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9-OCTADECEN-6-YNOIC ACID FROM *RICCIA FLUITANS*

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Key Word Index—*Riccia fluitans*; Ricciaceae; Hepaticae; acetylenic fatty acid.

Abstract—A new acetylenic fatty acid, 9-octadecen-6-ynoic acid, and the known compounds 9,12-octadecadien-6-ynoic acid and 9,12,15-octadecatrien-6-ynoic acid were isolated from the thalli of *Riccia fluitans*. The structure of the new compound was established by spectroscopic methods.

INTRODUCTION

In addition to the common C_{16} and C_{18} fatty acids, bryophytes contain large amounts of long-chain polyunsaturated fatty acids, particularly arachidonic and eicosapentaenoic acids [1–3]. Furthermore, a few musci are characterized by the presence of acetylenic fatty acids, especially in their triglyceride fraction: e.g. 9,12,15-octadecatrien-6-ynoic acid [4], 9,12-octadecadien-6-ynoic acid [5, 6] and 11,14-eicosadien-8-ynoic acid [5, 7]. To our knowledge no acetylenic fatty acid has been reported from the liverworts. This paper describes the isolation and elucidation of the structure of the major triglyceride component, a new acetylenic fatty acid, 9-octadecen-6-ynoic acid, from the thalli of the liverwort *Riccia fluitans* L.

RESULTS AND DISCUSSION

The triglyceride fraction of the total lipid extract from *R. fluitans* yielded three acetylenic fatty acids: 9,12,15-octadecatrien-6-ynoic acid, 9,12-octadecadien-6-ynoic acid and 9-octadecen-6-ynoic acid.

The hydrogenation product of the unknown fatty acid methylester was stearic acid methylester. The IR spectrum indicated a large CH_2 stretch at 2910 (alkyl chain), a nonterminal acetylenic group at 2310, C=O at 1740 (ester) and O–Me at 2840 cm^{-1}. The mass spectrum exhibited a molecular ion with *m/z* 292 and in addition an intensive peak at *m/z* 91 which is a characteristic fragment of acetylenic fatty acids.

The structure of 9-octadecen-6-ynoic acid methyl ester followed from high field ^1H NMR and ^{13}C NMR data. The ^{13}C NMR spectrum showed the presence of 19 carbons which were assigned by single frequency decoupling of protons and comparison with known chemical shifts (carbonyl, olefin, acetylene) and substituent effects (Table 1). The stereochemistry at the double bond and the position of the functional groups were deduced from ^1H NMR spin decoupling experiments, which further allowed the assignment of all hydrogens. *Z*-configuration of the olefinic protons was indicated by irradiation of hydrogens H-8 and H-11 (δ2.87 and 2.0) resulting in partial collapse of the vinylic multiplet (H-9, H-10 at δ5.4) to an ABX_2 pattern (H-9, δ5.36; H-10, δ5.44, J_{9-10} = 11.6 Hz). The triple bond was determined as being between C-6 and C-7 by long range coupling of H-5 (δ2.16) and H-8 (δ2.87). Further analysis of decoupling experiments allowed assignment of the residual chain protons (H-12, H-17). The presence of the ester methyl group was established by a singlet at δ3.65.

These results were compared with the ^1H and ^{13}C NMR spectroscopical data of 9,12,15-octadecatrien-6-ynoic acid, which differed only in the region of the two additional, methylene-interrupted double bonds (C-11 to C-17).

Table 1. NMR spectral data of 9-octadecen-6-ynoic acid

C	^1H NMR		^{13}C NMR
Me at C-1	3.65 *s*	3H	51.4
1	—		173.9
2	2.31 *t* (7.5)	2H	33.7
3	1.70 *m*	2H	24.2
4	1.49 *m*	2H	28.5
5	2.16 *tt* (2.5, 7)	2H	18.5
6	—		79.3
7	—		79.0†
8	2.87 *m*	2H	17.2
9	5.36 *m dd* (11.6)	1H	131.4†
10	5.44 *m*	1H	125.0†
11	2.00 *q* (6.7)	2H	27.2
12	1.24 *m*		29.3*
13	1.24 *m*		29.5
14	1.24 *m*	12H	29.4
15	1.24 *m*		29.3*
16	1.24 *m*		31.9
17	1.24 *m*		22.7
18	0.86 *t* (6.8)	3H	14.0

*Signals not evident.
†Indicates assignments may be reversed.
J (Hz) in parentheses.

†Deceased on January 10, 1986.

EXPERIMENTAL

General procedures. GC/MS: 70 eV. ^1H and ^{13}C NMR: 400 MHz, $CDCl_3$, TMS as internal standard.

Plant material. Thalli of *R. fluitans* received from and identified by Dr. H. D. Zinsmeister (Saarbrücken), were kept in axenic culture in our laboratory.

Culture. The floating thalli were grown for 21 days on 300 ml of a modified Bennecke-Medium in 1000 ml Fernbach flasks at 20°, under 2000 lx white light (Osram TL 40W/25 fluorescent tubes), in a 16 hr light–8 hr dark regime. The medium contained 82 μmol $CaCl_2 \cdot 2H_2O$, 789 μmol KNO_3, 1 mmol KH_2PO_4, 46 μmol $MgSO_4 \cdot 7H_2O$, 7 μmol $FeCl_3$ and 10 mmol glucose per 1; pH 5.8.

Extraction and isolation. Lipid extraction, separation and identification were performed as described previously [2]. Triglycerides were transesterified with 5% H_2SO_4 in MeOH for 4 hr at 80 and the resulting fatty acid methyl esters were analysed by capillary GLC [see 2]. The methyl esters of suspected fatty acids were isolated by preparative GLC with a split ratio of 1:25 between FID and trap outlet. A 180 cm × 4 mm i.d. column, packed with 15% DEGS-PS on Chromosorb W-AW was used, at an operating temperature of 200°. The identity and purity of the fraction was checked by capillary GLC. Hydrogenation was carried out in a 3 ml septum vial with Pt–asbestos as a catalyst; the vial was flushed with excess H_2 and shaken for 3 hr at room temp. The hydrogenation products were analysed by capillary GLC and GC/MS.

9-Octadecen-6-ynoic acid. IR γ_{max}^{KBr} cm^{-1}: 2910 (CH_2), 2840 (O–Me), 2310 (C≡C), 1740 (C=O), 1640 (C=C); MS *m/z* (rel. int.): 292 $[M]^+$ (7), 261 (20), 175 (11), 161 (35), 147 (22), 133 (36), 119 (51), 105 (51), 91 (100), 79 (98), 67 (67), 55 (70); ^1H and ^{13}C NMR see Table 1.

9,12-Octadecadien-6-ynoic acid. MS *m/z* (rel. int.): 290 $[M]^+$ (1), 259 (6), 173 (19), 159 (6), 145 (15), 131 (14), 119 (33), 117 (21), 105 (47), 91 (100), 79 (52), 67 (40), 55 (38).

9,12,15-Octadecatrien-6-ynoic acid. MS *m/z* (rel. int.): $-[M]^+$ ($-$), 257 (5), 173 (28), 159 (15), 145 (30), 131 (48), 119 (27), 117 (64), 105 (42), 91 (100), 79 (82), 67 (59), 55 (38); ^1H NMR (400 MHz, $CDCl_3$): δ0.95 (3H, *t*, *J* = 7.5 Hz, H-18), 1.49 (2H, *m*, H-4), 1.70 (2H, *m*, H-3), 2.05 (2H, *m*, H-17), 2.15 (2H, *tt*, *J* = 2.5, 7.1 Hz, H-5), 2.31 (2H, *t*, *J* = 7.5, H-2), 2.80 (4H, *m*, H-11 and H-14), 2.90 (2H, *m*, H-8), 3.65 (3H, *s*, Me at C-1), 5.27–5.43 (6H, *m*, H-9, H-10, H-12, H-13, H-15 and H-16); ^{13}C NMR (400 MHz, $CDCl_3$, protons decoupled): δ14.2 (C-18), 17.2 (C-8), 18.5 (C-5), 20.6 (C-17), 24.2 (C-3), 25.5 (C-11 and C-14), 28.4 (C-4), 33.6 (C-2), 51.4 (Me at C-1), 125.4—132.1 (C-9, C-10, C-12, C-13, C-15 and C-16), 173.9 (C-1).

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REFERENCES

1. Gellerman, J. L., Anderson, W. H., Richardson, D. G. and Schlenk, H. (1975) *Biochim. Biophys. Acta* **388**, 277.
2. Hartmann, E., Beutelmann, P., Vandekerckhove, O., Euler, E. and Kohn G. (1986) *FEBS Letters* **197**, 51.
3. Huneck, S. (1983) in *New Manual of Bryology*, Vol. 1, (Schuster, R. M. ed.) pp. 1–116. Hattori Botanical Laboratory, Nichinan.
4. Anderson, B., Anderson, W. H., Chipault, J. R., Ellison, E. C., Fenton, S. T., Gellerman, J. L., Hawkins, J. M. and Schlenk, H. (1974) *Lipids* **9**, 506.
5. Anderson, W. H., Gellerman, J. L. and Schlenk, H. (1975) *Lipids* **10**, 501.
6. Jamieson, G. R. (1975) *J. Chrom. Sci.* **13**, 491.
7. Jamieson, G. R. and E. H. Reid (1976) *Phytochemistry* **15**, 1731.