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LIPIDS OF *FONTINALIS ANTIPYRETICA*

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Key Word Index—*Fontinalis antipyretica*; moss; fatty acids; ketones; galactosyl diglycerides.

Abstract—Fatty acid compositions of the lipids of *Fontinalis antipyretica* shows the presence of octadeca-9, 12-dien-6-ynoic, octadeca-9,12,15-trien-6-ynoic and eicosa-11,14-dien-8-ynoic acids as the major components of the triglycerides. These acetylenic acids were not present in the galactosyl diglycerides. The moss lipids also contained relatively large amounts of two novel acetylenic methyl ketones with an unsaturation pattern analogous to that of the C_{18} acetylenic acids.

INTRODUCTION

Although several hundred natural acetylenes have been found in higher plants and fungi [1] only a few acetylenic long-chain fatty acids have been isolated, notably those derived from octadeca-9-ynoic (stearolic) acid from the seed oils of the Santalaceae and Olacaceae [2]. Octadeca-6-ynoic (tariric) acid and a few of its derivatives have been found in *Alvarado amorphoides* [3]. Recently Åndersson *et al.* [4] isolated octadeca-9,12,15-triene-6-ynoic acid from several mosses and showed that this acid was present only in the triglyceride fraction. Jamieson [5] reported the presence of octadeca-9,12-dien-6-ynoic acid in the moss, *Fontinalis antipyretica*, and Anderson, Gellerman and Schlenk [6] found this acid and its C20 homologue, eicosa-11,14-dien-8-ynoic acid in the same moss species.

In the present work the distribution of C_{18} and C_{20} acetylenic acids is investigated and an examination is made of the ketone fraction which comprises the major portion of the unsaponifiables of this moss species.

RESULTS AND DISCUSSION

Fatty acids

The fatty acid compositions of three samples of *F. antipyretica* are shown in Table 1. Although the compositions from the samples are the same qualitatively, there are considerable quantitative differences but very little variations in the fatty acid compositions in any one series. In both winter series moss was submerged in the fast-flowing stream, but in the summer of 1975 due to a very small rainfall in the area the stream almost dried up. The rocks to which the moss was attached were out of the water and only small amounts of moss was growing.

The total proportions of acetylenic acids in the winter series 1974–75, 1975–76, and the summer 1975 series were 44.1, 30.4 and 10.2% respectively and the ratios of C_{18} to C_{20} acetylenic acids were 3.3, 5.8 and 0.9 respectively. The major acetylenic acid in both winter series was octadeca-9, 12-dien-6-ynoic acid (32.0 and 24.1% respectively), but the amount of this acid was only 4% in the summer series. In our samples we found small amounts (0.8–2.0%) of octadeca-9,12,15-trien-6-ynoic acid. Anderson *et al.* [6] found 13% of this acid in a sample of *F. antipyretica* and up to 25% in other moss species. The proportions of the C_{20}, eicosa-11, 14-dien-8-ynoic acid in our samples varied from 4.5 to 10.1%.

The relative proportions of fatty acids, excluding the acetylenic acids were similar to those found by Nichols [7] in the moss, *Hypnum cupressiforme*. In the *F. antipyretica* samples the two winter series had total amounts of (16:3 ω-3 + 18:3 ω-3) of 24.5 and 42.0% respectively, and 17.6% in the summer series. The corresponding values for 18:2 ω-6 are 26.3, 15.1 and 27.0%. It has been shown [8–10] that, during the growing season of certain green plants, there are large variations in the relative proportions of these acids.

The fatty acid compositions of different lipid classes of *F. antipyretica* are shown in Table 2. As in other green plants [8–13] the (ω-3) acids are concentrated in the galactosyl diglycerides with the greatest concentration of 16:3 ω-3 in the MGDG fraction. There is an almost equal distribution of 20:5 ω-3 among the galactosyl diglycerides and the 'more polar lipids'. The distribution of this acid is similar to that found in some marine algae [12] and ferns [10]. Although the greatest concentrations of ω-6 acids were found in the 'more polar lipids' there were relatively high proportions in the galactosyl diglycerides. A similar distribution of ω-6 acids has been found in the lipids of some marine algae. It might be expected that 16:2 ω-6 would also be concentrated in the 'more polar lipids' but the greatest proportion of this acid was found in the MGDG fraction. Similar results were obtained for fern samples [10,14] and for a moss sample [7]. Similarly 16:3 ω-6 was found in greatest concentration in the MGDG fraction. This acid has also been found with a similar distribution in the lipids of a fresh-water [13] and marine algae [12]. The unusual *trans* acid, 16:1 ω-13*t*, was found only in the 'more polar lipids' and in this respect the moss lipids are similar to those of lipids from other green plants.

Acetylenic-acids were not detected in the galactosyl diglycerides but small amounts of the three acetylenic

Table 1. Fatty acid composition of *F. antipyretica* total lipids (% by weight)

	% by weight †	 ‡	 §	 †‖	 ‡‖	 §‖
Saturated and monoenoic acids						
12:0	tr	tr	tr	0.1	tr	tr
14:0	0.2	0.2	0.3	0.4	0.2	0.3
14:1	tr	0.1	0.1	tr	0.2	0.1
15:0	0.1	0.2	0.2	0.2	0.3	0.2
16:0	5.4	6.6	7.4	9.7	9.5	8.2
16:1 ω-7 + ω-9	2.1	1.5	1.9	3.8	2.2	2.1
16:1 ω-13t	0.4	0.4	0.8	0.7	0.7	0.9
18:0	0.6	0.6	1.3	1.0	0.9	1.4
18:1 ω-9*	2.3	1.6	3.7	4.1	2.3	4.1
20:0 + 20:1	1.4	1.5	4.7	2.5	2.2	5.2
22:0 + 20:1	0.8	1.0	1.6	1.4	1.4	1.8
24:0 + 20:1	0.9	0.8	1.0	1.6	1.1	1.1
	14.2	14.5	23.0	25.5	21.2	25.4
Dienoic acids						
16: ω-6	0.5	1.0	1.2	0.9	1.4	1.3
18:2 ω-6	14.7	10.5	24.2	26.3	15.1	27.0
20:2 ω-9	0.2	0.1	0.1	0.3	0.1	0.1
20:2 ω-6	1.1	0.6	1.8	2.0	0.9	2.0
	16.5	12.2	27.3	29.5	17.5	30.4
Trienoic acids						
16:3 ω-3	2.7	7.0	2.0	4.8	10.0	2.2
18:3 ω-6	3.5	4.6	5.6	6.3	6.6	6.2
18:3 ω-3	11.1	22.5	14.5	19.9	32.0	16.4
20:3 ω-6	2.2	1.6	4.2	3.9	2.3	4.7
	19.5	35.7	26.3	34.9	50.9	29.5
Tetraenoic acids						
18:4 ω-3	0.6	0.4	1.5	1.0	0.6	1.7
20:4 ω-6	2.3	3.1	3.3	4.1	4.5	3.7
20:4 ω-3	0.3	0.3	0.1	0.5	0.4	0.1
	3.2	3.8	4.9	5.6	5.5	5.5
Pentaenoic acids						
20:5 ω-3	2.5	3.4	8.3	4.5	4.9	9.2
Acetylenic acids						
18:3 6a,9,12	32.0	24.1	4.0	—	—	—
18:4 6a,9,12,15	2.0	1.8	0.8	—	—	—
20:3 8a,11,14	10.1	4.5	5.4	—	—	—
	44.1	30.4	10.2			

tr—trace; * other isomers present; † mean of four samples, Nov. 74—May 75; ‡ mean of four samples, Nov. 75–Feb. 76; § mean of three samples, June 75—Sept. 76; ‖ relative proportions excluding acetylenic acids.

acids were found in the 'more polar lipids'. The acetylenic acids accounted for 62.6% of the acids from the triglyceride fraction, the C_{18}:C_{20} ratio being 3.2:1. Anderson, Gellerman and Schlenk [6] found 75% acetylenic acids in the triglycerides of *F. antipyretica* and a C_{18}:C_{20} ratio of 0.9:1.

Methyl ketones

TLC examination of the unsaponifiable material of the *F. antipyretica* total lipids showed, as the major component, a band with a higher R_f value than the alcohols and sterols which were also present. This major component gave a positive test for a carbonyl group with 2,4-DNPH spray reagent, and charred very rapidly after spraying with ammonium bisulphate and heating to 150°. This rapid charring was similar to that shown by the acetylenic Me esters. The ketone fraction was separated by preparative TLC and the isolated components gave an IR spectrum which indicated an unsaturated acyclic Me ketone. IR and UV spectra showed that there was no conjugation or allenic double bonds and no *trans* configuration was detected. The ketone fraction on hydrogenation gave nonadecan-2-one.

When the ketone fraction was examined by GLC, a number of peaks were obtained and two of these peaks accounted for 86.5 and 6.7% respectively of the total peak area. The GLC characteristics of these components were not altered after treatment with AcOCl. The ketone fraction was oxidised with I_2–NaOH and the carboxylic acids obtained were converted to their Me esters. GLC of these Me esters gave two peaks which accounted for 87.0 and 6.3% respectively of the total peak area and the GLC characteristics of these Me esters were identical with those of 18:3 6a, 9,12 and 18:4 6a, 9,12,15 Me esters respectively.

Table 2. Fatty acid composition of different lipid classes (% by weight)

	 TG	% by weight MGDG	 DGDG	'more polar lipids'
Saturated and monoenoic acids				
12:0	0.3	tr	tr	0.1
14:0	0.5	0.1	0.6	0.5
14:1	0.2	tr	tr	0.1
15:0	0.2	tr	0.1	0.2
16:0	14.5	2.2	5.0	24.1
16:1 ω-7 + ω-9	1.7	3.0	2.3	4.8
16:1 ω-13t	—	—	—	2.1
18:0	2.9	0.2	0.5	1.8
18:1 ω-9*	5.6	1.3	1.8	3.2
20:0 + 20:1	1.0	0.1	0.1	0.2
22:0 + 22:1	0.9	0.4	0.3	0.2
24:0 + 24:1	0.8	0.2	0.2	0.4
	28.6	7.5	10.9	37.7
Dienoic acids				
16:2 ω-6	tr	4.0	0.9	0.4
18:2 ω-6	tr	11.6	15.8	21.8
20:2 ω-9	tr	tr	0.1	tr
20:2 ω-6	0.3	0.3	0.5	1.0
	0.3	15.9	17.3	23.2
Trienoic acids				
16:3 ω-6	tr	1.9	0.1	0.1
16:3 ω-3	tr	18.9	3.0	tr
18:3 ω-6	0.5	3.8	3.8	8.0
18:3 ω-3	0.3	44.1	52.7	13.8
20:3 ω-6	0.3	1.7	4.4	4.8
	1.1	70.4	64.0	26.7
Tetraenoic acids				
18:4 ω-3	0.1	1.6	0.8	1.2
20:4 ω-6	0.3	1.9	3.4	4.2
20:4 ω-3	tr	0.5	1.0	0.8
	0.4	4.0	5.2	6.2
Pentaenoic acids				
20:5 ω-3	tr	2.2	2.6	2.8
Acetylenic acids				
18:3 6a,9,12	51.7	—	—	1.9
18:4 6a,9,12,15	2.8	—	—	0.2
20:3 8a,11,14	15.1	—	—	1.4
	69.6	—	—	3.5
Total ω-3 acids	3.2	67.3	60.1	18.8
Total ω-6 acids	68.2	25.2	28.9	43.6

* Other isomers present; TG—triglycerides; MGDG monogalactosyl diglyceride; DGDG—digalactosyl diglyceride.

Table 3. Amounts of certain lipid constituents in air dried *F. antipyretica*

Constituent	mg/g air dried moss
Total lipids	71.4
Linoleic acid	1.3
Acetylenic acids	
18:3 6a,9,12	2.9
18:4 6a,9,12,15	0.2
20:3 8a,11,14	0.9
Acetylenic ketones	
C_{19} dien-yne	2.6
C_{19} trien-yne	0.2

These results indicate that the two major components of the ketone fraction are nonadeca-10,13-dien-7-yn-2-one and nonadeca-10,13,16-trien-7-yn-2-one, the Me ketones corresponding to the two C_{18} acetylenic acids found in the moss. The two Me ketones could also be obtained by GLC of the total moss lipids and, using an internal standard, it was possible to determine the amounts present in the moss (Table 3).

There is no previous record of the occurrence, in natural products, of unsaturated Me ketones of this C chain length and in such large amounts although Me ketones of short or moderate chain length are widespread in nature [15]. A number of Me ketones with C chain lengths of 17–37 have been found in peat and soil waxes [16,17] and long chain ketones (e.g. C_{29}), with the functional group near the middle of the molecule, occur in the cuticular wax of many plants [18]. Both saturated and monoenoic Me ketones C_{21}–C_{35} have been found in snake skin lipids [15]. In studies with microorganisms it has been established that Me ketones may be formed from fatty acids by β-oxidation followed by decarboxylation [20], but, in this study, there is no indication if this mechanism operates or, if it does operate, why the acetylenic acids favour this mechanism.

Chromatographic properties of the acetylenic compounds

TLC on Si gel with hexane–Et_2O as solvent gave R_f values of 0.56, 0.56, 0.45 for Me oleate, groundnut oil Me esters and acetylenic Me esters respectively. The separation factor for olefinic–acetylenic Me esters was 1.24 which compares with 1.25 obtained by Andersson *et al* using 18:4 6a, 9,12,15 [4]. When preparative-TLC was used to separate the acetylenic esters the acetylenic ester band contained 18:3 6a, 9,12 67.6%, 18:4 6a, 9,12,15 4.0% and 20:3 8a, 11,14 16.7%. After a second preparative separation the corresponding values were 76.3%, 4.5% and 16.7%. It is interesting to note that the main contaminants of the acetylenic esters after one preparative separation were 16:3, 18:3, 18:4, 20:4 and 20:5. This shows that the olefinic Me esters with the greatest number of olefinic bonds are found in the lower portion of the saturated/olefinic Me ester band.

TLC of the ketone fraction gave a band with R_f 0.44 which on hydrogenation gave R_f 0.55. This gives a separation factor of 1.26 similar to that of the corresponding Me esters.

Equivalent chain length (ECL) values were obtained for the moss acetylenic Me esters on polyester phases of different polarities. The variation of these ECL values with stationary phase polarity can be represented by

$$\mathrm{ECL}_X = A \times \mathrm{ECL}_{18:3\,n-3} - B \text{ [5,20].}$$

where

$X = 18:3\ 6a,9,12,15\ A = 1.51,\ B = -8.58;$

$X = 18:4\ 6a,9,12,15\ A = 1.75,\ B = -12.49;$

$X = 20:3\ 8a,11,14\ A = 1.50,\ B = -6.53.$

The mean deviations of calculated ECL values and experimental values (20 samples) are 0.04, 0.05 and 0.05 ECL units respectively.

The 'A' values of 1.51 and 1.50 for the triunsaturated Me esters can be compared with the mean value of 1.50 for Me esters with 5 methylene-interrupted olefinic bonds, and the 'A' value of 1.75 with the value of 1.78 for Me esters with 6 methylene-interrupted olefinic bonds. This indicates that the effect of an acetylenic bond on GLC retention characteristics of long-chain Me esters is similar to that of 3 olefinic bonds [21].

'A' values of 1.54 and 1.79 were obtained for the triunsaturated and tetraunsaturated Me ketones respectively but no further comparisons can be made since there is little published data on the GLC characteristics of long-chain Me ketones.

EXPERIMENTAL

Samples of *F. antipyretica* were collected from a stream at Rowbank Reservoir, Renfrewshire. Lipids were extracted and separated into classes by methods described previously [9–13]. GLC analysis of the total lipid Me esters and the Me esters from each of the lipid classes were carried out on a FID chromatograph using WCOT columns of different polarity [5,20]. TLC was carried out on 0.25 mm and 0.75 mm layers of Si gel in glass tanks lined with filter paper and hexane–Et_2O 8:2 as solvent. The ketone band obtained by preparative-TLC of the unsaponifiable matter from the total lipids gave an IR spectrum with main bands 3005, 2960, 2930, 2860, 1720, 1359, 1171 cm^{-1} indicating an acyclic unsaturated Me ketone. The ketone bond on hydrogenation over Pt gave a white solid which on recrystallization from aq EtOH gave white crystals mp 53.5–54° (lit 55°); IR 2960, 2930, 2860, 1720, 1359, 1171 cm^{-1}; PMR (60 MHz) 0.90 3H; 1.27 30H; 2.12 3H; 2.40 2H; I_2–NaOH gave stearic acid. These results indicate that the hydrogenated product was nonadecan-2-one. The unsaturated ketone in dioxane was heated to 50° with I_2–NaOH and the acid products of oxidation were converted to the Me esters. GLC analysis of the Me esters on columns of different polarity indicated that they were identical with the C_{18} acetylenic Me esters from the moss. The amounts of ketones present in a known wt of moss which had been air-dried for 18 hr were determined by adding a known amount of Me eicosanoate acid then direct GLC analysis.

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