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ON THE OCCURRENCE OF LIGNIN OR POLYPHENOLS IN SOME MOSSES AND LIVERWORTS

M. ERICKSON and G. E. MIKSCHE

Department of Organic Chemistry, Chalmers University of Technology and University of Göteborg, Fack, S-402 20 Göteborg, Sweden and Organic Chemistry 2, Chemical Center, Box 740, S-220 07 Lund, Sweden

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Key Word Index—Bryophyta; mosses; liverworts; lignin; phenolic polymers.

Abstract—Six species of mosses (Musci) and two species of liverworts (Hepaticae) have been investigated for the presence of lignin by oxidative degradation. The results clearly demonstrate that these species are devoid of lignin, but contain other types of phenolic cell wall material.

INTRODUCTION

IT IS well known that some species of the taxon Bryophyta contain substances that in some respects are similar to lignin. A product that appeared to be lignin thioglycolic acid was isolated by Holmberg[1] from *Polytrichum commune* and *Marchantia polymorpha*. Klason lignin has apparently been obtained from a number of mosses, and a "milled wood lignin" was prepared by Freudenberg and Harkin[2] from *Polytrichum* and *Sphagnum*. All the "moss lignins" that have been isolated have had very low methoxyl contents.

Nitrobenzene oxidation of "lignins" isolated from mosses as well as direct oxidation of pre-extracted moss meal gives minor amounts of *p*-hydroxybenzaldehyde,[3–7] vanillin,[3–5,7] and syringaldehyde.[3,5] The formation of these aldehydes may be indicative of the presence of lignin. On nitrobenzene and permanganate oxidation *Sphagnum* yields *p*-hydroxybenzaldehyde[3–6] and *p*-hydroxybenzoic acid[5] (anisic acid, I, from a methylated sample.)[8] This has been attributed to the presence of a lignin built up predominantly of *p*-hydroxyphenyl units.[3,5] Support for this hypothesis has been provided by the results of Reznikov and Sorokina[6] who obtained *p*-hydroxyphenylpropane, 4-hydroxy-3-methoxyphenylpropane and 3-(4-hydroxy-3-methoxyphenyl)-propanol on reduction of *Sphagnum medium* with sodium in liquid ammonia. Nilsson and Tottmar[9] on cupric oxide oxidation of polymeric material from *Sphagnum nemoreum* obtained *p*-hydroxyphenyl as well as guaiacyl compounds.

However, contradictory evidence has been provided by Sarkanen and Latif[10] who established the absence of Hibbert ketones in the reaction mixture obtained on ethanolysis of

[1] HOLMBERG, B. (1934) *Ing. Vetenskaps Akad. Handl.* **131**.
[2] FREUDENBERG, K. and HARKIN, J. M. (1964) *Holzforschung* **18**, 166.
[3] LINDBERG, B. and THEANDER, O. (1952) *Acta Chem. Scand.* **6**, 311.
[4] FARMER, V. C. (1953) *Research* **6**, 475.
[5] BLAND, D. E., LOGAN, A., MENSHUN, M. and STERNHELL, S. (1968) *Phytochemistry* **7**, 1373.
[6] REZNIKOV, V. M. and SOROKINA, N. F. (1968) *Zh. Prikl. Chim.* **41**, 176.
[7] SIEGL, S. M. (1969) *Am. J. Botany* **56**, 175.
[8] FARMER, V. C. and MORRISON, R. I. (1964) *Geochim. Cosmochim. Acta* **28**, 1537.
[9] NILSSON, E. and TOTTMAR, O. (1967) *Acta Chem. Scand.* **21**, 1558.
[10] SARKANEN, K. V. and LATIF, A. M. unpubl. results cited by SARKANEN, K. V. and HERGERT, H. L. (1971) in *Lignins* (SARKANEN, K. V. and LUDWIG, C. H. eds.), p. 81, Wiley-Interscience, New York.

Rhytidiadelphus loerus. This must imply that this moss at least is essentially free from lignin.

A more selective procedure for the characterization of lignin in plant material has recently been described.[11] In this method (in the present paper referred to as "oxidative degradation") the lignin of the pre-extracted, finely ground plant material is solubilized by heating at 170° with aq. $NaOH–Na_2S$ for 3 hr. The dissolved material is methylated with dimethyl sulfate and oxidized first with permanganate/periodate in dilute aq. sodium hydroxide containing *tert*-butyl alcohol, and then with H_2O_2 at pH 9–10. The resulting mixture of aryl carboxylic acids is methylated (CH_2N_2 in ether/methanol) and the yields of the major esters formed are determined by GLC. Their relative amounts indicate the type of lignin present in the material.

TABLE 1. OXIDATIVE DEGRADATION OF MOSSES AND LIVERWORTS. YIELDS OF METHYL ESTERS

Species*	Yields (mg ester per g of plant material) 1	2	3	4	5	8†	11†	12†	Remarks
Musci									
Bryales									
Dicranum bergeri Bland.‡	0·2	5·8	0·8	?	—	—	1·8	—	Stalks
Leptobryum pyriforme (Hedw.) Wils.§	0·8	4·9	0·8	0·9	—	—	1·4	—	Seta (not ground)
Ptilium crista-castrensis (Hedw.) DNot.‖	0·3	0·5	1·5	0·2	1·0	10	—	—	Stalks
Pogonatum urnigerium									
(Hedw.) P. Beauv.**	0·2	7·7	1·3	1·3	—	—	15	1·1	Stalks
(Hedw.) P. Beauv.**	0·1	11·0	1·3	2·1	—	—	18	4·7	Seta
Polytrichum commune Hedw.**	0·2	7·8	1·5	2·2	—	—	19	4·5	Stalks
Sphagnales									
Sphagnum spec.††	21	1·7	2·0	1·5	—	—	—	—	Stalks
Hepaticae									
Plagiochila asplenoides (L.) Dum.‡‡	2·0	1·7	0·2	0·2	0·4	11	—		Stalks
Scapania undulata (L.) Dum‡‡	0·3	2·0	0·5	0·4	—	—	0·4	—	Stalks + leaves

* Musci according to Nyholm,[12] Hepaticae according to Arnell.[13]
** Order Polytrichales.
† Yields not corrected.
†† Pertains to section *palustria*. Probably *S. palustre* L.
‡ Order Dicranales.
‡‡ Order Jungermanniales acrogynae.
§ Order Eubryales.
‖ Order Hypnobryales.

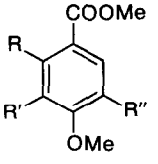
Lignin is an important structural component of higher plants. It is present in ferns and club-mosses, but the evidence for its presence in Bryophyta, which are among the earliest land plants, is conflicting. In view of these contradictory reports on the presence of lignin in mosses we decided to apply this method of oxidative degradation to some moss species. We also thought it to be of interest to investigate the possible presence of lignin in liverworts.

[11] ERICKSON, M., LARSSON, S. and MIKSCHE, G. E. (1973) *Acta Chem. Scand.* **27**, 127.
[12] NYHOLM, E. (1969) *Moss Flora of Fennoscandia* **2**, *Musci*. Vol. 1–5. Gleerups, Lund (1954–1965); Vol. 6, Natural Science Research Council, Stockholm.
[13] ARNELL, S. (1954) *Moss Flora of Fennoscandia* **1**, Hepaticae. Gleerups, Lund.

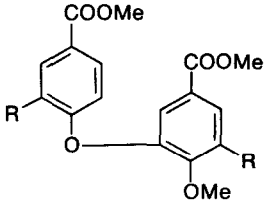
RESULTS AND DISCUSSION

Oxidative degradation of six mosses and two liverworts in each case yielded minor amounts of methyl veratrate(**2**), dimethyl isohemipate (**3**) and dimethyl metahemipate (**4**) (Table 1). These esters are the dominant components of the mononuclear ester fraction resulting from oxidative degradation of guaiacyl lignins (present in conifers and ferns).[14] Guaiacyl lignins in addition also give binuclear esters, mainly dimethyl 5,5′-dehydrodiveratrate (**9**) and dimethyl 2′,5,6-trimethoxydiphenylether-3,4′-dicarboxylate (**7**). It is generally accepted that lignification proceeds via coupling of phenoxy radicals.[15] Structures yielding **7** and **9** will thus have been formed by (6,6)- and (6,*O*)-coupling reactions, respectively.* None of esters **7** and **9** could, however, be detected among the methylated oxidation products from any of the investigated mosses and liverworts. This excludes the possible presence of a guaiacyl (or guaiacyl-syringyl) lignin.

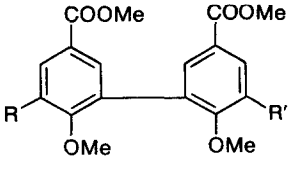
Further investigation showed that in fact the dimethoxylated esters **2**–**4** originate not from guaiacyl but from catechol structures. This was demonstrated by an experiment in which a sample of *Polytrichum commune* was methylated with hexadeuteriodimethyl sulfate prior to the permanganate/periodate oxidation.[16] All *O*-methyl groups introduced were trideuterated as shown by MS.



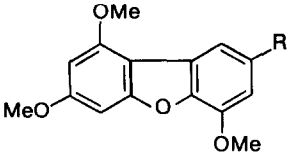
(1) R,R′,R″ = H
(2) R,R′ = H; R″ = OMe
(3) R = H; R′ = COOMe; R″ = OMe
(4) R = COOMe; R′ = H; R″ = OMe
(5) R,R′ = H; R″ = COOMe



(6) R = H
(7) R = OMe



(8) R,R′ = H
(9) R,R′ = OMe
(10) R = H; R′ = COOMe



(11) R = COOMe
(12) R = CH_2CH_2COOMe

No *p*-hydroxyphenyl lignin has been found in nature. However, oxidative degradation of an appropriate synthetic *p*-hydroxyphenyl lignin gave as major products the esters **1**, **5**, **6** and **8** (see Experimental). Of these methyl anisate (**1**) alone was present in the degradation products from all the plant samples examined, but, except for *Sphagnum Plagiochila* and *Ptilium crista-castrensis*, was present only in minute amounts (Table 1). Although

* For numbering of the carbon atoms see S. LARSSON and G. E. MIKSCHE (1971), *Acta Chem. Scand.* **25**, 647.

[14] ERICKSON, M. and MIKSCHE, G. E. (1974) *Holzforschung*. In press.

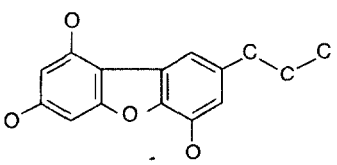
[15] HARKIN, J. M. (1967) in *Oxidative Coupling of Phenols* (TAYLOR, W. I. and BATTERSBY, A. R. ed.), Marcel Dekker, New York.

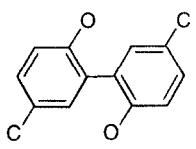
[16] ERICKSON, M. and MIKSCHE, G. E. (1974) *Acta Chem. Scand.* **28 1B**, 109.

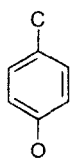
Sphagnum yielded appreciable amounts of methyl anisate (**1**), it gave no other degradation esters of the *p*-hydroxyphenyl type. Thus *Sphagnum* cannot contain a highly condensed polymer of *p*-hydroxyphenyl type as assumed[5] from earlier results. *Sphagnum magellanicum* has very recently been found[17] to give 4-hydroxy-β-carboxymethyl-cinnamic acid by solvent extraction.

Ptilium crista-castrensis and *Plagiochila asplenoides*, unlike the other species investigated, yielded dimethyl 5,5′-dehydrodianisate (**8**) as the predominant degradation product (Table 1). This does not, however, imply that these species contain true lignin. In fact, this is contradicted by the comparatively low yields of methyl anisate (**1**) and dimethyl 4-methoxyisophthalate (**5**) (cf. degradation of synthetic *p*-hydroxyphenyl lignin). Moreover, the important diphenyl ether type degradation ester **6** was totally missing from the degradation products in both cases.

The species that did not yield major amounts of degradation products derived from *p*-hydroxyphenyl nuclei, gave methyl 4,7,9-trimethoxy-2-dibenzofurancarboxylate (**11**) in varying amounts. In some cases it was accompanied by the related ester **12**. The isolation and structural determination of **11** and **12** from *Polytrichum commune* has been described elsewhere.[16] One half of the precursor of **11** and **12** possesses a phenylpropane skeleton. The other probably originates from acetate. This precursor structure, as well as the precursors of the other esters shown in Table 1, may be components of non-lignin cell wall polymers. The widespread presence of phenolic material in the cell walls of mosses and liverworts has been indicated by the investigations of Czapek.[18]


(A)


(B)


(C)

From the rather limited results presented here we can tentatively suggest three types of phenolic cell wall materials in bryophytes. The most frequent is that characterized by the occurrence of dibenzofuran structures (A). Two species, *Ptilium crista-castrensis* and *Plagiochila asplenoides*, contain a constituent rich in 2,2′-dihydroxy-4,4′-dialkylbiphenyl structures (2). Examples of types A and B are found both in Bryales and in Hepaticae. The phenolic cell wall material in *Sphagnum*[7] consists mainly of "uncondensed" *p*-hydroxyphenylalkyl structures (C). Materials of types A, B and C do not occur simultaneously. It may be concluded that in none of the Bryophyta species investigated was any lignin present.

EXPERIMENTAL

Plant material. Stalks of the mosses and liverworts were freed of leaves, "killed" with EtOH and ground. The resulting meal was extracted and subjected to oxidative degradation as previously described.[11] The mixture of aromatic esters was analyzed by GLC.

MS, Identification of compounds. Mass spectra of the methyl esters emerging from the GLC column were recorded by means of an AEI MS 20 mass spectrometer equipped with an electron impact source (70 eV). These spectra were compared with those of authentic samples.

[17] TUTSCHEK, R., RUDOLPH, H., WAGNER, P. H. and KREHER, R. (1973) *Biochem. Physiol. Pflanzen* **164**, 461.

[18] CZAPEK, F. (1899) *Flora* **86**, 361.

Preparation of DHP. The DHP (dehydrogenation polymer; 'synthetic lignin') was prepared by the method of "continuous" dehydrogenation.[19] Solutions of *p*-coumaryl alcohol (2·0 mMol) and peroxidase (8 mg) in 200 ml H_2O and 1·9 mMol H_2O_2 in 200 ml H_2O were added at an equal rate over 12 hr to a stirred solution of 2 mg peroxidase in 100 ml H_2O under N_2. After a further 6 hr, 25 ml 20% KCl solution was added and the precipitated DHP was centrifuged off, washed (H_2O) and dried (P_2O_5). Yield: 270 mg.

The DHP (200 mg) was oxidatively degraded. Ester yield (GLC), in mg per g DHP: **1**, 152; **5**, 119; **6**, 9; **8**, 74; **10**, 22. The yields of **6**, **8** and **10** have not been corrected by calibration curves. The ester **10**, which has not previously been observed, was identified by comparison of its mass spectrum with that of synthetic trimethyl 2,6'-dimethoxybiphenyl-3,3',5-tricarboxylate.

Synthesis of **10**. A small sample of **10** for MS analysis was prepared as follows. A mixture of methyl 3-bromoanisate (22 mg), dimethyl 4-methoxy-5-bromoisophthalate (130 mg) and Cu bronze (450 mg) was heated at 220° for 4 hr and then extracted with EtOAc. Evaporation of solvent and GLC fractionation of part of the remaining ester mixture yielded **10** as a colourless oil. Retention time of **10**: 1·09 (relative to **9**[11]). Precise mass determination of the molecular ion. Found: 388·115; $C_{20}H_{20}O_8$ requires 388·116. Mass spectrum (*m/e*, rel. int.): 388, 73; 373, 29; 357, 100; 355, 46; 341, 51; 329, 45; 325, 49; 311, 36; 297, 85; 179, 20; 163, 16; 149, 16. Only peaks with a rel. int. ≥ 10 at $m/e \geq 100$ have been included.

Acknowledgements—We are indebted for valuable suggestions to Prof. E. Adler, Göteborg, and to Dr. Brian Thomas, Lund, who also improved the English of this paper.

[19] ERICKSON, M. and MIKSCHE, G. E. (1972) *Acta Chem. Scand.* **26**, 3085.