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ESTERIFIED LONG-CHAIN HYDROXY ACIDS IN GREEN AND SENESCENT PARTS OF THE PEAT MOSS *SPHAGNUM FUSCUM*

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Key Word Index—*Sphagnum fuscum*; Sphagnales; peat moss; hydroxy acids; senescence.

Abstract—The bifunctional long-chain acids in extracts and alkaline hydrolysates of extractive-free tissues from the green and senescent parts of *Sphagnum fuscum* were investigated by GC and GC/MS. A series of C_{14}–C_{26} ω-hydroxy acids was liberated by alkaline hydrolysis of senescent tissue demonstrating the presence of insoluble polymeric lipid esters in the moss. In the corresponding hydrolysates of the topmost green parts of the shoot the amounts of these acids were significantly smaller. It is suggested that the presence of such polymeric esters in *S. fuscum* contributes to the cell-wall resistance of this peat-forming moss.

Senescence of *Sphagnum fuscum* shoots is accompanied by a loss of unsaturated fatty acids in the topmost yellow-green, yellow-brown and brown parts of the shoot [1]. Since lipoxygenase enzymes are known to be activated in senescent plant tissue [2] causing loss of unsaturated fatty acids, a further attempt was made to characterize the feasible enzyme-catalysed products, i.e. hydroperoxy acids and/or oxohydroxy acids [3]. However, no accumulation of these compounds, either in free or esterified form, was found in senescent, brown segments of *S. fuscum* shoots (9–12 cm beneath the top) by detailed GC/MS analysis of the $CHCl_3$–MeOH extract. The same analysis showed, on the other hand, the presence of small amounts of ω-hydroxy acids and α,ω-dicarboxylic acids (Table 1) released by saponification of the extract, and similar compounds were obtained from the green topmost part of the shoot (capitulum). Presumably these acids were originally present as oligomeric esters and were soluble enough to be dissolved from the disintegrated tissues by extraction with the neutral solvent mixture. No lactones, free hydroxy acids or free dicarboxylic acids could be detected in the $CHCl_3$–MeOH extracts from either green or brown (9–12 cm) segments of *S. fuscum*.

Since ω-hydroxy acids occur in higher plants as highly polymerized constituents of suberin and cutin [4], the finding of soluble esters of hydroxy acids in *S. fuscum* led us further to investigate the possible presence of highly polymerized esters of ω-hydroxy acids also in peat moss.

Detailed GC and GC/MS analysis of the methylated and silylated alkaline hydrolysate of the brown segment (9–12 cm) of *S. fuscum* tissue which had been pre-extracted with $CHCl_3$–MeOH and $CHCl_3$ (see Experimental) revealed the presence of the following series of saturated even-numbered *n*-aliphatic homologues: C_{16}–C_{28} acids, C_{18}–C_{28} alcohols and C_{20}–C_{24} α,ω-dicarboxylic acids. The compounds in these series were identified by comparison of GC R_ts and mass spectra (from GC/MS) with pure reference compounds. In addition, C_{14}–C_{26} homologues having identical mass spectra with published spectra for TMSi ethers of even-numbered *n*-alkanoic ω-hydroxy acid methyl esters [5] were identified. In general, the TMSi ethers of ω-hydroxy acid methyl esters give characteristic major ions at the upper end of their mass spectra [6] providing facile interpretation.

The dominant compound in the alkaline hydrolysate from the 9–12-cm segment could not be included as a member of any of the above homologous series from GC data. Its mass spectrum closely resembled the published spectrum of methyl 18-hydroxyoctadec-9-enoate TMSi ether [6]. Mass spectra of isomeric monoenoic fatty acid methyl esters do not, however, allow determination of double bond position [7]. The compound was therefore isolated, together with other oxidized long-chain acids in the 9–12 cm segment, by TLC and hydroxylated with OsO_4 to yield a trihydroxy compound. After methylation and silylation this compound gave a mass spectrum identical with published data for methyl 9,10,18-trihydroxyoctadecanoate *tris*-TMSi ether [6]. Thus the major compound in the fraction (Table 1) could be positively identified as 18-hydroxyoctadec-9-enoic acid. The TLC fraction hydroxylated with OsO_4 also contained a compound identical with methyl 9,10-dihydroxyoctadecane-1,18-dioate, *bis*-TMSi ether [6]. Since this compound was not present in the untreated fraction it was obviously the hydroxylation product of 9-octadecene-1,18-dioic acid. As expected a compound having a MW and mass spectral fragmentation pattern consistent with this acid was present. The GC of the alkaline hydrolysate also showed a peak with a R_t equal to that of 18-hydroxyoctadecanoic acid, but the mass spectrum revealed this monohydroxy acid to be present only in trace amounts. The major contribution to the peak was from a mixture of 9,16- and 10,16-dihydroxyhexadecanoic acids, the latter isomer being the dominating one as deduced from the mixed mass spectrum [8]. The alkaline hydrolysate further contained 9,10,18-trihydroxyoctadecanoic acid as a minor constituent. Its mass spectrum was identical with that of the OsO_4-hydroxylation product of 18-hydroxyoctadec-9-enoic acid.

Qualitative analysis of the alkaline hydrolysate from the green topmost segment of *S. fuscum* revealed the presence of the same compounds as in the 9–12-cm segment.

Table 1. Content (mg/g dry unextracted tissue) of *n*-aliphatic long-chain compounds in *Sphagnum fuscum*

Compound	$CHCl_3$–MeOH extract		KOH–EtOH-hydrolysate*	
	Segment 0–0.5 cm	Segment 9–12 cm	Segment 0–0.5 cm	Segment 9–12 cm
Hydroxy acids	0.06	0.19	0.29	1.93
14-Hydroxytetradecanoic	†–	tr‡	tr	tr
16-Hydroxyhexadecanoic	–	tr	0.02	0.07
9,16- and 10,16-Dihydroxyhexadecanoic§	tr	tr	0.11	0.24
18-Hydroxyoctadec-9-enoic	tr	0.01	0.10	1.03
9,10,18-Trihydroxyoctadecanoic	tr	0.02	0.01	0.04
20-Hydroxyeicosanoic	–	0.01	0.01	0.03
22-Hydroxydocosanoic	0.04	0.08	0.02	0.28
24-Hydroxytetracosanoic	0.02	0.07	0.02	0.20
26-Hydroxyhexacosanoic	tr	tr	tr	0.04
Dicarboxylic acids				
9-Octadecene-1,18-dioic		tr	0.01	0.08
C_{20}–C_{24}, saturated	–	tr	0.03	0.12
Fatty acids				
C_{18}, unsaturated	n.a.‖	n.a.	0.07	0.06
C_{16}–C_{28}, saturated	n.a.	n.a.	0.35	0.44
Fatty alcohols				
C_{18}–C_{28}, saturated	n.a.	n.a.	0.04	0.18

*Alkaline extract of pre-extracted ($CHCl_3$–MeOH) tissue.
† –, not detected.
‡ tr, less than 0.01 mg/g.
§ Includes small amounts of unresolved 18-hydroxyoctadecanoic acid.
‖ n.a., not analysed.

It is noteworthy that the total content of hydroxy- and dicarboxylic acids in the senescent tissues (9–12-cm segment) of *S. fuscum* is considerably higher than that in the topmost parts of the moss (Table 1). Hence it would seem that accumulation of the hydroxy- and dicarboxylic acid polymers in the peat moss is an age-dependent process. Microscopic examination of the tissue residues from $CHCl_3$–MeOH extraction showed that they were mostly made up of cell-wall remnants, further suggesting that the major part of the hydroxy- and dicarboxylic acid polymers cover or are impregnated in the cell walls of senescent peat moss. In addition, the tissue residues (Table 1) contained long-chain fatty acids, mostly saturated, and some saturated fatty alcohols, probably also as cell-wall constituents. Both fatty acids and fatty alcohols are considered typical constituents of suberin from higher plants [9]. In earlier studies concerning the cell-wall components of mosses, phenolic substances [10] have been reported. Very little information is available on lipid polymers of mosses; the leaves of two *Sphagnum* species, *S. palustre* and *S. cuspidatum*, have been reported to contain cutin acids primarily of C_{16} chain length [11]. Our contribution implicating shoot-age dependence of the content of lipid polymers in *S. fuscum* offers a new approach to studies of cell-wall structures of mosses.

In a recent study free and esterified polymeric ω-hydroxy and α,ω-dicarboxylic acids have been found to occur in *ca* 2000 year-old *Sphagnum* peat samples [12]. Such a finding suggests that the cell-wall polymers of *Sphagnum* spp. are environmentally highly resistant. Thus they may play an important role in peat accumulation and in the formation of peatland ecosystems, which are widespread and economically important especially in northern countries.

EXPERIMENTAL

S. fuscum (Schimp.) Klinggr. hummocks were collected in Oct. 1980 from the Karevanrahka bog in south-west Finland. The shoots were cut into two segments: (1) green top, 0–0.5 cm (capitulum) and (2) senescent brown portion, 9–12 cm beneath the top.

Two g of moss tissue was chopped up in a mortar and then extrd in ice-cold $CHCl_3$–MeOH (2:1) in a Potter–Elvehjem homogenizer for 15 min. A quantitative int. standard, 5α-cholestane, was added and the homogenate filtered through a glass sinter containing two fat-free filter papers and 1.5 cm sea sand (E. Merck AG). The tissue residue was re-extrd with $CHCl_3$–MeOH (2:1) followed by $CHCl_3$ at room temp.

The $CHCl_3$–MeOH extracts were divided into two which were evapd to dryness. One was methylated (CH_2N_2 in Et_2O–MeOH), dried and silylated for GC analysis of free long-chain compounds. The other was saponified (0.5 M KOH on 90% EtOH, 75°, 3 hr), diluted with H_2O (1:1) and acidified with 0.25 M H_2SO_4. The aq. suspension was extrd ×5 with Et_2O and the combined Et_2O layers washed (H_2O), dried (dry Na_2SO_4) and evapd to dryness. The dry residue was then methylated and silylated, as for the first portion, for GC analysis of total (free + esterified) long-chain compounds.

The tissue residues from filtration of the homogenates formed during $CHCl_3$–MeOH extractions were vacuum-dried and submitted to alkaline hydrolysis (0.5 M KOH in 90% EtOH, 75°, 6 hr, admixture of 5α-cholestane as int. standard). During hydrolysis the suspension was continuously mixed with a magnetic stirrer. The warm mixture was filtered through a glass sinter and the solid matter washed with warm 90% EtOH. The combined filtrates were diluted with H_2O (1:1) and acidified with 0.25 M H_2SO_4. The filtrate suspension was extrd × 5 with Et_2O and the combined Et_2O layers washed (H_2O), dried (dry Na_2SO_4) and evapd to dryness. The dry extract was methylated and silylated for GC analysis of liberated long-chain compounds.

About 10 g of dry, pre-extrd ($CHCl_3$–MeOH) tissue of the 9–12-cm segment was further submitted to alkaline hydrolysis (KOH–EtOH), in order to provide enough sample for preparative isolation of hydroxy- and other oxidized long-chain acids by prep. TLC (Si gel, 0.5-mm layer). The polar fraction at hR_f 2–45 [Et_2O–petrol (bp 40–60°)–HOAc, 80:20:1] was isolated and unsaturated compounds in the fraction were hydroxylated with OsO_4, methylated and silylated for the determination of double bond positions by GC/MS [13].

All GC analyses of methylated and silylated extract samples were performed using the same conditions: 30 m × 0.3 mm i.d. glass capillary column coated with SE-30; programmed heating, 150–290°, 4°/min. Quantitative calculations were based on peak areas relative to 5α-cholestane. MS of individual compounds were recorded with a low resolution GC/MS instrument using electron impact with a similar GC column as above and operated under the same conditions.

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