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Two Dibenzofurans Obtained on Oxidative Degradation of the Moss *Polytrichum commune* Hedw.

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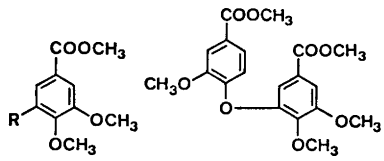
Stalks of the moss *Polytrichum commune* Hedw. were finely ground and the resulting meal was extracted and heated at 170° with aqueous NaOH-Na_2S for 3 h. The dissolved material was methylated with dimethyl sulfate, oxidized with $KMnO_4$-$NaIO_4$ in aqueous sodium hydroxide and with H_2O_2 in aqueous sodium carbonate. The crude mixture of products was treated with diazomethane. In addition to methyl veratrate and dimethyl isohemipate, two new esters were isolated and identified as methyl 4,7,9-trimethoxy-2-dibenzofurancarboxylate and methyl 3-(4,7,9-trimethoxy-2-dibenzofuranyl)-propanoate. Neither dimethyl 2′,5,6-trimethoxydiphenylether-3,4′-dicarboxylate nor dimethyl 5,5′-dehydrodiveratrate, two oxidation products characteristic of guaiacyl and guaiacyl-syringyl lignins, could be detected. This finding indicates that *Polytrichum commune* does not contain lignin.

A method for the structural characterization of lignins has recently been described.[1] This method (in this paper referred to as "oxidative degradation") comprises the solubilization of the lignin of the preextracted, finely ground plant material by heating with aqueous NaOH-Na_2S for 3 h at 170°, followed by methylation with dimethyl sulfate, and oxidation with permanganate-periodate in aqueous sodium hydroxide and with H_2O_2 in aqueous sodium carbonate. The resulting mixture of aryl carboxylic acids is treated with diazomethane in methanol-ether, and the major esters thus formed are measured by quantitative gas-liquid chromatography (GLC). Their relative amounts are indicative of the type of lignin originally present.

As the presence of lignin in non-vascular plants, notably the taxon Bryophyta, is still a matter of dispute (for a recent survey, see Ref. 2), we decided to investigate some mosses (Musci) and liverworts (Hepaticae) by this method. The present paper is restricted to the presentation of the results from the oxidative degradation of stalks of the moss *Polytrichum commune* Hedw.

Gas-liquid chromatography of the methyl esters obtained on oxidative degradation of preextracted *Polytrichum commune* meal (200 mg) indicated the presence of methyl veratrate (*1*) and dimethyl isohemipate (*2*), later confirmed by mass spectrometry (MS). Esters *1* and *2* have also been found to be the dominant members of the mononuclear ester fractions resulting from the oxidative degradation of a number of conifers [1,3] and ferns.[3]



1 R = H
2 R = $COOCH_3$

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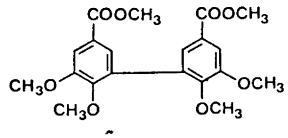
The composition of the binuclear ester fraction, as roughly defined by the volatility properties of its members in GLC analysis, was completely different from the composition of corresponding fractions obtained from guaiacyl

* Part XIII of the series "Gas Chromatographic Analysis of Lignin Oxidation Products". Part XII, see Ref. 13.

lignins (the lignins of most gymnosperms and Pteridophyta). Dimethyl 2′,5,6-trimethoxydiphenylether-3,4′-dicarboxylate (*3*) and dimethyl 5,5′-dehydrodiveratrate (*4*), the two major binuclear esters obtained from guaiacyl lignins, could not be detected among the degradation products of *Polytrichum commune* Hedw.



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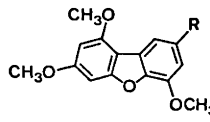
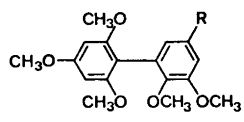
Instead, two unknown compounds, termed *A* and *B*, dominated the binuclear ester fraction from the moss. The mass spectra of *A* (molecular ion at $m/e = 316$, base peak) and of *B* (molecular ion at $m/e = 344$, base peak) were rather uninformative concerning the basic skeleton of these compounds. High resolution mass spectrometry indicated an elemental composition of $C_{17}H_{16}O_6$ for *A* and $C_{19}H_{20}O_6$ for *B*.

In order to obtain *A* and *B* in milligram quantities for NMR analysis, a total of 7 g of moss meal was oxidatively degraded. Separation of the products by preparative thin layer chromatography (TLC) yielded 15 mg of *A*, colourless crystals of m.p. 196 – 198°, and 19 mg of *B*, colourless crystals of m.p. 84.5 – 85.5°. The NMR spectrum of *A* revealed the presence of four methoxyl groups and four deshielded protons, coupled in pairs with coupling constants corresponding to *m*-positioned aromatic protons. The shift of the strongly deshielded proton at δ 8.27 ppm was indicative of in-plane deshielding by a neighbouring ring (*cf.* Ref. 4). On the basis of this spectroscopic evidence, structure *5*, methyl 4,7,9-trimethoxy-2-dibenzofurancarboxylate, was assigned to *A*. This assignment was confirmed by synthesis of *5 via* the biphenyls *8*, *9*, *10*, and *11*, and the dibenzofuran *7*.



5 R = $COOCH_3$
6 R = $CH_2CH_2COOCH_3$
7 R = CH_3



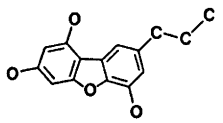
8 R = $COOH$
9 R = $COOCH_3$
10 R = CH_2OH
11 R = CH_3

The NMR spectrum of *B* indicated the same ring system as in *A*, but with the methoxycarbonyl substituent replaced by a 2-methoxycarbonylethyl side chain. This was supported by the prominent M – 73 fragment (CH_2-$COOCH_3$) in the mass spectrum of *B*. Furthermore, *B* was found to yield *A* on oxidative degradation. It thus could be concluded that *B* is represented by structure *6*, methyl 3-(4,7,9-trimethoxy-2-dibenzofuranyl)-propanoate. It seems very likely that, on oxidation of *Polytrichum commune* meal, *A* is formed *via B* or from a parent structure common to *A* and *B*.

It should be noted that this method for oxidative degradation originally had been optimized for the characterization of lignins in vascular plants. The reason why *B* is not completely oxidized to *A* in the experiments with moss meal must be due to an insufficiency of oxidant. This insufficiency may be caused by the comparatively large amounts of organic material dissolved by the NaOH-Na_2S pretreatment of the moss meal.

Since in the method used, methylation of phenolic hydroxyl groups precedes the oxidative degradation, nothing could be ascertained about the number of methylated phenolic hydroxyl groups originally present in the parent structures of type *12*. In a degradation experiment with *Polytrichum commune* meal, an answer to this question was found by using hexadeuterio-dimethyl sulfate in the methylation step. Mass spectra of deuterated components corresponding to *5* and *6* demonstrated the incorporation of three trideuterio-methyl groups (M + 9) per molecule. Thus all the methoxyl groups of *A* and *B* are derived from non-methylated phenolic hydroxyl groups originally present in parent structures of type *12*. By the same technique, it was furthermore demonstrated that the esters *1* and *2* are derived from non-methylated 4-alkyl and 3,5-dialkyl pyrocatechol units, respectively, rather than from the corresponding units of guaiacyl type as in guaiacyl and guaiacyl-syringyl lignins. The demethylation of methoxyl groups by the pretreatment with NaOH-Na_2S at 170° is comparatively slow and can be neglected.[5]

When *Polytrichum commune* meal was heated with NaOH-Na_2S, methylated with dimethyl



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sulfate, and treated with diazomethane, omitting the oxidation steps, compound *B* (*6*) could be detected while *A* (*5*) was absent. On the other hand, neither of them was found to be formed on mild alkaline hydrolysis of the plant material with aqueous KOH at 25°, or by boiling with 0.2 M HCl in dioxane-water (9:1, "acidolysis" [6]). To rule out the possibility of the precursor of *B* being an artefact formed from phloroglucinol and 4-alkylpyrocatechol structures eventually present, 4-methylcatechol and phloroglucinol were heated with aqueous NaOH-Na_2S at 170° and the reaction product methylated. No formation of *7* could be observed, however.

The biogenesis of the dibenzofuran structures yielding *A* and *B* remains to be properly explained. Obviously, one part of structure *6* is a phenylpropane, probably formed *via* a shikimic acid pathway. The substitution pattern of the other moiety is that of phloroglucinol and indicates formation by head–to-tail condensation of acetate units followed by cyclisation.

From the experiments described above it can thus be concluded that the moss *Polytrichum commune* Hedw. contains 3-(2-dibenzofuranyl)-propyl structures with oxygen functions in the 4-, 7-, and 9- positions (*12*). These entities are not connected with the bulk cell wall material by ester linkages. *Polytrichum commune* does not contain lignin, although structures partially derived from C_6C_3-units are present. This finding invalidates the postulation [7] that *Polytrichum commune* has a lignin of the *p*-hydroxyphenyl type.

EXPERIMENTAL

Plant material. Upper parts of stalks of *Polytrichum commune* Hedw., as long as covered with green leaves, were collected in Halland (southern Sweden). After removal of the leaves and fixation in ethanol, the stalks were ground and the resulting meal extracted and dried as described.[1]

Treatment of the moss meal with NaOH-Na_2S, methylation and oxidation. See Ref. 1.

GLC. See Ref. 1. *Retention times* (SE 30, relative to *4*, $t=240°$): *5*, 0.92; *6*, 1.43; *7*, 0.40; *9*, 0.60; *11*, 0.24.

MS. Identification: AEI MS 20. *High resolution:* AEI MS 902. *Electron energy:* 70 eV. *Source:* 200°. *Intensity relative to base peak (rel. int.):* ≥ 5 %. *Lower mass limit:* $m/e=100$.

NMR. 60 MHz, in $CDCl_3$, tetramethylsilane as internal standard, δ-values (ppm).

TLC. Silica gel HF 254, Merck. R_F*-values* (acetone-hexane 1:2): *1*, 0.29; *A*, 0.35; *B*, 0.25.

Isolation of compounds A *and* B. For this purpose, 7 g of preextracted moss meal was used. The permanganate oxidation was carried out in 7 batches. Appreciable amounts of MnO_2 precipitated during this reaction. The combined products were treated with H_2O_2 and then with diazomethane as described.[1] The resulting viscous oil (0.5 g) was chromatographed on preparative TLC plates (acetone-hexane 1:2). A major fraction contained compounds *1*, *A* and *B*. These components were separated by repeated TLC on analytical plates (toluene, acetone-hexane 1:2).

Compound *A*, *methyl 4,7,9-trimethoxy-2-dibenzofurancarboxylate* (5), colourless crystals (15 mg), m.p. 196–198° (ethyl acetate-hexane). (Found: C 64.67; H 5.16. Calc. for $C_{17}H_{16}O_6$ (316.32): C 64.55; H 5.10). A mixed m.p. with synthetic compound *5* showed no depression. *A* and synthetic *5* gave identical mass and NMR spectra. *NMR* (3 %): δ 3.86 (3) s, OCH_3; 3.96 (3) s, OCH_3; 4.00 (3) s, OCH_3; 4.07 (3) s, OCH_3; 6.42 (1) d, H_8; 6.71 (1) d, H_6; 7.56 (1) d, H_3; 8.27 (1) d, H_1. $J_{1,3}=1.5$ Hz. $J_{6,8}=1.9$ Hz. *MS. m/e, rel. int.*: 316, 100; 301, 11; 285, 22; 273, 19; 258, 11; 257, 9; 228, 5; 227, 8; 212, 10; 199, 5; 158, 11; 143, 10; 142.5, 24; 106, 11. *Exact mass of the molecular ion.* Found: 316.0936. Calc. for $C_{17}H_{16}O_6$: 316.0947.

Compound *B*, *methyl 3-(4,7,9-trimethoxy-2-dibenzofuranyl)-propanoate* (6), 19 mg, colourless needles, m.p. 84.5–85.5° (ethyl acetate). (Found: C 66.12; H 5.88. Calc. for $C_{19}H_{20}O_6$ (344.37): C 66.27; H 5.85). *NMR* (5 %): δ *ca.* 2.87 (4) m, CH_2CH_2; 3.68 (3) s, OCH_3; 3.85 (3) s, OCH_3; 3.98 (3) s, OCH_3; 4.01 (3) s, OCH_3; 6.35 (1) d, H_8; 6.69 (2) m, H_6 and H_3; 7.41 (1) broad d, H_1. $J_{6,8}=1.9$ Hz. *MS. m/e, rel. int.*: 344, 100; 316, 6; 313, 8; 301, 5; 285, 41; 284, 13; 271, 85; 270, 7; 256, 10; 241, 9; 227, 10; 226, 7; 213, 14; 167, 15; 149, 45; 142.5, 11. *Exact mass of the molecular ion.* Found: 344.1260. Calc. for $C_{19}H_{20}O_6$: 344.1260.

In addition to *A* and *B*, 37 mg of *methyl veratrate* (1), m.p. 56–57° (ether-hexane, Ref. 12, m.p. 58°) was isolated.

Oxidation of B. Dimethyl isohemipate (*2*, as internal standard) and *B* (4 mg) were oxidized with permanganate-periodate, the H_2O_2 step being omitted. After methylation of the products with diazomethane, analysis by GLC dem-

onstrated the formation of *A* in about 60 % yield and the presence of traces of *B* (identification by MS).

Acidolysis of moss meal. 200 mg of preextracted moss meal was acidolyzed.[6] The reaction product was methylated with diazomethane and analyzed by GLC. No *B* could be detected. Trimethylsilylation [Bis(trimethylsilyl)trifluoroacetamide, Pierce, in pyridine], followed by GLC, did not reveal the presence of binuclear degradation products.

Mild alkaline hydrolysis of moss meal. Moss meal (preextracted, 200 mg) was suspended in 25 ml of 2 M KOH under nitrogen for 26 h. After methylation with dimethyl sulfate, the solids were filtered off, the filtrate was neutralized, evaporated to dryness, and oxidized as usual. No *A* or *B* could be detected by GLC.

Treatment of moss meal with NaOH-Na_2S at 170°. The methylated (dimethyl sulfate) products were dissolved in methanol and methylated with ethereal diazomethane. GLC demonstrated the presence of minor amounts of *B*.

Treatment of phloroglucinol and 4-methylpyrocatechol with NaOH-Na_2S at 170°. A solution of 33 mg of 4-methylcatechol and 33 mg of phloroglucinol in 10 ml of aqueous NaOH-Na_2S was heated to 170° for 3 h. The solution was acidified, extracted with acetone-$CHCl_3$ (1:1), and the evaporation residue was methylated with dimethyl sulfate. *7* could not be detected by GLC.

Syntheses

2′,4′,5,6,6′-Pentamethoxybiphenyl-3-carboxylic acid (8). A mixture of bromophloroglucinol trimethyl ether[8] (3.8 g), methyl 5-bromoveratrate[9] (4.0 g), and copper bronze (10 g) was heated under nitrogen at 240° for 4 h. The ethyl acetate extract of the products was distilled *in vacuo* at 0.01 Torr. The fraction collected between 160 and 215° was saponified with KOH in MeOH-H_2O. From the alkaline solution, 1.1 g of *2,2′,4,4′,6,6′-hexamethoxybiphenyl*, m.p. 156° (Ref. 10, m.p. 156°), was isolated by extraction with ether. Neutralization afforded a mixture of 5,5′-dehydrodiveratric acid and *8*, from which *8* could be separated by soaking with acetone. Recrystallization from acetone yielded 480 mg of *8*, m.p. 238–239°. (Found: C 61.89; H 5.79. Calc. for $C_{18}H_{20}O_7$ (348.36): C 62.06; H 5.79).

Diazomethane and *8* (450 mg) gave *9* (440 mg), m.p. 145.5–146.5° (ethyl acetate-hexane). (Found: C 62.85; H 6.17. Calc. for $C_{19}H_{22}O_7$ (362.39): C 62.97; H 6.12). *NMR* (10 %): δ 3.65 (3) s, OCH_3; 3.68 (6) s, 2 OCH_3; 3.84 (6) s, 2 OCH_3; 3.92 (3) s, OCH_3; 6.19 (2) s, $H_{3'}$ and $H_{5'}$; 7.48 (1) d, H_4; 7.55 (1) broadened d, H_2. $J_{2,4}$ = 2.0 Hz.

2,2′,3,4′,6′-Pentamethoxy-5-hydroxymethylbiphenyl (10). Reduction of *9* (390 mg) with $LiAlH_4$ (180 mg) in tetrahydrofuran (40 ml) gave *10* as a colourless oil. *NMR* (10 %): δ *ca.* 2.3 (1) broad s, OH; 3.57 (3) s, OCH_3; 3.66 (6) s, 2 OCH_3; 3.83 (6) s, 2 OCH_3; 4.57 (2) s, $ArCH_2$; 6.18 (2) s, $H_{3'}$ and $H_{5'}$; 6.67 (1) d, H_6; 6.98 (1) d, H_4. $J_{4,6}$ = 2.0 Hz.

2,2′,3,4′,6′-Pentamethoxy-5-methylbiphenyl (11). Hydrogenolysis of *10* (Pd, 10 % on charcoal, in ethanol, 1 atm H_2) gave 243 mg of *11*, m.p. 122–123.5° (methanol). (Found: C 67.71; H 7.00. Calc. for $C_{18}H_{22}O_5$ (318.38): C 67.91; H 6.97). *NMR* (10 %): δ 2.30 (3) s, $ArCH_3$; 3.55 (3) s, OCH_3; 3.68 (6) s, 2 OCH_3; 3.82 (6) s, 2 OCH_3; 6.18 (2) s, $H_{3'}$ and $H_{5'}$; 6.52 (1) m, H_6; 6.67 (1) broad d, H_4. $J_{4,6}$ = 1.8 Hz.

1,3,6-Trimethoxy-8-methyl-dibenzofuran (7). For the method, see Ref. 11. The solution of 145 mg *11* in 10 ml HBr (48 %) and 10 ml glacial acetic acid in a thick walled, evacuated glass ampoule was heated to 110° for 24 h. The contents were neutralized with 40 % KOH and the solution extracted with ether (4 × 40 ml), then washed with dithionite and dried. The methylated (diazomethane) product was purified by TLC (acetone-hexane 1:2). Crystallization from methanol yielded 61 mg of *7*, m.p. 108.5–110.5. (Found: C 70.44; H 5.98. Calc. for $C_{16}H_{16}O_4$ (272.31): C 70.57; H 5.92). *NMR* (10 %): δ 2.46 (3) s, $ArCH_3$; 3.83 (3) s, OCH_3; 3.97 (3) s, OCH_3; 4.02 (3) s, OCH_3; 6.35 (1) d, H_2; 6.68 (1) m, H_7; 6.71 (1) d, H_4; 7.40 (1) m, H_9. $J_{2,4}$ = 1.9 Hz.

Methyl 4,7,9-trimethoxy-2-dibenzofurancarboxylate (5). 40 mg of *7* was oxidized, with omission of the H_2O_2 step. After methylation with CH_2N_2, 14 mg of *5*, m.p. 195–197°, were obtained (ethyl acetate). (Found: C 64.09; H 5.13. Calc. for $C_{17}H_{16}O_6$ (316.32): C 64.55; H 5.10).

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