Phytochemistry, Vol. 26, No. 2, pp. 429–431, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

STERYL ESTERS OF LIVERWORTS*

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(*Revised received* 14 *May* 1986)

Key Word Index—*Mylia taylorii, M. verrucosa*; Hepaticae; bryophyte; sterol fatty acid esters; sterols; fatty acids.

Abstract—Fatty acid steryl esters have been isolated from two liverwort species, and the nature of both the sterols and the fatty acids has been determined by analysis using GLC and GC/MS methods.

INTRODUCTION

Sterols are known to be widely distributed in the plant kingdom, existing as four types: (i) the free form, (ii) fatty acid esters, (iii) glycosides and (iv) esterified glycosides. Despite their widespread occurrence, the sterol types of the liverworts, which are considered to be an early stage in the evolution of terrestrial green plants, have hardly been investigated previously. There are a few reports on the isolation of fatty acid steryl esters [1, 2] and a sterol glycoside [3] from liverworts, although the occurrence of free sterols in many liverworts is well known following our initial report on the isolation of five sterols from certain liverworts [4, 5] and subsequent studies by several workers [6–8]. We have previously described our results on the chemistry of the sesqui- and diterpenoids from the two liverwort species *M. taylorii* (Hook.) S. Gray [9–11] and *M. verrucosa* Lindb. [12–14] of the genus *Mylia* belonging to the Jungermanniaceae of the Jungermanniales. In a continuation of our research upon the lipophilic constituents of these two liverworts, we have isolated fatty acid steryl esters. The present paper deals with the composition of both the sterols and fatty acids which form the steryl esters.

RESULTS AND DISCUSSION

The liverwort *Mylia taylorii* was extracted with ethanol and the neutral extract was chromatographed on a silica gel column to isolate the steryl ester fraction, together with the sesquiterpenoids described previously, in a yield of 14.0% of the ethanol extract. An aliquot of the steryl esters was subjected to methanolysis with methanolic hydrochloric acid to yield a mixture of fatty acid methyl esters and sterols. The composition of the fatty acid methyl esters was determined by GLC and GC/MS methods; identification was performed by comparing the retention times and mass spectral patterns with those of authentic specimens [15]. The results are shown in Table 1.

By the same procedure the steryl ester mixture from the other liverwort, *M. verrucosa*, was isolated in a yield of 7.9% of the extract, and fatty acid methyl esters were also identified by the above methods. The nature of the fatty acid components of these steryl esters obtained from the two liverwort species are shown in Table 1 together with their relative proportions calculated from the peak areas of the gas chromatograms.

In order to analyse the sterol constituents of the esters, the remainders of both steryl ester mixtures were reduced with lithium aluminium hydride in ether. The reduced products were then separated by preparative TLC to give two fractions of a mixture of free sterols and a mixture of aliphatic alcohols. The sterol mixtures were subjected to GLC and GC/MS analyses. On the basis of the coincidence of the retention times and mass spectral patterns with those of authentic specimens described in the previous papers [4, 5], the sterol constituents of the two liverworts were, respectively, shown to be 5-cholestenol, 24-methyl-5,22-cholestadienol, 24-methyl-5-cholestenol, 24-ethyl-5,22-cholestadienol and 24-ethyl-5-cholestenol. The relative contents were obtained from the peak areas of the gas chromatograms as shown in Table 2. However, we did not determine the stereochemistry at C-24 of these liverwort sterols, although Adler [16] and Patterson and co-workers [17] recently reported the configuration at C-24 of 24-methyl- and 24-ethyl-cholesterols in liverworts by using high-resolution ^{1}H NMR spectroscopy. They found that both 24-methyl-5-cholestenol and 24-ethyl-5-cholestenol of liverworts were a mixture of the 24α- and the 24β-epimers, although 24-ethyl-5,22-cholestadienol existed only as the 24α-epimer. GLC and TLC of the reduced products of *M. taylorii* showed the presence of an unidentified compound. The mixture was therefore submitted to preparative TLC, isolating the compound which was identified as cycloartenol, mp 114.5–116°; $[\alpha]_D$ + 46.3°, by coincidence of physical constants and spectral data (see Experimental) [18, 19].

All of the sterol and fatty acid components of the steryl esters are already known as liverwort constituents in their free states [8], except for cycloartenol, which is a rare compound in liverworts [20]. Furthermore, the com-

*Part of this work was reported at the 27th Symposium of Terpenes, Essential Oils and Aromatics, Nagasaki, 1983, *Symposium Papers*, p. 325.

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Table 1. Fatty acid composition of the steryl esters obtained from the two liverworts

Fatty acid	*M. taylorii*	*M. verrucosa*
Decanoic acid	+	16.5
Dodecanoic acid	+	8.6
Tetradecanoic acid	2.8	7.9
Hexadecanoic acid	9.3	10.1
Hexadecenoic acid	+	7.9
Octadecanoic acid	1.7	4.9
Octadeca-9-enoic acid	6.6	16.0
Octadeca-9,12-dienoic acid	43.6	17.0
Octadeca-6,9,12-trienoic acid	2.1	–
Octadeca-9,12,15-trienoic acid	23.9	+
Eicosanoic acid	+	2.5
Eicosenoic acid	+	–
Eicosadienoic acid	+	–
Eicosatrienoic acid	1.0	–
Eicosa-5,8,11,14-tetraenoic acid	6.7	–
Docosanoic acid	1.4	3.4
Tetracosanoic acid	–	5.1

Table 2. Distribution pattern of the sterol components of the fatty acid steryl esters

Sterol	*M. taylorii*	*M. verrucosa*
5-Cholestenol	5	2
24-Methyl-5,22-cholestadienol	15	6
24-Methyl-5-cholestenol	20	27
24-Ethyl-5,22-cholestadienol	25	35
24-Ethyl-5-cholestenol	35	30

positions of the two sterol mixtures are quite similar to each other and to the compositions of those which are present in the free state [6–8]. However, the fatty acid compositions exhibited different patterns: the fatty acid steryl esters of *M. taylorii* are comprised mainly of unsaturated fatty acids (the unsaturated fatty acid content is more than 80% of the total fatty acids). On the other hand, the proportion of unsaturated fatty acids of *M. verrucosa* is about 40%. It is also chemotaxonomically interesting that cycloartenol, a precursor of the 4-desmethyl sterols, exists in the steryl esters of the liverwort *M. taylorii* but not in the other liverwort, *M. verrucosa*.

EXPERIMENTAL

Mp are uncorr. Optical rotations were taken on an automatic polarimeter in $CHCl_3$ solns at 25°. IR spectra were recorded on a grating spectrometer for $CHCl_3$ solns and NMR spectra were determined at 60 MHz for $CDCl_3$ solns with TMS as internal standard. Mass spectra were obtained at 70 eV. For CC, Merck Kieselgel 60 was used; Merck Kieselgel PF_{254} was used for TLC and prep. TLC. Analytical plates were visualized under UV light or were sprayed with 10% H_2SO_4 in EtOH and then heated at 120° for 10 min.

GLC and GC/MS analyses of both mixtures of the sterols and fatty acid methyl esters. (1) *Analysis of fatty acid methyl esters*: GLC of the fatty acid methyl esters was carried out on a FID-type apparatus using a glass column packed with FFAP (10%) on Chromosorb AW (60–80 mesh) at a column temp. of 230°. For GC/MS analysis, a capillary column (Thermon 600T G-SCOT, 0.25 mm × 40 m) was used: column temp. 210°; total emission 20 eV.

(2) *Analysis of the sterols*: GLC of the sterols was carried out on a FID-type apparatus using with a glass column (3 mm × 3 m) packed with Silicon OV-1 (1.0%) on Shimalite (60–80 mesh). The column temp. was 255°. GC/MS analysis was performed using a single focus spectrometer under the following conditions: column Silicon OV-1 (1.0%) on Shimalite; column temp. 250°; total emission 70 eV. In order of increasing retention times, the following five sterols were identified: (a) 5-cholostenol: *m/z* 386 $[M]^+$, 371, 368, 353, 273, 255, 229 and 213; (b) 24-methyl-5,22-cholestadienol: *m/z* 398 $[M]^+$, 383, 380, 365, 355, 337, 300, 273, 271, 255, 229 and 213; (c) 24-methyl-5-cholestenol: *m/z* 400 $[M]^+$, 385, 382, 367 273, 255, 229 and 213; (d) 24-ethyl-5,22-cholestadienol: *m/z* 412 $[M]^+$, 397, 394, 379, 369, 351, 300, 273, 271, 255, 229 and 213; (e) 24-ethyl-5-cholestenol: *m/z* 414 $[M]^+$, 399, 396, 381, 273, 255, 229 and 213.

Isolation of the steryl ester fraction. The two liverworts, *M. taylorii* (440 g) and *M. verrucosa* (310 g), which have been described in previous papers [10, 13], were extracted with EtOH and the solvent was removed under red. pres. to obtain the extracts (20.0 and 9.3 g), respectively. Each fraction of the steryl esters (R_f 0.47; hexane–EtOAc, 25 : 1) was isolated by chromatography on a silica gel column using a mixed solvent of hexane–EtOAc (25:1) in yields of 14 and 7.9% of the neutral extracts, respectively.

Methanolysis of the steryl esters. About 5 mg each of the steryl ester fraction of both liverworts was refluxed with 5% HCl in MeOH (20 ml) for 2 hr. The reaction mixtures were poured into H_2O and extracted with hexane. The products were purified by prep. TLC (C_6H_6–EtOAc, 100:9) and then analysed by means of GLC and GC/MS.

Reduction of the steryl esters with lithium aluminium hydride. The steryl ester mixture (0.7 g) of *M. taylorii* was stirred with an excess of $LiAlH_4$ in Et_2O (15 ml) for 1 hr at room temp. The reaction mixture, after treatment in the usual way, was separated by prep. TLC with silica gel using a solvent system of C_6H_6–EtOAc (20:3) to give crude sterols (110 mg) and an aliphatic alcohol mixture (60 mg) corresponding to the above fatty acid methyl esters. By the same procedure, the steryl ester mixture (0.5 g) of the other liverwort, *M. verrucosa*, produced a mixture of free sterols (150 mg). Both sterol fractions were analysed by GLC and GC/MS to determine their compositions.

Isolation of cycloartenol. From the sterol mixture (110 mg) of *M. taylorii*, the dimethyl sterol cycloartenol (54 mg) was isolated by prep. TLC (R_f 0.50; C_6H_6–EtOAc, 20:3). Cycloartenol: $C_{30}H_{50}O$ ($[M]^+$ at *m/z* 426, base peak at *m/z* 69); mp 115.5–116°; $[\alpha]_D$ +46.3°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 3050, 1093, 1042, 1018 and 1000; ^1H NMR: δ0.32 and 0.40 (each 1H, *d*, *J* = 4.5 Hz), 0.80, 0.89, 0.96 and 0.96 (each 3H, *s*), 1.59 and 1.67 (each 3H, *br s*), 3.27 (1H, *m*, $W_{1/2}$ = 13.0 Hz) and 5.23 (1H, *m*).

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