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Fatty Acids of Lichen Species from Tian Shan Mountains

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ABSTRACT. Eight lichen species growing in the Tian Shan Mountains were investigated for their fatty acid composition using capillary GC–MS. Seventy-three fatty acids were identified: 24 saturated, 28 monoenoic, 5 dienoic, 11 trienoic and eight tetra-, penta- and hexaenoic. Very long-chain fatty acids, *viz*. 26:0, 26:1(11), 26:3(3), 27:1, 28:1 and 30:1 were also identified. For the first time 36 fatty acids were identified in these lichens.

Four mountain systems, including the Tian Shan, may be distinguished, in Central Asia according to the period during which the most intense folding took place. The Tian Shan was formerly regarded as a system of folded mountains formed during the Tertiary period and was considered to be of the alpine type. Traces of the glacial epoch are less in evidence in the Tian Shan than in the Alps (Berg 1950). Although the lipid composition of lichen species is well documented (Dembitsky 1992), there is only a limited amount of information on the lipids and fatty acids in lichens growing in the Tian Shan Mountains.

The presence of various chemical components in lichens has long been used as a chemotaxonomic marker providing useful information for taxonomic investigations, particularly with regard to aromatic compounds, carotenoids (Huneck and Yoshimura 1996) and fatty acids (Dembitsky 1992). Numerous reports on the fatty acid composition of lichens (Piervittori *et al*. 1994) including our previous analyses of fatty acids from some lichens growing in different locations suggest that lichens are organisms of great biochemical diversity (Dembitsky *et al*. 1991*a*,*b*, 1992*a*–*c*). Our recent reports on bromine-containing fatty acids found in the lichens (Řezanka *et al*. 1998; Řezanka and Dembitsky 1999) have indicated the need for further examination of various species from other families. The present work reports the results of a continued investigation on Tian Shan lichen fatty acids with a very long chain (more than 24 carbon atoms) and also oligounsaturated fatty acids using the GC–MS method.

MATERIALS AND METHODS

Lichens were harvested in August 1992 in the *Tian Shan Mountains Nature Reserve* near Kungei Ala Tau (Kirghizstan). Freshly collected lichens were thoroughly cleansed of extraneous matter, homogenized and extracted. Methyl esters were prepared by alkaline hydrolysis and reaction of free acids with BF_3–MeOH. Oxazolines were prepared by the method of Yu *et al*. (1988). Methyl esters were identified by GC–MS using a fused silica capillary column (60 m × 0.32 mm i.d./SPB-1, *i.e. Supelco* column), using splitless injection and He as carrier gas. Oven temperature was programmed from 100 to 320 °C at a gradient of 4 K/min. Ionization energy was 11 aJ (70 eV). Oxazolines were identified under conditions similar to those for methyl esters, the only difference being the column temperature, programmed from 150 to 330 °C at a gradient of 5 K/min.

RESULTS AND DISCUSSION

The fatty acid composition of the lichen species is shown in Tables I–III. We identified 76 fatty acids, including 24 saturated acids. The proportion of saturated fatty acids in the species examined varied from 16.8 to 22.1 %. 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. The lichen species were shown to contain pristanic acids, which constituted 0.15 % in *Peltigera canina* and 0.17 % in *Acarospora gobiensis*, respectively, and also phytanic acids which constituted 0.76, 0.29 and 0.18 % in *P. canina, Xantoria* sp., and *Lecanora fructulosa*, respectively (Table I). 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 some lichens collected in the Volga River Basin (Dembitsky *et al*.

Table I. Saturated fatty acid composition from lichens collected in the Tian Shan mountains[a]

Fatty acid	*A.g.*	*C.f.*	*L.f.*	*L.s.*	*Pa.c.*	*Pa.l.*	*Pe.c.*	*X.*sp.
i-12:0	0.29	0.30	0.32	0.20	0.18	0.19	0.25	0.19
12:0	1.08	0.86	0.53	1.33	0.42	0.43	0.98	0.72
i-13:0	0.54	0.06	0.06	0.18	0.17	0.17	0.46	0.17
i-14:0	0.22	0.02	0.02	0.53	0.49	0.51	0.19	0.49
14:0	1.51	1.06	2.33	2.17	2.86	3.20	0.71	0.75
i-15:0	0.64	0.34	0.37	0.37	0.35	0.36	0.55	0.35
ai-15:0	0.30	0.21	0.23	0.69	0.64	0.66	0.26	0.65
15:0	0.90	0.45	1.71	1.11	1.70	1.77	1.17	1.48
i-16:0	0.46	0.54	0.58	0.51	0.47	0.38	0.39	0.48
2,6,10,14-Me_4-15:0[b,d]	0.17	0.01	0.02	0.01	0.01	0.01	0.15	0.01
16:0	4.50	6.82	6.61	6.10	7.25	8.30	6.06	7.13
i-17:0	0.54	0.10	0.11	0.12	0.11	0.12	0.46	0.11
ai-17:0	0.28	0.61	0.66	0.29	0.27	0.28	0.24	0.27
3,7,11,15-Me_4-16:0[c,d]	0.11	0.01	0.01	0.18	0.01	0.01	0.76	0.29
17:0	0.57	0.34	0.37	0.32	0.30	0.31	0.48	0.30
18:0	2.17	5.47	5.32	2.03	1.82	2.37	3.85	3.11
19:0	1.12	0.19	0.20	0.73	0.67	0.70	0.95	0.68
20:0	0.81	0.44	1.16	1.49	2.17	2.54	1.45	1.87
21:0	0.26	0.13	0.14	0.13	0.12	0.12	0.22	0.12
22:0	0.82	0.27	0.29	0.74	0.82	0.27	0.56	0.77
23:0[d]	0.15	0.12	0.13	0.08	0.07	0.07	0.13	0.07
24:0[d]	0.39	0.32	0.35	0.45	0.41	0.12	0.33	0.11
26:0[d]	0.11	0.07	0.07	0.22	0.20	0.05	0.09	0.05
28:0[d]	0.22	0.02	0.02	0.11	0.10	0.02	0.19	0.02
Total saturated	18.2	18.8	21.6	20.1	21.6	23.0	20.9	20.2

[a] *A.g.* *Acarospora gobiensis*; *C.f.* *Cladonia furcata*; *L.f.* *Lecanora fructulosa*; *L.s.* *Leptogium saturninum*; *Pa.c.* *Parmelia comtschadalis*; *Pa.l.* *Parmelia tinctina*; *Pe.c.* *Peltigera canina*; *X.*sp. *Xanthoria* sp.
[b] Pristanic acid. [c] Phytanic acid. [d] Fatty acids isolated for the first time.

Table II. Monoenoic fatty acid composition from lichens collected in the Tian Shan mountains[a]

Fatty acid	*A.g.*	*C.f.*	*L.f.*	*L.s.*	*Pa.c.*	*Pa.l.*	*Pe.c.*	*X.*sp.
12:1	0.53	0.23	8.55	1.10	0.22	0.17	1.15	0.98
13:1	0.26	0.09	0.09	0.08	0.07	0.12	0.22	0.11
14:1(5)[d]	1.11	0.69	1.25	1.60	1.27	1.64	1.31	0.79
15:1(8)[d]	0.29	0.79	0.85	0.52	0.48	0.65	0.25	0.64
15:1(6)[d]	1.02	0.31	0.97	1.39	0.93	0.82	1.00	0.90
16:1(9)	6.45	4.97	6.78	2.96	3.47	2.97	5.88	4.36
16:1(7)[d]	1.78	1.15	4.07	2.63	3.22	3.54	0.88	2.21
trans-16:1(13)[d]	0.02	0	0	0	0	0.01	0.01	0.01
17:1(10)[d]	0.10	0.34	0.37	0.16	0.14	0.17	0.08	0.17
17:1(8)[d]	0.02	0.20	0.22	0.36	0.34	0.29	0.01	0.28
18:1(11)[d]	1.17	0.70	1.33	2.23	2.29	3.83	1.69	1.99
18:1(9)	9.67	11.4	5.83	7.41	4.98	4.25	14.3	9.87
18:1(7)[d]	0.71	4.10	1.23	1.27	1.40	3.23	0.60	0.75
19:1(8)[d]	0.26	0.25	0.27	0.09	0.08	0.09	0.22	0.09
20:1(11)[d]	0.43	0.56	0.61	0.60	0.55	0.72	0.37	0.70
20:1(9)	0.80	0.98	1.06	0.85	0.79	0.89	0.68	0.86
21:1(8)[d]	0.07	0.10	0.11	0.11	0.10	0.03	0.06	0.03
22:1(11)[d]	0.70	0.63	0.68	0.21	0.19	0.14	0.60	0.14
22:1(9)	0.39	0.25	0.28	0.61	0.06	0.07	1.03	0.73
23:1[d]	0.07	0.07	0.07	0.02	0.02	0.02	0.06	0.02
24:1(9)[d]	0.69	0.17	0.19	0.16	0.15	0.12	0.59	0.12
25:1(8)[d]	0.03	0.04	0.05	0.03	0.02	0.03	0.03	0.03
26:1(11)[d]	0.07	0.06	0.06	0.05	0.05	0.08	0.06	0.08
27:1[d]	0.02	0.01	0.02	0.03	0.03	0.02	0.01	0.02
28:1[d]	0.05	0	0	0.04	0.04	0.05	0.04	0.05
30:1[d]	0.02	0	0	0	0	0.02	0.01	0.02
Total monounsaturated	26.7	28.1	34.9	24.5	20.9	24.0	31.1	26.0

[a,d] See footnote to Table I.

1992*a*). According to Solberg (1987), who studied the chemistry of four lichen species, the fatty acids he identified ranged from C_6 to C_{28}.

Table III. Dienoic, trienoic and oligoenoic fatty acid composition from lichens collected in the Tian Shan mountains[a]

Fatty acid	*A.g.*	*C.f.*	*L.f.*	*L.s.*	*Pa.c.*	*Pa.l.*	*Pe.c.*	*X.*sp.
14:2(5)	0.14	0.30	0.32	0.75	0.70	0.48	0.12	0.47
16:2(4)	0.39	0.55	0.59	0.94	0.87	1.43	0.33	1.40
18:2(6)	9.12	10.32	7.65	7.25	7.52	6.11	6.25	7.34
20:2(6)	0.20	0.76	0.33	0.85	0.79	0.48	0.17	0.47
22:2(6)	0.06	0.17	0.18	0.15	0.14	0.57	0.05	0.56
Total diunsaturated	9.91	12.10	7.83	8.21	10.0	7.81	6.92	10.2
16:3(6)	2.64	1.43	1.41	0.99	1.12	1.61	1.61	1.28
16:3(3)	2.92	0.55	1.23	2.09	1.72	1.64	2.48	1.53
18:3(6)	6.91	1.12	1.14	1.13	0.91	1.13	6.58	2.56
18:3(3)	7.25	9.46	5.55	4.66	9.31	7.78	6.22	10.42
20:3(9)[d]	0.40	0.45	0.36	0.59	0.69	0.45	0.59	0.36
20:3(6)[d]	0.19	0.63	0.53	0.67	0.71	0.90	0.22	0.74
20:3(3)[d]	0.47	0.23	0.65	1.29	0.75	1.30	0.53	1.41
22:3(6)[d]	0.26	0.41	0.44	0.78	0.72	0.48	0.22	0.47
22:3(3)[d]	0.07	0.30	0.32	0.25	0.23	0.42	0.06	0.41
24:3(3)[d]	0.39	0.51	0.54	0.68	0.70	0.61	0.52	0.47
26:3(3)[d]	0.04	0.25	0.28	0.80	0.74	0.43	0.04	0.42
Total triunsaturated	21.5	15.3	12.5	13.9	17.6	16.8	19.1	20.1
16:4(3)	1.05	2.25	3.07	2.37	1.98	2.14	1.28	2.38
18:4(3)[d]	5.87	1.43	1.41	3.17	5.10	5.93	2.60	6.42
20:4(6)	2.39	2.52	3.64	4.17	3.43	2.92	1.47	1.90
20:4(3)[d]	3.24	1.57	4.02	3.73	2.74	2.10	3.08	2.63
20:5(3)	3.52	6.11	3.06	6.22	6.50	5.27	5.08	2.96
22:4(3)[d]	0.58	1.48	0.89	0.77	0.79	0.72	0.53	0.47
22:5(3)[d]	2.17	1.54	0.96	3.18	3.02	3.45	2.29	1.41
22:6(3)	3.85	7.73	4.88	7.92	4.16	4.75	4.80	3.84
Total oligounsaturated	22.7	24.6	21.9	31.5	27.7	27.3	21.1	22.0
Unknown	0.99	1.10	1.24	1.73	2.16	1.23	0.89	1.54

[a,d]See footnote to Table I.

Most of the acids identified in the course of the present study were monoenoic ranging from 12:1 to 30:1 (Table II). Three isomers, *viz*. 18:1(11), (9) and (7), represented C_{18} acids. The 16:1 acids were also represented by three isomers, 16:1(9), (7) and *trans*-16:1(3). Among the long-chain fatty acids, 25:1(8), 26:1(11), 27:1, 28:1 and 30:1 were identified. Similar types of acid have recently been identified in the genus *Cladonia* (Dembitsky *et al*. 1991*b*), *Parmelia* (Dembitsky *et al*. 1992*c*) and in the order *Lecanorales* (Dembitsky *et al*. 1992*b*). The 18:1 was one of the main fatty acids and showed a broad variation in concentration and position of the double bond, due to variations of the lichen substrates (Piervittori *et al*. 1994; Dertien *et al*. 1977; Kushnir *et al*. 1978). The proportion of monoenoic acids in the total fatty acid content in different lichen species was shown to range from 20.9 to 34.9 %.

Dienoic acids made up a small portion of the total content, ranging from 6.9 to 12.1 %. They were represented by five acids only, *viz*. 14:2(5), 16:2(4), 18:2(6), 20:2(6) and 22:2(6) (Table III). Eleven fatty acids were identified as trienoic, among which were the long-chain fatty acids 24:3(3) and 26:3(3), both found in only two lichen species.

The proportion of total trienoic acids varied widely, from 13.9 % in *L. fructulosa* to 21.6 % in *Xanthoria* sp. Among other interesting acids, we detected the 16:4(3) one, the presence of which is characteristic of green marine algae (Khotimchenko 1993), and 18:4(3), characteristic of brown marine algae (Zhukova and Svetashev 1999). It seems likely that the lichen phycobionts synthesize these acids. In *L. fructulosa,* the total level of 20:4(6) and 20:4(3) isomers was considerably higher than that from the other species examined, reaching 7.9 %. 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 (Dembitsky 1993). Small amounts of very long-chain fatty acids are reported to be present in certain photosynthetic microorganisms, particularly in the fresh-water autotrophic *Euglena gracilis*, which contains C_{24}–C_{26} (Řezanka *et al*. 1987).

Our study thus revealed a fairly broad diversity of fatty acids. More than thirty fatty acids, predominantly those with a long chain and oligo-unsaturated fatty acids were isolated in the above-mentioned lichens for the first time. As markers, we can use the earlier described unusual fatty acids, first of all, the brominated fatty acids isolated from all lichen species. These unusual metabolites are hidden in unknown components (Table III). The results of the fatty acid investigation demonstrated a marked difference between lipids from lichens, on the one hand, and lipids from fungi (cf. Řezanka *et al*. 1999) and/or algae, on the other.

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