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DISTRIBUTION OF ACETYLENIC ACIDS AND POLAR LIPIDS IN SOME AQUATIC BRYOPHYTES

VALERY M. DEMBITSKY* and TOMAS REZANKA

*Laboratory of Natural Products, Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel; Institute of Microbiology, Videnska 1083, Prague 142 20, Czech Republic

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Key Word Index—Liverworts; mosses; aquatic species; acetylenic fatty acids; triacylglycerols; DGTS; polar lipids.

Abstract—Two liverwort and three moss aquatic species from different regions of the former USSR were analysed for their fatty acid composition of total lipids and triacylglycerols (TAG). Diacylglyceryltrimethylhomoserine (DGTS) and phospholipid composition were also examined. The major polar lipids found were DGTS (20.4–39.6%), PC (18.2–25.5%), PG (13.9–25.8%) and PE (9.5–19.6%). Fatty acids were analyzed by GC-mass spectrometry in total lipid extracts and in TAG. Acetylenic fatty acids in TAG varied from 6.6% in the moss, *Calliergon cordifolium*, to 80.2% in the liverwort, *Riccia antipyretica*. Three acetylenic acids were identified among the monoenoics (6a-18:1 9a-18:1 and 12a-18:1) and dienoics (6a,9c-18:2, 9a, 12c-18:2 and 9c, 12a-18:2). Four acetylenic acids were identified amongst the polyenoics 6a, 9c, 12c-18:3, 8a, 11c, 14c-20:3, 6a, 9c, 12c, 15c-18:4 and 5a, 8c, 11c, 14c-20:4.

INTRODUCTION

Aquatic bryophytes are an interesting group of lower plants which have phylogenetic connections with green algae [1]. Some aquatic mosses and liverworts contain three major acetylenic acids, 9-octadecen-6-ynoic, 9,12-octadecadien-6-ynoic and 9, 12, 15-octadecatrien-6-ynoic [2].

Many taxonomists are interested in the distributions of secondary metabolites of plants. More than 700 acetylenic compounds, including fatty acids, from plants, fungi, micro-organisms and invertebrates are known [3,4]. The distribution of acetylenic fatty acids in bryophytes has some chemotaxonomic relevance [5, 6]. Detailed studies on acetylenic acids have been carried out on two aquatic bryophytes, the moss, *Fontinalis antipyretica* [7–9], and some liverworts from the genus *Riccia* [10, 11].

Polar lipids including betaine lipids and phospholipids from aquatic bryophytes are of particular interest, because they have not been examined so far. The present work is a detailed report on the fatty acids and polar lipids present in some aquatic bryophyte species and is a continuation of our earlier studies of bryophyte lipid composition [12–15].

RESULTS

All the species examined in this study were available as fresh material and they were collected from the different regions of the former USSR. The moss, *Calliergon cordifolium*, was collected from south-western Siberia, the moss, *Drepanocladus lycopodioides*, from north-western Russia, the moss, *Fontinalis antipyretica*, and the liverwort, *Riccia fluitans*, from the Volga river basin and the liverwort, *Pellia neesiana*, from the Tian-Shan mountains.

GC-mass spectrometry analysis of the fatty acids from total lipid extracts of the aquatic bryophytes showed that they contained small amounts of acetylenic acids. Among the identified fatty acids, we found seven major saturated acids which varied in moss species from 20.5–29.6% of the total lipid extract and from 8.3–30.6% in triacylglycerols (TAG); in liverwort species, they varied from 13.3–22.7% of the total lipid extract and from 13.6–17.4% in TAG (Table 1). Among the saturated acids, 16:0 (varying from 8.5–13.6% of the total lipid extract and from 4.7–18.3% in TAG) and 18:0 (1.6–6.1% of the total lipid extract and 1.8–7.2% in TAG) were the major ones with contents of over 1%.

Among the monoenoic fatty acids the major acids were 16:1 (*n*-9), 16:1 (*n*-7) and 18:1 (*n*-9) (Table 1). We have identified six isomers of 18:1, including the three acetylenic acids, 6a-18:1, 9a-18:1 and 12a-18:1. *Calliergon cordifolium* contained 0.3% of monoacetylenic acids and *F. antipyretica* contained the largest amounts of 9a-18:1 (9.4%), whilst *P. neesiana* contained the largest amounts of 6a-18:1 (2.7%) and 12a-18:1 (5.4%).

All the moss and liverwort species examined contained three major polyenoic fatty acids, 18:2 (*n*-6), 18:3 (*n*-6) and 18:3 (*n*-3) (Table 2). Ten fatty acids, including three acetylenic acids, from 29 monoenoic acids were

* Author to whom correspondence should be addressed.

Table 1. Saturated, monoenoic fatty acids of some aquatic bryophytes

	Mosses						Liverworts			
	Calliergon cordifolium		*Drepanocladus lycopodioides*		*Fontinalis antipyretica*		*Riccia fluitans*		*Pellia neesiana*	
Fatty acids	TL	TAG	TL	TAG	TL	TAG	TL	TAG	TL	TAG
12:0	0.7	1.3	0.8	0.9	1.5	0.3	0.2	0.5	0.5	0.6
14:0	0.1	0.1	0.5	0.7	0.4	0.7	0.1	0.2	0.3	0.8
15:0	0.6	0.4	0.5	0.6	1.2	0.2	0.3	—	0.3	0.5
16:0	10.2	12.7	11.0	18.3	13.6	4.7	8.5	9.3	12.0	6.9
17:0	0.6	0.3	0.7	0.4	1.3	—	0.3	—	0.5	0.3
18:0	3.4	4.7	6.1	7.2	5.6	1.8	1.6	3.0	4.1	5.0
20:0	0.4	2.0	0.6	1.1	1.0	0.4	0.2	0.3	0.4	1.3
TMSFA*	4.5	1.5	4.0	1.4	5.0	0.2	2.1	0.3	4.6	2.0
Saturated	20.5	23.0	24.2	30.6	29.6	8.3	13.3	13.6	22.7	17.4
13:1 (*n*-5)	0.7	0.1	0.5	0.1	1.5	—	0.7	—	0.5	—
15:1 (*n*-9)	0.6	0.2	0.4	0.1	0.8	—	0.7	—	0.4	0.1
16:1 (*n*-9)	3.2	2.6	5.9	3.9	2.5	1.1	1.1	0.4	3.8	2.3
16:1 (*n*-7)	1.8	1.1	2.5	1.2	1.7	0.6	1.5	0.3	2.0	0.6
17:1 (*n*-9)	0.6	0.2	0.6	0.1	0.6	—	0.7	—	0.6	0.1
18:1 (*n*-11)	0.8	1.3	1.4	3.2	1.3	0.3	0.9	0.1	0.1	0.4
18:1 (*n*-9)	6.1	10.3	10.7	14.3	13.4	10.2	3.2	0.5	10.0	8.4
18:1 (*n*-7)	0.8	1.0	1.0	0.2	0.4	2.2	0.9	0.2	0.9	0.3
6a-18:1	0.1	0.1	0.1	1.1	0.6	2.0	0.8	1.4	0.1	2.7
9a-18:1	0.1	0.1	0.2	1.2	2.5	9.4	1.6	7.0	0.6	5.8
12a-18:1	0.1	0.2	0.3	2.1	1.5	4.4	1.5	6.7	0.5	5.4
TMMFA†	3.8	1.8	4.0	0.9	3.9	0.2	10.4	0.5	6.2	0.3
Monoenoic	18.6	19.1	28.7	28.3	30.1	30.4	23.2	16.6	26.7	26.4
Acetylenic	0.3	0.4	0.6	4.4	4.6	15.8	3.9	15.1	1.2	13.9

*TMSFA, total minor saturated fatty acids: 13:0, 19:0, 21:0, 22:0, 23:0, 24:0, 25:0, 26:0, *i*12:0, *i*13:0, *i*14:0, *i*15:0, *i*16:0, *i*17:0, *ai*13:0, *ai*15:0, *ai*17:0, *ai*19:0, pristanic and phytanic.

†TMMFAs, total minor monoenoic fatty acids: 12:1 (*n*-5), 14:1 (*n*-5), 15:1 (*n*-7), *trans*-16:1 (*n*-13), *i*17:1 (*n*-9), *ai*17:1 (*n*-9), 17:1 (*n*-7), 19:1 (*n*-8), 20:1 (*n*-11), 20:1 (*n*-9), 22:1 (*n*-11), 22:1 (*n*-9), 21:1 (*n*-8), 24:1 (*n*-11), 24:1 (*n*-9), 25:1 (*n*-9), 26:1 (*n*-11), 26:1 (*n*-9).

TL = Total lipids; TAG = triacylglycerols.

identified in *F. antipyretica*. Low levels of polyenoic acetylenic acids (6.2%) were detected in *C. cordifolium* but this moss had a high content of arachidonic (all isomers) acid, (7.1%). The two liverworts (*R. fluitans* and *P. neesiana*) and the moss *F. antipyretica*, contained the highest amounts of acetylenic acids in TAG, *viz*., 80.2%, 57.3% and 63%, respectively (Table 2).

The results obtained from the quantitative estimation of lipids in the bryophyte species are given in Table 3; total lipids varied from 4.4–9.4% of the dry wt. Neutral lipids were the most abundant lipid fraction in mosses (44.9–57.3%). Glycolipids made up more than 30% of the total lipids in all species, with polar lipids varying from 9.4–28.1%.

Amongst the polar lipid classes, only five phospholipids were identified, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylserine (PS) and phosphatidylinositol (PI), together with two polar lipids, diacylglyceryltrimethylhomoserine (DGTS) and X. The results from analyses of the polar lipid fractions are shown in Table 3. DGTS was the major lipid in four species and it varied from 20.4% in *F. antipyretica* to 39.6% in *P. neesiana*.

DISCUSSION

The fatty acid composition of *C. cordifolium*, *D. lycopodioides* and *P. neesiana* is reported for the first time. The first identification of 9,12-octadecadien-6-ynoic and 11,14-eicosadien-8-ynoic acids in *F. antipyretica* was made 20 years ago by Anderson *et al.* [7]. Jamieson and Reid [8, 9] later reported the presence of four acetylenic acids in *F. antipyretica*, 6a,9-18:2, 6a,9,12-18:3, 6a,9,12,15-18:4 and 8a,11,14-20:3. Monoacetylenic acids, however, were not reported by these authors, but we have identified three in this moss, 6a-18:1, 9a-18:1 and 12a-18:1 (Table 2).

Kohn *et al.* [11] have reported interesting results on the fatty acid composition of acyl lipids from 17 liverwort species belonging to the genus *Riccia*. They found high 18:1A levels (varying from 12.5–61.7%) in the subgenus *Ricciella*, including *R. fluitans* (61.7%); in our study, we found 18% (all isomers) of this acid in the same species. These authors, however, did not report the presence of the six acetylenic acids, 6a-18:1, 9a-18:1, 12a-18:1, 8a,11,14-20:3, 6a,9,12,15-18:4 and 5a,8,11,14-20:4 in *R. fluitans*. We have detected 1.4, 7.0, 6.7, 6.3, 5.1 and

Table 2. Polyenoic and acetylenic acids of some aquatic bryophytes

	Mosses						Liverworts			
	Calliergon cordifolium		*Drepanocladus lycopodioides*		*Fontinalis antipyretica*		*Riccia fluitans*		*Pellia neesiana*	
Fatty acids	TL	TAG	TL	TAG	TL	TAG	TL	TAG	TL	TAG
18:2 (*n*-6)	8.5	9.8	9.0	5.6	7.7	1.3	9.1	0.6	6.9	3.1
18:2 (*n*-3)	0.8	0.1	0.9	—	—	—	1.2	0.2	0.9	2.3
20:2 (*n*-6)	0.5	0.7	0.5		—	—	1.0	0.1	0.7	—
22:2 (*n*-6)	4.2	0.5	1.0	—	9.6	—	1.4	—	1.3	—
16:3 (*n*-6)	0.6	0.2	0.5	0.3	—	—	0.6	0.1	0.9	0.1
16:3 (*n*-3)	1.0	0.6	0.5	0.2	—	—	1.7	0.3	0.9	0.2
18:3 (*n*-6)	10.9	11.2	2.9	1.4	3.8	5.0	5.6	1.2	4.4	1.3
18:3 (*n*-3)	14.7	19.7	11.0	16.0	9.0	6.1	6.2	2.2	9.3	3.9
20:3 (*n*-9)	0.3	0.1	0.7	0.1	—	—	1.5	—	1.0	0.1
20:3 (*n*-6)	0.5	0.3	0.6	—	—	—	1.6	—	1.0	0.2
22:3 (*n*-6)	1.7	—	2.0			—	1.5	—	1.1	—
22:3 (*n*-3)	0.4	—	0.6	—	—	—	1.4	—	0.7	—
16:4 (*n*-3)	1.1	1.4	1.0	0.2	—	—	2.3	—	1.4	—
18:4 (*n*-3)	3.2	5.0	2.2	0.6	—	—	3.1	—	2.6	—
20:4 (*n*-6)	7.0	0.5	6.5	0.4	—	—	2.1	0.1	5.3	1.0
20:4 (*n*-3)	0.1	—	0.5	—	0.4	1.1	1.8	0.1	1.0	0.2
22:4 (*n*-3)	1.8	—	0.7	—	—	—	2.1	—	1.2	—
20:5 (*n*-3)	2.4	1.1	2.6	1.3	—	—	2.3	0.1	3.4	0.4
6a,9-18:2	0.1	0.3	0.1	0.8	3.2	16.3	2.9	9.9	0.4	2.6
9,12a-18:2	0.1	0.6	0.2	1.2	2.5	9.0	1.1	4.7	0.4	4.4
9a,12-18:2	0.1	0.8	0.2	1.1	0.1	1.9	0.9	3.4	0.4	4.1
6a,9,12-18:3	0.1	0.3	1.2	6.3	0.1	2.2	5.3	24.2	3.1	14.1
8a,11,14-20:3	0.1	0.5	0.1	1.0	0.1	1.4	1.4	6.3	0.5	5.2
6a,9,12,15-18:4	0.3	3.3	0.6	3.1	3.1	15.2	1.0	5.1	1.4	9.2
5a,8,11,14-20:4	0.1	0.4	0.2	1.1	0.5	1.2	2.6	11.5	0.4	3.8
Polyenoic	60.9	57.9	47.1	41.1	39.9	61.3	63.5	69.8	50.6	56.2
Acetylenic	0.9	6.2	2.6	14.6	9.6	47.2	15.2	65.1	6.6	43.4
Total acetylenic fatty acids	1.2	6.6	3.2	19.0	14.2	63.0	19.1	80.2	7.8	57.3

Minor polyenoic fatty acids: 14:2 (*n*-5), 16:2 (*n*-4), 20:3 (*n*-3), 24:3 (*n*-3).
For abbreviations see Table 1.

11.5%, respectively, of these fatty acids in this species (Table 2). Kohn *et al.* [11] reported 3% of 6a,9,12–18:3 whereas we found 24.2% of this acid.

Styme and Beutelmann [16] discovered an uncommon pathway for acetylenic acid biosynthesis in the moss, *Ceratodon purpureus.* The authors concluded that 18:3A (6,9,12-octadecatrien-6-ynoic acid), the main fatty acid *Ceratodon* TAG was synthesized from 18:2 by two Δ6-desaturation steps, followed by Δ15-desaturation. Since 18:2A was found exclusively in cell TAG, the last desaturation step either takes place in the TAG molecule, or 18:2A has to be detached, desaturated and reincorporated into the TAG. These results present evidence that, contrary to general belief, plant TAG are not metabolically inert molecules during the cellular stage of TAG deposition.

The betaine lipids (DGTS and DGTA) represent a prominent group of polar lipids and have been detected in most cryptogamic plants [17–19] and other organism species [17]. However, DGTS has not yet been detected in seed plants (angiosperms or gymnosperms) [20]. It is interesting to note that organisms that accumulate high levels of DGTS do not contain or contain only very low PC levels. This reciprocity between DGTS and PC can be interpreted in terms that DGTS has been progressively replaced by PC during the evolution of green plants. The apparent reciprocity of DGTS and PC abundance among different lower plants and algae which has been described recently [18, 19], suggests that DGTS and PC are similar in their chemical and physical properties and are approximately interchangeable with each other in their roles within the cell.

DGTS has been reported previously in some bryophytes, including moss species [12–15]. Sato and Furuya [20] have studied DGTS distribution in nonvascular green plants and found this betaine lipid in two liverworts, *Bazzania yoshinagama* and *Marchantia paleacea*, and in three mosses, *Dicranum scoparium, Polytrichum formosum* and *Pleurozium schreberi*; DGTS varied from 7 to 36 nmol mg^{-1} total lipids. Recently, Dembitsky and Rezanka [21] have reported on the presence of DGTS and other phospholipids in 13 moss species.

Table 3. Lipid composition of some aquatic bryophytes

	Mosses			Liverworts	
	Calliergon cordifolium	*Drepanocladus lycopodioides*	*Fontinalis antipyretica*	*Riccia fluitans*	*Pellia neesiana*
TL	5.8	4.4	6.3	4.4	9.4
Lipid fractions					
Neutral	57.3	44.9	47.2	30.8	19.4
Glyco	10.1	18.2	19.4	9.4	28.1
Polar	32.6	36.9	33.4	59.7	52.5
Polar lipids					
DGTS	25.6 ± 2.4	26.8 ± 3.1	20.4 ± 2.2	34.3 ± 3.8	39.6 ± 4.0
PC	21.4 ± 0.8	23.6 ± 0.9	25.5 ± 1.1	21.9 ± 0.5	18.2 ± 0.4
PG	24.3 ± 0.5	23.7 ± 0.6	25.8 ± 0.7	17.3 ± 0.5	13.9 ± 0.5
PE	19.6 ± 0.6	15.6 ± 0.5	18.9 ± 0.3	10.6 ± 0.7	9.5 ± 0.4
PS	3.6 ± 0.3	4.8 ± 0.3	5.2 ± 0.2	10.3 ± 0.2	11.2 ± 0.8
PI	2.9 ± 0.2	3.6 ± 0.4	2.5 ± 0.4	4.1 ± 0.3	3.5 ± 0.2
X	2.6 ± 0.5	1.9 ± 0.7	1.7 ± 0.9	1.2 ± 0.6	4.1 ± 0.9

TL, total lipids, % of dry wt; fractions, as % of total lipids.
DGTS = diacylglycerotrimethylhomoserine; PC = phosphatidylcholine; PG = phosphatidylglycerol; PE = phosphatidylethanolamine; PS = phosphatidylserine; PI = phosphatidylinositol; X = unidentified polar lipid.

DGTS was the major polar lipid in eight species and it varied from 13.3% in *Pleurozium medium* to 38.3% in *Sphagnum nemoreum*. PC was the major polar lipid in five species; this varied from 7.5% in *Sphagnum squarrosum* to 57.5% in *Climacium dendroides*.

EXPERIMENTAL

Calliergon cordifolium (Hedw.) Kindb. was sampled in August 1992, from the Primorsky nature reserve near Lake Baikal. *Drepanocladus lycopodioides* (Brid.) Warnst. = *Pseudocalliergon lycopodioides* was collected in March 1991 from north-western Russia near St Petersburg. *Fontinalis antipyretica* Hewd. was collected in September 1991 from the Samarskaya Luka nature reserve park near Togliatti. *Riccia fluitans* (L.) A.Br. was collected in July 1992 from the Samarskaya Luka nature reserve park near Togliatti (Volga river basin). *Pellia neesiana* (Gott.) Limpr. was collected in September 1992 from the nature reserve park near Lake Issyk-Kul, Tian-Shan, Middle Asia, Kirgizstan Republic. Freshly collected bryophyte species were thoroughly cleansed of extrageneous matter and homogenized in a high-speed unit. Lipids were extracted immediately after collection.

Extraction of lipids, their analyses and quantification, and the identification of individual phospholipid classes were carried out as described elsewhere [13, 14]. DGTS was determined as described in ref. [22]. Qualitative determination of phospholipids, as well as DGTS, used the methods given in refs [12, 13]. A total of 3–5 replicate determinations was made for each sample. Fatty acids, as the corresponding trimethylsilyl-oxazoline derivatives, were prepd and analysed by capillary GC-MS as described in refs [12–14]. The values given in Tables 1–2 are the means of 3 expts.

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REFERENCES

1. Graham, L. E., Delwiche, C. F. and Mishler, B. D. (1991) *Adv. in Bryology* **4**, 213.
2. Dembitsky, V. M. (1993) *Prog. Lipid Res.* **32**, 281.
3. Sorensen, N. A. (1968) *Recent Advances in Phytochemistry*, **1**, 187.
4. Bohlmann, F., Burkhardt, T. and Zdero, C. (1973) In *Naturally Occurring Acetylenes*, Academic Press, New York.
5. Anderson, W. H., Hawkins, J. M., Gellerman, J. L. and Schlenk, H. (1974) *J. Hattori Bot. Lab.* **38**, 99.
6. Karunen, P. (1990) In *Bryophytes: Their Chemistry and Chemical Taxonomy* (Zinsmeister, H. D., and Mues, R., Eds). pp. 121–141. Clarendon Press, Oxford.
7. Anderson, W. H., Gellerman, J. L. and Schlenk, H. (1975) *Lipids* **10**, 501.
8. Jamieson, G. R. and Reid, E. H. (1976) *J. Chromatogr.* **128**, 193.
9. Jamieson, G. R. and Reid, E. H. (1976) *Phytochemistry* **15**, 1731.
10. Kohn, G., Vierengel, A., Vandekerkhove, O. and Hartmann, E. (1987) *Phytochemistry* **26**, 2101.

11. Kohn, G., Vandekerkhove, O., Hartmann, E., and Beutelmann, P. (1988) *Phytochemistry* **27**, 1049.
12. Dembitsky, V. M., Rezanka, T., Bychek, I. A. and Afonina, O. M. (1993) *Phytochemistry* **33**, 1009.
13. Dembitsky, V. M., Rezanka, T., Bychek, I. A. and Afonina, O. M. (1993) *Phytochemistry* **33**, 1021.
14. Dembitsky, V. M., Rezanka, T., Bychek, I. A. and Afonina, O. M. (1994) *J. Hattori Botan. Lab.* **75**, 161.
15. Dembitsky, V. M. and Rezanka, T. (1994) *Phytochemistry* **36**, 685.
16. Styme, S. and Beutelmann, P. (1994) In *11th International Meeting of Plant Lipids*, Paris. Abstract, L43.
17. Dembitsky, V. M., *Prog. Lipid Res.*, in press.
18. Eichenberger, W. (1993) *Plant Physiol. Biochem.* **31**, 213.
19. Sato, N. (1992) *Botan. Mag. Tokyo* **105**, 185.
20. Sato, N. and Furuya, M. (1984) *Phytochemistry* **23**, 1625.
21. Dembitsky, V. M. and Rezanka, T. (1995) *Biochem. Syst. Ecol.*, **23**, 71.
22. Kabara, J. J. and Chen, J. S. (1976) *Analit. Chem.* **48**, 814.