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CHEMICAL PROPERTIES OF MILLED WOOD LIGNIN OF GRASSES

T. HIGUCHI, Y. ITO, M. SHIMADA and I. KAWAMURA

Laboratory of Wood Chemistry, Faculty of Agriculture, and Laboratory of Chemistry,
Faculty of Engineering, Gifu University, Kakamigahara, Gifu, Japan

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Abstract—Ester linkages between *p*-coumaric and ferulic acids and lignin have been confirmed in milled wood lignin (MWL) of grasses by analytical and spectrophotometric procedures. The MWL gave absorption bands at 315 nm and 280 nm, but the former absorption disappeared on saponification of the MWL with concomitant release of the *p*-coumaric and ferulic acids. An absorption band at 1730 cm^{-1} in the i.r. spectrum, which is attributed to the ester groups, also disappeared after saponification of the MWL of grasses. These results conclusively indicate that the *p*-hydroxyphenylpropane portion of grass lignin is mainly due to the *p*-coumaric acid esterified with lignin.

INTRODUCTION

IN PREVIOUS papers[1, 2] it has been shown that the *p*-hydroxyphenylpropane portion of grass lignins can be mainly ascribed to *p*-coumaric acid esterified with the lignins and that the participation of *p*-hydroxyphenylglycerol-β-aryl ether structure in the grass lignins is of minor importance.

In the present paper, using milled wood lignin (MWL), further confirmatory evidence supporting the results in the previous paper, and the chemical properties of the MWL of grasses, are described.

RESULT AND DISCUSSION

The yield of each aldehyde in the lignin oxidation products of grass MWL determined by gas–liquid chromatography is shown in Table 1. Except for *Miscanthus tinctorius*, the amount of total aldehydes was less than that produced from stem powder in both grasses and other monocotyledons, and the amounts of vanillin in *M. sacchariflоyus*, *Coix lachryma-jobi*, *Phragmites communis* and *Smilax china* decreased remarkably compared with those from plant stems. On the other hand, the decrease in amount of *p*-hydroxybenzaldehyde was rather less in all the plants tested. Exceptionally, in *Trachycarpus excelsa*, the amount of syringaldehyde decreased remarkably. Thus the molecular ratio of syringaldehyde and *p*-hydroxybenzaldehyde to vanillin increased generally compared with that from stem powder.

These facts indicate that during preparation of MWL some of the ester linkages between ferulic acid and grass lignins are split and/or a compound producing vanillin in the alkaline nitrobenzene oxidation, which is removed in preparation of MWL, is contained in some grasses.[3] However, the ester linkage between *p*-coumaric acid and grass lignins is stable and almost no release of *p*-coumaric acid occurs during preparation of MWL.

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[1] T. HIGUCHI and I. KAWAMURA, *Holzforschung* **20**, 16 (1966).

[2] T. HIGUCHI, Y. ITO and I. KAWAMURA, *Phytochem.* **6**, 875 (1967).

[3] J. E. STONE, M. J. BLUNDELL and K. G. TANNER, *Can. J. Chem.* **29**, 734 (1951).

TABLE 1. YIELD OF AROMATIC ALDEHYDES IN ALKALINE NITROBENZENE OXIDATION OF GRASS MWL

	Lignin oxidation products* (%)			Molecular ratio†	
	V	S	*p*-HB	S	*p*-HB
Miscanthus sacchariflorus	5·7	7·6	5·4	1·12	1·20
M. condensatus 1	11·2	9·8	5·3	0·70	0·59
M. condensatus 2	5·8	4·5	2·9	0·65	0·62
M. tinctorius	5·9	6·2	4·4	0·89	0·95
Coix lachryma-jobi	4·8	8·6	5·1	1·45	1·33
Phragmites communis	6·2	5·6	2·3	0·75	0·46
Arundo donax	7·4	9·9	3·3	1·11	0·56
Asparagus officinalis	9·0	6·9	0·4	0·64	0·06
Trachycarpus excelsa	5·2	16·3	0·7	2·63	0·17
Smilax china	3·5	10·6	‡	2·56	‡

* Per cent of lignin. V: vanillin; S: syringaldehyde; *p*-HB: *p*-hydroxybenzaldehyde.
† Vanillin = 1. ‡ Trace.

Table 2 shows the yield of each aldehyde obtained by nitrobenzene oxidation of the saponified MWL. In all the grasses and in some other monocotyledons the yield of *p*-hydroxybenzaldehyde and vanillin, especially the former, decreased considerably, and the percentage decrease of both was comparable to that found in the oxidation of saponified stem powder.[2]

TABLE 2. YIELD OF LIGNIN OXIDATION PRODUCTS FROM ALKALI EXTRACTED MWL

	Lignin oxidation products* (%)			Molecular ratio†	
	V	S	*p*-HB	S	*p*-HB
Miscantus sacchariflorus	5·3	7·6	2·0	1·22	0·49
M. condensatus 1	10·9	9·8	2·5	0·75	0·29
M. condensatus 2	5·5	4·5	0·9	0·68	0·20
M. tinctorius	5·7	6·2	1·6	0·89	0·37
Coix lachryma-jobi	4·7	8·6	1·7	1·53	0·45
Phragmites communis	6·1	5·5	1·3	0·76	0·27
Arundo donax	6·9	9·9	1·9	1·19	0·33
Asparagus officinalis	8·1	6·9	0·3	0·71	0·04
Trachycarpus excelsa	5·1	16·3	0·4	2·70	0·08
Smilax china	3·5	10·6	‡	2·56	‡

* Per cent of lignin. V: vanillin; S: syringaldehyde; *p*-HB: *p*-hydroxybenzaldehyde.
† Vanillin = 1. ‡ Trace.

As in the case of stem powder, *p*-coumaric and ferulic acids were isolated from the alkali extract of MWL and identified by paper chromatography and by mixed melting points.

Table 3 shows the amounts of *p*-coumaric and ferulic acids obtained by alkali extraction. As seen in the table, the amount of *p*-coumaric acid was 50–100 per cent larger than that from plant stems, whereas the amount of ferulic acid was about one-half or one-tenth of that obtained from stems. The calculated amounts of *p*-hydroxybenzaldehyde and vanillin from both acids correspond closely to the decreased amount of both aldehydes by alkali extraction, confirming the conclusion of the previous paper[2] (Table 4).

TABLE 3. YIELD OF *p*-COUMARIC AND FERULIC ACIDS OBTAINED BY ALKALI EXTRACTION

	Ferulic acid (%)*	*p*-Coumaric acid (%)*
Miscanthus sacchariflоyus	0·55	9·75
M. condensatus 1	0·98	8·66
M. condensatus 2	0·76	5·84
M. tinctorius	0·56	7·93
Coix lachryma-jobi	0·17	9·20
Phragmites communis	0·19	2·58
Arundo donax	0·08	3·81
Asparagus officinalis	†	0·30
Trachycarpus excelsa	†	0·15
Smilax china	†	†

* Per cent of lignin.
† Trace.

TABLE 4. RELATION BETWEEN THE DECREASED AMOUNT OF *p*-HYDROXYBENZALDEHYDE AND VANILLIN AND THE CALCULATED AMOUNT OF BOTH ALDEHYDES FROM *p*-COUMARIC AND FERULIC ACIDS

	Decreased amount (%)*		Calculated amount (%)*	
	V	*p*-HB	V	*p*-HB
Miscanthus sacchariflоyus	0·41	3·40	0·17	2·90
M. condensatus 1	0·32	2·81	0·31	2·58
M. condensatus 2	0·40	2·00	0·24	1·74
M. tinctorius	0·15	2·83	0·18	2·36
Coix lachryma-jobi	0·13	3·50	0·05	2·74
Phragmites communis	0·10	1·03	0·06	0·77
Arundo donax	0·50	1·40	0·03	1·14
Asparagus officinalis	0·90	0·10	†	0·09
Trachycarpus excelsa	0·10	0·30	†	0·04
Smilax china	†	†	†	†

* Per cent of lignin. V: vanillin; *p*-HB: *p*-hydroxybenzaldehyde.
† Trace.

The occurrence of a much smaller amount of ferulic acid in the alkali extract of grass MWL might indicate that the ester linkage between ferulic acid and grass lignins has minor significance.

It has been shown that the u.v. spectrum of grass lignins gives a peak or a shoulder around at 315 nm and this absorption is rather specific for the grass lignins. Stevens and Nord[4] showed that Brauns native lignin of bagasse exhibits a plateau at 282–295 nm and a maximum at 315 nm and the maximum is shifted to 355 nm in 0·02 N sodium hydroxide. They explained that the shift is due to the presence of substituted *p*-hydroxyphenylketone groups. However, Smith[5] found that Brauns native lignin of sugar-cane gives an u.v. spectrum with a broad

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[4] G. DESTEVENS and F. F. NORD, *J. Am. Chem. Soc.* 73, 4622 (1951).
[5] D. C. C. SMITH, *J. Chem. Soc.* 2347 (1955).

distinctive maximum at 355 nm in *N* sodium hydroxide and the lignin solution contains a considerable amount of *p*-coumaric acid as shown by acidification and extraction with ether. Thus the distinctive absorption bands of sugar-cane lignin have been attributed to the presence of *p*-coumaric acid ester groups.

Figures 1–3 show the u.v. spectra of MWL and the alkali extracted MWL from both a grass and a non-grass species. As clearly seen from Fig. 1, MWL from *Phragmites communis* showed absorption peaks at 315 nm and 280 nm, but the former peak almost completely disappeared on alkali extraction. Such behaviour is consistent with Smith's results, and it seems reasonable to conclude that ester linkages between *p*-coumaric and probably ferulic acids and grass lignins generally occurs, and that the alkali-labile absorption at about 315 nm is due to this fact.

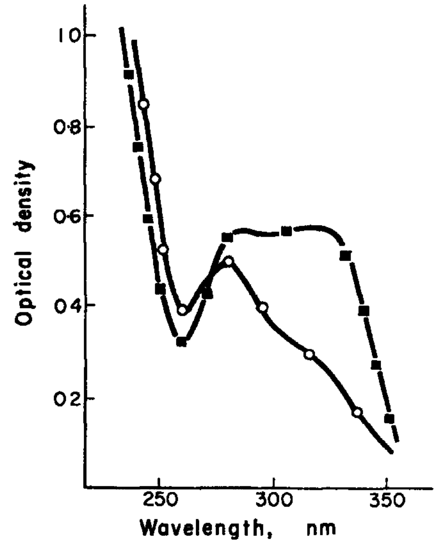

Fig. 1. U.V. spectra of MWL of *Phragmites communis*.
■—MWL; ○—alkali-extracted MWL; solvent—methylcellosolve.

As shown in Fig. 2 the u.v. spectrum of the MWL of *Smilax china* gave an absorption peak at 280 nm only, and the spectrum did not change after treatment with alkali, indicating that no ester groups are present in this MWL.

Figure 3 shows the u.v. spectra of MWL, alkali-extracted MWL and the ether-soluble fraction of the alkali extract of *P. communis* all in 0·05 *N* sodium hydroxide. The MWL gave a broad distinctive maximum at 355 nm as well as at 280 nm but the alkali extracted MWL gave single absorption at 280 nm and the ether-soluble fraction, which contains *p*-coumaric and ferulic acids, gave absorption at about 355 nm as found by Smith.

The occurrence of ester groups in MWL was also shown by the i.r. spectrum which exhibited a band at 1730 cm^{-1}. The i.r. spectra of MWL of both *P. communis* and *S. china* had strong absorption at 1730 cm^{-1}, which almost completely disappeared in the alkali-extracted MWL indicating that the ester carbonyl groups were split off by the alkali extraction. However, the absorption at 1730 cm^{-1} is found in the MWL prepared from many other species in which no ester group has been found and thus carbonyl groups other than ester group may also be responsible for this absorption.

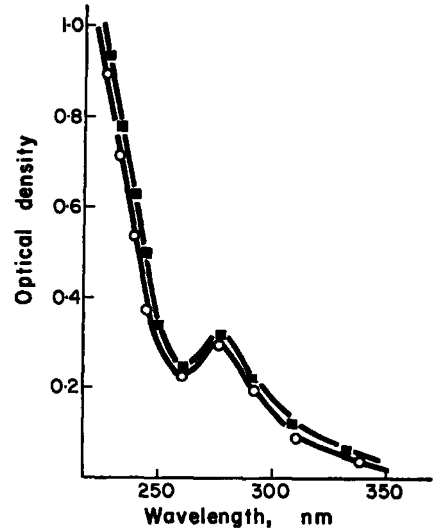

FIG. 2. U.V. SPECTRA OF MWL *Smilax china*.
■—MWL; ○—alkali-extracted MWL; solvent—methylcellosolve.

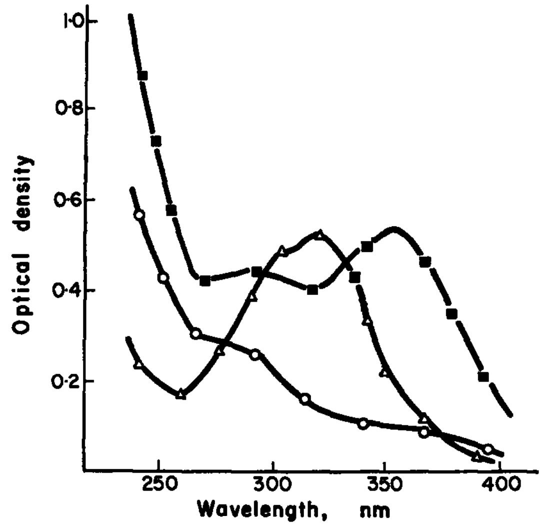

FIG. 3. U.V. SPECTRA OF MWL AND THE ETHER-SOLUBLE FRACTION OF ALKALI EXTRACT OF MWL OF *Phragmites communis*.
■—MWL; ○—alkali-extracted MWL; △—ether-soluble fraction of alkali extract of MWL; solvent—0·05 *N* sodium hydroxide.

Quite a bit of information on the mode of ester linkages between the acids and lignin has been obtained. The acids may be attached to aliphatic hydroxyl groups of lignin as suggested by Smith. Freudenberg and Geiger[6] have shown that, during preparation of dehydrogenation polymers of coniferyl alcohol, pinoresinolid and its isomer are formed, and these lactones give absorption at 1730 cm^{-1}. This type of lactone might occur in grass lignin.

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[6] K. FREUDENBERG and H. GEIGER, *Chem. Ber.* 96, 1265 (1963).

EXPERIMENTAL

The species of grasses and monocotyledons and the procedure for preparation of their stem powders were the same as described in a previous paper.[2]

Preparation of MWL

Ten grammes of the extractive free powder of plant stems was ground for 48 hr in dry toluene by using a vibratory ball mill (Siebtechnik G.M.B.H. Mülheim) and the MWL was extracted with dioxane containing 10 per cent water and purified according to the standard method of Björkman.[7]

Saponification of MWL

MWL (50 mg) was dissolved in *N* NaOH (0·5 ml) and the solution was kept for 24 hr at 20°. The solution was acidified (pH 2) with HCl and shaken with 1·5 ml of ether. The ether extracts were separated and evaporated. The residues were dissolved in 0·5 ml of acetone and analysed by gas-liquid and paper chromatography.[8]

Ultra-violet and Infra-red Spectra of MWL and Saponified MWL

A suitable amount of MWL or saponified MWL was dissolved in methyl-cellosolve and their u.v. spectra determined by a Hitachi–Perkin Elmer 139 U.V.-VIS Spectrometer. In some cases the lignins were dissolved in 0·05 *N* NaOH and their spectral behaviour in the alkaline solution was also determined. I.R. spectra of the MWL and the saponified MWL were determined by KBr disk method using a Nihon Denshi Recording I R. Spectrometer.

Nitrobenzene Oxidation

MWL and the saponified MWL (0·1 g) was subjected to alkaline nitrobenzene oxidation as described previously,[1] and aromatic aldehydes formed were analysed by gas–liquid chromatography.

[7] A. BJÖRKMAN, *Svensk Papperstid.* **59**, 477 (1956).

[8] T. HIGUCHI, N. KIMURA and I. KAWAMURA, *J. Japan Wood Res. Soc.* **12**, 173 (1966).