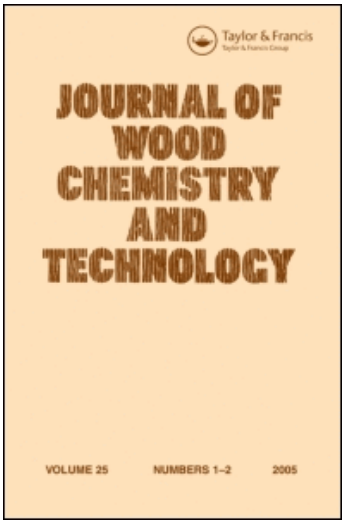

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CHEMISTRY OF DELIGNIFICATION DURING KRAFT PULPING OF BAMBOOS

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ABSTRACT

Milled Bamboo Lignin (MBL) from Phyllostachys pubescens (Bambuseae) was found to contain p-hydroxycinnamic acid (PC), guaiacylpropane and syringylpropane units in an approximate molar ratio of 1:4:3 on the basis of chemical and spectroscopic analyses. In addition, the MBL is comprised of a guaiacyl-syringyl type lignin-core and PC type peripheral groups. At least 9 PC type units/100 C_9-units were chemically bonded to the lignin-core in the form of ester linkages at C-α and C-γ, and the remainder in the form of aryl ether linkages at C-α. Bamboo kraft lignin (BKL) was found to be a lignin of the guaiacyl-syringyl type, having a higher degree of condensation and a higher phenolic hydroxyl content than the MBL. The major low-molecular-weight constituents of bamboo kraft black liquor were determined to be acetosyringone, syringic acid, acetoguaiacone and vanillic acid. Structural change in bamboo lignin during kraft cooking are discussed on the basis of the observed results.

INTRODUCTION

Bamboos (Bambuseae) are perennial grasses with woody stalks, belonging to the family Gramineae. The tribe consists of about 45 genera which are widely distributed throughout the tropic, subtropic and temperate regions of the earth.[1,2] Bamboos are among the most important renewable forest resources for

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manufacture of pulp and paper in the developing countries in southern and southeastern Asia. In addition, bamboos are used in the third world as raw materials for house construction and for manufacture of objects of art.

Lignins in bamboos are composed of a lignin-core and peripheral groups.[3-13] The lignin-core consists mainly of guaiacylpropane and syringylpropane units, the quantity of p-hydroxyphenylpropane units being negligible. Thus, the lignin-core is a guaiacyl-syringyl type lignin. The peripheral groups include p-hydroxycinnamic acid and ferulic acid units, the former being the major component of the peripheral groups. These units are bonded chemically to the lignin-core, mostly in the form of esters with hydroxyl groups at C-α and C-γ of side chains in the lignin-core, and less in the form of aryl ether at C-α. The molar ratio of lignin-core to peripheral groups in terms of phenylpropane units depends on the nature of bamboo species, generally in the range of 4:1 to 19:1.

In spite of the importance of bamboos as raw materials for the pulp and paper industries in the third world, no comprehensive research has been conducted so far on the nature of lignins in bamboos and technical bamboo lignins.[14,15] In order to develop an economically viable pulp industry based on bamboos, it is of primary importance to investigate the chemistry of delignification during pulping of bamboos as well as the potential use of technical bamboo lignins thus obtained as chemical feedstocks. The objective of this investigation is, therefore, twofold: (a) to study comparatively the chemical nature of milled bamboo lignin (MBL) and bamboo kraft lignin (BKL), and (b) to identify the low-molecular-weight constituents in the black liquor, produced from degradation of bamboo lignins during the kraft pulping of bamboos. Evaluation of the results will eventually lead to elucidation of mechanisms for the delignification of bamboos during the kraft pulping process as well as potential applications of bamboo kraft lignins as chemical feedstocks.

EXPERIMENTAL

Preparation of Milled Bamboo Lignin (MBL)

Stalks of a 2-year old, healthy bamboo (Phyllostachys pubescens Mazel ex H. De Lehaie) were ground with a Wiley mill to pass a 60 mesh screen. The air-dried bamboo meal was extracted continuously for 48 hrs with benzene: 95% EtOH (2:1, v/v), then percolated with hot water at 50-60°C for 24 hrs. The extractives-free bamboo meal was air-dried, then dried over P_2O_5 under vacuum for several days. The MBL was prepared from the dried, extractives-free bamboo meal according to the Bjorkman procedure,[16] and purified according to the Lundquist procedure.[17,18] Yield: 20%/bamboo.

Preparation of Bamboo Kraft Lignin (BKL)

The BKL was prepared from a concentrated bamboo kraft pulping black liquor (the content of total organics: 112 g/l) provided by the Paper and Chemical Industries Corp., Rangoon, Burma. The concentrated black liquor was acidified to pH 2 by dropwise addition of 3M H_2SO_4. The reaction mixture was heated in a water bath at 80°C for 1 h to promote coagulation of the precipitate (PPT). After cooling, the PPT was centrifuged, suspended in dil. H_2SO_4 solution (pH 3) and again centrifuged. This operation was repeated three times. Finally, the PPT was suspended in deionized water, stirred mechanically for 1 h, centrifuged, suspended in deionized water again and then freeze-dried. The crude BKL was then dried over P_2O_5 under vacuum, and dissolved in a minimum volume of 96% aqueous dioxane. After removal of insolubles by filtration, the dioxane solution was added dropwise into 10 times the volume of Et_2O under mechanical stirring. The PPT was centrifuged, washed twice with deionized water, suspended in deionized water, then freeze-dried. The purified BKL was dried over P_2O_5 under vacuum. A yield of ca. 29.6%/total organics in the black liquor was obtained.

Acetylation of MBL and BKL

To 30 ml of pyridine-Ac_2O (2:1, v/v) was added 1 g of purified lignin-preparation. The reaction mixture was kept at

room temperature for 48 h, then poured into a mixture containing 100 g of crushed ice and 10 ml of concentrated HCl. The PPT was filtered off, washed with cold diluted HCl until the PPT was free of pyridine, then washed with cold deionized water. The product was dried over P_2O_5 under vacuum. Yield: 80%

Nitrobenzene Oxidation

A dried, purified lignin-preparation (50 mg), 7 ml of 2M NaOH and 0.4 ml of nitrobenzene were placed in a 10 ml stainless steel bomb. The bomb was sealed tightly with a Teflon gasket and screw cap, and was heated at 170°C for 2.5 h with occasional shaking. After cooling the bomb with cold water, the reaction mixture was transferred to a $CHCl_3$-extractor, and was extracted continuously for 6 h with $CHCl_3$ to remove excess nitrobenzene and its reduction products. The reaction mixture was then acidified to pH 4 with conc. HCl, and was further extracted continuously with $CHCl_3$ for 48 h. The $CHCl_3$ solution was dried over $MgSO_4$, and the solvent was removed at 40°C under reduced pressure. The resulting residue was dissolved in CH_2Cl_2 for further quantitative determination of the oxidation products according to the Chen procedure.[19]

Determination of p-Hydroxycinnamic Acid Units in Lignins

To 25 ml of 1M NaOH solution was added 100 mg of a lignin-preparation. The resulting solution was kept at room temperature for 48 h. under N_2-atmosphere with mechanical stirring. The reaction mixture was acidified to pH 2 with 1M HCl. The PPT was centrifuged, washed with deionized water thoroughly, then dried with P_2O_5 under vacuum. The supernatant and washings were combined and extracted continuously for 48 h with Et_2O to obtain the alkaline hydrolysate. The dried PPT was dissolved in 50 ml of 2M HCl-dioxane (1:9, v/v). The resulting solution was refluxed for 1 h. The reaction mixture was poured into 50 ml of water, and the PPT was centrifuged, then washed thoroughly with water. After removal of dioxane, the supernatant was combined with washings, and extracted continuously for 48 h with Et_2O to give the acidic hydrolysate. After removal of the solvent from the Et_2O-

extractives, the p-hydroxycinnamic acid and ferulic acid contents in both alkaline and acidic hydrolysates were quantitatively determined by means of high performance liquid chromatography (HPLC) with a C-18 column, using vanillin as internal standard and a mixture of CH_3CN-H_2O (1:8, v/v) containing 1% HAc as eluent. The effluents were monitored by an UV detector at λ 280 nm. The procedure for the determination of p-hydroxycinnamic acid units in lignins is based on the procedure reported by Scalbert et al.[20] with some modifications.

^{13}C NMR Spectra

The ^{13}C NMR spectra were recorded with a JEOL FX 60 spectrometer, operating at 14.9 MHz for ^{13}C nuclei frequency with the wide-band ^{1}H decoupling mode. Deuterated dimethylsulfoxide (DMSO-d_6) was used as the solvent. The spectra were run in 10 mm (OD) sample tube with concentration of the samples being about 400 mg in 2 ml of DMSO-d_6 (ca. 20%, w/v) at 50°C with a 60° pulse angle corresponding to a 20 μsec pulse width, a repetitive time of 2 sec, and a scan number of ca. 20,000. Tetramethylsilane (TMS) was used as the internal chemical shift (δ in ppm) reference.

Isolation of Low-Molecular-Weight Fractions

The concentrated bamboo kraft pulping black liquor (250 ml) was extracted continuously for 24 h with $CHCl_3$ at pH 12.7 to obtain the $CHCl_3$-soluble neutral fraction. The black liquor was adjusted to pH 8 with conc. H_2SO_4, then extracted continuously for 24 h with $CHCl_3$ to give the $CHCl_3$-soluble phenolic fraction. The black liquor was finally acidified to pH 2 with conc. H_2SO_4, then again extracted continuously for 24 h with $CHCl_3$ to yield the $CHCl_3$-soluble acidic fraction. The three $CHCl_3$ solutions were dried over $MgSO_4$, then the solvent was removed at 40°C under reduced pressure. Each of the resulting neutral, phenolic and acidic residues was dissolved in a minimal volume of $CHCl_3$, added dropwise into 10 times the volume of Et_2O, and the PPT was centrifuged. The resulting Et_2O solutions were dried over $MgSO_4$, and the solvent was removed at 40°C under reduced pressure to give the Et_2O-soluble neutral, phenolic and acidic fractions with yields of 0.4, 3.2 and 2.7%/total organics, respectively.

Identification of the Constituents of Ether-Soluble Fractions by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS)

Sample Preparation: Each of the Et_2O-soluble neutral, phenolic and acidic fractions (100 mg) was dissolved in 4 ml of pyridine-Ac_2O (1:1, v/v), and the mixture was kept at room temp. for 24 h. The reaction mixture was poured into ca. 20 g of crushed ice, acidified to pH 2 with 3N HCl, then extracted continuously for 24 h with $CHCl_3$. The $CHCl_3$-solution was dried over $MgSO_4$, and the solvent was removed at 40°C under reduced pressure. The resulting O-acetylated mixture was dissolved in 5 ml of CH_3OH, then treated with ethereal CH_2N_2. After removal of the solvent, the residue was dissolved in 5 ml of CH_2Cl_2, and the solution was filtered through Millipore membrane HVHP to remove any high-molecular-weight contaminants. The resulting solution was used as the sample solution for GC and GC-MS analyses.

GC Operation: Injected 0.5 µl of the sample solution into a Hewlett-Packard Model 5880A GC system with a DB-5 bonded phase, fused silica capillary column (30 m x 0.32 mm ID; film thickness, 0.25 m; J & W Scientific) and a flame ionization detector (temp., 270°C). Carrier gas: He (column flow rate, 2 ml/min). Temperature profile: hold for 1 min at 60°C; 60-140°C (15°C/min); 140-255°C (10°C/min); final temp., 255°C (10 min); post value, 260°C (10 min).

GC-MS Operation: The sample solution (1 µl) was injected into a Hewlett-Packard Model 5985B GC-MS system with a DB-5 bonded phase, fused silica capillary column (30 m x 0.32 mm ID; film thickness, 0.25 m; J & W Scientific). The temperature profile: hold for 1 min at 60°C; 60-255°C (10°C/min). The MS conditions were EI mode (70 eV) and scanning rate of ca. 80 scans/min.

O-Acetylguaiacol (1a): GC-Retention time, 7.6 min; MS (70 eV), m/z (rel. int.) 166 (M+, 8.7), 125 (7.4), 124 (100), 110 (4.6), 109 (68.4), 81 (17), 43 (13.8).

O-Acetyl-4-ethylguaiacol (3a): GC-Retention time, 8.1 min; MS (70 eV), m/z (rel. int.) 194 (M+, 8.6), 153 (7.5), 152 (80.5), 138 (8.6), 137 (100), 122 (3.9), 43 (8.3).

4-O-Acetyl-4-hydroxyacetophenone (11a): GC-Retention time, 10.3 min; MS (70 eV), m/z (rel. int.) 178 (M+, 11.1), 136 (28), 122 (7.5), 121 (100), 93 (9), 43 (16.9).

O-Acetylsyringol (2a): GC-Retention time, 10.6 min; MS (70 eV), m/z (rel. int.) 196 (M+, 5.6), 155 (9.6), 154 (100), 139 (26.6), 111 (9.6).

α-(4-O-Acetyl-4-hydroxyphenyl)acetone (7a): GC-Retention time, 11.2 min; MS (70 eV), m/z (rel. int.) 192 (M+, 4.7), 151 (9.6), 150 (22.6) 108 (11.8), 107 (100), 77 (8.6) 43 (18.7).

4-O-Acetylacetoguaiacone (12a): GC-Retention time, 12.6 min; MS (70 eV), m/z (rel. int.) 208 (M+, 4.4), 167 (4.6), 166 (44.1), 152 (8.3), 151 (100), 123 (9.3), 43 (17).

O-Acetyl-4-ethylsyringol (4a): GC-Retention time, 12.8; MS (70 eV), m/z (rel. int.) 224 (M+, 6.2), 183 (10.5), 182 (100), 168 (7.1), 167 (82.5), 77 (5.6), 43 (9.8).

4-O-Acetylvanillic Acid Methyl Ester (14a): GC-Retention time, 13.0 min; MS (70 eV), m/z (rel. int.) 224 (M+, 5), 183 (9.4), 182 (94.6), 152 (8.9), 151 (100), 123 (8.7), 43 (12.1).

α-(4-O-Acetylguaiacyl)acetone (8a): GC-Retention time, 13.4; MS (70 eV), m/z (rel. int.) 222 (M+, 2.9), 180 (23.3), 138 (9.9), 137 (100), 122 (5.3), 43 (13.5).

4-O-Acetylacetosyringone (13a): GC-Retention time, 15.8 min; MS (70 eV), m/z (rel. int.) 238 (M+, 2.8), 197 (7.4), 196 (68.4), 182 (9.1), 181 (100), 153 (6.9), 150 (5.8), 43 (20.6).

Palmitic Acid Methyl Ester (17a): GC-Retention time, 16.8 min; MS (70 eV), m/z (int. rel.) 270 (M+, 11.7), 241 (2.3), 239 (4.7), 227 (8.8) 199 (3.2), 185 (3.5), 171 (2.9), 157 (1.9), 143 (14.5), 129 (6.1), 101 (5.5), 97 (6.4), 88 (5.9), 87 (68), 75 (18.3), 74 (100).

4-O-Acetylsyringic Acid Methyl Ester (15a): GC-Retention time, 17.7 min; MS (70 eV), m/z (rel. int.) 254 (M+, 3.5), 223 (2.1), 213 (10.6), 212 (100), 197 (6.4), 182 (5.9), 181 (48.6), 153 (5), 43 (10).

α-(4-O-Acetylsyringyl)acetone (9a): GC-Retention time, 18.0 min; MS (70 eV), m/z (rel.int.) 252 (M+, 2), 211 (3.3), 210 (27.8), 168 (10.5), 167 (100), 43 (11.7).

3-(4-O-Acetylguaiacyl)propyl Acetate (5a): GC-Retention time, 18.6 min; MS (70 eV), m/z (rel. int.) 266 (M+, 8.3), 225 (10.3), 224 (89.3), 165 (13.8), 164 (100), 151 (8.1), 150 (7.6), 149 (40), 138 (12), 137 (89.3), 123 (4.5), 43 (66.7).

1-(4-O-Acetylsyringyl)ethyl Acetate (10a): GC-Retention time, 19.2 min; MS (70 eV), m/z (rel. int.) 282 (M+, 3), 240 (10.2), 181 (14.1), 180 (100), 167 (13.8), 165 (12.8), 43 (18.8).

3-(4-O-Acetylsyringyl)propyl Acetate (6a): GC-Retention time, 20.2 min; MS (70 eV), m/z (rel. int.) 296 (M+, 11.3), 255 (17.9), 254 (100), 195 (5.7), 194 (35.4), 181 (10.6), 180 (8.7), 179 (10), 168 (18.5), 167 (46.4), 153 (5.6), 43 (57.2).

1,1-Bis(4-O-acetylguaiacyl)ethane (16a): GC-Retention time, 21.5 min; MS (70 eV), m/z (rel.int.) 358 (M+, 5), 317 (14.5), 316 (83.8), 301 (8.3), 275 (5), 274 (32.1), 273 (3.5), 260 (15.7), 259 (100), 43 (29.5).

Elemental Analyses

Elemental Analyses for C, H, S, N and Ac were carried out by E & R Microanalytical Laboratory, Inc. (Corona, NY). Values for O% were obtained by difference, i.e., O% = 100 - (C% + H% + S% + N%). Methoxyl contents were determined by in-house analytical laboratory of this Department.

RESULTS AND DISCUSSION

1. Nature of Lignins in Bamboos

Tables 1 and 2 show the results of elemental and carbohydrate analyses of bamboo lignins, including the purified milled bamboo lignin (MBL) from Phyllostachys pubescens, a bamboo species widely distributed in southern China, particularly in the region southeast of the Yangtze river. The data in Tables 1 and 2 indicate that the purified MBL contains about 5% carbohydrates and about 7% proteins. The purified MBL was obtained from the corresponding crude MBL according to the Lundquist procedure.[17,18] This procedure was developed for purification of crude milled wood lignins (MWLs) from soft- and hardwood species by removal of carbohydrate and low-molecular-weight phenol contaminants in the

Table 1. Chemical Composition of Bamboo Lignin Preparations (%/Lignin)

Lignin Preparations	Carbohydrate Content (%)	Protein Content (%)	C %	H %	O %[a]	S %	OCH_3 %	Ac %	N %
Milled Bamboo Lignin	5	6.9[b]	61.66[c]	5.53[c]	32.81[c]	----	19.42[c]	----	1.11
Bamboo Kraft Lignin	-	-	61.81	5.25	31.26	1.68	15.99	----	----
Acetylated Milled Bamboo Lignin	6	1.4[b]	60.67[c]	5.37[c]	33.96[c]	----	15.02[c]	24.46[c]	0.23
Acetylated Bamboo Kraft Lignin	-	-	62.41	5.05	31.19	1.35	13.19	18.63	----

[a] By difference (see Experimental).
[b] Protein content calculated as 6.25 times the observed nitrogen %.
[c] Corrected for carbohydrate and protein contents.

Table 2. C_9-Unit Formulae of Bamboo Lignin Preparations

Lignin Preparations	C_9-Unit Formulae	C_9-Unit Weight
Milled Bamboo Lignin	$C_9H_{7.20}O_{2.84}(OCH_3)_{1.25}$[a]	199.6
Bamboo Kraft Lignin[b]	$C_9H_{7.12}O_{2.80}S_{0.08}(OCH_3)_{1.00}$[b]	194.3[b]
Acetylated Milled Bamboo Lignin	$C_9H_{5.69}O_{2.81}(OCH_3)_{1.27}(COCH_3)_{1.49}$[a]	262.3
Acetylated Bamboo Kraft Lignin[b]	$C_9H_{5.61}O_{2.52}S_{0.10}(OCH_3)_{0.98}(COCH_3)_{1.00}$[b]	230.7[b]

[a] Corrected for carbohydrate and protein contents.
[b] These lignin preparations do not have C_9 structure; these C_9-unit are presented only for the purpose of comparsion.

crude MWLs. Moreover, most of the protein contaminants in the purified MBL were removed during the acetylation of the MBL but not the carbohydrates. Thus, the experimental data suggest that the carbohydrates in the MBL could be chemically bonded to the lignin in the form of a lignin-carbohydrate-complex. In general, purified milled grass lignins (MGLs) have a rather high carbohydrate content, in the range of 5-10%/lignin.[21-23] The high protein content of the BML is attributable to the fact that the lignin was prepared from stalks of 2 year old saplings of P. pubescens. However, the protein content of the acetylated MBL was sufficiently low to permit further characterization of the MBL. As given in Table 3, the results of functional group analyses of bamboo lignins show that the MBL has a relatively higher phenolic hydroxyl content as compared to MWLs from hardwood species, about 36 versus 20-30/100 C_9-units. This could be in part attributable to the presence of a considerable quantity of p-hydroxycinnamic acid ester moieties in the BML.

The UV spectrum of the BML showed intense absorption bands at λ281 and 315 nm. These bands shifted to λ 300 and 364 nm on addition of base. The bands at λ281 and 315 nm, therefore, correspond to the long-wave benzenoid (L.E.) bands of 4-alkylphenols and p-hydroxycinnamic acid esters, respectively.[24] As shown in Table 4, the MBL gave about 9 mole % of p-hydroxycinnamic acid (PC)/C_9-unit on saponification with dilute alkaline solution at room temperature, and further about 3 mole % of PC/C_9-unit on subsequent acidolysis of the resulting lignin residue. Only a trace amount of ferulic acid was detected in the alkaline hydrolysate. Furthermore, none of the PC type structures of bamboo lignins survived under the conditions of kraft pulping as evidenced by the results of 2-stage hydrolysis and nitrobenzene oxidation as given in Tables 4 and 5, respectively. Thus, the MBL is comprised of about 12 units of PC type structures/100 C_9-units, of which at least 9 units are connected to the lignin-core at C-α and C-γ in the form of ester linkages. Most of the remaining PC units are probably present in the lignin in the form of α-aryl

Table 3. Functional Groups of Bamboo Lignins

Lignin Preparations	Groups/100 C9-Units			
	OCH_3	Ph-OH*	Aliph. OH	Total OH
Milled Bamboo Lignin	126	36	113	149
Bamboo Kraft Lignin	100	44	56	100

* Determined by PMR of the corresponding acetylated lignins.

Table 4. Yields of p-Hydroxycinnamic Acid and Ferulic Acid on Saponification and Subsequent Acidolysis (Mole %/C9-Unit)*

Lignin Preparations	Saponification		Acidolysis	
	PC Acid*	FE Acid*	PC Acid*	FE Acid*
Milled Bamboo Lignin	8.8	+	3.4	-
Bamboo Kraft Lignin	-	-	+	-

* PC Acid = p-Hydroxycinnamic Acid; FE Acid = Ferulic Acid; "+" = Trace Amount; "-" = Not Detected.

Table 5. Yields of Aromatic Aldehydes on Nitrobenzene Oxidation (Mole %/C9-Unit)*

Lignin Preparations	p-Hydroxy-benzaldehyde	Vanillin	Syring-aldehyde	Total Aldehydes	Molar Ratio (H:V:S)
Milled Bamboo Lignin	7.9	19.0	25.7	52.6	0.4 : 1 : 1.4
Bamboo Kraft Lignin	+	7.2	4.5	11.7	1 : 0.6

* "+" = Trace Amount; "-" = Not Detected.

ether. On nitrobenzene oxidation (Table 5), the MBL produced p-hydroxybenzaldehyde, vanillin and syringaldehyde in an approximate molar ratio of 0.4:1:1.4 with a total aldehyde yield of about 53 mole %/C_9-unit. On the basis of the experimental data discussed above, one can estimate that the MBL consists of p-hydroxycinnamic acid, guaiacylpropane and syringylpropane units in an approximate molar ratio of 1:4:3, taking the methoxyl content of 1.26 groups/C_9-unit, an average of the values for the MBL and its acetate. Thus, the MBL from P. pubescens is composed of a lignin-core of the guaiacyl-syringyl type and peripheral groups of the p-hydroxycinnamic acid (PC) type unit in an approximate molar ratio of 7:1. The PC type peripheral groups are bonded to the lignin-core mostly in the form of ester at C-α and C-γ. Only less than one-fourth of the PC units are linked to the lignin-core in the form of α-aryl ether. The lignin-core consists of guaiacylpropane and syringylpropane units in an approximate molar ratio of 1:0.8. In addition about 5% of carbohydrates/lignin are linked to the lignin-core in the form of lignin-carbohydrate-complex.

The ^{13}C NMR spectrum of the MBL (Figure 1A) provides further evidence for the nature of the MBL discussed previously. Table 6 shows assignment of signals in the spectra of bamboo lignins. The presence of p-hydroxycinnamic acid ester structures in the MBL is revealed by moderately intense signals 4, 5, 11, 16, 19, 21 and 23 corresponding to ester -C=O in C-γ, C-4, C-α, C-2/C-6, C-1, C-3/C-5 and C-β of p-hydroxycinnamic acid esters, respectively. The guaiacyl-syringyl nature of the lignin-core is evidenced by intense signals 7, 8, 15, 20, 22 and 24 corresponding to C-3, C-4, C-1, C-6, C-5 and C-2 of etherified, uncondensed guaiacylpropane units, respectively, and by intense signals 6, 12, 13 and 26 corresponding to C-3/C-5, C-1, C-4 and C-2/C-6 of etherified, uncondensed syringylpropane units, respectively.[25-27] Signals 28-29, 33-34 and 37-38 correspond to oxygenated C-β, C-α and C-γ in side chains of both guaiacyl- and syringylpropane units, respectively. Furthermore, the presence of carbohydrates in the

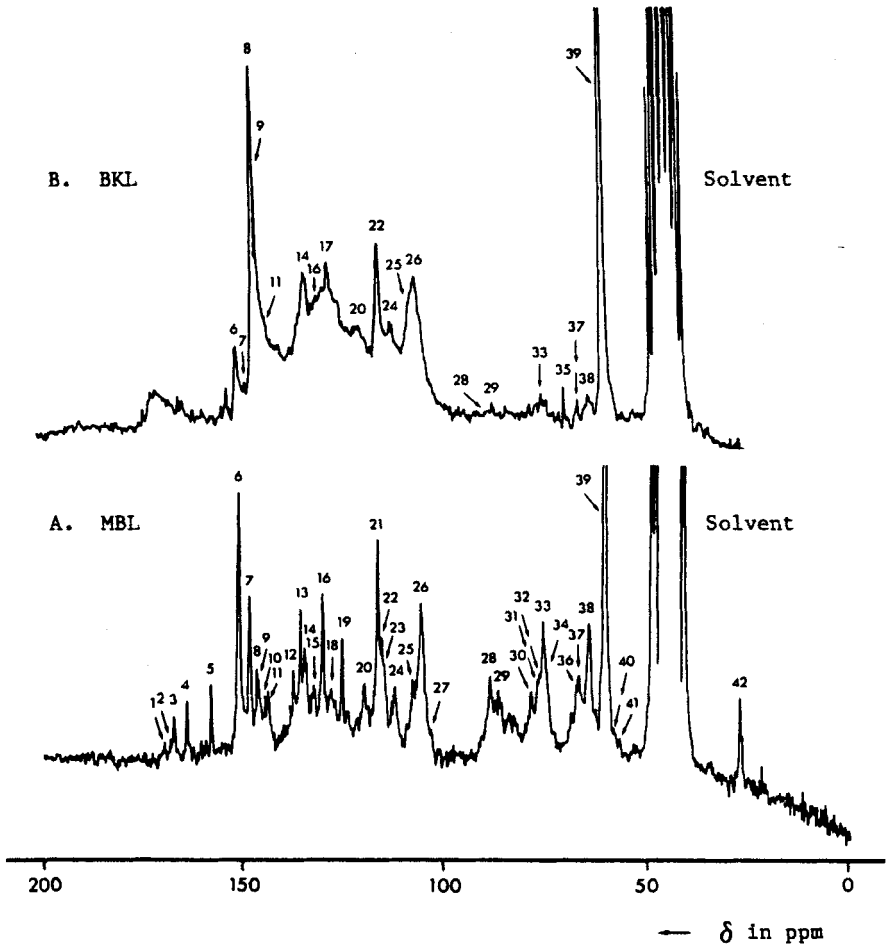


Figure 1. Routine ^{13}C NMR Spectra of Milled Bamboo Lignin (MBL) from Phyllostachys pubesecens (Spectrum A) and Bamboo Kraft Lignin (BKL) (Spectrum B). Solvent: DMSO-d_6.

MBL is evidenced by signals 3, 27, 30, 31-32, 33, 36 and 42 corresponding to -C=O of O-acetyls, C-1, C-4, C-3, C-2, C-5 and $-CH_3$ of O-acetyls in xylan, respectively.

2. The Nature of Bamboo Kraft Lignin and Structural Changes Occurred in Bamboo Lignins during Kraft Pulping of Bamboos

Tables 1 and 2 also show the results of the elemental and functional group analyses of the purified bamboo kraft lignin

(BKL). It was observed that the BKL has lower methoxyl and total hydroxyl contents than the purified milled bamboo lignin (MBL), although the BKL contains substantially higher phenolic hydroxyl content but lower aliphatic hydroxyl content per both C_9-unit and OCH_3 than the MBL. Evidently, the lignins in bamboos underwent extensive degradation during kraft pulping.

As shown in Tables 4 and 5, the BKL gave only trace amount of p-hydroxycinnamic acid on two stage hydrolysis, and a negligible amount of p-hydroxybenzaldehyde on nitrobenzene oxidation. These results can be interpreted as further evidence for the occurrence of the base-catalyzed hydrolysis of PC ester structures and cleavage of PC type α-aryl ether structures via a quinonemethide intermediate under the conditions of kraft pulping. Moreover, the results from nitrobenzene oxidation provide further evidence for the guaiacyl-syringyl nature of the BKL. In addition to the rather low yield of the aldehydes, the molar ratio of vanillin to syringaldehyde is about 1:0.6 for the BKL on nitrobenzene oxidation as compared to about 1:1.4 for the MBL. Thus, syringyl type uncondensed β-aryl ether structures are evidently more susceptible to the base-catalyzed cleavage than the corresponding guaiacyl type structures. It follows that the BKL should have a higher phenolic hydroxyl content than the MBL, which is supported by the results from the functional group analyses as shown in Table 3. The low yield of the aldehydes on nitrobenzene oxidation of the BKL could be also attributable to the occurrence of condensation among lignin fragments during kraft pulping.

A comparison between ^{13}C NMR spectra of the MBL and BKL (Figure 1) provides further evidence that bamboo lignins undergo base-catalyzed hydrolysis of p-hydroxycinnamic acid (PC) ester linkages and base-catalyzed cleavages of α- and β-aryl ether bonds with concomitant condensation of the resulting aromatic fragments and degradation of side chains under the conditions of kraft pulping. The hydrolysis of PC ester bonds is indicated by the absence of signals characteristic of PC ester structures in the spectrum of the BKL (Figure 1B) as compared to that of the MBL

Table 6. Assignment of Signals in ^{13}C NMR Spectra of Milled Bamboo Lignin (MBL) and Bamboo Kraft Lignin (BKL)

Signal Number	Chemical Shift (δ in ppm)	Intensity* MBL	Intensity* BKL	Assignment of Signals
1	172.0	vw	w	-C=O of Aliphatic Carboxylic Acids
2	170.2	vw	w	
3	169.6	m	-	-C=O of O-Acetyls in Xylans
4	166.3	m	-	-C=O of p-Hydroxycinnamic Acid Esters
5	159.8	m	-	C-4 of p-Hydroxycinnamic Acid Esters
6	152.2	vs	m	C-3/C-5 of etherified S C-3/C-3' of etherified 5-5'
7	149.5	s	w	C-3 of etherified G
8	147.5	m	vs	C-4 of etherified G C-3/C-5 of non-etherified S
9	147.0	m	m	C-3 of non-etherified G
10	145.5	w	w	C-4 of non-etherified G
11	144.6	w	-	C-α of p-Hydroxycinnamic Acid Esters
12	138.0	m	vw	C-1 of etherified S
13	136.1	s	w	C-4 of etherified S
14	135.0	m	s	C-1 of etherified G C-4 of non-etherified S
15	132.2	w	m	C-1 of non-etherified G and S
16	130.2	s	m	C-2/C-6 of p-Hydroxycinnamic Acid Esters C-1 of non-etherified G and S
17	128.3	-	s	C-α of p,o'-Stilbenes -CH=CH- in Olefins
18	128.0	w	m	C-α/C-β of Ar-CH=CH-CH_2OH C-2/C-6 of H -CH=CH- in Olefins
19	125.1	m	w	C-1 of p-Hydroxycinnamic Acid Esters
20	119.3	m	m	C-6 of G

Table 6. (Continued)

Signal Number	Chemical Shift (δ in ppm)	Intensity* MBL	Intensity* BKL	Assignment of Signals
21	115.9	vs	–	C-3/C-5 of p-Hydroxycinnamic Acid Esters
22	114.6	s	s	C-5 of G
23	113.8	m	–	C-β of p-Hydroxycinnamic Acid Esters
24	112.3	m	m	C-2 of G
25	106.8	m	m	C-2/C-6 of S with α-C=O
26	104.5	vs	vs	C-2/C-6 of S
27	101.9	w	–	C-1 of Xylans
28	86.2	m	vw	C-β of S β-O-4 (erythro)
29	84.0	m	w	C-β of G β-O-4 (erythro)
30	75.5	m	–	C-4 of Xylans
31	74.1	m	–	C-3 of Xylans
32	73.3	m	vw	C-α of S β-O-4 (erythro)
33	72.4	s	w	C-α of G β-O-4 (erythro) C-2 of Xylans
34	71.6	m	w	C-α of G and S β-O-4 (threo)
35	66.5	–	w	$-CH_2-O-$ of Dioxane (Artifact)
36	65.3	m	–	C-5 of Xylans
37	62.6	m	w	C-γ of G and S β-O-4 with α-C=O C-γ of G and S β-5' and β-1'
38	60.3	s	w	C-γ of G and S β-O-4 (erythro and threo)
39	56.0	vs	vs	$-OCH_3$ of G and S Ar-OCH_3
40	54.2	w	vw	C-β of G and S β-β'
41	53.5	vw	vw	C-β of G and S β-5'
42	20.9	s	–	$-CH_3$ of O-Acetyls in Xylans

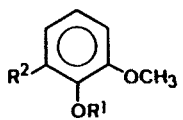
* vs = Very Strong; s = Strong; m = moderate; w = Weak; vw = Very Weak.

** H = p-Hydroxyphenyl; G = Guaiacyl; S = Syringyl.

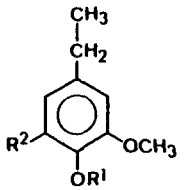
(Figure 1A). The splitting of α- and ß-aryl ether bonds is evident from the significant reduction in the relative intensity of signals characteristic of etherified guaiacyl- and syringylpropane structures in the aromatic region of the spectra. The relative intensities of signals 6 and 7 in the spectrum of the BKL are significantly weaker than that of the corresponding signals in the spectrum of the MBL. The signals 6 and 7 correspond to C-3/C-5 of etherified syringylpropane units and C-3 of etherified guaiacylpropane units, respectively. Moreover, as compared to the spectrum of the MBL, the near absence of signal 7 and increase in the relative intensity of signals 8 and 14 in the spectrum of the BKL suggest that BKL contains more non-etherified guaiacyl and syringyl type structures than the MBL. The signal 8 corresponds to C-4 of etherified guaiacyl groups, C-3 of non-etherified guaiacyl groups and C-3/C-5 of non-etherified syringyl groups, while the signal 14 corresponds C-4 of non-etherified syringyl groups.

3. Low-Molecular-Weight Constituents in Black Liquor from Kraft Pulping of Bamboos

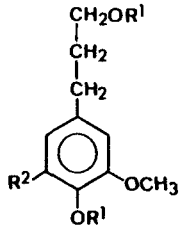
The black liquor from kraft pulping of bamboo contained ether-soluble neutral, phenolic and acid fractions in about 0.4, 3.2 and 2.7% per total organics in the black liquor, respectively. Thus, the organics in the combined ether-soluble fractions was about 6.3% of the total organics in the black liquor which was about 112 g per liter. The ether-soluble phenolic and acidic fractions from the black liquor were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry after O-acetylation with pyridine-acetic anhydride and subsequent O-methylation with ethereal diazomethane. The analysis of the mass spectra thus obtained resulted in elucidation of the structure of 17 compounds. Among the compounds identified, compounds 1a-16a are aromatic compounds with a guaiacyl or syringyl group, and must be derived from degradation of bamboo lignins during the kraft pulping. The remaining one is identified as palmitic acid methyl ester (17a). Except for compound 16a, the identity of these



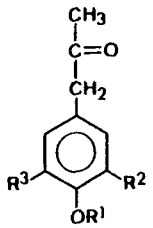
1, $R^1 = R^2 = H$
1a, $R^1 = Ac$; $R^2 = H$
2, $R^1 = H$; $R^2 = OCH_3$
2a, $R^1 = Ac$; $R^2 = OCH_3$



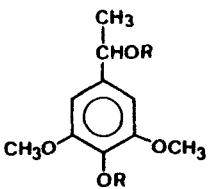
3, $R^1 = R^2 = H$
3a, $R^1 = Ac$; $R^2 = H$
4, $R^1 = H$; $R^2 = OCH_3$
4a, $R^1 = Ac$; $R^2 = OCH_3$



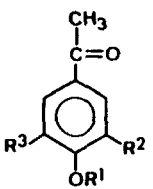
5, $R^1 = R^2 = H$
5a, $R^1 = Ac$; $R^2 = H$
6, $R^1 = H$; $R^2 = OCH_3$
6a, $R^1 = Ac$; $R^2 = OCH_3$



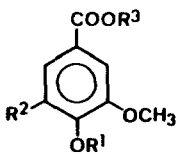
7, $R^1 = R^2 = R^3 = H$
7a, $R^1 = Ac$; $R^2 = R^3 = H$
8, $R^1 = R^3 = H$; $R^2 = OCH_3$
8a, $R^1 = Ac$; $R^2 = OCH_3$; $R^3 = H$
9, $R^1 = H$; $R^2 = R^3 = OCH_3$
9a, $R^1 = Ac$; $R^2 = R^3 = OCH_3$



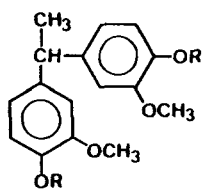
10, R = H
10a, R = Ac



11, $R^1 = R^2 = R^3 = H$
11a, $R^1 = Ac$; $R^2 = R^3 = H$
12, $R^1 = R^3 = H$; $R^2 = OCH_3$
12a, $R^1 = Ac$; $R^2 = OCH_3$; $R^3 = H$
13, $R^1 = H$; $R^2 = R^3 = OCH_3$
13a, $R^1 = Ac$; $R^2 = R^3 = OCH_3$



14, $R^1 = R^2 = R^3 = H$
14a, $R^1 = Ac$; $R^2 = H$; $R^3 = CH_3$
15, $R^1 = R^3 = H$; $R^2 = OCH_3$
15a, $R^1 = Ac$; $R^2 = OCH_3$; $R^3 = CH_3$



16, R = H
16a, R = Ac

Table 7. Yield and GC-Retention Time of Compounds Identified as the Constituents of O-Acetylated, O-Methylated Phenolic and Acidic Ether-Soulble Fractions of Black Liquor from Kraft Pulping of Bamboos

Compounds Identified	GC-Retention Time (min)*	Yield (%)**
O-Acetylguaiacol (1a)	7.6	0.7
O-Acetyl-4-ethylguaiacol (3a)	8.1	0.1
4-O-Acetyl-4-hydroxyacetophenone (11a)	10.3	0.2
O-Acetylsyringol (2a)	10.6	0.4
α-(4-O-Acetyl-4-hydroxyphenyl)-acetone (7a)	11.2	0.1
4-O-Acetylacetoguaiacone (12a)	12.6	2.4
O-Acetyl-4-ethylsyringol (4a)	12.8	0.2
4-O-Acetylvanillic Acid Methyl Ester (14a)	13.0	1.5
α-(4-O-Acetylguaiacyl)acetone (8a)	13.4	0.2
4-O-Acetylacetosyringone (13a)	15.8	6.5
Palmitic Acid Methyl Ester (17a)	16.8	0.6
4-O-Acetylsyringic Acid Methyl Ester (15a)	17.7	3.4
α-(4-O-Acetylsyringyl)acetone (9a)	18.0	0.2
3-(4-O-Acetylguaiacyl)propyl Acetate (5a)	18.6	0.3
1-(4-O-Acetylsyringyl)ethyl Acetate (10a)	19.2	0.2
3-(4-O-Acetylsyringyl)propyl Acetate (6a)	20.2	0.3
1,1-Bis(4-O-acetylguaiacyl)-ethane (16a)	21.5	0.2
Total Yield of the Compounds Identified		17.5

* GC Conditions: See Experimental.

** Yield per Total Organics of the Combined O-Acetylated, O-Methylated Neutral, Phenolic and Acidic Ether-Soluble Fractions of the Black Liquor (About 8.2 g from the Total Organics in the Black Liquor, i.e., 112 g per Liter).

compounds was further established by comparison of GC-retention times and mass spectra with those of authentic samples. Table 7 summarizes GC-retention times and yields of compounds 1a-17a. The major constituents were 4-O-acetyl-acetosyringone (13a), 4-O-acetylsyringic acid methyl ester (15a), 4-O-acetylacetoguaiacone (12a) and 4-O-acetylvanillic acid methyl ester (14a) with yields of about 6.5, 3.4, 2.4 and 1.5% of total organics of the combined O-acetylated, O-methylated ether-soluble fractions, respectively. The yields of the other compounds were less than 1%. The molar ratio of guaiacyl type to syringyl type degradation products is approximately 1:2. Since the molar ratio of guaiacylpropane to syringylpropane is estimated to be about 50:38 (≒ 1:0.8), the result further supports the observation that syringyl type β-aryl ether structures in bamboo lignins are more susceptible to base-catalyzed cleavage of β-aryl ether linkage than the corresponding guaiacyl type structures.

As mentioned previously, compounds 1a-17a were identified as the constituents of either the O-acetylated, O-methylated ether-soluble phenolic or acidic fraction. It follows that the compounds originally present in either the ether-soluble phenolic or acid fraction are guaiacol (1), syringol (2), 4-ethylguaiacol (3), 4-ethylsyringol (4), 3-guaiacylpropanol (5), 3-syringylpropanol (6), α-(4-hydroxyphenyl)acetone (7), α-guaiacylacetone (8), α-syringylacetone (9), 1-syringylethanol (10), 4-hydroxyacetophenone (11), acetoguaiacone (12), acetosyringone (13), vanillic acid (14), syringic acid (15), 1,1-diguaiacylethane (16) and palmitic acid (17). Except compounds 14 and 15, compounds 1-16 have been identified as the low-molecular-weight constituents of black liquors from kraft pulping of soft- and hardwoods.[28-34] However, reaction mechanisms leading to formation of vanillic acid (14) and syringic acid (15) are not well established. Further investigation is required to determine whether these acids are produced directly from degradation of bamboo lignins or result from redox reactions of lignin degradation products under the conditions of kraft pulping.

Neither p-hydroxycinnamic acid nor ferulic acid was detected as a low-molecular-weight constituent of the black liquor. Evidently, after release of p-hydroxycinnamic acid (PC) and ferulic acid (FE) from the lignin-core via base-catalyzed hydrolysis of PC and FE type ester and α-aryl ether structures, these acids must undergo further degradation and condensation with lignin fragments under the conditions of kraft pulping. Treatment of ethyl ferulate under the conditions of kraft pulping resulted not only in disappearance of the substrate and ferulic acid produced on saponification of the substrate but also in formation of over 30 degradation and condensation products. The results of this investigation are being evaluated and will be published elsewhere in the near future.

CONCLUSIONS

Milled bamboo lignin (MBL) from Phyllostachys pubescens is found to be composed of p-hydroxycinnamic acid, guaiacylpropane and syringyl-propane units in an approximate molar ratio of 1:4:3 on the basis of chemical and spectroscopic analyses. The guaiacylpropane and syringyl-propane units constitute a lignin-core, to which the p-hydroxycinnamic acid (PC) units are bonded as the peripheral groups. More than three-fourths of the PC units are bonded to the lignin-core in the form of ester linkages with hydroxyl groups at C-α and C-γ. The remaining PC units are bonded to the lignin-core in the form of α-aryl ether linkages. On the basis of chemical and spectroscopic analyses, the PC type ester linkages are found to be preferentially saponified during kraft pulping. The resulting p-hydroxycinnamic acid then undergoes further degradation and condensation with lignin fragments. In addition, the uncondensed syringylpropane units in the form of α- and β-aryl ether linkages are found to be more susceptible to base-catalyzed cleavage of aryl ether bonds than the corresponding guaiacylpropane units.

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