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STEROLS AND FATTY ACIDS OF THE FRESHWATER *MYRIOPHYLLUM VERTICILLATUM**

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Key Word Index—*Myriophyllum verticillatum*; Haloragaceae; fatty acids; sterols.

Abstract—The fatty acids and sterols of *Myriophyllum verticillatum* are reported. Only 4α-methyl sterols have been detected in free and esterified forms. 4α,24*S*-Dimethyl-5α-cholesta-7,25(27)-dien-3β-ol and 4α,24*S*-dimethyl-5α-cholesta-8(14),25(27)-dien-3β-ol have been isolated for the first time. The major fatty acids are 16:1, 16:2, 16:3 and two isomeric hydroxy 16:3.

INTRODUCTION

In connection with our interest in the study of the chemical composition of the aquatic plants distributed in Italy, we have so far examined *Lemna minor* L. [1], *Lemna trisulca* L. [2] and *Elodea canadensis* L. C. H. Richard [3]. Despite the well defined different taxonomic classification of these species, they seem to have similar lipid profiles with an interesting presence of cyclic and acyclic oxygenated fatty acids.

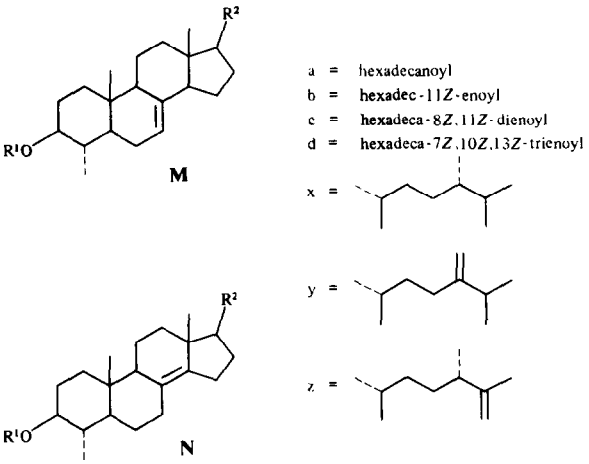
In this paper we report the fatty acid and sterol composition of *Myriophyllum verticillatum* L., which has already been partially investigated [4].

RESULTS AND DISCUSSION

The acidic fraction obtained using the previously described procedures [5] was esterified with ethereal diazomethane and then chromatographed on a silica gel column to afford a mixture of unsaturated fatty acid methyl esters beside two isomeric hydroxy fatty acid methyl esters which, after separation and purification, were identified as methyl-(7*S*)-hydroxyhexadeca-8*E*, 10*Z*, 13*Z*-trienoate [6] and methyl-(10*R*)-hydroxyhexadeca-7*Z*,11*E*,13*Z*-trienoate [7] by comparison with authentic samples. The mixture of unsaturated lipids was rechromatographed on silica gel–$AgNO_3$ to give mainly methyl hexadeca-11*Z*-enoate, methyl hexadeca-8*Z*,11*Z*-dienoate and methylhexadeca-7*Z*,10*Z*,13*Z*-trienoate.

Column chromatography on silica gel of the neutral extract gave a mixture of sterylacyl esters and a mixture of free sterols besides the already described compounds [4]. The mixture of steryl esters **1M**, **2M**, **3M**, **4M**, **1N** and **2N** was chromatographed on HPLC to give three fractions. Each fraction was later separated through preparative TLC (silica gel–$AgNO_3$) into its components which were then saponified. The acyl residues were identified by comparison (GLC) with authentic samples while sterols were identified on the basis of their mass spectra, ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) data. The mixture of free sterols **5M**, **6M**, **7M**, **3N** and **4N** was chromatographed on silica gel–$AgNO_3$ to give two fractions which were then separated into their components by reversed phase argentation HPLC [8].

Whereas **5M**, **6M** and **3N** have been already isolated from several marine [9] and terrestrial sources [10], this is the first report of the isolation of 4α,24*S*-dimethyl-5α-cholesta-7,25(27)-dien-3β-ol (**7M**) and 4α,24S-dimethyl-5α-cholesta-8(14),25(27)-dien-3β-ol (**4N**). The mass spectra of **7M** and **4N** showed a molecular ion at *m/z* 412 ($C_{29}H_{48}O$) and fragments indicative of the presence of a C_{20} nucleus with a C_9 side chain. The ^{1}H NMR spectra with a broad triplet at δ3.13 and a doublet at δ0.99 suggested the presence of a 3β-hydroxyl and a 4α-methyl group. The unsaturation of the nucleus in **7M** was located



	1M	2M	3M	4M	5M	6M	7M	1N	2N	3N	4N
R^1	a	b	b	d	H	H	H	a	c	H	H
R^2	x	x	y	x	x	y	z	y	z	y	z

*Part 10 in a series of studies on aquatic plants distributed in Italy. For Part 9 see ref. [3].

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Table 1 ^{1}H NMR spectral data of sterols

H	5M	6M	7M	3N	4N
3	3.13 *m*	3.13 *m*	3.13 *m*	3.11 *m*	3.13 *m*
7	5.18 *m*	5.18 *m*	5.18 *m*		
18	0.54 *s*	0.54 *s*	0.54 *s*	0.85 *s*	0.85 *s*
19	0.83 *s*	0.83 *s*	0.83 *s*	0.71 *s*	0.71 *s*
21	0.93 *d* (6.5)	0.95 *d* (6.5)	0.98 *d* (6.3)	0.97 *d* (6.6)	1.02 *d* (6.5)
25		2.23 *m*		2.23 *m*	
26	0.79 *d* (6.8)	1.02 *d* (6.8)	4.67 *d* (1.1)	1.02 *d* (6.8)	4.67 *d* (1.1)
27	0.87 *d* (6.9)	1.03 *d* (6.9)	1.64 *s*	1.03 *d* (6.9)	1.64 *s*
28	0.79 *d* (6.8)	4.66 4.71 *ss*	1.00 *d* (6.8)	4.65 4.71 *ss*	1.00 *d* (6.8)
4-Me	0.99 *d* (6.3)	0.99 *d* (6.3)	0.99 *d* (6.3)	0.98 *d* (6.4)	0.98 *d* (6.4)

The *J* values (Hz) are reported in parentheses.

at C-7 on the basis of the chemical shifts of the olefinic proton (δ 5.18) and the C-18 methyl (δ 0.54) whereas the lack of olefinic protons and the presence of the C-18 and C-19 methyls at δ 0.83 and 0.71, respectively, suggested a $\Delta^{8(14)}$-unsaturation in **4N**. With regard to the side chain the presence of an olefinic methylene centred at δ 4.62 and a vinylic methyl suggested a $\Delta^{25(27)}$-unsaturation with a methyl at C-24.

Tentatively the *S*-configuration at C-24 was hypothesized on the basis of the close similarity of the ^{13}C NMR data of **7M** and **4N** with those reported for the side chain of cyclolaudenol by Laonigro *et al.* [11] even if the ^{1}H NMR data of **7M** were identical with those of the 4α,24-dimethyl-5α-cholesta-7,25(27)-dien-3β-ol recently isolated from *Polypodium niponicum* by Agata and Arai [12] which had been attributed a 24*R*-configuration on the basis of CD measurements.

However, hydrogenation of **7M** and **4N** gave the already known 4α,24*S*-dimethyl-5α-cholest-7-en-3β-ol (**5M**) and 4α,24*S*-dimethyl-5α-cholest-8(14)-en-3β-ol [13] thus unequivocally confirming the stereochemistry of these compounds.

M. verticillatum, as well as the other freshwater plants so far examined, contain as major lipids C_{16} fatty acids with a significative abundance of oxygenated compounds. In the light of the great taxonomic differences among these species this lipid distribution seems to reflect an environmental adaptation. As far as the free and esterified sterols are concerned it is noteworthy that the abundant presence in this species of 4α-methyl sterols with a Δ^7-or a $\Delta^{8(14)}$-unsaturation are the main constituents whereas the more usual Δ^5-sterols are not present in detectable amounts.

EXPERIMENTAL

Isolation of fatty acids. The acidic fraction (220 mg) was esterified with ethereal CH_2N_2 and then chromatographed on silica gel; hexane gave the mixture of fatty acid methyl esters (126 mg) which was resolved through chromatography on silica gel–$AgNO_3$ using hexane with increasing percentages of Et_2O as eluent. Hexane–Et_2O (7:3) gave the hydroxy fatty acid methyl esters (82 mg) which were separated and purified by prep. TLC (hexane–Et_2O 9:1, 2 runs).

Isolation of free and esterified sterols. The neutral fraction (500 mg) was chromatographed on silica gel; hexane eluted the mixture of steryl esters (100 mg) whereas hexane–Et_2O (7:3) gave the free sterols (68 mg). The mixture of steryl esters, chromatographed on HPLC (Lichrosorb Si-60 7 μm, 250 mm × 10 mm i.d., hexane-isopropyl ether, 97:3) gave three fractions which were worked-up by prep. silica gel–$AgNO_3$ TLC (C_6H_6–Et_2O, 19:1) to afford mainly compounds **1M** (13 mg), **2M** (9 mg), **3M** (15 mg), **4M** (20 mg), **1N** (14 mg) and **2N** (17 mg). The

Table 2 ^{13}C NMR spectral data of sterols

C	5M	6M	7M	3N	4N	C	5M	6M	7M	3N	4N
1	36.95 *t*	36.97 *t*	36.96 *t*	36.76 *t*	36.75 *t*	16	27.91 *t*	27.91 *t*	27.92 *t*	27.00 *t*	27.00 *t*
2	30.90 *t*	30.92 *t*	30.90 *t*	31.10 *t*	31.10 *t*	17	55.94 *d*	55.98 *d*	55.97 *d*	56.72 *d*	56.70 *d*
3	76.15 *d*	76.17 *d*	76.14 *d*	76.24 *d*	76.26 *d*	18	11.80 *q*	11.82 *q*	11.79 *q*	18.11 *q*	18.08 *q*
4	40.24 *d*	40.20 *d*	40.21 *d*	40.39 *d*	40.36 *d*	19	14.17 *q*	14.13 *q*	14.15 *q*	14.12 *q*	14.13 *q*
5	46.55 *d*	46.60 *d*	46.56 *d*	50.12 *d*	50.15 *d*	20	36.60 *d*	36.13 *d*	36.04 *d*	36.08 *d*	36.03 *d*
6	26.61 *t*	26.60 *t*	26.63 *t*	26.03 *t*	26.06 *t*	21	18.99 *q*	18.36 *q*	18.39 *q*	18.51 *q*	18.37 *q*
7	117.35 *d*	117.41 *d*	117.43 *d*	29.07 *t*	29.04 *t*	22	33.63 *t*	35.09 *t*	34.07 *t*	35.03 *t*	34.05 *t*
8	139.07 *s*	139.09 *s*	139.06 *s*	126.13 *s*	126.11 *s*	23	30.66 *t*	29.72 *t*	31.69 *t*	29.71 *t*	31.70 *t*
9	49.59 *d*	49.59 *d*	49.56 *d*	49.01 *d*	49.04 *d*	24	39.02 *d*	156.70 *s*	41.69 *t*	156.68 *s*	41.71 *t*
10	34.77 *s*	34.80 *s*	34.81 *s*	37.13 *s*	37.11 *s*	25	31.41 *d*	33.90 *d*	150.30 *s*	33.57 *d*	150.33 *s*
11	21.38 *t*	21.33 *t*	21.35 *t*	19.95 *t*	19.98 *t*	26	20.51 *q*	21.91 *q*	18.69 *q*	21.88 *q*	18.67 *q*
12	39.49 *t*	39.44 *t*	39.45 *t*	37.54 *t*	37.51 *t*	27	17.55 *q*	22.02 *q*	109.37 *t*	22.07 *q*	109.34 *t*
13	43.25 *s*	43.31 *s*	43.33 *s*	42.71 *s*	42.72 *s*	28	15.40 *q*	106.01 *t*	20.16 *q*	106.07 *t*	20.19 *q*
14	54.97 *d*	54.89 *d*	54.91 *d*	142.35 *s*	142.36 *s*	4 Me	15.13 *q*	15.13 *q*	15.17 *q*	15.12 *q*	15.18 *q*
15	22.95 *t*	22.89 *t*	22.87 *t*	25.65 *t*	25.67 *t*						

mixture of sterols was chromatographed on silica gel–$AgNO_3$ (hexane–Et_2O, 7:3) and gave **5M** (6 mg) whereas hexane–Et_2O (3:2) gave a mixture of four sterols which were separated through reversed phase argentation HPLC (Lichrosorb RP-18, 7 μm, 250 mm × 10 mm i.d. MeOH–H_2O, 17:3, 0.1 N $AgNO_3$). **7M** (11 mg) had MS *m/z* (rel. int.): 412 (50), 397 (27), 379 (10), 328 (13), 285 (100), 269 (30), 245 (10), 241 (15), 227 (23). **4N** (8 mg) had MS *m/z* (rel. int.): 412 (42), 397 (100), 379 (12), 328 (15), 285 (65).

Hydrogenation of **7M** and **4N**. Pure samples of **7M** (6 mg) and **4N** (5 mg) dissolved in EtOAc were treated with H_2 over PtO_2 for 2 hr to give the monounsaturated sterols **5M** and 4α,24*S*-dimethyl-5α-cholest-8(14)en-3β-ol.

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REFERENCES

1. Previtera, L. and Monaco, P. (1987) *J. Nat. Prod.* **50**, 807.
2. Monaco, P. and Previtera, L. (1987) *Phytochemistry* **26**, 745.
3. Monaco, P., Parrilli, M. and Previtera, L. (1987) *Tetrahedron Letters* **28**, 4609.
4. Monaco, P., Della Greca, M., Onorato, M. and Previtera, L. (1988) *Phytochemistry* **27**, 2355.
5. Lanzetta, R., Monaco, P., Previtera, L. and Simaldone, A. (1988) *Phytochemistry* **27**, 887.
6. Previtera, L., Merola, D. and Monaco, P. (1985) *Phytochemistry* **24**, 1838.
7. Previtera, L. and Monaco, P. (1983) *Phytochemistry* **22**, 1445.
8. Vonach, B. and Schomburg, G. (1978) *J. Chromatog.* **149**, 417.
9. Withers, N. (1983) *Marine Natural Products*. Academic Press, New York.
10. Banerji, R., Misra, G. and Nigam, S. K. (1987) *Phytochemistry* **26**, 2644.
11. Laonigro, G., Adinolfi, M., Barone, G., Lanzetta, R. and Mangoni, L. (1982) *Gazz. Chim. Ital.* **112**, 273.
12. Ageta, H. and Arai, Y. (1984) *Phytochemistry* **23**, 2875.
13. Wright, J. L. C., McInnes, A. G., Shimizu, S., Smith, D. G. and Walter, J. A. (1978) *Can. J. Chem.* **56**, 1898.

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2,5-DIMETHYL-4-HYDROXY-3(2*H*)-FURANONE GLUCOSIDE: ISOLATION FROM STRAWBERRIES AND SYNTHESIS

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Key Word Index—*Fragaria ananassa*; Rosaceae; glucoside; 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone glucoside.

Abstract—2,5-Dimethyl-4-hydroxy-3-(2*H*)furanone β-glucoside has been isolated from strawberry juice and synthesized. Both the natural and synthetic material exist as a mixture of diastereoisomers.

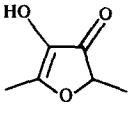
INTRODUCTION

For more than 20 years Furaneol® [2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, **1**] has been known as an important aroma compound of many fruits, notably pineapples [1] and strawberries [2], in which it occurs to the extent of 2.2–7.4 ppm, depending on the source and length of time of storage [3]. It seemed likely that this compound could occur in the plant as a glycoside, and this paper presents the successful outcome of a search for Furaneol glycoside in strawberries.

RESULTS AND DISCUSSION

Recently a reverse-phase HPLC method was described for use in the quantification of Furaneol in pineapple and grapefruit juice [4], although the peak assigned to Furaneol was not checked spectroscopically by isolation. We based our search for Furaneol glucoside (**2**) in strawberry juice on a similar method.

The juice was prepared by macerating commercial strawberries with a small amount of water followed by centrifugation. The supernatant was subjected to a series of membrane filtrations, using decreasing pore sizes (if fine-bore membranes are used immediately, they become rapidly clogged). A final lyophilization yielded 21.6 g of solid residue from 725 g of strawberries. This material



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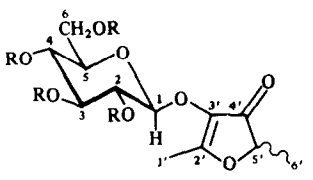

2 R = H
3 R = Ac

*The ^1H NMR values, particularly for the protons H-6 and H-1 are close to those of phenolic β-glucosides [5].