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ACETYLENIC FATTY ACIDS IN THE RICCIACEAE (HEPATICAE)

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Abstract—Twelve species of the liverwort genus *Riccia* were analysed for their fatty acid composition and in all species acetylenic fatty acids were detected In the species of the subgenus *Ricciella*, 9-octadecen-6-ynoic acid, and in the subgenus *Euriccia*, 9,12,15-octadeatrien-6-ynoic acid were the most abundant components. 9,12-octadecadien-6-ynoic acid was present in low amounts in both subgenera. In other genera, for example, *Marchantia*, *Corsinia*, *Oximitra* and *Ricciocarpos*, which are related to *Riccia*, no acetylenic fatty acids could be detected.

INTRODUCTION

More than 600 acetylenic compounds are known from higher plants, fungi and microorganisms [1]. The distribution of these compounds has, to a certain extent, some chemotaxonomic relevance [2]. There is evidence that this is also true for the acetylenes of bryophytes which have been identified exclusively as long chain fatty acids with a characteristic arrangement of triple and double bonds. This arrangement has not been found in acetylenes from other natural sources [3, 4] The fatty acids 9,12-octadecadien-6-ynoic acid (18:2A), 9,12,15-octadecatrien-6-ynoic acid (18 3A) and 11,13-eicosadien-8-ynoic (20 2A) acid have been isolated and identified from different mosses [5–7]. 9-octadecen-6-ynoic acid (18:1A) which was assumed to occur in the moss *Fontinalis antipyretica* Hedw. [8] has recently been isolated and identified from the liverwort *Riccia fluitans* (L.) A Br. [9]

The purpose of the present study is to analyse a number of species of the Ricciaceae and related families in order to find out whether the distribution of acetylenic fatty acids can provide any useful chemotaxonomic information on this group of liverworts

RESULTS AND DISCUSSION

Twelve species of the liverwort genus *Riccia* were examined for their fatty acid composition and all of them were found to contain up to three different acetylenic fatty acids in various amounts. All species showed one of two typical patterns of acetylenic fatty acids which clearly correspond with the two subgenera *Ricciella* and *Euricca* into which the genus is subdivided [10, 11]. In the subgenus *Riccciella* 18 1A is the major acetylenic compound 18 2A is also present in all species examined, but in amounts less than 5 mol% 18 3A was detected only in two species in similar low amounts In three of the four species analysed 18·1A was also the principal fatty acid. Only in *Riccia huebeneriana* Lindenb was the amount of 18·1A exceeded by that of 18:0 and 18 1 (Table 1)

The distribution of the fatty acids of the main lipid classes in *Riccia duplex* Lorbeer is shown in Table 2; the patterns of the fatty acids of the other members of the subgenus are very similar Acetylenic fatty acids were found not only in the triacylglycerols, as occurs in mosses [4, 5, 12, 13], but also in considerable amounts in the glycolipids, particularly in the monogalactosyl diglycerides The occurrence of an unusual fatty acid which is found in particularly high amounts in the phosphoditylcholine fraction is also remarkable The compound has been tentatively identified as 18 2ω9 Its GC R_t is slightly shorter than that of 18·2ω6. Catalytic hydrogenation of the compound yielded 18.0 GC-MS analysis revealed a $[M]^+$ of m/z 294 and a mass spectrum almost identical to that of linoleic acid Because of the structural relationship, this compound is likely to be connected with the presence of the acetylenic acid 18 1A

In the subgenus *Euriccia* the major acetylenic fatty acid is 18 3A and in five of the eight species examined, it was the most abundant compound (Table 1). 18·2A is present in amounts very similar to that of the subgenus *Ricciella*, whereas 18 1A is found in very low amounts or was absent.

The family Ricciaceae consists of two genera, *Ricciocarpos* and *Riccia* The genus *Ricciocarpos* is monotypic with *Ricciocarpos natans* (L.) Corda which is free of acetylenic fatty acids The fatty acid pattern of *Ricciocarpos* is very similar to that of the Marchantiaceae, with 18:3ω3 being the principal component with large amounts of 20:5ω3 and 20 4ω6 [14], (Table 1) The flavonoid pattern as well as the occurrence of oil bodies suggest a close relationship of *Ricciocarpos* to *Marchantia* [15]. The fatty acid pattern resembles that of *Oxymitra paleacea* Bischoff, representing the family Oxymitraceae which, together with the Ricciaceae, forms the suborder Ricciinae Also *Corsinia coriandrina* (Spreng) Lindb of the Corsiniaceae, a family which is supposed to be closely related with the Ricciaceae [16] was found to be free of acetylenic fatty acids.

Our results indicate that the occurrence of acetylenic fatty acids is a genus-specific character of *Riccia*, which distinguishes the genus not only from related genera and families of the order Marchantiales, but also from most

*Deceased on 10 January 1986

Table 1. Distribution of the major fatty acids in genus *Riccia* and in related genera (the values represent mol% of the total fatty acid content)

Species	Fatty acid 16:0	18:0	18:1ω9	18:2ω6	18:3ω3	20:4ω6	20:5ω3	18:1A	18:2A	18:3A
Monocleales										
Monocleaceae										
Monoclea forsteri Hook.	9.3	6.5	6.2	5.1	9.0	4.9	3.7	57.4	0.5	0.9
Marchantiales										
Marchantiaceae										
Marchantia polymorpha L. emend. Burgeff	23.1	6.4	4.9	3.8	27.3	4.6	11.0			
Corsiniaceae										
Corsinia coriandrina (Spreng.) Lindb.	21.8	6.7	8.2	13.0	10.3	7.1	3.2			
Oxymitraceae										
Oxymitra paleacea Bischoff	30.1	4.1	3.8	11.2	19.1	2.9	0.8			
Ricciaceae										
Ricciocarpos natans (L.) Corda	26.4	8.8	15.5	14.5	16.5	7.6	6.3			
Subgenus *Ricciella*										
Riccia huebeneriana Lindenb.	11.6	5.3	20.6	29.9	2.8	1.7	3.9	12.5	4.2	1.2
Riccia fluitans (L.) A. Br.	7.6	1.8	10.2	5.1	3.4	0.8	0.9	61.7	2.6	3.0
Riccia duplex Lorbeer	10.9	1.4	8.4	5.1	4.0	0.8	1.7	59.9	1.8	
Riccia canaliculata Hoffm.	14.2	1.6	10.2	10.8	9.1	5.2	2.1	35.6	1.9	
Subgenus *Euriccia*										
Riccia bifurca Hoffm.	28.6	9.3	11.8	6.2	5.2		4.4	1.1	3.6	23.5
Riccia ciliifera Link	15.1	5.9	16.3	7.3	7.0		5.0		5.4	27.9
Riccia glauca L.	19.9	7.1	9.6	3.5	7.6		9.0	0.8	4.7	31.8
Riccia sorocarpa Bisch.	26.2	5.0	7.4	6.1	21.4	2.5	12.5	0.3	1.4	10.6
Riccia warnstorfii Limpr.	32.3	11.5	11.8	7.7	7.0	1.0	4.9	1.2	4.9	10.2
Riccia michelii Raddi	20.9	6.3	14.1	8.6	5.0		4.0		2.4	31.7
Riccia papillosa Morris.	17.5	4.4	5.7	5.6	5.3	0.5	1.7	0.5	3.6	49.2
Riccia zachariae Lorbeer	17.6	6.1	18.3	7.5	7.7	0.5	4.8	5.2	2.8	25.1

Table 2. Fatty acid pattern of the major acyl lipids of *Riccia duplex* (the values are mol% of the total fatty acid content of the respective lipid fraction)

Fatty acid	TG	MDGD	DGDG	SL	PG	PI	PE	PC
16:0	14.8	8.8	37.6	51.5	35.7	52.4	26.7	39.4
16:1	2.1	0.7	1.1	5.7	10.5	1.6	1.4	0.7
18:0	3.6	3.5	3.7	5.6	8.9	3.2	2.1	1.3
18:1ω9	7.9	1.8	5.5	11.3	12.4	10.5	8.0	10.6
16:3ω3		16.2	2.7					
18:2ω9	0.7	0.9	0.6	2.6	1.7	6.0	2.7	16.3
18:2ω6	1.5	1.1	3.7	9.7	10.6	9.1	5.4	4.4
18:3ω6	0.5	0.5	0.7	-	0.5	1.4	1.4	2.0
18:3ω3	1.2	22.2	37.0	14.2	10.3	8.7	3.8	2.9
20:3ω6	-	-	-	-	2.0		7.5	1.3
20:4ω6	0.4	0.7	1.6		3.2	2.5	13.4	6.3
20:5ω3	0.7	1.9	1.7	-	4.3	4.6	23.4	12.5
18:1A	57.6	19.6	4.1					
18:2A	3.3	2.5		--				
18:3A	4.5	18.7	-	–	-			
nmol FA/mg DW	22.0	20.3	9.9	2.3	5.9	3.7	6.2	14.8

TG triacylglycarols, MDGD monogalactosyl diglycarides, DGDG digalactosyl diglycarides, SL sulphoquinovosyl diglycaride, PG phosphatidyl glycasol, PI phosphaditytl inositol, PE phosphatidyl ethanolamine PC phosphatidyl choline

Hepaticae. Among 70 species from 27 families out of five orders which were analysed, we found, besides the *Ricciae*, only one species, namely *Monoclea forsteri* Hook., in which acetylenic fatty acids were present, with 18:1A being the major component.

In mosses acetylenic fatty acids are found more frequently than in liverworts, but they are also restricted to certain families or genera, e.g. Ditrichaceae, Dicranaceae, *Bryum* and *Fontinalis* [4, 6, 7] and they obviously possess chemotaxonomic significance.

Since the position of the triple bond relative to the double bonds (6-ynoic in C^{18} and 8-ynoic in C^{20} acids) is the same in all acetylenic acids identified in bryophytes, it can be assumed that all these acids are synthesized by the same desaturation system. The availability of different substrates possibly regulates the resulting composition of the acetylenic acid pattern. Thus, the different patterns by which the subgenera *Ricciella* and *Euriccia* are readily distinguished, might be determined by varying concentrations of the possible precursors 18 1, 18:2 and 18·3. *Ricciella* species prefer moist or aquatic habitats whereas *Euriccia* species are more adapted to a dry environment [10] Thus, there, appears to be a correlation between habitat requirements and fatty acid pattern, but it cannot be decided whether the difference in adaptation is the reason for or the result of the difference in fatty acid composition

It seems unlikely that a highly specific triple bond-generating system developed twice independently during evolution in related taxa The enzyme systems responsible for the synthesis of acetylenic fatty acids in both mosses and liverworts can therefore be regarded as homologous and to be controlled by genes which must have already been present in a common ancestor of mosses and liverworts. This could also support the hypothesis that those taxa which are characterized by acetylenic fatty acids, have not evolved too far from such a common ancestor. The Monocleales, for example, are in several respects regarded as primitive members of the Hepaticae, from which the Marchantiales with the Ricciaceae probably have derived [16] For *Fontinalis*, an acetylenic fatty acid-containing moss genus, it is known that the segmentation pattern of the apical cell is exactly like that in Hepatics [17] Also the branching modalities and the leaf arrangement show obvious similarities with liverwort species [16, 18] These observations indicate relatively little divergence from a common ancestor The order Dicranales which is also characterized by acetylenic fatty acids is also regarded to be a less advanced taxon of the mosses [19–21]

Our results indicate that the study of the fatty acid composition, particularly the search for acetylenic fatty acids, can provide new information on taxonomy and phylogeny of bryophytes. Further research in this field is necessary and should be promising

EXPERIMENTAL

Plant material Ricciocarpos natans, Oxymitra paleacea, Riccia huebeneriana, R bifurca, R ciliifera, R. glauca, R sorocarpa, R warnstorfii, R michelii, R papillosa, R zachariae and *Corsinia coriandrina* were obtained from the 'Algarum, Hepaticarum Muscorumque in Cullturis Collectio' in Prague, Czechoslovakia *Riccia duplex* was supplied by Dr W Schier (University of Wurzburg, F.R G), and *Riccia fluitans* and *R canaliculata* from Prof H D Zinsmeister (University of Saarbrucken, F R G) *Marchantia polymorpha* and *Monoclea forsteri* are kept in the botanical garden of the University of Mainz The nomenclature is used according to refs [10] and [11]

The liverworts, except for *Marchantia* and *Monoclea* which were kept on soil in a greenhouse, were grown at a constant temp of 20° under white light (2000 lux, Osram TL 40 W/25) in a 16 hr light, 8 hr dark illumination regime A liquid or agar medium in penicillin flasks was used, containing 82 µmol/l $CaCl_2$ $2H_2O$, 789 µmol/l KNO_3, 1 mmol/l KH_2PO_4, 46 µmol/l $MgSO_4$ $7H_2O$, 7 µmol/l $FeCl_3$ and 10 mmol/l glucose The pH was adjusted to 5 8

Lipid analysis Extn and sepn was carried out according to ref [22] GC analysis and identification of the various fatty acids have been described previously [4, 9, 22]. The data shown are the mean values of at least three dets

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