p-Hydroxyphenyl:Guaiacyl:Syringyl Ratio of Lignin in Some Austral Hardwoods Estimated by CuO-Oxidation and Solid-State NMR

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Summary

The white-rot fungus *Ganoderma australe* causes extensive decay of hardwood in Chilean rain forest, producing a delignified material used as cattle feed. Since lignin composition can influence the delignification efficiency, it was investigated by CuO alkaline degradation and solid-state NMR of wood. The lignin syringyl/guaiacyl ratio estimated by both techniques presented high values in the two woods, i.e., *Eucryphia cordifolia* and *Nothofagus dombeyi*, which showed the most selective delignification and the highest digestibility after fungal decay. On the other hand, a reduced S/G ratio was found in the *Aextoxicon punctatum* wood, which presented a low biodegradability. An additional peculiarity of the two former hardwoods is the relatively high yield of non-cinnamic *p*-hydroxyphenyl compounds after CuO alkaline degradation.

Introduction

Since the first report by Philippi (1893), considerable scientific interest arose by the natural process of fungal transformation of wood into a partially delignified material (“huempe” or “palo podrido”) used as cattle feed in Chilean rain forest (Zadrazil *et al.* 1982; Dill and Kraepelin 1986). The ligninolytic basidiomycete *Ganoderma australe* (Fr.) Pat. is responsible for this unique phenomenon of wood delignification, although complex microbial populations develop at the latter stages of decay (González *et al.* 1986). Wood from several arboreous species can be delignified by *G. australe* at the Chilean forest, but that of *Eucryphia cordifolia* Cav. is the most frequently found. The wood from the latter species and from *Nothofagus dombeyi* (Mirb.) Oerst. present high *in vitro* digestibility after fungal decay (Zadrazil *et al.* 1982). The first information on the chemical composition of the Austral woods studied have been recently reported (Martínez *et al.* 1990a).

Detailed models of hardwood (Nimz 1974) and softwood (Adler 1977) lignin have been obtained from a limited number of plant species. However, considerable variation in lignin composition has been demonstrated between different woods (Sarkanen and Hergert 1971; Glasser *et al.* 1983; Higuchi 1990). On

the other hand, it has been shown that differences in hardwood lignin characteristics can affect the decay pattern produced by fungi (Blanchette *et al.* 1988). Lignin composition in the Austral hardwoods studied was analyzed by solid-state NMR and CuO alkaline degradation of whole wood samples. Alkaline oxidations with CuO or nitrobenzene produce a similar depolymerization of lignin. The latter has been extensively utilized for studying the chemical structure of different lignins, but it produces reaction by-products that interfere with the detection of lignin oxidation compounds. CuO degradation is a relatively mild procedure and its utilization for lignin depolymerization has been optimized by Hedges and Ertel (1982). Cinnamic acids, involved in lignin-polysaccharides linkages (Iiyama *et al.* 1990), are transformed into the corresponding aldehydes by nitrobenzene. However, both types of compounds can be quantified after CuO degradation. Lignin composition in different woods and other lignin-containing materials has been studied by CuO alkaline degradation (Hedges and Mann 1979; Hedges *et al.* 1988).

The ¹³C NMR of solid lignocellulosic samples by using the “cross polarization” and “magic-angle spinning” (CPMAS), can provide information on the different wood constituents. The conditions for obtaining quan-

titative high resolution ^{13}C NMR spectra of lignocellulosic materials in the solid state were obtained and different woods analyzed by this technique (Kolodziejewski *et al.* 1982; Haw *et al.* 1984; Hartfield *et al.* 1987; Fründ and Lüdemann 1989a). Moreover, the solid-state NMR enables "direct" estimation of syringyl/guaiacyl (S/G) ratio in lignins (Manders 1987), overcoming some problems (e.g. partial degradation of lignin, and uncontrolled side-reactions) produced during chemical degradations of lignin for S/G calculation.

Experimental

Samples studied

Wood from *Eucryphia cordifolia* Cav. (Eucryphiaceae, Saxifragales), *Laurelia philippiana* Looser (Monimiaceae, Laurales), *Nothofagus dombeyi* (Mirb.) Oerst. (Fagaceae, Fagales), *Gevuina avellana* Mol. (Proteaceae, Proteales) and *Aextoxicon punctatum* Ruiz et Pavón (Aetoxicaceae, Euphorbiales) were studied. Samples from beech (*Fagus sylvatica* L.) and pine (*Pinus sylvestris* L.) wood and wheat straw (*Triticum aestivum* L.) were analyzed for comparison.

Wood analysis

Samples were milled, passed throughout a 40-mesh screen and dried overnight (50°C under vacuum) before analysis. Ash content was determined at 750°C for 6 h. Nitrogen content in wood was estimated by the Kjeldahl method. Extractives were removed with benzene-ethanol (2:1), 12 h at 60°C. Water-soluble was extracted at 60°C, 12 h. Klason lignin was estimated after hydrolysis of polysaccharides in the extracted substrates and deduction of ash and protein in the residue (Effland 1977). Sugars in polysaccharide hydrolysates were analyzed by gas chromatography (GC) as alditol acetates, and quantified using inositol as internal standard.

Cupric oxide degradation

Fifty mg of wood extracted with light petroleum (24 h) were degraded at 170°C (3 h in N_2 atmosphere) using teflon bombs containing 2 g of CuO , 200 mg of $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ and 14 ml of boiled 2N NaOH . Lignin-derived products were recovered at acid pH with peroxide-free ether, dried, weighted, suspended in 100 μl of pyridine (with 33 μg of ethylvaniline as internal standard) and derivatized with bis(trimethylsilyl)-trifluoroacetamide. The trimethylsilyl derivatives were analyzed in a Perkin-Elmer Sigma-3

chromatograph with a SP-2100 fused silica capillary column (30 m $\times$ 0.25 mm i.d.), programmed from 100°C to 270°C at 4°C/min, identified using a Perkin-Elmer electron-impact mass detector (ITD) and quantified with a flame ionization detector (FID). Response factors were determined from mixtures of standards of the different compounds (1 mg/ml of pyridine) and quantifications were based on the internal standard. The extraction procedure was not exhaustive, and the total yields of lignin-derived products were mainly used with comparative purposes.

Solid-state NMR

High resolution solid-state ^{13}C NMR spectra were obtained with the CPMAS technique at 75.4 MHz in a Bruker MSL 300 spectrometer (Fründ and Lüdemann 1989b). The pulse repetition rate was 5 s, and the cross polarization contact time was 1 ms. The sweep width was 31.25 kHz, the filter width was set to 37.5 kHz and the acquisition time was 0.016 s. Magic-angle spinning was performed at 4 kHz in the commercial Bruker double bearing probes in phase stabilized zirconium dioxide rotors. The chemical shift scale was calibrated with glycine.

Results and Discussion

Wood characteristics

The composition of the samples studied is presented in Table 1. A relatively high lignin content, slightly lower than in the pine wood, was found in the *A. punctatum* wood. It presents other remarkable characteristics, like high ash and low xylan contents. The xylan content in most of the Chilean woods studied was lower than generally found in hardwoods. The influence of the low nitrogen content of some Chilean woods on their extensive delignification by *G. australe* has been suggested by Dill and Kraepelin (1986). Low N values, similar to those reported by Dill *et al.* (1984), were found in some of the woods studied, i.e. those of *E. cordifolia* (0.07% N) and *Nothofagus dombeyi* (0.06% N), when compared with *L. philippiana* (0.25% N) or *F. sylvatica* (0.19% N). However, comparatively low N values have been reported in many other woods, and the extensive delignification of *E. cordifolia* wood by *G. australe* can not be explained only from differences in N content.

Table 1. Composition¹⁾ of *Eucryphia cordifolia*, *Nothofagus dombeyi*, *Gevuina avellana*, *Laurelia philippiana*, *Aextoxicon punctatum*, *Fagus sylvatica* and *Pinus sylvestris* woods and *Triticum aestivum* straw.

	ASH	EXT	WSO	LIG	GLU	XYL	ARA	MAN
<i>E. cordifolia</i>	0.3	5.4	1.9	25.6	44.6	10.8	2.3	3.0
<i>N. dombeyi</i>	0.2	3.3	1.5	23.5	49.1	9.9	1.7	2.4
<i>G. avellana</i>	1.4	3.6	4.5	24.0	40.1	12.5	2.9	3.5
<i>L. philippiana</i>	0.5	4.5	2.4	21.6	44.9	9.9	2.1	5.4
<i>A. punctatum</i>	2.5	1.8	1.7	27.0	46.9	6.1	2.2	3.1
<i>F. sylvatica</i>	0.7	1.9	1.5	20.3	45.5	15.9	3.4	3.0
<i>P. sylvestris</i>	0.3	7.8	1.4	28.5	38.7	3.9	2.0	11.1
<i>T. aestivum</i>	6.9	9.4	11.8	15.6	35.8	10.2	4.5	0.8

¹⁾ ASH = ash; EXT = extractives; WSO = water soluble; LIG = lignin; sugars after polysaccharide hydrolysis: GLU = glucose, XYL = xylose, ARA = arabinose, MAN = mannose (all as dry-weight percentages).

Lignin-derived products from chemical degradation of wood

The molar abundances ($\mu\text{mol/g}$ of sample) after GC (FID) of the aromatic compounds from lignin depolymerization, the total yields after alkaline CuO oxidation of the Chilean woods, and the molar *p*-hydroxyphenyl:guaiacyl:syringyl (H:G:S) ratios are presented in Table 2. The abundances are compared with those from beech and pine woods and from wheat straw. The woods from *E. cordifolia* and *A. punctatum* showed quite different lignin compositions and the

Fig. 1. Gas chromatography after CuO degradation of the woods from *Eucryphia cordifolia* (a), *Aextoxicon punctatum* (b), and *Fagus sylvatica* (c) (ITD detector). Peak identification: 1 = *p*-hydroxybenzaldehyde; 2 = *p*-hydroxyacetophenone; 3 = vanillin; 4 = ethylvanillin; 5 = acetovanillone; 6 = *p*-hydroxybenzoic acid; 7 = syringaldehyde; 8 = acetosyringone; 9 = vanillic acid; 10 = syringic acid; 11 = *p*-coumaric acid; 12 = ferulic acid.

chromatographic separation of the products from their alkaline CuO degradation, and those from the beech wood, are presented in the Figure 1.

The total yield (mg/g of sample) after alkaline degradation (Table 2) is limited by the degree of condensation of lignin through C—C linkages (Neyroud and Schnitzer 1974). Relatively high degradation yields, similar to that found in the beech wood, were found in *N. dombeyi* and *E. cordifolia* but a very low yield, close to that from wheat straw, was obtained with the *A. punctatum* wood. The variation of lignin condensation degree in different plants and the high condensation of wheat straw lignin had been demonstrated by different analytical techniques (Lapierre *et al.* 1989; Nimz *et al.* 1981). The *A. punctatum* wood showed the lowest S/G molar ratio (1.92) whereas high ratios were found in the *N. dombeyi* (5.17) and *E. cordifolia* woods (4.04). The relation between degradation yield and S/G ratio in wood lignin shows the participation of guaiacyl units in CuO-resistant C₅—C₅ linkages.

A noteworthy characteristic of the *E. cordifolia* and *N. dombeyi* woods is the comparatively great yields of *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid and cinnamic acids (Table 2). A similar result was found after CuO alkaline degradation of the pine wood, but these compounds were not obtained from the beech wood. Small amounts of *p*-hydroxybenzaldehyde were obtained from conifers (specially from the families Pinaceae and Taxodiaceae) after nitrobenzene alkaline degradation but none, or only minute amounts, from most hardwoods (Brauns and Brauns 1960). The *p*-hydroxybenzaldehyde is abundant after nitrobenzene degradation of lignin from grasses but a low content, similar to that obtained by thioacidolysis (Lapierre *et al.* 1989), was found after CuO alkaline degradation of wheat straw. The relative contents of non-cinnamic *p*-hydroxyphenyl compounds in the *E. cordifolia* and *N. dombeyi* woods were near 50% of that found in wheat straw, although the relative abundance of cinnamic acids were many times lower.

CPMAS ¹³C NMR of wood

Main signals in the CPMAS ¹³C NMR spectra of the woods studied (Fig. 2) can be assigned to cellulose: C₁ (105 ppm), C₄ (89 and 84 ppm), C₂, C₃, C₅ (75 and 73 ppm) and C₆ (65 and 62 ppm) and hemicellulose: acetyl (21 ppm) and carbonyl carbons from uronic acids (172 ppm). The higher intensity of the latter signals in the beech wood spectra is in accordance with the relatively low hemicellulose content in the Chilean woods studied (Table 1). High acetylation of hemicellulose (46–0 ppm region in Table 3) was observed in the wheat straw. The relative intensity of the C₄ signals at 89 ppm (crystalline cellulose) and 84 ppm (amorphous cellulose) suggests a higher degree of cellulose crystallinity in *E. cordifolia* and *A. punctatum* when compared with beech wood. The high content of crystalline cellulose, more resistant to fungal attack, could be involved in the preferential degrada-

Table 2. Abundances ($\mu\text{mol/g}$ of wood or straw) of the different aromatic compounds, total yields, and molar ratios in lignin (excluding cinnamic compounds) after CuO degradation (FID detector).

	pOH-Phenyl			Guaiacyl			Syringyl			Cinnamic		Total yield (mg/g)	Molar Ratios (H:G:S)
	1*)	2	6	3	5	9	7	8	10	11	12		
<i>E. cordifolia</i>	5	0	16	46	8	2	181	40	46	1	1	63.75	6:19:75
<i>N. dombeyi</i>	11	1	16	40	6	8	210	36	34	2	1	64.73	8:15:77
<i>G. avellana</i>	1	0	0	21	3	1	88	16	2	0	0	23.61	0:19:80
<i>L. philippiana</i>	1	0	1	52	7	12	151	33	31	0	3	52.20	1:25:74
<i>A. punctatum</i>	0	0	0	23	3	0	44	8	0	0	0	13.87	0:34:66
<i>F. sylvatica</i>	0	0	0	64	8	13	187	37	32	0	0	60.94	0:25:75
<i>P. sylvestris</i>	2	0	1	185	32	54	1	0	1	1	2	43.73	1:98:0
<i>T. aestivum</i>	2	0	0	11	2	2	8	6	2	4	7	7.87	14:50:36

1*) = *p*-hydroxybenzaldehyde; 2 = *p*-hydroxyacetophenone; 3 = vanillin; 5 = acetovanillone; 6 = *p*-hydroxybenzoic; 7 = syringaldehyde; 8 = acetosyringone; 9 = vanillic acid; 10 = syringic acid; 11 = *p*-coumaric acid; 12 = ferulic acid.

tion of lignin during decay of the Chilean woods by *G. australe* because cellulase activity on microcrystalline cellulose was not found in this fungus.

Lignin signals correspond to: aromatic C linked to O in S units (153 ppm); aromatic C linked to alkyl chains in S units (137 ppm); unsubstituted aromatic C (around 120 ppm); and methoxy C (56 ppm) (Fig. 2). The shoulder at 148 corresponds to aromatic C linked to O in G units and was considered for S/G estimation as described later. In the spectrum of wood from *E. cordifolia* it was partially overlapped with the signal attributed to tannins at 145 ppm (Fig. 2a) and it showed a greater intensity in the other woods, mainly in that of *A. punctatum* (Fig. 2b). The signal from aromatic C linked to alkyl chains in G units (134 ppm) is included in the 137 ppm signal and produced a slight broadening in the case of *A. punctatum*.

Integration of the areas corresponding to different carbon types in the CPMAS ^{13}C NMR spectra is shown in Table 3, and the results were compared with those reported by Fründ and Lüdemann (1989a). The values of Klason lignin (Table 1) and lignin carbon (including methoxy groups) from CPMAS ^{13}C NMR were similar. Among the woods studied, the highest values corresponded to *A. punctatum* and the lowest to beech. The 56 and 153 ppm signals showed greater intensity in the *E. cordifolia* spectrum in agreement with the high S/G ratio in this wood.

Estimation of the S and G components in the aromatic region of the CPMAS ^{13}C NMR spectra was carried out by digital subtraction of a cedar wood spectrum (G-lignin). After cedar spectrum scaling and obtention of a zero subtraction value in the 146–148 ppm region, S and G components were obtained (Fig. 3). The S/G ratios presented in Table 4 were estimated from the area integration of both components, normalized to the same number of substituted aromatic carbons.

Fig. 2. CPMAS ^{13}C NMR spectra of wood from *Eucryphia cordifolia* (a), *Aextoxicon punctatum* (b), and *Fagus sylvatica* (c). See Results and Discussion for assignation of signals.

Table 3. Lignocellulose composition from integration of CPMAS ^{13}C NMR spectra (% of the total C).

	Carboxyl-C 210–160 ppm	Aromatic-C 160–110 ppm	Carbohydrate-C 110–46 ppm ¹⁾	Aliphatic-C 46–0 ppm
<i>E. cordifolia</i>	2.5	14.5	80.1	2.9
<i>A. punctatum</i>	2.3	17.1	78.5	2.1
<i>F. sylvatica</i>	2.7	13.4	80.8	3.2
<i>T. aestivum</i>	3.8	10.3	80.3	6.5

¹⁾ oxygen-linked aliphatic C, including lignin methoxy groups. **Fig. 3.** Syringyl (a–c) and guaiacyl (d–f) components of the substituted aromatic region (125–165 ppm) obtained by digital treatment of the CPMAS ^{13}C NMR spectra of the woods from *Eucriphia cordifolia* (a, d), *Aextoxicon punctatum* (b, e) and *Fagus sylvatica* (c, f) shown in Fig. 2.

Lignin S/G ratio

The S/G ratios obtained from alkaline CuO degradation showed 3 times higher values than those from CPMAS ^{13}C NMR (Table 4). The latter gives a more realistic information of lignin composition, and the results are comparable to literature values that were obtained by a combination of methoxyl and elemental analysis of lignin (Sarkanen and Hergert 1971). High ratios after chemical degradation are due to the lower degradability of lignin condensed by $\text{C}_5\text{--C}_5$ linkages between guaiacyl units. A similar result was found by Manders (1987) using nitrobenzene alkaline degradation and CPMAS ^{13}C NMR of different woods.

Sound and decayed wood from *E. cordifolia* was analyzed by Mulder *et al.* (1990) using pyrolysis-mass spectrometry (Py–MS), and the results are compared with Py–MS analyses of other woods (Genuit *et al.* 1987; Saíz-Jiménez *et al.* 1987). Changes in lignin markers were observed and the S/G molar ratio was tentatively estimated from the intensities of the m/z 210 (4(propanone)-syringol) and m/z 180 (4(propanone)-guaiacol) (Table 4). The values obtained were close to those from CPMAS ^{13}C NMR and suggest the possibility of S/G estimation by this analytical technique.

The lignin S/G ratio in some Chilean woods, like *E. cordifolia*, was comparatively higher than those reported for most hardwoods (Manders 1987). This wood experiments an extensive delignification when degraded by *G. australe* and a progressive decrease of the S/G ratio (from CPMAS ^{13}C NMR, Py–MS and alkaline degradation) was observed during fungal decay (Martínez *et al.* 1990b–c). The low nitrogen content, the crystallinity degree of cellulose, and the particular composition of the hemicellulose fraction of *E. cordifolia* wood, as so as ecological factors of Austral rain forests, could be involved in this noteworthy degradation pattern. However, it must be emphasized that the high S/G ratio probably confers a high biodegradability to lignins from *E. cordifolia* and other Chilean woods, and promotes their extensive delignification by fungi. On the other hand, strong resistance to fungal biodegradation by *Lentinus degener* was found during *in vitro* decay of wood from *A. punctatum* (Martínez *et al.* 1990b), showing a low S/G ratio in lignin.

Table 4. Comparison of syringyl/guaiacyl ratios from CuO degradation, CPMAS ^{13}C NMR and pyrolysis-MS of wood.

	CuO	CPMAS	Py–MS
<i>E. cordifolia</i>	4.04	1.18	1.51 ¹⁾
<i>A. punctatum</i>	1.92	0.75	—
<i>F. sylvatica</i>	2.99	1.05	0.61 ²⁾

¹⁾ From Mulder *et al.* (1990).²⁾ From Genuit *et al.* (1987).

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