Two Dibenzo-furans 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-Na2S for 3 h. The dissolved material was methylated with dimethyl sulfate, oxidized with KMnO4-NaIO4 in aqueous sodium hydroxide and with H2O2 in aqueous sodium carbonate. The crude mixture of products was treated with diazomethane. In addition to methyl veratratre and dimethyl isohemipate, two new esters were isolated and identified as methyl 4,7,9-trimethoxy-2-dibenzo-furan carboxylate and methyl 3-(4,7,9-trimethoxy-2-dibenzo-2-furyl)-propanoate. Neither dimethyl 2',5,6-trimethoxy-diphenylether-3,4'-dicarboxylate nor dimethyl 5,5'-dehydroveratratoate, 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. 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-Na2S for 3 h at 170°, followed by methylation with dimethyl sulfate, and oxidation with permanganate-periodate in aqueous sodium hydroxide and with H2O2 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 (Muscis) 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 pre-extracted Polytrichum commune meal (200 mg) indicated the presence of methyl veratratre (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 conifers1 and ferns.2

\[ \text{COOH}_3 \]
\[ \text{CH}_3 \text{O} \]
\[ \text{OCH}_3 \]

1. R = H
2. R + COOH3

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
lignins (the lignins of most gymnosperms and Pteridophyta). Dimethyl 2',5,6-trimethoxydiphenylether-3,4'-dicarboxylate (3) and dimethyl 5,5'-dehydrodigeratrate (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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\begin{array}{c}
\text{COOCH}_3 \\
\text{CH}_3 \\
\text{OCH}_3 \\
\end{array}
\quad
\begin{array}{c}
\text{COOCH}_3 \\
\text{CH}_3 \\
\text{OCH}_3 \\
\end{array}
\]

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_{11}H_{16}O_4 for A and C_{12}H_{20}O_4 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 methoxy 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-dibenzo-furancarboxylate, was assigned to A. This assignment was confirmed by synthesis of δ via

\[
\begin{array}{c}
\text{CH}_3 \\
\text{OCH}_3 \\
\end{array}
\quad
\begin{array}{c}
\text{CH}_3 \\
\text{OCH}_3 \\
\end{array}
\]

The NMR spectrum of B indicated the same ring system as in A, but with the methoxy-carbonyl substituent replaced by a 2-methoxy-carbonyl-ethyl side chain. This was supported by the prominent M — 73 fragment (CH_2COOCH_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-dibenzo-furanyl)-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 δ and 6 demonstrated the incorporation of three deuterio-methyl groups (M + 9) per molecule. Thus all the methoxy 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 methoxy groups by the pretreatment with NaOH-Na_2S at 170° is comparatively slow and can be neglected.

When *Polytrichum commune* meal was heated with NaOH-Na_2S, methylated with dimethyl...
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, "acidiolysis") 

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-Na2S 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-dibenzofuran-yl)-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 C4-C units are present. This finding invalidates the postulation 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.

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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 pre-extracted moss meal was acidolized. The reaction product was methanolized with diazo-methane and analyzed by GLC. No B could be detected. Trimethylsilylation [Bis(trimethyl-silyl)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-Na₂S 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-Na₂S at 170°. A solution of 33 mg of 4-methylpyrocatechol and 33 mg of phloroglucinol in 10 ml of aqueous NaOH-Na₂S was heated to 170° for 3 h. The solution was acidified, extracted with acetone-CHCl₃ (1:1), and the evaporation residue was methylated with dimethyl sulfate. C could not be detected by GLC.

Syntheses

2',4',5',6'-Pentamethoxybiphenyl-3-carboxylic acid (8). A mixture of bromophloroglucinol trimethyl ether * (3.8 g), methyl 5-bromoverratrate * (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-H₂O. From the alkaline solution, 1.1 g of 2,2',4,4',5,6'-hexamethoxybiphenyl, m.p. 156° (Ref. 10, m.p. 156°), was isolated by extraction with ether. Neutralization afforded a mixture of 5,5'-dehydroverratic acid and 8, from which 8 could be separated by boiling with acetone. Recrystallization from acetone yielded 480 mg of 3, m.p. 235°-236°. (Found: C 61.89; H 5.73. Calcd. for C₈H₁₄O₃ (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. Calcd. for C₉H₁₄O₃ (362.39): C 62.97; H 6.12. NMR (10 %): δ 3.65 (3) s, OCH₃; 3.68 (6) s, 2 OCH₃; 3.84 (6) s, 2 OCH₃; 3.92 (3) s, OCH₃; 6.19 (2) s, H₃ and H₄; 7.48 (1) d, H₅; 7.55 (1) broadened d, H₂. J₄,₅ = 2.0 Hz.)

2',3',5',6'-Pentamethoxy-5-hydroxymethylbiphenyl (10). Reduction of 9 (390 mg) with LiAlH₄ (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, OCH₃; 3.66 (6) s, 2 OCH₃; 3.83 (6) s, 2 OCH₃; 4.57 (2) s, ArCH₂; 6.18 (2) s, H₃ and H₄; 6.67 (1) d, H₂; 6.98 (1) d, H₄. J₃,₄ = 2.0 Hz.

REFERENCES


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