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ACETYLENIC FATTY ACIDS OF THE DICRANACEAE

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Key Word Index—Dicranaceae; mosses; acetylenic fatty acids.

Abstract—Eight moss species of the family Dicranaceae were analysed for their fatty acid composition. They were collected from different regions of Russia and all species contained acetylenic fatty acids. Fatty acids were examined by GC-MS in total lipid extracts; 86 fatty acids were identified, including 27 saturated ones (both *iso*- and *anteiso*-). Three acetylenic acids were identified among monoenoic (6a–18:1, 9a–18:1, 12a–18:1) and dienoic acids (6a,9c–18:2, 9a,12c–18:2 and 9c,12a–18:2). Two more acetylenic acids were identified among the trienoic and tetraenoic fatty acids (6a,9c,12c–18:3, 8a,11c,14c–20:3, 6a,9c,12c,15c–18:4 and 5a,8c,11c,14c–20:4).

INTRODUCTION

At present, many taxonomists are interested in the distribution of secondary metabolites in plants. In many cases, one is confronted with the problem of classifying the genus or the species by classical morphological methods. More than 700 acetylenic compounds from plants and primitive animals are known [1–7] and the distribution of these compounds has some chemotaxonomic relevance [1, 8]. There is evidence that this is also true for the acetylenes of Bryophytes which have been identified exclusively as long chain fatty acids with characteristic locations of triple and double bonds. This feature has not been found among acetylenes from other natural sources [9–12]. Recently, Kohn *et al.* [13] have identified two main acetylenic long-chain fatty acids in moss species belonging to the Dicranales, namely, 9c,12c,15c-octadecatrien-6-ynoic and 9c,12c-octadecadien-6-ynoic.

The present work reports the fatty acid composition of some moss species from the family Dicranaceae and continues our earlier study on Bryophyte lipids [12, 14].

RESULTS

All species in this study were available as fresh material, but the moss species collected from northwestern Russia, namely, Dicranum *congestum*, *D. majus* and one from three species of *D. polysetum* were harvested together with moist soil substance and kept in the dark for three months before lipid analysis. The other moss species were extracted immediately after collection.

The GC-MS analysis of the total lipid extracts from the mosses showed the domination of acetylenic acids in species growing on sunlight. Among the identified fatty acids we found 27 saturated acids ranging from C_{12} to C_{26}. We also observed the presence of prystanic, phytanic, *iso*- and *anteiso*-acids. Among the saturated acids, 16:0 (varying from 6.49 to 16.51%) and 18:0 (1.73 to 10.43%) were the major ones with contents over 1% (Table 1). Considerable differences were found between the saturated fatty acids from the sunlight and dark-growing samples. Saturated fatty acids varied from 32.74 in *D. bonjeanii* to 12.70% in *D. majus*.

Among the monoenoic fatty acids, the major acids were 16:1 (n-9), 16:1 (n-7) and 18:1 (n-9) (Table 2). We have identified three isomers for 16:1, four isomers, including *iso*- and *anteiso*-, for 17:1 and six isomers including acetylenic acids for 18:1. The greatest amount of monoenoic (over 40%) acids was found in *D. polysetum*. Three acetylenic acids were identified amongst the monoenes, 6a–18:1, 9a–18:1 and 12a–18:1. High percentages of acetylenic acids were detected in all moss species which were extracted immediately after collection on a sunny day (12.49, 10.65, 5.01, 12.34 and 8.99%). Moss species growing for three months under dark conditions contained only small percentages of monoenoic acetylenic FAs (2.03, 2.0 and 1.91%, see Table 2).

From moss species growing in darkness, 10 dienoic, 12 trienoic and eight tetra- and pentaenoic acids were identified (Table 3). The major dienoic acids were 18:2 (n-6) (varying from 0 to 10.85%) and all three acetylenic acids, 6a,9c–18:2, 9a,12c–18:2 and 9c,12a–18:2.

We have found considerable differences between the acetylenic acids among mosses growing in darkness and sunlight conditions. For example, *D. polysetum* which was kept for three months in the darkness had 0.90% of 6a,9c,12c–18:3 acid, whereas *D. polysetum* (samples 1 and 3) collected in a sunny day had 3.48 and 9.42% of these acids, respectively. The other dark-growing species examined yielded smaller percentages (1.09% in *D. congestum*

Table 1. Saturated fatty acids of total lipids from mosses of the family Dicranaceae

Species	*D. boj*	*D. con*	*D. fra*	*D. fla*	*D. maj*	*D. poly*		
Collection places:	1	2	1	1	2	2	1	3
12:0	0.95	0.46	1.03	0.46	0.65	0.62	0.96	0.52
13:0	0.28	0.07	0.30	0.07	0.07	0.06	0.28	0.10
14:0	0.54	0.40	0.58	0.39	0.45	0.43	0.54	0.40
15:0	0.42	0.21	0.45	0.21	0.23	0.22	0.42	0.27
16:0	16.51	9.85	12.67	7.00	7.05	8.33	14.29	6.49
17:0	0.81	0.41	0.88	0.40	0.45	0.43	0.82	0.50
18:0	9.82	2.23	10.43	2.84	1.73	2.30	9.87	2.43
19:0	0.14	0.18	0.15	0.17	0.20	0.19	0.14	0.20
20:0	0.68	0.23	0.73	0.22	0.22	0.21	0.68	0.39
21:0	0.42	0.04	0.45	0.04	0.04	0.04	0.42	0.10
22:0	0.28	0.08	0.30	0.08	0.10	0.09	0.28	0.12
23:0	0.14	0.04	0.15	0.04	0.07	0.07	0.14	0.09
24:0	0.28	0.06	0.30	0.06	0.04	0.04	0.28	0.10
25:0	0.14	0.01	0.15	0.01	0.01	0.01	0.14	0.05
iso-12:0	0.28	0.03	0.30	0.04	0.03	0.03	0.28	0.08
iso-13:0	0.28	0.08	0.30	0.08	0.07	0.07	0.28	0.11
iso-14:0	0.42	0.24	0.45	0.24	0.24	0.23	0.42	0.26
iso-15:0	0.00	0.11	0.06	0.11	0.12	0.12	0.00	0.12
iso-16:0	0.02	0.16	0.00	0.16	0.12	0.12	0.00	0.10
iso-17:0	0.07	0.10	0.00	0.10	0.11	0.11	0.03	0.10
anteiso-13:0	0.14	0.10	0.15	0.10	0.10	0.09	0.14	0.15
anteiso-15:0	0.05	0.04	0.00	0.04	0.04	0.04	0.00	0.05
anteiso-17:0	0.07	0.02	0.00	0.02	0.01	0.01	0.02	0.03
anteiso-19:0	0.00	0.09	0.00	0.09	0.09	0.08	0.00	0.10
Pristanic	0.00	0.12	0.00	0.12	0.23	0.22	0.00	0.09
Phytanic	0.00	0.12	0.00	0.12	0.23	0.22	0.40	0.09
Total saturated	32.74	15.48	29.83	13.21	12.70	14.38	30.83	13.04

Abbreviations: *D. boj*, *D. bojeanii*; *D. con*, *D. congestum*; *D. fra*, *D. fragilifolium*; *D. maj*, *D. majus*; *D. poly*, *D. polysetum*; *O. fla*, *Orthodicranum flagellare*.

1. Southwestern Siberia.
2. Northwestern Europian Russia.
3. Zhuguli Natural Park (central Russia).

and 1.18% in *D. majus*) (Table 3). Two species, *D. bonjeanii* and *D. fragilifolium* did not contain these acids, but they have high levels of 20:3 (n-6) (5.20 and 7.53%, respectively) and 8a,11c,14c–20:3. We found high amounts (*ca* 14%) of 18:3 (n-3) and 18:3 (n-6) in dark-growing moss species only. The major tetraenoic acid in the total lipids was 6a,9c,12c,15c–18:4.

DISCUSSION

Anderson *et al.* [9] isolated 9c,12c,15c-octadecatrien-6-ynoic acid from several moss species and showed that this acid was present only in the triacylglycerol fraction. Jamieson and Reid [11] have reported the presence of 9c,12c-octadecadien-6-ynoic acid in the moss, *Fontinalis antipyretica*, and Anderson *et al.* [15] found this acid and its C_{20} homologue, 11c,14c-eicosadien-8-ynoic acid, in the same moss species. Kohn *et al.* [16] identified a new acetylenic, fatty acid as 9c-octadecen-6-ynoic acid from the liverwort, *Riccia fluitans*.

Kohn *et al.* [13] have reported rather interesting results on the fatty acid composition of the acyl lipids of 38 moss species belonging to four families of the order Dicranales. They found two acetylenic acids only, namely, 9c,12c-octadecadienoic-6-ynoic and 9c,12c,15c-octadecatrien-6-ynoic. In the Ditrichaceae and the Dicranaceae, numerous species were found to contain acetylenic fatty acids esterified as triacylglycerols. 9c,12c,15c-Octadecatrien-6-ynoic acid was the major component, often accounting for more than 80 mol%, whereas 9c,12c-octadecadien-6-ynoic acid was found in small amounts of less than 5 mol%. In some genera, all the species examined contained acetylenic compounds, e.g. *Dicranella* and *Dicranum*, whereas in the genus *Campylopus* all five species tested lacked acetylenic compounds. Two genera, *Ditrichum* and *Dicranoweisia*, were found to have a non-homogeneous distribution of acetylenic fatty acids. The same authors also made a comparative study of fresh and non-fresh material. Samples of *C. strumiferum* were extracted fresh, after dry storage for three days and after a period of one year. It was observed that the total triacylglycerol content decreased markedly on storage; this is probably due to the polymerization and peroxidation of the lipids. Changes

Table 2. Monoenoic fatty acids of total lipids from mosses of the family Dicranaceae

Species	*D. boj*	*D. con*	*D. fra*	*D. fla*	*D. maj*	*D. poly*		
Collection places:	1	2	1	1	2	2	1	3
12:1 (n-5)	0.42	0.30	0.45	0.29	0.42	0.40	0.42	0.34
13:1 (n-5)	0.28	0.15	0.30	0.14	0.15	0.14	0.28	0.30
14:1 (n-5)	0.54	0.37	0.58	0.37	0.42	0.40	0.54	0.50
15:1 (n-9)	0.00	0.11	0.00	0.11	0.12	0.12	0.00	0.20
15:1 (n-7)	0.42	0.37	0.45	0.37	0.39	0.37	0.42	0.12
16:1 (n-9)	1.63	2.32	1.77	2.29	3.76	3.62	1.64	5.81
16:1 (n-7)	1.09	1.16	1.19	1.14	0.91	0.87	1.10	2.10
trans-16:1 (n-13)	0.03	0.10	0.00	0.10	0.20	0.19	0.07	0.11
iso-17:1 (n-9)	0.28	0.17	0.30	0.17	0.16	0.15	0.28	0.20
anteiso-17:1 (n-9)	0.00	0.09	0.00	0.09	0.09	0.08	0.00	0.10
17:1 (n-9)	0.42	0.42	0.45	0.41	0.47	0.46	0.42	0.40
17:1 (n-7)	0.28	0.24	0.30	0.23	0.25	0.24	0.28	0.20
18:1 (n-11)	0.81	0.52	0.88	0.51	0.48	0.46	0.82	0.48
18:1 (n-9)	14.78	16.20	17.18	18.32	16.03	16.34	21.29	8.37
18:1 (n-7)	0.28	0.51	0.30	0.50	0.45	0.43	0.28	0.38
6a–18:1a,b	3.67	0.68	3.99	0.67	0.67	0.64	3.69	3.01
9a–18:1a,b	3.66	0.95	1.05	0.94	0.93	0.89	3.46	4.19
12a–18:1b,d	5.16	0.40	5.61	3.40	0.40	0.38	5.19	1.79
19:1 (n-8)	0.00	0.17	0.00	0.17	0.15	0.14	0.00	0.25
20:1 (n-11)	0.14	0.18	0.15	0.18	0.22	0.21	0.14	0.20
20:1 (n-9)	0.28	0.09	0.30	0.09	0.10	0.09	0.28	0.11
21:1 (n-8)	0.14	0.05	0.15	0.05	0.05	0.05	0.14	0.07
22:1 (n-11)	0.14	0.05	0.15	0.05	0.05	0.05	0.14	0.09
22:1 (n-9)	0.28	0.03	0.30	0.03	0.03	0.03	0.28	0.10
24:1 (n-11)	0.14	0.01	0.15	0.01	0.01	0.01	0.14	0.03
24:1 (n-9)	0.14	0.01	0.15	0.01	0.01	0.01	0.14	0.00
26:1 (n-11)	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00
26:1 (n-9)	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
Total monoenoic	35.01	25.09	36.15	30.66	26.92	26.77	41.44	29.45
Total acetylenic acids	12.49	2.03	10.65	5.01	2.00	1.91	12.34	8.99

a,b and d: See footnote on Table 3 for explanation.

in the composition of the fatty acids were not very striking. Thus, even after one year of storage, the characteristic composition, with the dominant acetylenic acid, 9c,12c,15c-octadecatrien-6-ynoic, was retained.

Some investigators have studied the effects of temperature, light [17–25], age [26, 27] and nitrogen starvation [28] on the lipid and fatty acid compositions of some Bryophytes. Karunen *et al.* [25] observed that decreasing light intensity and decreasing daylength are the major environmental factors limiting the accumulation of triacylglycerol-containing acetylenic acids in the sub-Arctic moss, *Dicranum elongatum*. We have also found very similar data for moss species growing under dark conditions.

Higher proportions (more than 60%) of homo-α-linolenic acid [20:3 (n-3)] have been found in the total lipids of brown and green parts of the gametophore of two other moss species, *Pogonatum urnigerum* and *Fissidens adianthoides* [29]. This acid (22.5%) was also isolated from digalactosyldiacylglycerol only in *Cynodontium strumiferum* (Dicranaceae) [13]. Our examined moss species, contained only a small percentage of 20:3 (n-3) (from 0 to 0.32%). Phosphatidylethanolamine and phosphatidylcholine from the moss, *Leptobryum pyriforme*, was shown to contain 5.6 and 3.3% of 20:3 (n-6), respectively [30].

EXPERIMENTAL

Moss species. (1) *Dicranum bojeanii* De Not., (2) *Dicranum congestum* Brid., (3) *Dicranum fragilifolium* Lindb., (4) *Dicranum majus* Sm., (5–7) *Dicranum polysetum* Sw., (8) *Orthodicranum flagellare* (Hedw.) Loeske. Species 1,3,6 and 8 were collected from a forest near the Biological Station of the Irkutsk State University 'Bolshye Koty' (southwestern part of Lake Baikal, Siberia) in August 1992. Species 2,4 and 5 were collected from a forest near St Petersburg (northwestern Europian Russia) in July 1992. Moss 7 came from the Zhiguli Natural Park (near Togliatti) in May 1992 (see also footnote Table 1). Freshly collected mosses were thoroughly cleansed of extraneous matter and homogenized in a high-speed unit. Mosses 2,4 and 5 were kept in the darkness for 3 months before lipid extraction. Lipids were extracted and fatty acids were analysed by GC-MS as described in refs [12, 14].

Table 3. Polyenoic fatty acids of total lipids from mosses of the family Dicranaceae

Species	*D. boj*	*D. con*	*D. fra*	*D. fla*	*D. maj*		*D. poly*	
Collection places:	1	2	1	1	2	2	1	3
14:2 (n-5)	0.00	0.04	0.00	0.03	0.02	0.02	0.00	0.07
16:2 (n-4)	0.00	0.29	0.00	0.29	0.25	0.24	0.00	0.21
18:2 (n-6)	0.00	10.85	6.85	9.50	10.72	10.54	5.05	4.83
18:2 (n-3)	5.02	0.54	0.00	0.54	0.67	0.65	0.00	0.80
9c,12a–18:2a,d	0.00	0.13	4.40	0.13	0.12	0.12	1.64	0.54
9a,12c–18:2b,d	4.05	0.16	1.77	0.16	0.16	0.15	2.05	0.71
6a,9c–18:2c,d	1.63	1.64	2.22	1.62	1.61	1.55	4.07	7.25
20:2 (n-6)	2.04	0.35	0.00	0.35	0.53	0.51	0.00	0.20
20:2 (n-3)	0.00	0.35	0.00	0.00	0.53	0.51	0.00	0.00
22:2 (n-6)	0.00	0.11	0.00	0.11	0.11	0.11	0.00	0.23
Total dienoic	12.74	14.46	15.74	12.73	14.72	14.40	12.81	14.84
Acetylenic acids	5.68	1.93	8.39	1.91	1.89	1.82	7.76	8.50
16:3 (n-6)	0.00	0.43	0.00	0.42	0.44	0.43	0.00	0.40
16:3 (n-3)	0.00	0.38	0.00	0.37	0.33	0.32	0.00	0.40
18:3 (n-6)	1.92	14.82	1.86	5.51	14.36	14.99	2.45	2.35
18:3 (n-3)	5.86	17.29	4.16	8.44	18.11	16.90	5.88	5.63
6a,9c,12c–18:3c,d	0.00	1.09	0.00	8.11	1.18	0.90	3.48	9.42
20:3 (n-9)	0.00	0.21	0.00	0.21	0.21	0.20	0.00	0.40
20:3 (n-6)	5.20	0.40	7.53	0.39	0.47	0.46	0.00	0.28
20:3 (n-3)	0.00	0.31	0.00	0.31	0.32	0.31	0.00	0.23
8a,11c,14c–20:3c,d	1.33	0.17	0.85	0.17	0.17	0.16	0.50	0.77
22:3 (n-6)	0.00	0.17	0.00	0.16	0.19	0.18	0.00	0.45
22:3 (n-3)	0.00	0.10	0.00	0.10	0.11	0.11	0.00	0.23
24:3 (n-3)	0.00	0.07	0.00	0.07	0.03	0.03	0.00	0.17
Total trienoic	14.31	35.44	14.40	24.26	35.92	34.99	12.31	20.73
Acetylenic acids	1.33	1.26	0.85	8.28	1.35	1.06	3.98	10.19
16:4 (n-3)	0.00	0.75	0.00	0.74	0.88	0.85	0.00	0.57
18:4 (n-3)	0.00	2.22	0.00	2.19	2.67	2.57	0.00	0.59
6a,9c,12c,15c–18:4c,d	3.47	1.64	2.37	11.98	1.47	1.51	1.74	16.08
20:4 (n-6)	0.00	1.78	0.00	1.76	1.88	1.81	0.00	2.63
20:4 (n-3)	0.00	0.44	0.00	0.40	0.41	0.39	0.00	0.16
5a,8c,11c,14c–20:4b,d	1.73	0.33	1.51	0.33	0.32	0.31	0.87	1.46
22:4 (n-3)	0.00	0.20	0.00	0.19	0.10	0.09	0.00	0.10
20:5 (n-3)	0.00	1.57	0.00	1.55	2.01	1.93	0.00	0.37
Total tetra- and pentaenoic acids	5.20	8.93	3.88	19.14	9.74	9.46	2.61	21.96
Acetylenic acids	5.20	1.97	3.88	12.31	1.79	1.82	2.61	17.54
Total of acetylenic acids	24.70	7.19*	23.77	27.51	7.03*	6.61*	26.69	45.22

*Moss species kept in darkness for three months.
a Known in nature.
b Prepared only by synthesis.
c Known in some mosses and/or liverworts.
d Structure confirmed by GC-MS of trimethylsilyloxazolines after preseparation.

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