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STERYL AND WAX ESTERS IN *DICRANUM ELONGATUM*

RAINER EKMAN* and PIRJO KARUNEN†

*Institute of Wood Chemistry and Cellulose Technology, Åbo Akademi, SF 20500 Turku 50, Finland; †Department of; Botany, University of Turku, SF 20500 Turku 50, Finland

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Key Word Index—*Dicranum elongatum*; Dicranaceae; moss; sterols; prenols; fatty alcohols; fatty acids; phylogeny.

Considerable amounts of steryl and wax esters have been found in green moss shoots, spores and protonemata [1–4]. The earlier investigations have dealt with the component fatty acids, fatty alcohols and prenols. In this paper we report, in addition, the composition of the esterified sterols in shoots of the subarctic moss *Dicranum elongatum* Schleich.

The amount of steryl and wax esters from *D. elongatum* was 9 mg/g dry tissue and the steryl esters made up about 85% of the fraction. The major fatty acids obtained by acid methanolysis of the fraction were 16:0 (7.5%), 18:0 (3.1%), 18:2ω6 (28.2%), 18:3ω6 (6.1%), 18:3ω3 (15.4%), 20:4ω6 (26.6%) and 20:5ω3 (2.4%). The arctic Alaskan mosses, *Hylocomium splendens* var. *alaskanum* and *Pleurozium schreberi* have similarly been found to contain notable amounts of steryl esters [1]. The aliphatic alcohols liberated by alkaline hydrolysis of the esters included fatty alcohols and prenols. 1-Octadecanol, phytol and geranylgeraniol were the major components (Table 1). Fatty alcohols [5, 6] and phytol [1, 4, 5] have been found in mosses but geranylgeraniol, although present in spores and protonemata [4], has not previously been reported to occur in moss shoots.

Table 1. Alcohols and sterols from *Dicranum elongatum* esters

Compound	Percentage composition	RR_t on SE-30 TMSi ethers* 210°	TMSi ethers* 270°	Acetates† 280°
1-Octadecanol	2.7	1.00		
Phytol	4.9	1.07		
Geranylgeraniol	1.3	1.34		
Cholesterol	<1		0.66	0.67
Campesterol	9.1		0.83	0.83
Stigmasterol	9.2		0.88	0.89
Unidentified	9.8		0.98	0.93
Sitosterol	3.4		1.00	1.00
Cycloartenol	46.2		1.13	1.08
24-Methylene cycloartanol	3.3		1.28	1.23
Other	10.0			
	100.0			

* Retention times of TMSi ethers calculated relative to 1-octadecanol for aliphatic ethers and to sitosterol for steryl ethers.

† Retention times of acetylated sterols calculated relative to sitosterol.

The predominant esterified sterol in *D. elongatum* was cycloartenol (4,4-dimethyl sterol) (Table 1). Campesterol and stigmasterol were present in almost equal amounts, and sitosterol and 24-methylene cycloartanol (4,4-dimethyl sterol) were minor compounds. Three sterols remained unidentified.

Cycloartenol, which is the first intermediate for phytosterol biosynthesis [7], has not previously been found in mosses, although several other sterols with one or two methyl groups at the C-4 position have been reported [8–13]. Since ergosterol has previously been found in mosses [8, 10, 11], it is feasible that mosses could contain lanosterol, which is considered to be the precursor in ergosterol biosynthesis [7]. In *D. elongatum*, however, the amount of a compound, which had the same retention time as the acetylated lanosterol, was too small (<0.1%) for unambiguous identification.

EXPERIMENTAL

Frozen shoot tissue of *D. elongatum* was collected in October 1978 in Finnish Lapland (69°45'N). The tissue was crushed with liquid N_2 and then homogenized in ice-cold $CHCl_3$–MeOH (2:1). The steryl and wax ester fraction (hR_f 69–74) was isolated on TLC (Si gel G) with the solvent system hexane–Et_2O–HOAc (90:10:1). The esters were hydrolysed for 1 hr at 90° with dry 2 N HCl in MeOH [5]. Acidic methanolysis was necessary to prevent the isomerization of phytenic acid present in small amounts in the esters. The unsaponifiables and fatty acid methyl esters were separated on TLC with the solvent system hexane–Et_2O (80:20). The fatty acid methyl esters were analysed by GLC on FFAP and BDS capillary columns [14].

For analysis of the alkyl moieties of the steryl and wax ester fraction samples were refluxed for 12 hr in N KOH in 90% EtOH. Alkaline hydrolysis was necessary because of the presence of prenyl moieties apt to be destroyed under acidic hydrolysis [5]. The unsaponifiables were analysed as TMSi ethers on a 25m × 0.3mm i.d. glass capillary column coated with SE-30. Column temp. was programmed from 170 to 280° at a rate of 4°/min. The amounts of TMSi ethers were calculated from peak areas. Relative retention times (RR_t) were determined by isothermal GLC runs (Table 1). MS were recorded with an LKB-9000 GC–MS instrument. Compound identifications were made by direct comparison of RR_t values and MS of the TMSi ethers and acetylated (Ac_2O–Py, 4:1) derivatives with authentic samples. The TMSi ether of an

unidentified compound had the same retention time as the TMSi ether of lanosterol (RR_t 0.98), but the acetylated derivative (RR_t 0.93) was separated from the acetylated lanosterol (RR_t 0.99). Cycloartenol was completely separated from 31-norcyclolaudenol (RR_t of acetylated derivative 1.03) and 24-methylene cycloartanol from cyclolaudenol (RR_t of acetylated derivative 1.20).

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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. Swanson, E. S., Anderson, W. H., Gellerman, J. L. and Schlenk, H. (1976) *Bryologist* **79,** 339.
3. Karunen, P. and Liljenberg, C. (1978) *Physiol. Plant.* **44,** 417.
4. Liljenberg, C. and Karunen, P. (1978) *Physiol. Plant.* **44,** 369.
5. Gellerman, J. L., Anderson, W. H. and Schlenk, H. (1975) *Lipids* **10,** 656.
6. Huneck, S. (1971) *Phytochemistry* **10,** 3282.
7. Goad, L. J. (1977) in *Lipids and Lipid Polymers in Higher Plants* (Tevini, M. and Lichtenthaler, H. K., eds.) pp. 146–168. Springer, Berlin.
8. Marsili, A. and Morelli, I. (1968) *Phytochemistry* **7,** 1705.
9. Marsili, A. and Morelli, I. (1970) *Phytochemistry* **9,** 651.
10. Huneck, S., Schreiber, K. and Jänicke, S. (1973) *Phytochemistry* **12,** 2533.
11. Catalano, S., Marsili, A., Morelli, I. and Pacchiani, M. (1976) *Phytochemistry* **15,** 1178.
12. Marsili, A., Morelli, I. and Iori, A. M. (1971) *Phytochemistry* **10,** 432.
13. Marsili, A., Morelli, I., Bernardini, C. and Pacchiani, M. (1972) *Phytochemistry* **11,** 2003.
14. Karunen, P. (1977) *Physiol. Plant.* **40,** 239.

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OBTUSILOBICININ, A NEW SAPONIN FROM *ANEMONE OBTUSILOBA*

K. P. Tiwari* and M. Masood

Department of Chemistry, University of Allahbad, Allahabad-211002, India

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Key Word Index—*Anemone obtusiloba;* Ranunculaceae; obtusilobicinin; olean-12-ene-28-oic-3-*O*-(α-L-arabinofuranosyl 1→2) (α-L-arabinofuranosyl 1→2-*O*-α-L-rhamnopyranosyl 1→4)-β-D-glucopyranoside.

INTRODUCTION

Several saponins [1–6] have been isolated from different species of the genus *Anemone*, but, surprisingly, complete structures of very few of them have been elucidated [5, 6]. The presence of two saponins, obtusilobin and obtusilobinin [6], has already been reported in *Anemone obtusiloba* [7].

RESULTS AND DISCUSSION

Obtusilobicinin (**1**), $C_{52}H_{84}O_{20}$, gave all the tests of saponins [8]. Hydrolysis with 7% H_2SO_4 yielded oleanolic acid (IR, ^{1}H NMR, MS) [9–12], D-glucose, L-rhamnose and L-arabinose (co–PPC). The sugars were found to be present in the ratio of 1:1:2 as revealed by colorimetric estimation [13] and the genin content was found to be 44.0% (quantitative hydrolysis) against 44.35% calculated for one unit of oleanolic acid and four units of sugars per molecule of obtusilobicinin. Thus, a molecule of obtusilobicin contains one unit each of oleanolic acid, D-glucose, L-rhamnose and two units of L-arabinose.

It is evident from the structure of oleanolic acid that the —OH at C-3 and the —COOH at C-17 are available for glycosidic linkage with sugar residues. The saponin could not be hydrolysed with 5 N NH_4OH, which is a specific reagent [14] for the hydrolysis of sugar esters, indicating that sugars were not present in ester combination with the —COOH group of oleanolic acid. This led to the conclusion that all the sugar units were linked as a tetroside unit to the —OH

* Present address: Department of Community Health and Environmental Medicine, Institute for Hygiene OG Social Medicine, Odeuse University, J. B. Winsløwsvej-19, D.K. 5000, Odeuse C, Denmark.