8	0.3
n-19:0	0.2	0.1	0.1	0.4	0.3	—	0.1	—
n-20:0	1.1	0.2	0.3	0.6	—	0.2	0.2	0.2
n-21:0	0.3	—	—	0.1	—	—	—	—
n-22:0	0.4	0.1	0.1	—	—	0.2	0.1	0.2
n-23:0	0.2	—	—	0.1	—	—	0.1	—
n-24:0	0.3	0.1	0.6	0.3	0.2	0.2	—	0.2
n-26:0	0.2	0.1	—	0.1	0.1	—	—	—
n-28:0	0.2	—	—	—	—	—	—	—
Total saturated	25.4	18.7	17.6	17.3	17.6	3.8	16.2	3.2
12:1	0.3	0.2	—	0.1	0.2	—	0.3	—
13:1	—	0.1	—	—	—	—	0.1	—
14:1 (n-5)	0.6	1.4	0.9	1.0	0.9	—	—	—
15:1 (n-8)	0.4	1.0	0.4	0.4	0.2	0.2	0.6	0.2
15:1 (n-6)	0.9	0.9	0.3	0.1	0.5	—	0.8	—
16:1 (n-9)	1.8	7.0	5.2	8.6	4.4	1.3	2.1	0.5
16:1 (n-7)	2.7	5.2	0.9	3.2	7.0	0.5	0.7	1.4
17:1 (n-11)	0.3	0.2	0.5	—	0.3	—	0.1	—
17:1 (n-8)	0.6	0.1	0.6	0.4	0.8	—	1.2	0.2
18:1 (n-11)	2.5	1.0	0.3	1.3	3.1	0.2	1.4	—
18:1 (n-9)	6.8	7.9	16.4	9.1	2.4	15.5	0.8	1.4
18:1 (n-7)	1.7	1.5	1.1	2.2	3.6	1.2	15.2	0.8
19:1 (n-8)	0.3	—	0.3	0.2	0.1	—	—	—
20:1 (n-11)	1.3	0.4	0.3	0.2	0.1	—	—	0.3
20:1 (n-9)	2.0	1.9	1.1	0.8	1.7	0.5	0.6	0.2
21:1 (n-8)	0.3	—	0.1	0.1	0.2	—	—	—
22:1 (n-11)	0.2	—	0.3	—	—	—	—	—
22:1 (n-9)	0.7	0.1	0.9	0.1	—	0.2	0.2	0.5
24:1 (n-9)	0.4	0.1	0.3	0.3	0.6	0.2	0.1	—
25:1 (n-8)	0.2	0.2	0.1	—	—	—	0.1	—
26:1 (n-11)	0.6	—	—	0.2	—	—	0.1	—
28:1	0.2	0.1	—	0.1	—	—	—	—
30:1	0.2	—	—	0.1	—	—	—	—
Total monoenes	24.8	29.3	30.0	28.5	26.1	19.8	24.4	5.5
14:2 (n-5)	—	—	—	—	—	—	0.4	—
16:2 (n-4)	0.7	0.9	0.9	0.7	0.5	0.2	0.4	0.3
18:2 (n-6)	8.1	5.8	7.3	16.2	10.4	6.2	6.6	8.0
20:2 (n-6)	1.1	0.4	0.5	0.6	0.5	0.3	—	0.2
22:2 (n-6)	0.2	0.1	0.4	1.0	1.6	0.3	—	0.5
Total dienes	10.1	7.2	9.1	18.5	13.0	7.0	7.4	9.0
16:3 (n-6)	1.8	1.4	3.5	1.9	1.8	0.5	0.9	1.1
16:3 (n3)	1.8	2.3	3.3	3.2	2.4	0.7	1.7	5.0
18:3 (n-6)	3.1	1.5	0.6	1.0	1.4	20.3	14.4	0.5
18:3 (n-3)	13.4	17.7	17.7	13.5	19.0	1.3	0.8	18.2

Table 1. *Continued*

Fatty acids	Cladonia sp.1	C.sp.2	C.sp.3	C.sp.4	C.sp.5	C.sp.6	C.sp.7	C.sp.8
20:3 (n-9)	0.6	0.2	0.6	1.0	0.6	0.2	0.2	0.3
20:3 (n-6)	1.1	0.5	1.1	0.9	0.8	0.3	0.3	1.3
22:3 (n-6)	0.6	0.5	0.9	0.9	0.8	0.2	0.3	1.1
24:3 (n-3)	1.1	1.3	1.5	1.0	1.1	0.7	—	—
26:3 (n-3)	0.7	0.9	0.3	0.2	0.5	—	—	—
Total trienes	24.0	26.4	29.3	23.6	28.3	24.2	18.6	27.4
16:4 (n-3)	3.0	2.1	0.9	0.9	2.1	2.2	0.8	10.4
18:4 (n-3)	1.7	1.7	2.9	7.0	3.2	4.9	17.9	5.4
20:4 (n-6)	1.8	0.9	1.4	1.0	1.4	4.4	2.3	9.8
20:4 (n-3)	2.7	0.9	2.0	0.4	0.8	7.1	0.9	1.1
20:5 (n-3)	3.0	3.5	2.3	1.5	2.0	8.9	4.4	8.3
22:4 (n-3)	1.5	2.7	2.6	0.6	3.6	0.8	1.4	1.6
22:5 (n-3)	1.1	5.2	1.1	0.3	1.6	5.4	2.8	7.7
22:6 (n-3)	0.9	1.4	0.8	0.4	0.3	11.5	2.9	10.6
Total tetra-, penta- and hexaenes	15.7	18.4	14.0	12.1	15.0	45.2	33.4	54.9

288°. Quantitative evaluation was based on the total ion current. The accuracy of repeated analysis was ±0.01%.

Mass spectra. Energy of ionizing electrons, 70 eV; ionizing current, 1 mA; temp. of the direct inlet system, 20–200°; composition of ions at high resolution was determined with an error smaller than 4 ppm. This method was used for Me esters of linoleic and linolenic acids (standards; Lachema, Brno, CSFR). The same mixture of fatty acid Me esters was analysed using an HP-1 fused-silica capillary column (Hewlett-Packard) (25 m × 0.2 mm i.d.) with a film thickness 0.25 μm. The injector temp. was 280° (splitting ratio 1:50) and the oven temp. was increased from 35 to 250° at 3° min^{-1}. The temp. of the GC-MS interface was 270° and the EI energy was 70 ev.

The positions of double bonds in the Me esters were determined, based on the splitting of pyrrolidines in the mass spectrum [41]. Me esters of fatty acids with more than 21 carbons were separated by HPLC [42]. Individual Me esters of monoenoic fatty acids were oxidatively degraded [43] and the resulting mono- and dicarboxylic acids were converted into Me esters by reaction with CH_2N_2 and identified by GC-MS [43, 44]. Me esters of mono- and dicarboxylic acids were analysed according to refs [43, 44].

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REFERENCES

1. Yamamoto, Y. and Watanabe, A. (1974) *J. Gen. Appl. Microbiol.* **20**, 83.
2. Kushnir, E., Tietz, A. and Galun, M. (1978) *Protoplasma* **97**, 47.
3. Dertien, B. K., De Kok, L. J. and Kuiper, P. J. (1977) *Physiol. Plant* **40**, 175.
4. Beltman, I. N., De Kok, L. J., Kuiper, P. J. C. and van Hasselt, P. R. (1980) *OIKOS* **35**, 321.
5. Solberg, Y. (1986) *J. Hattori Bot. Lab.* **60**, 391.
6. Solberg, Y. (1987) *J. Hattori Bot. Lab.* **63**, 357.
7. Dembitsky, V. M., Bychek, I. A., Shustov, M. V. and Rozentsvet, O. A. (1991) *Phytochemistry* **30**, 837.
8. Dembitsky, V. M., Shustov, M. V. and Bychek, I. A. (1991) *Khim. Prir. Soedin.* (in press).
9. Rosenberg, A. (1963) *Biochemistry* **2**, 1148.
10. Douglas, A. G., Douraghi-Zadeh, K. and Eglinton, G. (1969) *Phytochemistry* **8**, 285.
11. Gillan, F. T., McFadden, G. I., Wetherbee, R. and Johns, R. B. (1981) *Phytochemistry* **20**, 1935.
12. Rezanka, T., Vokoun, J., Slavicek, J. and Podojil, M. (1983) *J. Chromatogr.* **268**, 71.
13. Hung, J. G. C. and Walker, R. W. (1970) *Lipids* **5**, 720.
14. Takayama, K., Qureshi, N. and Schnoes, H. K. (1978) *Lipids* **13**, 575.
15. Shaw, R. (1966) *Adv. Lipid Res.* **4**, 107.
16. Fisher, D. J., Holloway, P. J. and Richmond, D. V. (1972) *J. Gen. Microbiol.* **72**, 71.
17. Uchida, K. (1974) *Biochem. Biophys. Acta* **348**, 86.
18. Zviagintseva, I. S., Pitriuk, I. A., Babyeva, I. P. and Ruban, E. L. (1975) *Mikrobiologia* **44**, 625.
19. Wassef, M. K. (1977) *Adv. Lipid Res.* **15**, 159.
20. Rezanka, T., Klanova, K., Podojil, M. and Vanek, Z. (1984) *Folia Microbiol.* **29**, 217.
21. Litchfield, C., Greenberg, A. J., Noto, G. and Morales, R. W. (1976) *Lipids* **11**, 567.
22. Litchfield, C. and Marcantonio, E. E. (1978) *Lipids* **13**, 194.
23. Litchfield, C., Tyszkiewichz, J. and Dato, V. (1980) *Lipids* **15**, 200.
24. Dembitsky, V. M. and Chelomin, V. P. (1985) *Izvestya AN SSSR, Ser. biol.* **1**, 53.
25. Dembitsky, V. M. (1981) *Khim. Prir. Soedin.* **4**, 511.
26. Dembitsky, V. M. (1981) *Khim. Prir. Soedin.* **4**, 513.
27. Dembitsky, V. M., Kashin, A. G. and Karaganova, M. (1991) *Comp. Biochem. Physiol* (in press).
28. Morales, R. W. and Litchfield, C. (1976) *Biochim. Biophys. Acta* **431**, 206.
29. Morales, R. W. and Litchfield, C. (1977) *Lipids* **12**, 570.
30. Ayanoglu, E., Walkup, R. D., Sica, D. and Djerassi, C. (1982) *Lipids* **17**, 617.

31. Walkup, R. D., Jamieson, G. C. and Ratcliff, M. R. (1981) *Lipids* **16**, 631.
32. De Rosa, M., Minale, L. and Sodano, G. (1973) *Comp. Biochem. Physiol.* **45B**, 883.
33. Lawson, M. P., Bergquist, P. R. and Cambie, R. C. (1986) *Tissue Cell* **18**, 19.
34. Lankelma, J., Ayanoglu, E. and Djerassi, C. (1983) *Lipids* **18**, 853.
35. Lawson, M. P., Thompson, J. E. and Djerassi, C. (1988) *Lipids* **23**, 741.
36. Dembitsky, V. M. and Nebylitsyn, B. D. (1980) *Bioorgan. Khim.* **6**, 1542.
37. Welch, J. W. and Burlingame, A. L. (1973) *J. Bacteriol.* **115**, 464.
38. Nurminen, T. and Suomalainen, H. (1971) *Biochem. J.* **125**, 963.
39. Diab, M. A., Popojil, M., Wurst, M. and Prokes, B. (1976) *Folia Microbiol.* **21**, 294.
40. Anderson, B. A. (1978) *Progr. Chem. Fats Other Lipids* **16**, 279.
41. Anderson, B. A. and Holman, R. T. (1974) *Lipids* **9**, 185.
42. Rezanka, T. and Podojil, J. (1985) *J. Chromatogr.* **346**, 453.
43. Rezanka, T. and Podojil, J. (1986) *J. Chromatogr.* **362**, 399.
44. Veden, I. and Rezanka, T. (1987) *J. Chromatogr.* **408**, 145.

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Identified of lichen species:
Cladonia sp.1 = Cladonia deformis (L.) Hoffm,
Cladonia sp.2 = C.cervicornis (Ach.) Flot.
subsp. verticillata (Hoffm.) Ahti,
Cladonia sp.3 = C.cornuta (L.) Hoffm.,
Cladonia sp.4 = C.pyxidata,
Cladonia sp.5 = C.phyllophora Hoffm.,
Cladonia sp.6 = C.subulata (L.) Wigg.,
Cladonia sp.7 = C. fimbriata (L.) Fr.,
Cladonia sp.8 = C.anomea (Ach.) Athi et P.James.
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