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Inhomogeneities in the Chemical Structure of Sugarcane Bagasse Lignin

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Sequential treatments of dewaxed bagasse with distilled water, 0.5 M NaOH, 0.5, 1.0, 1.5, 2.0, and 3.0% H_2O_2 at pH 11.5, and 2 M NaOH at 55 °C for 2 h solubilized 2.8, 52.5, 14.9, 3.3, 5.5, 5.0, 2.8, and 2.2% of the original lignin, respectively. The eight isolated lignin fractions were subjected to a comprehensive structural characterization by UV, FT-IR, and ^{1}H and ^{13}C NMR spectroscopies and thermal analysis. The nitrobenzene oxidation method was also applied to the in situ lignins. The seven lignin fractions, isolated successively with alkali and alkaline peroxide, were all SGH-type lignins, with a small amount of esterified *p*-coumaric acid and mainly etherified ferulic acid. No significant differences were found in the weight-average molecular weights (1680−2220 g/mol) of the seven alkali and alkaline peroxide dissolved lignins. However, the first four lignin fractions, isolated with 0.5 M NaOH and 0.5, 1.0, and 1.5% H_2O_2 at pH 11.5, were rich in syringyl units and contained large amounts of noncondensed ether structures, whereas the last three lignin fractions, isolated sequentially with 2.0 and 3.0% H_2O_2 at pH 11.5 and 2 M NaOH at 55 °C for 2 h, had a higher degree on condensation and were rich in guaiacyl lignins.

KEYWORDS: Bagasse lignins; sugars; molecular weight; UV; FT-IR; ^{1}H and ^{13}C NMR; nitrobenzene oxidation; phenolic acids

INTRODUCTION

In recent years, there has been an increasing trend toward more efficient utilization of agroindustrial residues such as sugarcane bagasse (generally known as "bagasse"), sugar beet pulp, and coffee pulp. These lignocellulosic materials are abundant and renewable, and they are expected to be used as alternatives to fossil resources. About 54 million dry tons of bagasse, the fibrous byproduct remaining after sugar extraction from sugarcane, is produced annually throughout the world (*1*). About 40−50% of the dry residue is the glucose polymer cellulose, much of which is in a crystalline structure. Another 25−35% is hemicelluloses, an amorphous polymer usually composed of xylose, arabinose, galactose, glucose, and mannose. The remainder is mostly lignin plus lesser amounts of minerals, waxes, and other compounds (*2*). Application of agroindustrial residues in pulping processes and alcohol production on the one hand provides alternative substrates and, on the other hand, help in solving pollution problems, which their disposal may otherwise cause. However, during the treatment process, not only the quantity but also the quality of lignin strongly affects the properties of the products and the cost efficiency. Thus, the removal of lignin is a very important issue not only for the pulping industry but also for utilizing polysaccharides as starting material to produce sugars and alcohols on an industrial scale.

Lignin is formed by dehydrogentive polymerization of hydroxycinamyl alcohols (monolignols) of three different types; *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (*3*−*5*). During the polymerization, the monolignols are oxidized by peroxidases and/or laccases to form phenoxy radicals that exist in the main mesomeric forms (*6*). The most abundant interunit linkage in all lignins is the β-aryl ether bond (β-*O*-4). In softwoods, the content of this linkage type has been estimated at 48%, whereas for hardwoods (birch) this type of linkage amounts to 60% (*7*). 4-*O*-5 (diaryl ether), 5-5′ (biphenyl), β-5 (phenylcoumaran), β-β, and β-1 structures are also frequent linkage types (*8*). In addition, lignin is extremely complicated, and its structure has not yet been completely elucidated. Lignin in situ has no structural regularity. Unlike most natural polymers, which consist of a single inter-monomeric linkage, lignin is a network polymer made up of many carbon-to-carbon and ether

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linkages. The tight physical binding and chemical linkages between lignin and cell wall polysaccharides also practically prevent its isolation in unaltered form. This makes it very difficult to use degradative or nondegradative methods for structural determination (*9*). Therefore, most of our present knowledge about the molecular structure of lignin is based on fractional isolation and analysis of monomers and dimers of chemically degraded lignins. Over the years, several lignin degradation methods have been developed within this field, for example, alkaline nitrobenzene oxidation, acidolysis, and thioacidolysis, for the analysis of monomeric degradation products (*10*−*12*). It is, however, very difficult to obtain a complete understanding of the structural variability of lignin in different morphological regions of the woody sample by means of such investigation (*13*).

It is known that the structural features of lignin, such as the ratio of syringyl nuclei to guaiacyl nuclei and the amount of ring-conjugated carbonyl groups, have a great influence on the rate of the delignification reaction and the quality of the final products in the pulping process (*14*). Thus, development of a rapid, nondestructive, and simple procedure to isolate lignin from the cell walls of lignocellulosic materials would be beneficial to estimate the structural features of lignin in a woody matrix. More importantly, during the past decade, increased concern for the environment has caused the pulp and paper industry to reconsider the use of chlorine as the initial delignifying agent in a bleaching sequence. Conventional bleaching sequences are being replaced by various elemental chlorine-free and totally chlorine-free sequences. Whereas these changes will continue to evolve as new technologies become available, peroxygen-based regents such as hydrogen peroxide and peroxy acids are expected to play an important role (*15*). As a bleaching agent, hydrogen peroxide offers several major advantages; it is environmentally friendly, relatively inexpensive, and easy to retrofit into existing mills (*16*). The various decompositions of hydrogen peroxide result in the formation of hydroxyl radicals, oxygen, and superoxide ions. These may attack reactive lignin structures, that is, phenolic units bearing no carbonyl group. Alkaline hydrogen peroxide also causes the oxidative degradation of phenolic lignin units, accounting for the lignin dissolution effects observed during the peroxide stage. Thus, the use of alkaline peroxide has been proposed as a delignifying and bleaching system for pulps (*17*, *18*). The alkaline peroxide treatment was therefore characterized with respect to the H_2O_2 concentration affecting delignification efficiency because its application gave results comparable to those of other delignifying agents. In this study, eight fractional lignin preparations were therefore isolated by sequential treatments of dewaxed bagasse with distilled water, 0.5 M NaOH, 0.5, 1.0, 1.5, 2.0, and 3.0% H_2O_2 at pH 11.5, and 2 M NaOH at 55 °C for 2 h. The chemical composition and physicochemical properties of the lignins were comparatively studied by both degradation methods such as alkaline nitrobenzene oxidation and nondestructive techniques, for example, ultraviolet (UV), Fourier transform infrared (FT-IR), and gel permeation chromatography (GPC). The thermal stability of the lignin fractions was also investigated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC).

MATERIALS AND METHODS

Materials. Sugarcane bagasse was obtained from a local sugar factory (Guangzhou, China). It was first dried in sunlight and then cut into small pieces (1−3 cm). The cut bagasse was ground to pass a 0.8-mm screen. The composition (percent, w/w) of the bagasse was cellulose, 43.6%, hemicelluloses, 33.5%, lignin, 18.1%, ash, 2.3%, and wax (mainly lipophilic extractives), 0.8%, on a dry weight basis.

Fractionation of Lignins. The dried powder (10 g) was first extracted with toluene/ethanol (2:1, v/v) in a Soxhlet apparatus for 6 h, and the meal was dried in an oven at 60 °C for 16 h. The dewaxed bagasse meal was then soaked in 300 mL of distilled water at 55 °C for 2 h under stirring. After isolation of the water-soluble hemicelluloses by precipitation of water extracts with 3 volumes of 95% ethanol, a water-soluble lignin preparation was obtained by reprecipitation at pH 1.5, adjusted by 6 M HCl, from the supernatant solution, and labeled as water-soluble or acid-insoluble lignin fraction L_1. A sample free of wax and water solubles was successively treated with 300 mL of 0.5 M NaOH, 200 mL of 0.5, 1.0, 1.5, 2.0, and 3.0% H_2O_2 at pH 11.5 adjusted with 6 M NaOH, and 200 mL of 2 M NaOH at 55 °C for 2 h. After the indicated period of treatment, the insoluble residue was collected by filtration, washed with distilled water until the pH of the filtrate was neutral, and then dried at 60 °C. Each of the supernatant fluids was adjusted to pH 5.5 with 6 M HCl and then concentrated to ∼30 mL under reduced pressure. The released hemicelluloses were precipitated by pouring the concentrated supernatant fluid into 120 mL of 95% ethanol. The solubilized lignins were obtained from the corresponding supernatants by precipitation at pH 1.5−2.0. Each of the lignin fractions was rinsed with acidified water (pH 2.0), freeze-dried, and designated acid-insoluble lignin preparation L_2, L_3, L_4, L_5, L_6, L_7, or L_8, respectively. Triplicate runs were done for each lignin preparation. The relative standard deviation, determined by dividing the standard deviation by the mean value, was <3.5%.

Characterization of Acid-Insoluble Lignin Fractions. The monomeric composition of the noncondensed monomeric units of these lignin fractions was characterized by nitrobenzene oxidation, and the resulting aromatic aldehydes and acids were characterized by high-performance liquid chromatography (HPLC) as previously reported (*19*). The hemicellulosic moieties associated with lignin fractions were hydrolyzed with 2 M trifluoroacetic acid for 2 h at 120 °C. Liberated neutral sugars were analyzed as their alditol-acetate derivatives by gas chromatography (*20*). The methods for determination of molecular weights and thermal analysis of the acid-insoluble lignin preparations have been described in a previous paper (*19*). The standard errors or deviations were observed to be 6.2−13.8%. Other analyses were run at least twice. The error for the sugar analysis was <0.06% for all of the same lignin preparation. For molecular average weights, the average relative error in all analyses was ∼2.2−4.5%, and the maximum error was ∼6.2%.

UV−vis spectra were obtained using a Hewlett-Packard 8452A diode array spectrophotometer. FT-IR spectroscopy was performed on a Nicolet 510 spectrophotometer using a KBr disk containing 1% finely ground samples. The ^{1}H NMR spectrum of the lignin in solution was recorded on a Bruker MSI-250 spectrometer using 25 mg of lignin in 1.0 mL of DMSO-d_6. For each sample, 1000 scans were collected. The solution ^{13}C NMR spectrum was recorded on a Bruker MSI-300 spectrometer at 74.5 MHz from 250 mg of sample dissolved in 1.0 mL of DMSO-d_6 after 28000 scans. A 70° pulse flipping angle, a 10 μs pulse width, and a 15 s delay time between scans were used.

RESULTS AND DISCUSSION

Fractional Yield and Purity of Lignin. Table 1 gives the yields of total solublized lignin, acid-insoluble lignin, acid-soluble lignin (lignin solubilized in the pH 1.5−2.0 solution), and lignin associated with the solubilized hemicelluloses, obtained from the various treatment procedures. As can be seen, sequential treatment of the dewaxed bagasse with distilled water, 0.5 M NaOH, 0.5, 1.0, 1.5, 2.0, and 3.0% H_2O_2 at pH 11.5, and 2 M NaOH at 55 °C for 2 h solubilized 0.5, 9.5, 2.7, 0.6, 1.0, 0.9, 0.5, and 0.4% lignin (percent dry starting material), corresponding to the release of 2.8, 52.5, 14.9, 3.3, 5.5, 5.0, 2.8, and 2.2% of the original lignin, respectively. Meanwhile, the successive treatment also released 4.1, 12.0, 3.8, 1.4, 2.5, 1.0, 0.9, and 4.6% hemicelluloses (percent dry starting material), corresponding to the dissolution of 12.2, 35.8, 11.3, 4.2, 7.5,

Table 1. Yield of Lignin (Percent Dry Matter) Solubilized during the Successive Treatments of Dewaxed Sugarcane Bagasse with Distilled Water, 0.5 M NaOH, Various Concentrations of Alkaline Peroxide at pH 11.5, and 2 M NaOH at 55 °C for 2 h

	lignin fraction								
	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	total
total solubilized lignins	0.5	9.5	2.7	0.6	1.0	0.9	0.5	0.4	16.1
acid-insoluble lignins[a]	0.05	6.6	2.0	0.3	0.7	0.6	0.3	0.2	10.8
acid-soluble lignins[b]	0.05	2.2	0.6	0.2	0.2	0.3	0.2	0.2	4.0
lignin associated with solubilized hemicelluloses	0.4	0.6	0.1	0.06	0.06	0.01	0.02	0.02	1.3

[a] Lignin fractions obtained by precipitation of the supernatant solution at pH 1.5–2.0 after isolation of the solubilized hemicelluloses. [b] Lignin fractions still solubilized in the pH 1.5–2.0 supernatant after precipitation of the acid-insoluble lignin fractions and obtained by difference (total solubilized lignin − acid-insoluble lignin − lignin associated in the solubilized hemicelluloses).

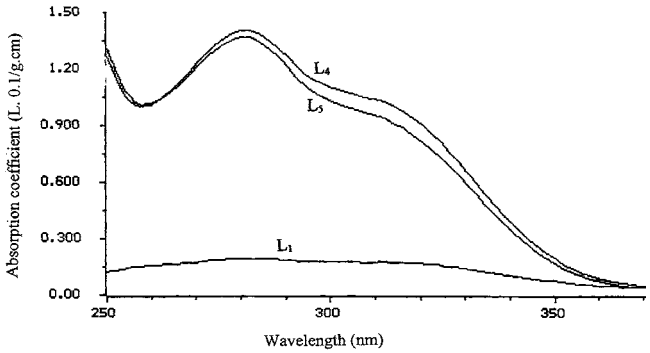

Figure 1. UV spectra of acid-insoluble lignin fractions L_1, L_4, and L_5.

3.0, 2.7, and 13.7% of the original hemicelluloses, respectively (data not shown). The total yield of eight lignin fractions accounted for 89.0% of the original lignins in the cell walls of bagasse, indicating that substantial amounts of lignin were extracted sequentially with sodium hydroxide and alkaline peroxide in increasing the concentration from 0.5 to 3.0% under the conditions used. In addition, as shown in **Table 1**, the isolated acid-insoluble lignin, obtained by alkali and alkaline peroxide, was the major lignin fraction, comprising 50.0−74.1% of the total solubilized lignins, whereas the corresponding lignin fraction, associated with the solubilized hemicelluloses, accounted for only 1.0−6.3% of the total released lignins. In contrast, 80% of the water-soluble lignin fraction was found to be associated with the released hemicelluloses. This observation indicated that the alkali and alkaline peroxide treatment under the conditions used significantly cleaved the linkages between lignin and hemicelluloses from the cell walls of bagasse.

Although UV−vis spectroscopy of lignins is not well-suited for structure elucidation because of absorption band overlapping from the different chromophores found in the macromolecule, it is useful for a preliminary characterization (*21*). In this study, UV−vis absorption measurements of the acid-insoluble lignin fractions were carried out using a dioxane/water mixture, which solubilizes the lignins but which is limited to wavelengths above 240 nm. Spectra of acid-insoluble lignin fractions L_1 solubilized during the water treatment, L_4, and L_5 released in 1.0 and 1.5% H_2O_2 extraction, respectively, are given in **Figure 1**. The maximum absorption near 280 nm originates from nonconjugated phenolic groups in the lignin. The presence of a second characteristic region of lignin absorption around 310 nm can be assigned to the presence of both ferulic and *p*-coumaric acids (*22*). Obviously, the lower absorption coefficient of the water-soluble lignin fraction L_1 was undoubtedly due to the higher amounts of bound polysaccharides such as hemicelluloses and other nonlignin materials.

The treatment with alkali or alkaline peroxide, although leading to minor modification of the lignin structure, allows recovery of the lignin in large quantities and in high purity. This is in contrast to the water treatment that does not significantly affect the lignin structure but leads to low yields and contamination of the lignin by polysaccharide residues. **Table 2** shows the composition of neutral sugars in eight acid-insoluble lignin preparations. The lignin fraction released during the water treatment contained significant amounts of hemicelluloses as shown by the neutral sugar content of 24.3%, whereas all lignin preparations solubilized during the alkali and alkaline peroxide treatment had rather low amounts of bound polysaccharides as shown by 0.81−1.3% neutral sugar contents. This indicates that the treatments of bagasse with alkali and alkaline peroxide substantially cleaved the linkages between lignin and hemicelluloses in the cell walls of bagasse in addition to saponification of hydroxycinnamic esters, such as between *p*-coumaric acid and lignin or between ferulic acid and hemicelluloses. Interestingly, increases in alkali concentration from 0.5 to 2 M and percentage of alkaline peroxide from 0.5 to 3.0% resulted in decreases in the level of associated polysaccharides from 1.3 to 0.98% and from 1.2 to 0.81%, respectively. These data revealed that an increase in alkali and alkaline peroxide concentration could peel more lignin from most of the neighboring polysaccharide moieties. In addition, a relatively high amount of xylose (0.41−7.8%) together with noticeable quantities of arabinose (0.17−3.7%), galactose (0.11−3.4%), and glucose (0.12−3.1%) in all of the lignin fractions implied that these bound polysaccharides mainly originated from the hemicelluloses such as xylan in the secondary cell walls of bagasse, not from the pectic polysaccharides in the middle lamella.

Table 2. Content of Neutral Sugars (Percent Dry Weight, w/w) in Isolated Acid-Insoluble Lignin Fractions

	lignin fraction							
sugar	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8
rhamnose	0.96	Tr[a]	Tr	Tr	Tr	ND[b]	ND	ND
arabinose	3.68	0.26	0.25	0.22	0.19	0.17	0.17	0.17
xylose	7.78	0.59	0.56	0.53	0.48	0.44	0.41	0.49
mannose	5.32	Tr	Tr	ND	ND	ND	ND	ND
galactose	3.42	0.17	0.17	0.14	0.13	0.11	0.11	0.12
glucose	3.12	0.27	0.24	0.22	0.18	0.16	0.12	0.20
total	24.28	1.29	1.22	1.11	0.98	0.88	0.81	0.98

[a] Tr, trace. [b] ND, not detectable.

Composition of Phenolic Monomers. Alkaline nitrobenzene oxidation analysis can be used for characterization of the structural lignin, and amounts and relative distribution of degradation products can then be used to derive information about the composition of the original polymer (*23*). Results obtained by alkaline nitrobenzene oxidation at 170 °C for 3 h are shown in **Table 3**. In comparison, a much lower yield of phenolic acids and aldehydes in the water-soluble lignin fraction was due to the substantial amounts of non-lignin material such

Table 3. Yield (Percent Lignin Sample, w/w) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of the Acid-Insoluble Lignin Fractions

	lignin fraction							
phenolic acids and aldehydes	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8
p-hydroxybenzoic acid	3.32	0.89	0.90	1.17	1.53	0.95	0.90	0.41
p-hydroxybenzaldehyde	0.41	6.29	3.02	3.66	3.77	0.48	0.32	0.25
vanillic acid	0.14	0.25	0.38	0.68	0.91	0.33	0.31	0.25
vanillin	1.52	14.16	11.53	14.87	16.23	6.90	5.56	5.68
syringic acid	0.36	0.96	0.54	0.55	0.40	0.28	0.23	0.22
syringaldehyde	1.44	22.40	14.43	19.30	24.00	4.20	5.22	5.52
acetovanillone	Tr[a]	1.51	0.70	1.13	1.97	0.85	0.42	0.36
acetosyringone	ND[b]	1.53	0.76	1.17	2.41	0.89	0.61	0.55
p-coumaric acid	0.98	0.41	0.16	0.21	0.38	0.23	0.43	0.22
ferulic acid	0.52	0.58	0.29	0.22	0.33	0.21	0.26	0.16
total	8.69	31.09	32.71	42.96	51.93	15.32	14.26	13.62
molar ratio (S:V:H)[c]	0.35:0.39:1	2.3:1.8:1	2.8:2.6:1	3.0:2.8:1	3.5:3.0:1	2.7:4.9:1	3.6:4.5:1	6.9:8.2:1

[a] Tr, trace. [b] ND, not detectable. [c] S, relative sum of total moles of syringaldehyde, syringic acid, and acetosyringone; V, relative sum of total moles of vanillin, vanillic acid, and acetovanillone; H, relative sum of total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid.

as hemicelluloses and ash coextracted in L_1. However, the total yields of phenolics in 0.5 M NaOH-soluble lignin fraction L_2 (31.1%), 0.5, 1.0, and 1.5% H_2O_2-soluble lignin fractions L_3 (32.7%), L_4 (42.3%), and L_5 (51.9%) were much higher than those of the lignin preparations L_6 (15.3%), L_7 (14.3%), and L_8 (13.6%) isolated with 2.0 and 3.0% H_2O_2 at pH 11.5 and 2 M NaOH at 55 °C for 2 h. Thus, a decrease in its yield suggested an increase in the condensed-type linkages of lignin in the fractions L_6, L_7, and L_8 because alkaline nitrobenzene oxidation products would be used to measure the noncondensed types of the lignin present. The results in **Table 3** indicate that the lignin fractions isolated with a high concentration of peroxide and alkali were rich in the condensed type linkages of lignin.

As the data in **Table 3** show, the dominant oxidation products in water-soluble lignin fraction L_1 were found to be *p*-hydroxybenzoic acid, vanillin, and syringaldehyde. The presence of large amounts of *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde was considered to be indicative of noncondensed *p*-hydroxyphenyl units, indicating the incorporation of *p*-hydroxycinnamyl alcohol in bagasse lignin. The occurrence of almost equal amounts of vanillin and syringaldehyde originated from noncondensed guaiacyl and syringyl units. The relative molar ratios of S (the relative total moles of syringaldehyde, syringic acid, and acetosyringone) to V (the relative total moles of vanillin, vanillic acid, and acetovanillone) and to H (the relative total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid) in this fraction appeared to be 0.35:0.39:1.0. This high ratio of *p*-hydroxyphenyl units in the L_1 fraction revealed that the water-soluble lignin resulted mainly from the secondary wall of bagasse because the secondary wall lignin was suggested to originate from a higher concentration of *p*-hydroxyphenyl type lignin (*8*). In addition, a higher content of *p*-hydroxybenzoic acid obtained in this fraction was presumed to be largely due to the partial oxidation of *p*-coumaric acid by nitrobenzene. Furthermore, it is very likely that the water-soluble lignin fraction may contain noticeable amounts of *p*-hydroxybenzoic acid, which is linked to lignin or hemicelluloses by ester or ether bonds. Similar results have been found in fast-growing poplar wood in our previous study, in which *p*-hydroxybenzoic acid was found to be linked to lignin by means of ester linkages in the cell walls (*24*).

There are significant differences in the chemical structure of lignin depending on its morphological origin (*8*). As expected, the molar ratios of S:V:H in lignin fractions L_2, L_3, L_4, and L_5 were found to be 2.3:1.8:1, 2.8:2.6:1, 3.0:2.8:1, and 3.5:3.0:1, whereas these ratios decreased to 2.7:4.9:1 in L_6, 3.6:4.5:1 in L_7, and 6.9:8.2:1 in L_8, respectively. These higher amounts of noncondensed syringyl units in the L_2, L_3, L_4, and L_5 fractions indicated that the acid-insoluble lignin fractions isolated by 0.5 M NaOH and 0.5, 1.0, and 1.5% H_2O_2 arose from the secondary wall lignin. In the L_6, L_7, and L_8 lignin fractions extracted by 2.0 and 3.0% H_2O_2 and 2 M NaOH, the concentrations of noncondensed guaiacyl units were much higher than those of syringyl units, indicating that these lignins were attributed to the middle lamella lignin because the secondary wall lignin contains many more syringyl units than the middle lamella lignin (*25*). Overall, it is clear that syringyl units are more reactive to alkali and alkaline peroxide delignification conditions than guaiacyl lignin units, and syringyl β-*O*-4 ether lignin units are more reactive than the corresponding guaiacyl structures. In addition, although considerable amounts of *p*-coumaric and ferulic acids had been converted into *p*-hydroxybenzaldehyde or *p*-hydroxybenzoic acid and vanillin or vanillic acid, respectively, during the alkaline nitrobenzene oxidation at 170 °C, the remaining occurrence of small amounts of *p*-coumaric acid (0.16−0.98%) and ferulic acids (0.16−0.58%) implied that these two hydroxycinnamic acids are linked to lignin and/or hemicelluloses in the cell walls of bagasse.

Table 4. Weight-Average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersity (M_w/M_n) of the Acid-Insoluble Lignin Fractions

	lignin fraction							
	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8
M_w	3020	2180	2010	2180	2220	2200	1930	1680
M_n	1820	1460	1320	1370	1390	1360	1010	780
M_w/M_n	1.66	1.49	1.52	1.59	1.60	1.62	1.91	2.15

Molecular Weight. Weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of the eight acid-insoluble lignin fractions are summarized in **Table 4**. It can be seen that the differences in average molecular weights of the alkali and alkaline peroxide-soluble lignin preparations were not significant (M_w 1680−2220 g/mol). However, the M_w between water-soluble and alkali and alkaline peroxide-soluble lignins appeared to be significantly different. Due to the associated substantial amounts of hemicelluloses in L_1 fraction, the molecular weight of the water-soluble lignin fraction (M_w 3020 g/mol) was higher than was observed for alkali and alkaline peroxide-soluble lignins. The much lower

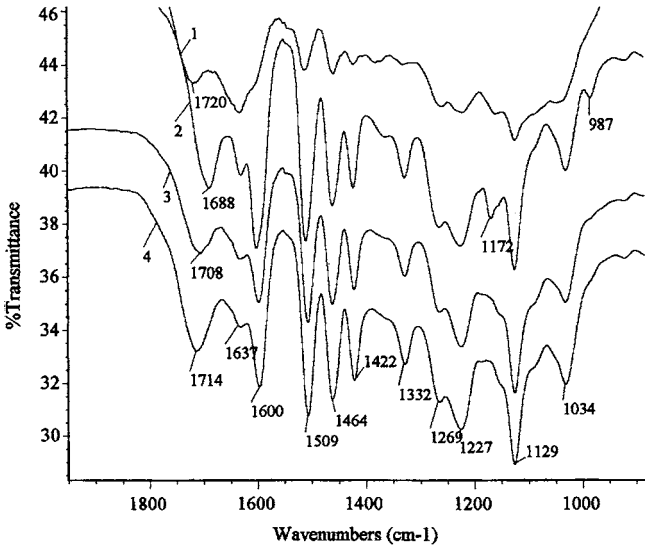

Figure 2. FT-IR spectra of acid-insoluble lignin fractions L_1 (spectrum 1) L_2 (spectrum 2), L_4 (spectrum 3), and L_5 (spectrum 4) isolated from dewaxed sugarcane bagasse.

values of M_w between L_2 and L_8 verified that the bagasse lignin was extensively degraded by alkali and alkaline peroxide under the conditions used. It was found that the lignin fraction L_8 isolated with a relatively high concentration of alkali (2 M NaOH) had the lowest value of M_w (1680 g/mol), strongly suggesting that the level of degradation of the released lignin is very high at a relatively high concentration of alkali. By contrast, the polydispersity value of L_8 is in the reverse order. Higher polydispersity is observed when the alkali or alkaline peroxide is increased.

FT-IR Spectra. Figure 2 illustrates FT-IR spectra of acid-insoluble lignin fractions L_1 (spectrum 1), L_2 (spectrum 2), L_4 (spectrum 3), and L_5 (spectrum 4) isolated sequentially with water, 0.5 M NaOH, and 1.0 and 1.5% H_2O_2 at pH 11.5. As can be seen from the diagram, the relative intensities, assigned at 1600, 1509, 1464, and 1422 cm^{-1}, were very similar in spectra 2−4, which confirmed that the "core" of the lignin structure did not change significantly during the alkali and alkaline peroxide treatment processes. However, the changes of the carbonyl absorption region might enable the evaluation of the effects of the alkaline peroxide treatment. The bands at 1708 and 1714 cm^{-1} in spectra 3 and 4 correspond to the carbonyl and unconjugated ketone and carboxyl group stretching, whereas the band at 1688 cm^{-1} in spectrum 2 relates to conjugated carbonyl stretching in the alkali-soluble lignin fraction L_2. The absorption at 1637 cm^{-1} in all four spectra might originate from an enol structure in lignins (*26*). Obviously, a remarkable increase of carboxyl absorption observed in spectra 3 and 4 demonstrated that a noticeable oxidation of the lignin structure did occur during the alkaline peroxide treatment. Moreover, syringyl and condensed guaiacyl absorptions are clearly seen at 1332 cm^{-1}, whereas guaiacyl ring breathing appears at 1269 cm^{-1}. Similarly, an intensive band at 1227 cm^{-1} is indicative of syringyl and guaiacyl ring breathing with C=O stretching, and an increasing intensity of this band in spectra 3 and 4 indicated again a noticeable increase in carbonyl groups in lignin fractions by alkaline peroxide oxidation. The strong band at 1129 cm^{-1} is assigned to C−O and C−C stretching vibrations, C−O deformation in secondary alcohols, or aromatic C−H in-plane deformation, whereas the C−O deformation in primary alcohols exhibits a peak at 1034 cm^{-1}. The carbonyl stretching of conjugated ester groups can be observed in spectrum 2 at 1172 cm^{-1} and is typical of *p*-hydroxyphenyl structures of grass lignin (*24*). In addition, it should be noted that the intensities for aromatic skeleton vibration in water-soluble lignin fraction L_1 (spectrum 1) are rather weak because of the substantial bound polysaccharides. In addition, occurrence of an important ester absorption band at 1720 cm^{-1} in the L_1 fraction suggests that the hemicelluloses associated with the water-soluble lignin fraction are rich in acetyl, hydroxycinnamate, or uronate ester groups.

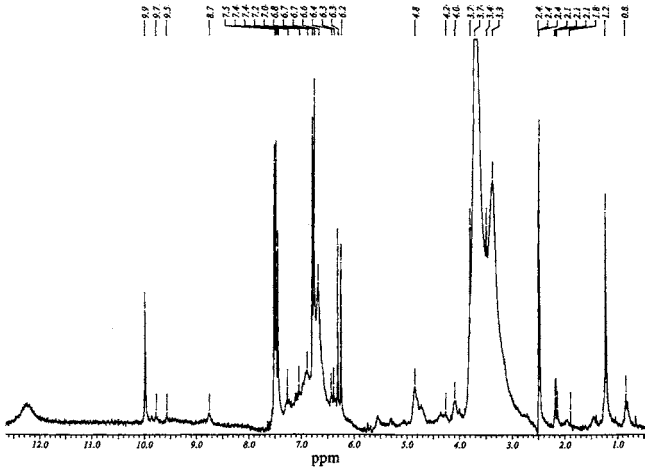

Figure 3. ^1H NMR spectrum of acid-insoluble lignin fraction L_2.

^1H and ^{13}C NMR Spectra. The ^1H NMR spectrum of lignin fraction L_2, isolated with 0.5 M NaOH, is presented in **Figure 3**. The integrals of signals centered around 6.70 and 6.79 ppm are assigned to aromatic protons in syringylpropane and guaiacylpropane structures, respectively (*27*), suggesting the presence of similar relative contents of syringyl and guaiacyl units in the lignin. The signals around 7.4−7.5 ppm are attributed to the aromatic protons in positions 2 and 6, in structures containing a C_α=O group, to aromatic protons in positions 2 and 6 of *p*-hydroxyphenyl units conjugated with a double bond, to the proton in C_α=C_β structures, and to aromatic protons in *p*-coumaric and ferulic acids, confirming the presence of *p*-hydroxyphenyl units, C_α=O groups, and *p*-coumaric and ferulic acids in the lignin fraction (*28*). The H_α and H_β in *β-O*-4 structures give two signals at 5.6 (data not shown) and 4.8 ppm, respectively. Methoxyl protons (−OCH_3) produce a strong signal at 3.7 ppm. The signal at 3.3 ppm is due to protons in water in DMSO. An intense signal at 2.4 ppm corresponds to protons in DMSO. Protons in aliphatic groups give signals between 0.8 and 2.1 ppm. The peak at 9.9 ppm is probably due to the protons in benzaldehyde units (*29*).

To gain a more complete understanding of the structures in the isolated lignins, a qualitative ^{13}C NMR spectrum of the 0.5 M NaOH-soluble lignin fraction L_2 was obtained (**Figure 4**). Most of the observed signals have been previously assigned in straw and wood lignin spectra (*30*, *31*). The most striking characteristic of the ^{13}C NMR spectrum is the almost complete absence of typical polysaccharide signals between 57 and 101 ppm. The spectrum does show two very small signals at 63.0 (C-5, Xyl internal unit) and 175.0 ppm (C-6 in methyl uronates) for the associated hemicelluloses; however, the peak intensity is rather weak, indicating trace amounts of contaminating polysaccharides.

The region between 103.8 and 168.4 ppm relates to the aromatic part of the lignin. The syringyl (S) residues were verified by signals at 152.8 and 152.6 (C-3/C-5, S etherified), 147.9 and 147.4 (C-3/C-5, S nonetherified), 138.5 (C-4, S

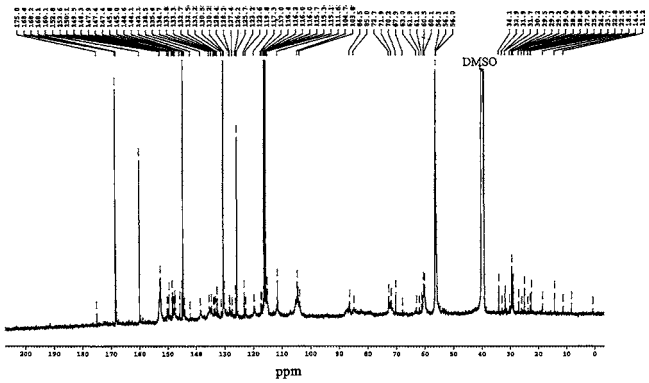

Figure 4. ^{13}C NMR spectrum of acid-insoluble lignin fraction L_2.

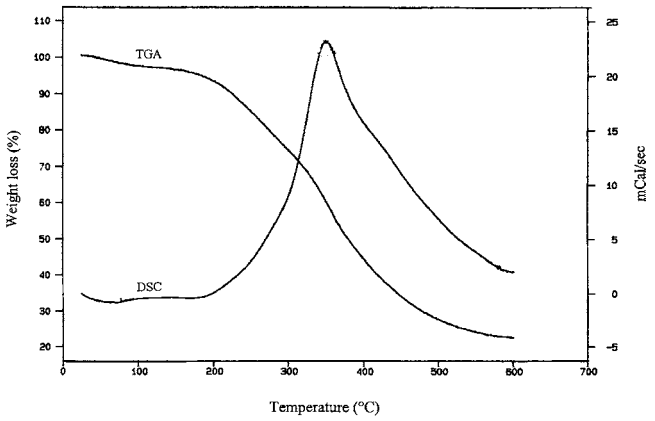

Figure 5. TGA/DSC curves of acid-insoluble lignin fraction L_4.

etherified), 134.7 (C-1, S etherified), and 104.7 and 103.8 ppm (C-2/ C-6, S). Guaiacyl (G) residues were identified by signals at 149.5 and 148.3 (C-3, G etherified), 147.9 and 147.4 (C-4, G etherified), 145.8 and 145.0 (C-4, G nonetherified), 134.7 (C-1, G etherified), 119.8 (C-6, G), and 111.6 ppm (C-2, G). The *p*-hydroxyphenyl (H) residues were found at 128.4 and 128.2 ppm (C-2/C-6, H). These signals confirmed that the lignin fraction could be justified as SGH grass lignin. The signals at 168.4 and 168.2 [C-γ, *p*-coumaric acid (PC) ester], 160.1 (C-4, PC ester), 144.6 (C-α PC ester), 130.5 and 130.2 (C-2/C-6, PC ester), 125.7 (C-1 PC ester), and 115.7, 115.3, and 115.1 ppm (C-3/C-5, PC ester) originated from esterified *p*-coumaric acid. Etherified ferulic acids give signals at 168.4 [C-γ, ferulic acid (FE) ether], 144.1 (C-α, FE ether), and 122.2 ppm (C-6, FE ether). Esterified ferulic acid was detected with a weak signal at 122.6 ppm (C-6, FE ester). These observations revealed that *p*-coumaric acid is linked to lignin by ester bonds, whereas ferulic acid is linked to lignin by both ether and ester bonds.

As shown in **Figure 4**, the spectrum also verified that β-*O*-4 linkages (C-α in β-*O*-4, 72.7 and 71.9 ppm; C-β in β-*O*-4, 86.5 and 85.5 ppm; C-γ in β-*O*-4, 60.5 and 60.1 ppm) were the major linkages between lignin structural units. In addition, certain signals belonging to condensed structures can easily be distinguished in the spectrum. The signal at 126.2 ppm is indicative of C-5/C-5′ in 5-5′ structures. Other common carbon−carbon linkages, such as β-β (C-γ in β-β units, 70.2 ppm) and β-5 (C-4 in β-5 units, 144.1 ppm), were also identified in the spectrum. These observations demonstrated that the bagasse lignin is mainly composed of β-*O*-4 ether bonds together with small amounts of 5-5′, β-β, and β-5 carbon−carbon linkages. The strong signals at 56.3, 56.1, and 56.0 ppm arise from the OCH_3 group in syringyl and guaiacyl units. The signals representing the γ-methyl and α- and β-methylene groups in *n*-propyl side chains occur in the regions of 11.2−14.4 and 18.5−34.1 ppm, respectively.

Thermal Stability. Figure 5 gives thermograms of the acid-insoluble lignin fraction L_4 isolated with 1% H_2O_2 under pH 11.5 for 2 h at 55 °C. As shown, the lignin sample began to decompose at 186 °C. At 10% weight loss the decomposition temperature of the lignin fraction occurred at 257 °C. When weight loss reached 50%, the temperature increased to 381 °C. It is clear that after ~300 °C, the thermal degradation of the lignin took place rapidly. Char residue decreased dramatically to 23% when the temperature rose to 600 °C. The DSC curve of the lignin fraction gave a large exothermic peak centered at 348 °C, due to exothermic reactions of the lignin.

These results represent the first comprehensive study on the fractionation of lignins from sugarcane bagasse. It is concluded that bagasse lignins are typical grass lignins composed of syringyl, guaiacyl, and a small amount of *p*-hydroxyphenyl units. *p*-Coumaric is linked to lignin by ester bonds, whereas ferulic acid is linked to lignin by both ether and ester bonds. It was found that β-*O*-4 ether bonds are the major linkages between the lignin structural units together with common carbon−carbon linkages such as β-β, 5-5′, and β-5. The syringyl-rich lignins from the secondary wall were found to be easily released during the initial alkali or alkaline peroxide treatment at lower concentrations and contained a higher amount of noncondensed ether linkages, whereas the middle lamella lignin, which is rich in guaiacyl lignin and seems to contain fewer ether structures, can be extracted only during the latter alkali or alkaline peroxide treatment at higher concentrations. The complete characterization of the lignins is important for the understanding of the lignin structural features as well as for possible utilization of such natural materials.

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