Phytochemistry, 1968, Vol. 7, pp. 1373 to 1377. Pergamon Press. Printed in England

THE LIGNIN OF *SPHAGNUM*

D. E. BLAND, A. LOGAN and M. MENSHUN

Division of Forest Products, C.S.I.R.O., South Melbourne, 3205, Australia

and

S. STERNHELL

Department of Organic Chemistry, University of Sydney, 2006, Australia

(*Received* 22 *November* 1967, *in revised form* 21 *February* 1968)

Abstract—The existence of lignin in *Sphagnum* moss has been investigated. A vibratory ball milling technique gave a low yield (0·9 per cent) of a presumed lignin. This substance was low in methoxyl, aromatic hydrogen and ether oxygen. It gave an acetate containing 36·5 per cent $COCH_3$ and nitrobenzene oxidation of this acetate gave small amounts of *p*-hydroxybenzaldehyde, vanillin and syringaldehyde. Permanganate oxidation of the acetate gave *p*-hydroxybenzoic acid and traces of other acids. These properties show that the substance is basically a highly condensed C—C linked polymer of methoxyl-free units with some methoxylated ether-linked units. This substance may therefore reasonably be classified as a lignin.

INTRODUCTION

IN INVESTIGATIONS of the lignins of different Phyla by Kratzl and Eibl,[1] vanillin was not detected in the oxidation products of *Sphagnum*. A sulphuric acid determination of lignin left a residue which they therefore considered to be "pseudolignin". The aromatic content of the cell wall of *Sphagnum* was ascribed by Manskaya[2] to the presence of a phenolglycoside and only small amounts of aromatic aldehydes, rather than to lignification.

Recently the cell wall pigments of various species of *Sphagnum* has been the subject of several detailed investigations. Bendz, Martensson and Nilsson[3] by the use of methanol-hydrogen chloride extraction, followed by chromatography on cellulose powder were able to separate three distinct pigments from *Sphagnum*. In their studies of moss pigments Nilsson and Tottmar[4] distinguished red and brown cell wall polymers which yielded *p*-hydroxybenzaldehyde and vanillin. Nilsson[5] also recognized a violet pigment and found considerable evidence for the anthocyanidin character of its chromophore.

Two species of *Sphagnum* were found by Lindberg and Theander[6] to give about 5 per cent Klason lignin which contained about 1 per cent methoxyl. Nitrobenzene oxidation of the moss, which had been extracted with water and an organic solvent, yielded 0·6 per cent aldehydes of which 90 per cent was *p*-hydroxybenzaldehyde; small amounts of vanillin and syringaldehyde were also present. They concluded that *Sphagnum* contained a lignin built chiefly of *p*-hydroxyphenyl units.

---

[1] K. KRATZL and J. EIBL, *Mitt. Öesterr. Ges Holzforsch.* 3, 77 (1951).

[2] S. M. MANSKAYA, in *Biochem. Wood IV. Internat. Kong. Biochem. Wien* (1958), p. 215. Pergamon (1959).

[3] G. BENDZ, O. MARTENSSON and E. NILSSON, *Ark. Kem.* **25**, 215 (1966).

[4] E. NILSSON and O. TOTTMAR, *Acta. Chem. Scand.* **21**, 1558 (1967).

[5] E. NILSSON, *Acta. Chem. Scand.* **21**, 1942 (1967).

[6] B. LINDBERG and O. THEANDER, *Acta. Chem. Scand.* **6**, 311 (1952).

Farmer[7] found that the cell walls in *Sphagnum* were stained by diazotized sulphanilic acid-sodium carbonate treatment. It should be pointed out that this reagent while giving a positive reaction with lignin does so with phenolic substances generally. Farmer found that dilute acid hydrolysis of *Sphagnum* gave a product which it was necessary to treat by further cautious hydrolysis to remove carbohydrates and leave a slightly altered lignin which yielded 5·5 per cent *p*-hydroxybenzaldehyde. It appears from this that acid hydrolysis liberated a lignin carbohydrate compound.

By methylation and permanganate oxidation Farmer and Morrison[8] obtained a yield of 4 per cent anisic acid from *Sphagnum*. Nitrobenzene oxidation gave up to 0·8 per cent *p*-hydroxybenzaldehyde. Attempts to prepare ethanol and dioxan lignins from *Sphagnum* have not been successful, but were found by Farmer and Morrison to give a black, altered product.

Ball milling of wood followed by extraction with inert solvents has been demonstrated to give an unaltered lignin. Brownell[9] demonstrated that dry ball milling could successfully be employed. Bland and Menshun[10] used dry vibratory ball milling followed by acetone extraction for the isolation of lignin from *E. regnans* wood. We have attempted to isolate lignin from *Sphagnum* by this technique thus avoiding acids or alkalis in order to ascertain if this procedure yielded a product which the methods of lignin chemistry would give results supporting its classification as a lignin.

It might be expected that material extracted in this way would be a lignin and not the cell wall pigments isolated by the use of acids or alkalis. On the other hand in view of the known differences between lignins of different classes of the plant kingdom close resemblance to wood lignin of angiosperms or gymnosperms would seem unlikely.

RESULTS AND DISCUSSION

Analysis of ethanol-extracted *Sphagnum* showed 0·63 per cent methoxyl, 0·57 per cent nitrogen and 7·96 per cent pentosan. Application of a sulphuric acid lignin determination gave a residue of 34·5 per cent. This contained 1·60 per cent methoxyl, 0·89 per cent nitrogen and 1·27 per cent ash.

The ethanol extract contained traces of *p*-hydroxybenzoic and vanillic acids. Alkaline hydrolysis of the ethanol-extracted *Sphagnum* gave small amounts of *p*-hydroxybenzoic, *p*-coumaric and ferulic acids. Nitrobenzene oxidation of the ethanol-extracted material gave *p*-hydroxybenzaldehyde 0·09 per cent, vanillin 0·07 per cent and syringaldehyde 0·04 per cent.

Dioxan extraction of the moss after vibratory ball milling gave a considerable yield of dioxan-soluble material but as this was almost completely insoluble in 1,2-dichloroethane-ethanol (2:1) purification via this solvent was not possible. Direct acetylation of the total dioxan-soluble material from the moss gave a yield of 7·88 per cent. The analysis of this material however showed a hydrogen content greatly exceeding that of any known lignin. Although it appeared to give a homogeneous solution of approximately 15 per cent concentration in CD_3SOCD_3, its proton magnetic resonance spectrum showed only an extremely broad signal extending from $\delta = 0$ to 6 ppm. This indicates that either the material was not in true solution or that it contained paramagnetic impurities.

---

[7] V. C. FARMER, *Research* **6**, 475 (1953).
[8] V. C. FARMER and R. I. MORRISON, *Geochim. Cosmochim. Acta* **28**, 1537 (1964).
[9] H. H BROWNELL, *Tappi* **48**, 513 (1965).
[10] D. E. BLAND and M. MENSHUN, *Appita* **21**, 17 (1967).

Extraction of milled moss with 80 per cent acetone gave a considerable yield of material, some of which dissolved in dichloroethane-ethanol leaving a residue of 4·7 per cent. The soluble fraction was precipitated into diethyl ether to give a yield of 0·9 per cent of ether-insoluble material.

The analysis of this material C, 59·72; H, 6·08; O, 32·51; N, 0·69; OCH_3, 3·26 corresponds to $C_9H_{10.43}O_{3.59}(OCH_3)_{0.20}$ (corrected for protein). It was not sufficiently soluble to permit determination of its PMR spectrum. Acetylation of it gave a product of composition C, 61·53; H, 5·79; O, 30·7; N, 0·83; OCH_3, 2·91; $COCH_3$, 36·5. Despite its high acetyl content the yield on acetylation was only 108 per cent on a weight basis, indicating considerable loss. The analysis corresponds to $C_9H_{7.43}O_{2.62}(OCH_3)_{0.27}(COCH_3)_{2.46}$ (corrected for protein) which in turn corresponds to a composition of $C_9H_{7.43}O_{0.16}(OCH_3)_{0.27}(OH)_{2.46}$ for the presumed lignin. Comparison of this with the composition of the original ether-insoluble material shows an increase in methoxyl, a striking decrease in oxygen and some decrease in hydrogen. Considered together with the low yield of acetate the data show the original material to have been contaminated with carbohydrate which was removed during acetylation. The original material contained 1·3 per cent pentosan, the acetylated product 0·6 per cent. The 0·16 atom of oxygen per C_9 unit could be ether oxygen, carbonyl oxygen or possibly carboxyl oxygen, but it could also belong to carbohydrate remaining in the acetylated product. In any case it is clear that the amount of ether oxygen in the *Sphagnum* "lignin" is very low.

Infrared spectra confirmed the possibility of contamination by carbohydrate as the spectrum of the original ether insoluble material was very diffuse, showing a broad band centred at about 1050 cm^{-1}, which is also present in the curves of Farmer and Morrison.[8] There were also definite bands at about 1740 cm^{-1} and 1660 cm^{-1} corresponding to bands observed in *Sphagnum* and *Sphagnum* peat lignin by Farmer and Morrison[8] and in artificial lignin prepared from *p*-coumaric acid by Bland and Logan.[11]

The u.v. absorption curve lacked any definite features only a weak band at 280 nm being barely distinguishable. This is similar to the result of Farmer and Morrison for alkaline extract of *Sphagnum*. The spectrum of the acetylated product showed more definite peaks, the acetyl bands at 1735 cm^{-1} and 1220 cm^{-1} being the dominating features and the band at 1050 cm^{-1} being greatly reduced. A small residual peak at 3370 cm^{-1} indicated that some free hydroxyl groups remained. Nitrobenzene oxidation of the ether-insoluble material gave less than 0·1 per cent of *p*-hydroxybenzaldehyde, 0·9 per cent vanillin and 0·9 per cent syringaldehyde, whereas the acetylated product gave yields of 0·1, 1·1 and 1·1 per cent respectively. This again confirms the higher purity of the acetylated product.

Permanganate oxidation of the acetylated product gave *p*-hydroxybenzoic acid (1·5 per cent) small amounts (0·1 per cent) of vanillic and syringic acids and traces of 4-hydroxyisophthalic and 5-carboxy-vanillic acids. The yield of *p*-hydroxybenzoic acid being higher than the yield of *p*-hydroxybenzaldehyde shows the presence of uncondensed methoxyl-free units in which the α carbon atom of the propane side chain is not linked to an oxygen atom. Units in which this type of linkage occurs are known to give the corresponding aromatic aldehyde by nitrobenzene oxidation (Wacek and Kratzl[12]).

The ether-insoluble material was not sufficiently soluble in any suitable solvents to permit examination of it by proton magnetic resonance spectroscopy. However the acetylated

---

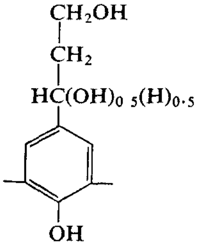
[11] D. E. Bland and A. F. Logan, *Biochem. J.* **95**, 315 (1965).

[12] A. V. Wacek and K. Kratzl, *Öesterr. Chemiker-Ztg* **48**, 36 (1947). Cited in F. E. Brauns, *Chemistry of Lignin* p. 557, Academic Press (1952).

product was sufficiently soluble to permit this. The procedure was essentially that employed by Bland and Sternhell.[13]

The PMR spectrum differed from the spectra of acetylated wood lignins[13] in not showing strong, discrete signals associated with either aromatic protons (i.e. in the region $\delta = 6{\cdot}5$–$7{\cdot}7$ ppm) or with methoxyl groups (i.e. in the region $\delta = 3{\cdot}5$–$4{\cdot}2$ ppm). A conspicuous feature in the region $\delta = 1{\cdot}8$–$2{\cdot}2$ ppm must be assigned to the acetyl methyl groups, while signals near 0·9 and 1·25 ppm are thought to be due to a lipid-type impurity which accompanied the lignin through the purification processes.

All the evidence shows *Sphagnum* "lignin" to be a highly condensed polymer consisting mainly of methoxyl-free units. As it contains very little ether oxygen it must therefore be mainly a C—C linked polymer. This accounts for its resistance to nitrobenzene oxidation and its failure to give an ethanol lignin (Ref. 8). Its resemblance to the artificial lignin prepared from *p*-coumaric acid by Bland and Logan[11] both in its i.r. spectrum and in its failure to give any considerable yield of *p*-hydroxybenzaldehyde by nitrobenzene oxidation appears to be very significant. The substance here investigated was isolated as a lignin insoluble in ether and is probably distinct from lower molecular weight cell wall pigments and phenolic substances isolated by other workers from various species of *Sphagnum*.[3–5] Although wood lignin is a polymer linked by both C—C and C—O—C bonds, this material appears to be basically a C—C linked polymer of units of structure (I) and may reasonably be classed as a lignin.



(I)

The presence of vanillin and syringaldehyde and the corresponding acids in the oxidation products suggests that some of the methoxylated units occur as ether-linked groups of the polymer. The high degree of condensation of methoxyl-free units is in accordance with the properties of these units observed in artificial lignin and appears to be a result of the readiness with which these units condense rather than to any peculiarity of the enzyme system of *Sphagnum*. Comparison of the characteristics of this *Sphagnum* lignin with lignins of plants belonging to other classes is in accordance with the concept that all lignins are *p*-hydroxyphenyl propane polymers. The different degrees of condensation in different lignins appears to be determined by the degree of substitution of the phenyl propane units. These conclusions are not really in disagreement with Kratzl and Eibl[1] but they do extend their concept of lignin. The methoxyl free units in wood lignin are usually present in the condensed form, compression wood is exceptional in this respect.[14] The difficulty of obtaining the lignin free from carbohydrate indicates that most of it is combined, presumably with polysaccharide as suggested by Farmer.[7]

Freudenberg and Harkin[15] found the composition of *Sphagnum* lignin in terms of their

---

[13] D. E. BLAND and S. STERNHELL, *Australian J. Chem.* **18**, 401 (1965).

[14] D. E. BLAND, *Holzforschung* **15**, 102 (1961).

[15] K. FREUDENBERG and J. M. HARKIN, *Holzforschung* **18**, 166 (1964).

general formula for lignins to be $C_9\,H_{7\cdot96}\,O_2\,(H_2O)_{0\cdot90}\,(OCH_3)_{0\cdot25}$; expressed in the same way our result becomes $C_9\,H_{8\cdot65}\,O_2\,(H_2O)_{0\cdot62}\,(OCH_3)_{0\cdot27}$.

EXPERIMENTAL

Material

Sphagnum moss was air-dried, ground to pass 20 mesh and extracted with ethanol in a Soxhlet type extractor for 24 hr. It was analysed for pentosan, methoxyl and nitrogen. The lignin determination was carried out by the Tappi method modified by increasing both the time of standing with 72 per cent H_2SO_4 and the time of boiling with 3 per cent acid to 6 hr. The resulting lignin was analysed for ash, nitrogen and methoxyl.

Preparation and Acetylation of Lignin

Samples of oven-dry, ethanol extracted moss were milled in a vibratory ball mill for 48 hr. The milled moss was extracted by stirring with 90 per cent dioxan for 24 hr and an attempt made to obtain a pure lignin by dissolving the extracted material in dichloroethane-ethanol (2:1) and precipitating it in ether according to Bjorkman[16]; the yield was negligible. The entire dioxan-soluble material was then acetylated by being allowed to stand in solution in ten times its weight of pyridine-acetic anhydride (1:1) for 24 hr at 20°. The mixture was poured into a large volume of water and the acetylated material recovered by filtration, washing and drying under reduced pressure at 45°.

Further samples of lignin were prepared by extraction of the milled moss with 80 per cent acetone. The acetone-soluble material was treated with dichloroethane-ethanol and the soluble part precipitated into diethyl ether. Part of the ether precipitate was treated with the pyridine-acetic anhydride mixture and the acetylated product recovered as described above.

Oxidation of Lignin

Nitrobenzene oxidation was carried out on a semi-micro scale as used by Bland[17] and the permanganate oxidation as described by Bland and Logan.[11]

Proton Magnetic Resonance Spectra

The spectra were obtained for a 15 per cent solution in deuterochloroform by using a Varian A60 spectrometer.

Acknowledgements—We thank Dr. A. J. Michell for determination of and assistance with interpretation of i.r. spectra. The determinations of C, H, O, OCH_3 and $COCH_3$ were made by the Australian Microanalytical Service.

---

[16] A. Bjorkman, *Svensk Papperstid.* **59**, 477 (1956).
[17] D. E. Bland, *Biochem. J.* **75**, 195 (1960).