Extraction and Characterization of Lignins from Maize Stem and Sugarcane Bagasse

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ABSTRACT: Lignins were isolated from maize stem and sugarcane bagasse by using mild dioxane or acidic dioxane solution. The result of nitrobenzene oxidation of the isolated lignins shows that there is a high proportion of *p*-hydroxyphenyl alcohol in the lignins of maize stem and sugarcane bagasse. The lignins isolated from maize stem and sugarcane bagasse have relatively same value of the weight-average ($\overline{M}_w$ = 3405–3868 g mol^{-1}) and number-average ($\overline{M}_n$ = 1411–1612 g mol^{-1}) molecular weights, and polydispersity ($\overline{M}_w/\overline{M}_n$ = 2.24–2.51). Acidic dioxane treatment did attack the β-aryl ether structures in lignins, in particular for β-aryl syringyl ethers, and broke the ester bonds between arabinose and ferulic acid that etherified to lignins, and it also cleaved lots of bonds in hemicellulosic polymer. The proportion of β-*O*-4 (threo) guaiacyl units is higher than that of β-*O*-4 (erthreo) guaiacyl units. The phenyl glycoside and benzyl ether linkages between lignin and hemicelluloses are also demonstrated in NMR analysis. J Appl Polym Sci 120: 3587–3595, 2011

Key words: maize stem; sugarcane bagasse; lignin; *p*-hydroxyphenyl alcohol; NMR

INTRODUCTION

Lignin is one of the most abundant organic polymer and renewable resources on earth, and it accounts for approximately 18% of the dry matter in sugarcane bagasse (SCB),[1] and 15% in maize stem (MS).[2] Industry first began to use lignins in the 1880s when lignosulfonates were used in leather tanning and dye baths. Since then, they have even found applications in food products, serving as emulsifiers in animal feed and as raw material in the production of vanillin (vanillin is widely used as an ingredient in food flavors, in pharmaceuticals and as a fragrance in perfumes and odor-masking products).[3] Lignin uses have expanded into literally hundreds of applications—impacting on many facets of our daily lives. Technologically, the main thrust of lignin chemistry has been directed toward the goal of eliminating lignin in the production of paper and related products—i.e., delignification. As a potentially useful renewable resource, lignin has become the subject of increasing interest; an improvement in the quality of knowledge on the detailed chemical structural characteristics of lignin is an important scientific goal.[4]

Lignin is a complex three-dimensional macromolecule present in all vascular plants, including herbaceous species, which is synthesized from dehydrogenative polymerization of three phenylpropane-type alcohols, namely, trans-*p*-coumaryl alcohol (H), guaiacyl alcohol (G), and syringyl alcohol (S).[4–6] The most frequent interunit linkage is the β-*O*-4 linkage in lignin. It is also the one most easily cleaved chemically, providing a basis for industrial processes, such as chemical pulping, and several analytical methods. The other linkages are β-5, β-β, 5-5, 5-*O*-4, and β-1, all more resistant to chemical degradation. Figure 1 shows the chemical structures of three phenylpropane-type alcohols and some linkages in lignin. The relative abundance of the different linkages depends on the contribution of a particular monomer to the polymerization process. For example, lignins composed mainly of G units, such as conifer lignins, contain more resistant (β-5, 5-5, 5-*O*-4) linkages than lignins incorporating S units because of the availability of the C_5 position for coupling. Besides some 20 different types of linkages present within the lignin itself, lignin seems to be particularly associated with the hemicellulosic

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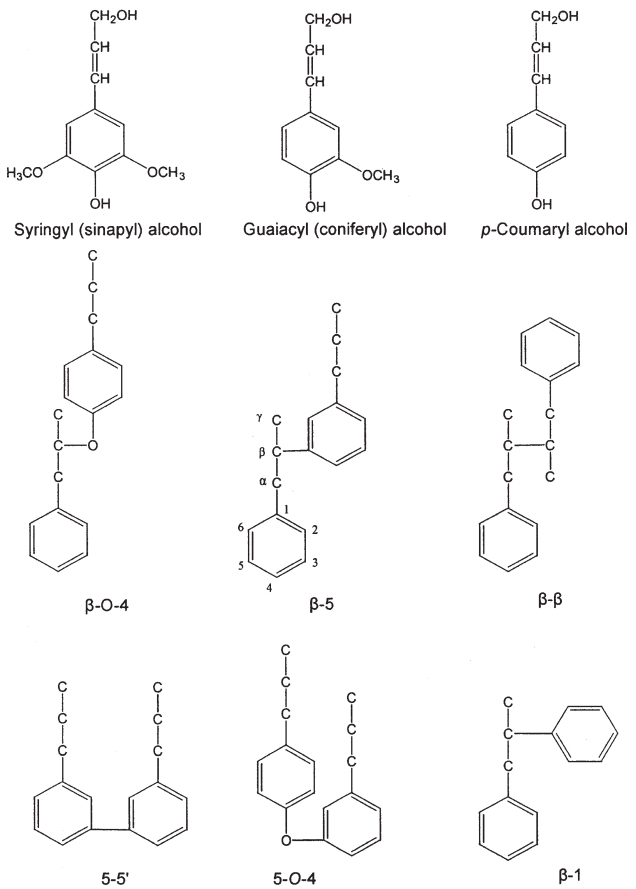


Figure 1 The chemical structures of three phenylpropane-type alcohols and some linkages in lignin.

polysaccharides.[7,8] Some covalent linkages have also been proposed between lignin and other structural polymers of the cell wall, e.g., proteins.[9] In contrast to all other organic building blocks of the cell wall, lignin has no optical activity. Owing to its reticulation, lignin *in situ* is usually insoluble in all solvents, unless it is degraded by physical or chemical treatments.

Isolation of lignin has been investigated using alkali or acidic solutions and organic solvents as well as steam explosion from cereal straws, such as wheat straw[10,11] and rice straw,[12] and these strong physic and chemical methods have resulted in the structural change of macromolecule, therefore they cannot used as a standard method for isolation and characterization of lignin polymer. MS and SCB are the most abundant residues from agricultural industry in the world. The complex nature of lignins and the difficulty of isolation of relatively pure lignin from MS and SCB have made progress in obtaining structural information on these lignins slower than progress on wood lignins. The Björkman procedure has been extensively employed in the isolation of a milled wood lignin from plant tissue, and has proved to be a successful method of isolation of relatively pure wood lignins.[7] However, when it is applied to other plant material having lower lignin content, such as MS, the results have not been as successful.[13]

The lignins of MS and SCB were isolated in this study, using mild dioxane or acidic dioxane treatment. Utilization of acidic dioxane to isolate lignin was primarily targeted to reduce the level of contamination. Chemical properties of the lignin fractions isolated were studied using a combination of several destructive and nondestructive techniques. Spectroscopy techniques such as FT-IR, UV, ^{13}C NMR, and ^{1}H NMR were employed for structural characterization of lignins.

EXPERIMENTAL

Materials

MS and SCB were obtained from the farm located in Yangling and Guangzhou, respectively. Maize hybrid (*Zea mays* var. *rugosa*, F349XP25) was planted on June 12, 2007, and harvested on October 10, 2007. Sugarcane hybrid (*Saccharum officenarum L.*, CP65-357×F172) was planted on September 22, 2006, and harvested on October 26, 2007. All the weights and the calculations were made on oven-dried material (60°C, 16 h). The composition (w/w) of MS and SCB were determined in triplicate according to Lawther et al.[14] MS used was cellulose 38.5%, hemicelluloses 28.0%, lignin 15.0%, wax 3.6%, ash 4.2%, pectin 0.5% and others (water-soluble substances) on a dry weight basis,[2] and SCB is a compact structure of three main polymers of cellulose (43.6%), hemicelluloses (33.5%), and lignin (18.1%).[1] The deviations of these contents from their respective means were all less than 1.8%. All chemicals used were of analytical or reagent grade.

Extraction of lignin fractions

Dewaxed MS and SCB (10 g) were ground in 1-gallon porcelain jar under a nitrogen atmosphere using porcelain balls, respectively, after 5 mL toluene was added to each. The jar was then placed on a rotary mill for period of four days. Ball-milled SCB substrate was directly suspended in 200 mL dioxane-water (80 : 20 v/v) solution with a concentration of 0.05 mol/L of hydrochloric acid and refluxed at 85°C under nitrogen for 4 h. The resulting mixture solution was filtered and collected. The solid residue was washed with fresh dioxane until the filtrate was clear. Ball-milled MS substrate was firstly suspended in dioxane-water (90 : 10 v/v) with a ratio of solid to liquid of 1 : 20 and refluxd at 85°C under nitrogen for 3 h. The resulting mixture was filtered and collected. The solid residue was washed with fresh

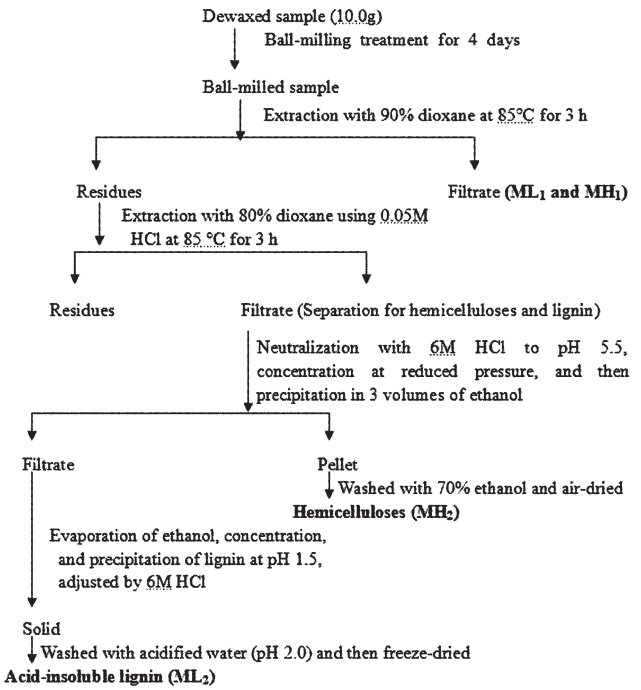


Figure 2 Scheme for the extractions of hemicelluloses and lignins from maize stem.

dioxane. After dried at 60°C overnight, the solid residue was then treated with acidic dioxane-water (80 : 20 v/v) with 0.05 mol/L HCl and refluxed at 85°C for 3 h, and then solid residue and filtrate were collected. Figure 2 shows the scheme for extraction of lignins from MS.

All the filtrated solutions were then rotary-evaporated at 30°C to about 10 mL. Then 20-mL distilled water was added, followed by evaporation under vacuum and allowed complete elimination of dioxane with a pH solution above 1. The solubilized lignin fraction was obtained by precipitation at pH 1.5–2.0 after isolation of the degraded hemicelluloses in three volumes of 95% ethanol. The lignin fraction was then purified by washing with acidified water (pH 2.0) and freeze-dried.

Characterization of lignin fractions

To determine the neutral sugar composition, lignin fraction was hydrolyzed with 2*M* trifluoroacetic acid at 120°C for 2 h. The hydrolysates from lignin fraction was reduced, acetylated, and analyzed as their alditol acetates by gas chromatography (GC) according to the method of Blakeney et al.[15] The chemical composition of phenolic acids and aldehydes, liberated from alkaline nitrobenzene oxidation of lignin fraction at 185°C for 2.5 h, were determined on a Hichrom H5ODS HPLC column of dimensions 250 $\times$ 4.6 mm^2 (Phenomenex Co., England). The identification of the individual compounds was detected at 280 nm by computer comparison of retention times and peak areas with the authentic phenolics. Measurement of the molecular weight of the lignin is according to the method described by Chen et al.[16]

UV spectrum was recorded on Hewlett–Packard 8452A diode array spectrophotometer. Lignin sample (5 mg) was dissolved in 95% (v/v) dioxane-water (10 mL). A 1-mL aliquot was diluted to 4 mL with 50% (v/v) dioxane-water, and the absorbance between 260 and 350 nm were measured. FT-IR spectra were obtained on a FT-IR spectro-photometer (Nicolet 510) using a KBr disc containing 1% finely ground samples. ^{13}C and ^{1}H NMR spectra of lignin fractions were recorded on a Bruker Avance 500 MHz spectrometer from 200 mg of sample dissolved in DMSO-d6 (1.0 mL).

RESULTS AND DISCUSSION

Yields and chemical compositions of lignin fractions

The treatment of ball milled SCB with acidic dioxane-water solution solubilized 10.1% lignin (% dry matter, SL), corresponding to 55.8% of the original lignin fractions. This indicated that acidic dioxane treatment led to the substantial dissolution of lignin from all cell wall parts. The dissolutions of 2.5% lignin (ML_1) and 2.2% hemicelluloses (MH_1) from MS were obtained by using 90% mild dioxane solution, and the treatment of MS with 80% acidic dioxane solution released 5.2% lignin (ML_2) and 3.1% hemicelluloses (MH_2). Obviously, acidic condition resulted in the separation of lignin and hemicelluloses.

TABLE I
The Content of Neutral Sugars (% Dry Lignin, w/w) in the Isolated Lignin Fractions

Components	Content (%)		
	ML_1[a]	ML_2[b]	SL[c]
Rhamnose	0.22	0.58	0.95
Arabinose	3.75	4.02	3.92
Xylose	1.54	4.15	6.80
Mannose	ND[d]	ND	0.33
Galactose	0.05	ND	0.28
Glucose	0.44	0.34	0.42
Total	5.80	9.0	12.7
Ara/Xyl ratio	2.44	0.97	0.58

[a] ML_1 represents lignin fraction solubilized during treatment with 90% dioxane from maize stem at 85°C for 3 h.
[b] ML_2 represents lignin fraction solubilized during treatment with 80% dioxane solution contaning 0.05*M* HCl from maize stem at 85 °C for 3 h.
[c] SL represents lignin fraction solubilized during treatment with 80% dioxane solution contaning 0.05*M* HCl from SCB at 85°C for 4 h.
[d] ND, not detectable.

TABLE II
The Yields (% Sample, w/w) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of the Isolated Lignin Preparations

Phenolic acids and aldehydes	Lignin preparations		
	ML_1[a]	ML_2[a]	SL[a]
p-Hydroxybenzoic acid	0.68	0.45	1.71
p-Hydroxybenzaldehyde	14.03	10.35	18.15
Vanillic acid	8.19	5.13	11.14
Vanillin	9.02	6.80	17.66
Syringic acid	2.30	0.63	3.49
Syringaldehyde	19.31	13.68	30.42
Acetovanillin	2.74	1.04	6.43
Acetosyringone	1.90	0.94	0.50
Cinnamic acid	0.24	0.17	0.55
Total	58.41	39.19	90.05
G:S:H ratio	13 : 13 : 12	8 : 8 : 9	22 : 19 : 16

[a] Corresponding to the fractions in Table I.

The data on neutral sugar composition in lignin fractions is given in Table I. Obviously, xylose and arabinose were major sugar constituents in lignin fractions. The analysis of the hemicellulosic fractions isolated also showed that hemicelluloses of MS and SCB are mainly arabinoxylans in which xylose and arabinose were major sugar constituents but the ratios of arabinose to xylose were lower than that found in the lignins of SCB and MS. This monosaccharide analysis revealed that lignin is linked to arabinoxylans. As shown in Table I, the high ratio of arabinose to xylose in lignin fraction (ML_1) reveals that arabinose plays an important role in the cross-links between hemicelluloses and lignin, whereas the decrease of the content of arabinose in lignin fractions (ML_2 and SL) indicated that the acidic dioxane treatment resulted in the breaking of covalent linkages between lignin and arabinoses. It is interesting to note that lignin fractions (ML_2 and SL) contained relatively high amounts of chemically linked polysaccharides, as shown by 9.0% and 12.7% neutral sugars, indicating that the acidic dioxane-water treatment also cleaved lots of bonds, possibly α-glycosidic bonds, in hemicellulosic macromolecule, giving rise to some sugar fragments that tightly attached to lignin.

Alkaline nitrobenzene oxidation has been widely used for assaying and identifying the structure of lignins. The high yield (90.05%) of noncondensed phenolic compounds with the lignin fraction (SL) suggested that SCB lignin isolated with acidic dioxane solution had a relatively low degree of condensation, whereas MS lignin had relatively low amount of noncondensed phenolic compounds (58.41% and 39.19%). As shown in Table II, the predominate oxidation products from lignin fractions were identified to be vanillin (9.02–17.66%), vanillic acid (6.80–17.66%), and syringaldehyde (12.68–30.42%), resulting from the oxidation of guaiacyl (G) and syringyl (S) units involved in the noncondensed structure of lignin, respectively. The high content of vanillic acid suggested that guaiacyl units are easier oxidized to acids and more etherified in the lignins of SCB and MS. The presences of *p*-hydroxylbenzaldehyde (10.35–18.15%) and *p*-hydroxybenzoic acid (0.45–1.71%) were considered most probably to be indicative of noncondensed *p*-hydroxyphenyl (H) units, showing the incorporation of a high proportion of *p*-hydroxyphenyl alcohol in the lignins of MS and SCB that is in contrast to the lignin of wheat straw.[17] The molar ratio of G/S/H was calculated as 13 : 13 : 12 for ML1, and 8 : 8 : 9 for ML2. However, the lower concentrations of *p*-hydroxylbenzaldehyde (2.48%) and *p*-hydroxybenzoic acid (0.82%) were detected for the lignin fraction isolated from MS with alkali solution.[2] The analysis of SL showed that the molar ratio of G/S/H was 22 : 19 : 16. On the basis of previous study on SCB lignin,[1] we found that the ratio of H united was reduced with the increase of treatment intensity. The high ratio of H units in the lignin fractions isolated with dioxane solutions reveals that ML_1, ML_2, and SL would represent the original lignin of MS and SCB.

Molecular mass

The value of the weight-average ($\overline{M}_w$ = 3405–3868 g mol^{-1}) and number-average ($\overline{M}_n$ = 1411–1612 g mol^{-1}) molecular weights and the polydispersity ($\overline{M}_w/\overline{M}_n$ = 2.24–2.51) of three lignin preparations are given in Table III. As shown in Table III, the lignin fractions isolated with dioxane solutions from MS and SCB did not show a significant difference in their molecular weights. This phenomenon reveals that the lignin fractions isolated with dioxane solutions have same molecular weight distribution. In previous studies, the value of $\overline{M}_w$ of the alkali-soluble lignin from MS is 3680 g mol^{-1},[2] and the $\overline{M}_w$ of the lignin preparations isolated with alkali and alkaline peroxide from SCB ranged between 1680 and 2220 g mol^{-1}.[1] These data indicated that the treatment with mild or acidic dioxane did not significantly break the structure of lignin polymer.

TABLE III
Weight-Average $\overline{M}_w$ and Number-Average $\overline{M}_n$ Molecular Weights and Polydispersity ($\overline{M}_w/\overline{M}_n$) of the Isolated Lignin Preparations

	Lignin preparations		
	ML_1[a]	ML_2[a]	SL[a]
$\overline{M}_s$	3405	3610	3868
$\overline{M}_n$	1411	1612	1543
$\overline{M}_w/\overline{M}_n$	2.41	2.24	2.51

[a] Corresponding to the fractions in Table I.

UV absorption spectra

Figure 3 shows UV absorption spectra of lignin fractions from MS (Spectrum a) and SCB (Spectrum b). All spectra exhibit an absorption maximum between 280 nm and 320 nm. The UV absorption of a specific lignin sample depends on both the concentrations of various structural units of lignin, and the extinction coefficient of each structural unit. In the UV spectra of lignins in gramineous monocotyledons, the absorption in the 280 nm region is mainly assigned to the polylignol, a dehydrogenative copolymer of G, S and H units, and the absorption in 310–320 region mainly to hydroxylcinnamic acid esters, that is, ferulic acid ester and *p*-coumaric acid ester (Fig. 4).[18] All of these structural moieties give different absorption maximum and extinction coefficient. The value of A_{320}/A_{280} was 1.27 in ML_2 curve and 1.25 in ML_1 curve, respectively. The higher value of A_{320}/A_{280} in ML_2 than ML_1 suggested that the lignin extracted with acidc dioxane solution contained a high amount of hydroxylcinnamic acids, in the other words, acidic condition resulted in the cleavage of hydroxylcinnamic acid ester from the connected hemicelluloses. It is found that A_{320}/A_{280} isalmost directly proportional to the increase of the content of hydroxylcinnamic acid ester.[18] This evidence and the result on the decrease of arabinose content seems to indicate that acidic dioxane treatment led to break the ester bonds between arabinoses and ferulic acids that etherified to lignin [Fig. 4(a)]. UV absorption spectrum of lignin fraction from SCB is shown in Figure 3(b), and the absorption maximum at 313 nm

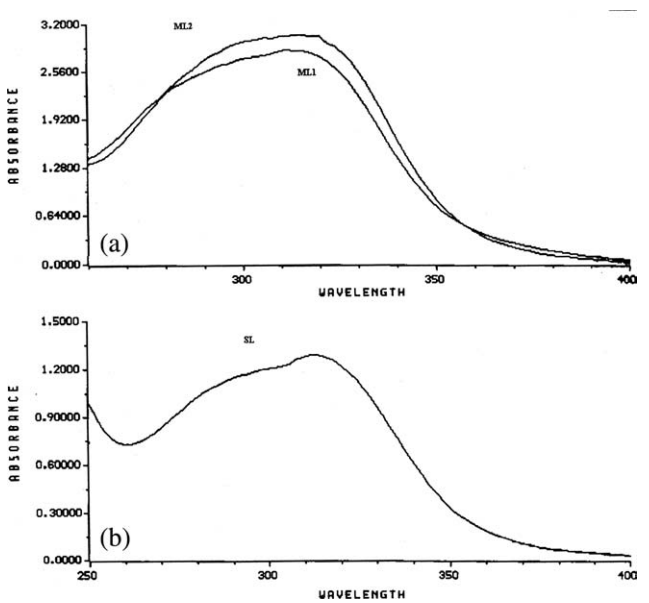
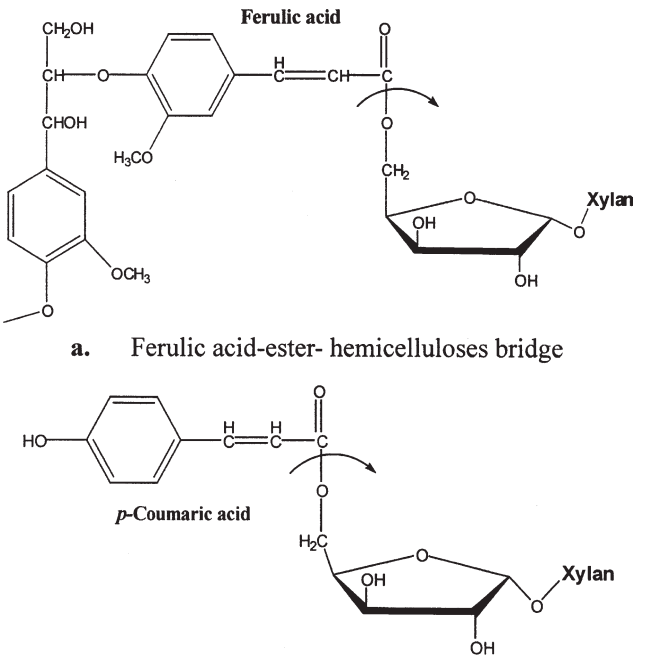


Figure 3 UV spectra of lignin preparations isolated from maize stem (a) and sugarcane bagasse (b).



a. Ferulic acid-ester- hemicelluloses bridge

b. *p* -Coumaric acid-ester-hemicelluloses bridge

Figure 4 Hydroxylcinnamic acid esters (the arrows indicate the cleavage of hydroxylcinnamic acid esters).

was exhibited. The absorption at 313 nm indicated that more *p*-coumaric acids are present in SCB lignin than ferulic acids, because the absorption would be shifted to lower than 318 nm if the content of *p*-coumaric acid ester [Fig. 4(b)] is higher than ferulic acid ester [Fig. 4(a)].[18]

FT-IR spectra

The FT-IR spectra of lignin fractions reflect the overall view of the chemical structure. As shown in Figure 5, the major peaks showed up in the spectra are the broad band at 3426 cm^{-1}, as attributed to hydroxyl groups in phenolic and aliphatic structures, and the band at 2935 cm^{-1} predominantly arising from C—H stretching in aromatic methoxyl group and methylene groups. The shoulders at 1703 and 1629 cm^{-1} are originated from the conjugated carbonyl stretching, possibly indicating the occurrence of hydroxylcinnamic acids, and are of particular interest since ball milling treatment should result in a slight increase in these bands.[19] Aromatic skeletal vibrations give three strong peaks at 1600, 1510, and 1419 cm^{-1}. Further bands are located at 1459 (asymmertric C—H deformations), 1362 (symmetric C—H bending), 1328 (syringyl ring breathing with C—O stretching), 1263 (guaiacyl ring breathing with C=O stretching), 1229 (aromatic ring breathing with C—O and C=O stretching), 1161 (typical for HGS lignins; C=O in ester groups), 1124 (aromatic

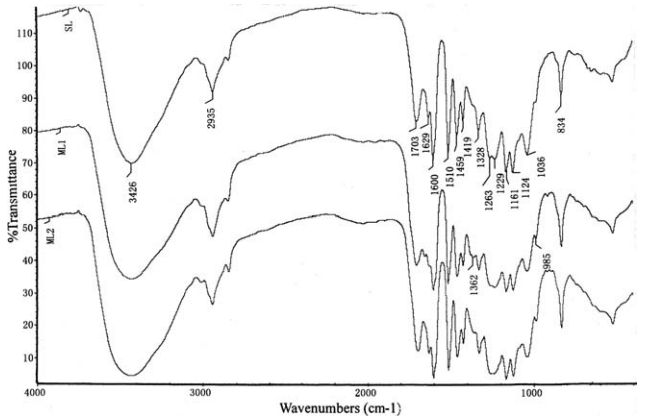


Figure 5 FT-IR spectra of lignin preparations isolated from maize stem and sugarcane bagasse.

C—H in-plane deformation, syringyl type), 1036 (aromatic C—H in-plane deformation plus C—O in primary alcohols, guaiacyl type), 985 (—HC=CH— out of plane deformation), and the band at 834 cm^{-1} (aromatic C—H out of plane deformation). The bands at 985 and 1161 cm^{-1} show the presence of *p*-coumaric esters, which are stronger in MS lignin than in SCB lignin, and that is typical for GSH lignins.[20,21] In addition, it was found that the content of condensed guaiacyl units is relatively low in the isolated lignin fractions, because the signals at 1600 and 1229 cm^{-1} are relatively weak.[21] In short, FTIR spectra of lignin fractions show that the lignins of MS and SCB are GSH type with small content of hydroxycinnamic acids.

^{13}C spectra of lignin fractions

To obtain the precise knowledge of the composition and structural features of polymer, the lignin fractions isolated from MS (Fig. 6) and SCB (Fig. 7) were investigated by ^{13}C NMR spectroscopes. Most of the observed signals have been previously assigned in wood and straw lignin spectra.[22,23] The most striking characteristic of the two ^{13}C NMR spectra is the presence of high content of *p*-hydroxyphenyl (H) units. This is clearly shown by strong signals at 115.7 and 129.4 or 130.0 ppm assigned to C3, 5 and C2, 6 and 159.2 or 159.7 ppm assigned to C4 in the aromatic nuclei of H units. The content of *p*-comarate ester is also high as shown by the signals (C-γ, 166 ppm; C-4, 159 ppm; C-1, 124 ppm; C-β, 115 ppm).[24] The etherified ferulic acid was observed with two signals at 167 (C-γ, FE ether) and 144 ppm (C-α, FE ether). It is, therefore, very likely that *p*-comarate is linked to lignin by ester bonds, which the ferulic acid is linked to lignin by ether bonds. It is worth commenting on the relative intensity of signals for aromatic carbons in the region between 103 to 160 ppm. Characteristic aromatic carbon signals of etherified and nonetherified syringyl and guaciacyl units are also detected in ^{13}C NMR spectra. The syringyl units (S) are identified by signals at 152

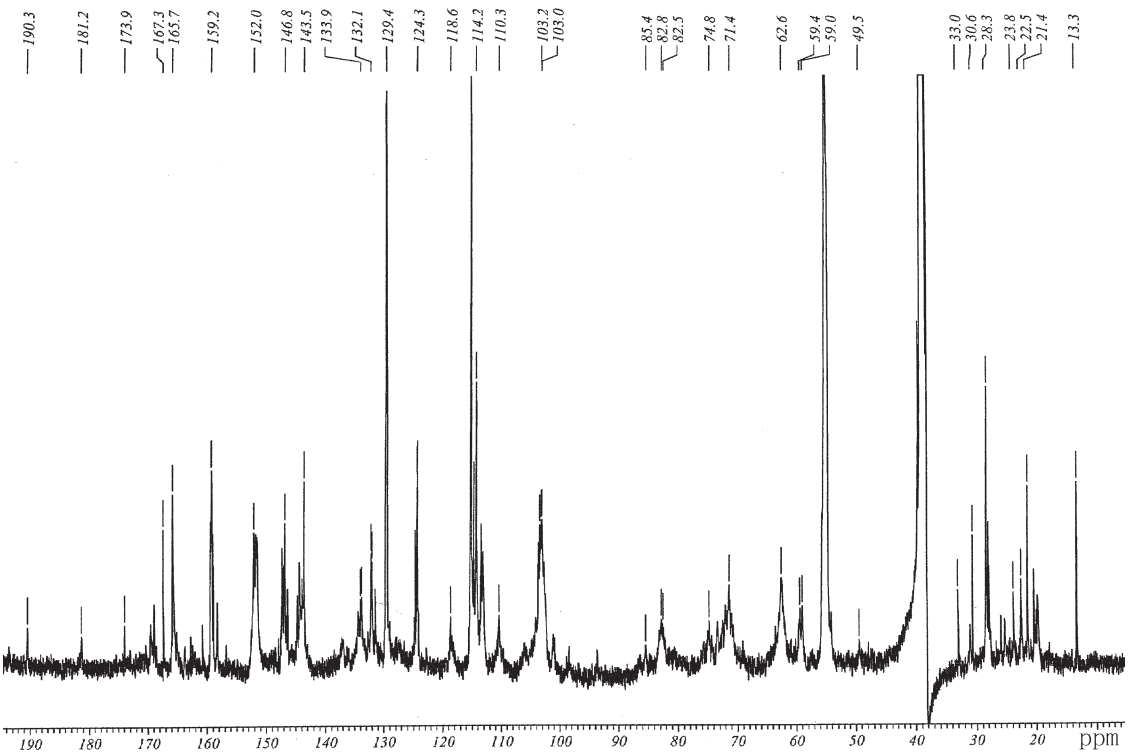


Figure 6 ^{13}C NMR spectrum of lignin isolated with 90% dioxane from maize stem.

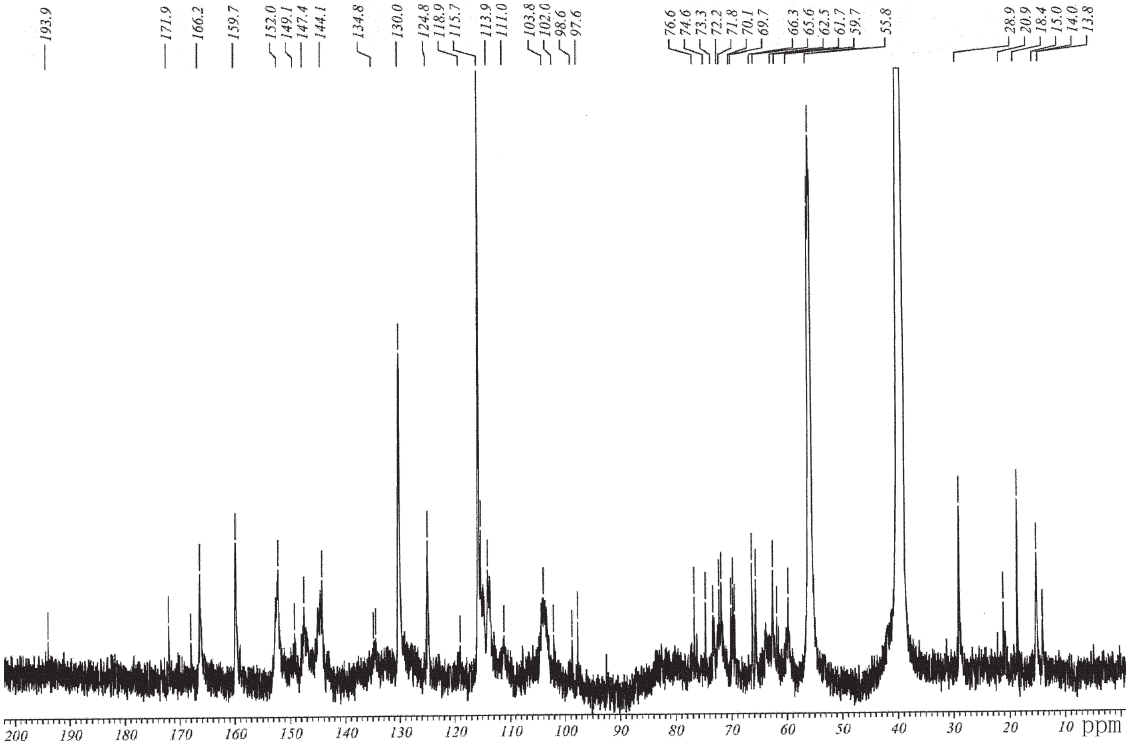


Figure 7 ^{13}C NMR spectrum of lignin isolated with 80% acidic dioxane from sugarcane bagasse.

(C-3/C-5, S), 134 (C-1, S etherified), and 103 ppm (C-2/C-6, S). Guaiacyl units (G) are verified by the signals at 149 (C-3, G etherified), 147 (C-4, G etherified), 134 (C-1, G etherified), 118 (C-6, G), and 111 ppm (C-2, G). The signals of etherified syringyl units are relatively weak in the lignin of SCB compared to

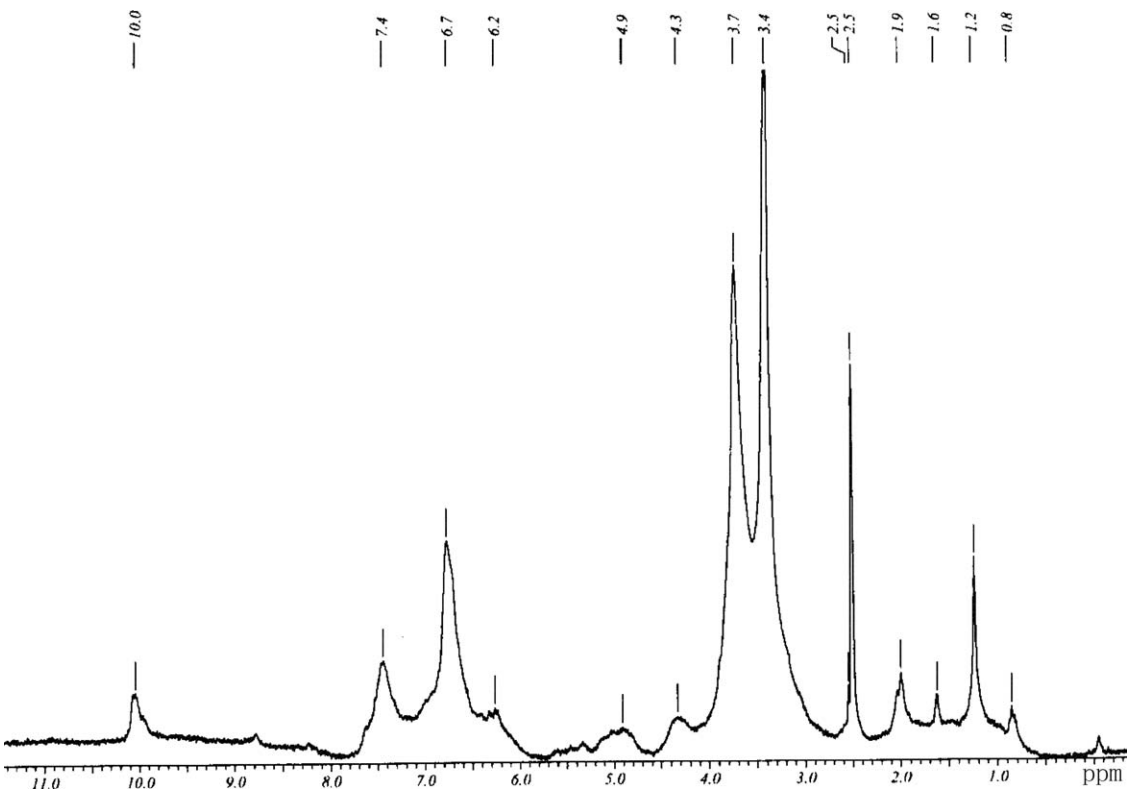


Figure 8 ^{1}H NMR spectrum of lignin isolated with 90% dioxane from maize stem.

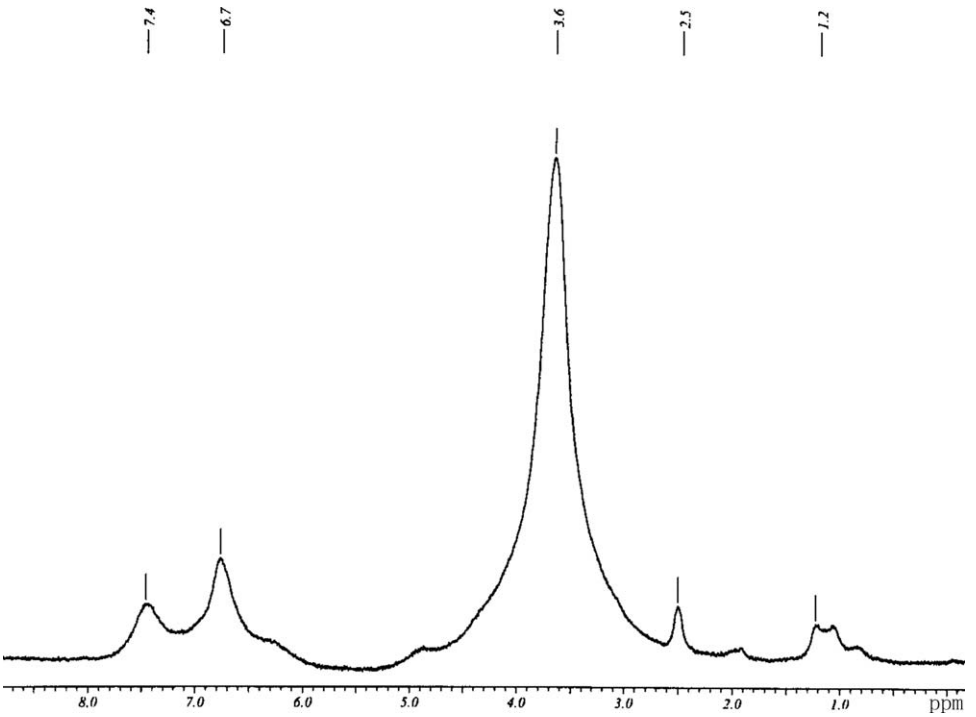


Figure 9 ^{1}H NMR spectrum of lignin isolated with 80% acidic dioxane from sugarcane bagasse.

the lignin of MS, suggesting that the treatment of SCB with acidic dioxane solution resulted in the cleavage of syringyl ether structures. It is, however, found that syringyl units are more etherified than guaiacyl units in the lignins of MS and SCB. The ^{13}C NMR spectrum of lignin isolated from MS gives three signals at 82.8, 74.8, and 62.6 ppm (very strong), which are assigned to C-β in β-*O*-4 with α-carbonyl group, C-α and C-γ in β-*O*-4, respectively. The absence or weakening of these signals in SCB lignin declared that the treatment with acidic dioxane solution under the condition given did attack the β-aryl ether structures. The absence of β-aryl syringyl ethers is possibly due to its easier cleavage than β-aryl guaiacyl ethers during treatment.[25] Interestingly, by the comparison in the intensity of signals, the proportion of β-*O*-4 (threo) guaiacyl units is higher than that of β-*O*-4 (erthreo) guaiacyl units. β-β Structure is also found by the two signals at 85.4 (C-α) and 71.4 (C-γ) ppm in the lignin of MS, and the signal at 49.5 ppm could be assigned to β-1 structure.[26] In addition, the occurrence of acetyl groups showed up by these signals between 20 and 25 ppm and at 171.9 ppm implied that some of monolignols were acetylated during lignification of straw, which have been studied by Lu et al.[27] It is found that the content of acetyl groups is lower in SCB lignin than in MS lignin, indicating that the treatment with acidic dioxane solution removed some acetyl groups. As shown in Figure 7, the signal at 193.9 ppm embodies carbon atoms of carbonyl groups in coniferyl aldehyde. The vanillin moieties and quinone structure are found by the signals at 190.3 and 181.2 ppm, respectively, (Fig. 6). The signals at 28.3 and 33.0 ppm are related to C-α and C-β in dihydroconiferyl alcohol. A very strong signal at 56 ppm is due to the methoxyl groups in syringyl and guaiacyl units.

The linkages between lignin and hemicelluloses are demonstrated in the spectrum of SCB lignin. The arabinoxylan residues attached to lignin are clearly shown by these signals at 102.0 (C-1, xylan in phenyl glycoside type), 98.6 (C-1, MeGlcA), 97.6 (C-1 in the reducing end xylose), 76.6 (C-4, xylan), 73.3 (C-2, xylan), 65.6 (C-5 in the esterified arabinose), and 61.7 (C-5, xylan). Three appreciate signals at 69.5, 69.7, and 70.1 ppm are assigned to benzyl ether lignin-carbohydrate linkages involving primary hydroxyl groups of carbohydrate, such as those at C-5 of arabinofuranosyl units and xylopyranosyl units.[28] The uronic acids or esters in arabinoxylan are found by the signals at 173.9 and 59.4 ppm in the spectrum of MS lignin, which represent C-6 in methyl urinates and 4-*O*-methoxyl group of uronic acid residues.

^{1}H spectra of lignin fractions

In ^{1}H NMR spectra of lignin fractions isolated from MS (Fig. 8) and SCB (Fig. 9), a broad peak centered at 3.7 ppm was assigned to the resonance of the methoxy and side-chains protons in various structures, such as β-β, β-1 forms, and aliphatic hydroxyl

groups. The signals from 6.6 to 7.6 ppm are attributed to the resonance of aromatic protons of the lignin. It has been observed that lignin fractions arise from all cell wall parts, not only from middle lamella that is considered to have a more condensed lignin structure.[29] The two signals at 2.5 and 3.4 ppm arise from DMSO-d_6 and HDO, respectively. The signal at 7.4 ppm can be assigned to the aromatic protons in positions 2 and 6, in structures containing a C_α=O group, and to aromatic protons in positions 2 and 6 units conjugated with a double bond, to the proton in HC_α=C_β structure, confirming the presence of *p*-courmarate-type structure and hydroxylcinnamic acid in lignin. Quantification of β-*O*-4, β-1, β-β types, and aliphatic hydroxyl groups is difficult in the ^{1}H-NMR spectrum, due to the overlapping of signals. H-β and H-α in β-*O*-4 are documented by the signals at 4.3 and 4.9 ppm,[30] respectively, and the signal of H-γ in β-*O*-4 was overlapped with that of HDO. The protons of aliphatic acetate are found by a signal at 1.9 ppm in MS lignin. Moreover, the small peak at 10.0 ppm is attributed to *p*-coumarate groups in MS lignin, and the signal at 6.2 ppm is assigned to H-β in hydroxycinnimic acids,[30] and these signals indicated that MS lignin contained relatively high amount of hydroxycinnimic acids.

CONCLUSIONS

The treatments with mild and acidic dioxane solutions led to the releases of 7.7% lignin from MS and 10.1% lignin from SCB, and it is found that the acidic condition also cleaved some bonds in hemicellulosic polymer. The results of nitrobenzene oxidation show that there are a high proportion of *p*-hydroxyphenyl alcohol in the lignin of MS and SCB. The lignin fractions isolated from MS and SCB by using dioxane solution have relatively same value of the weight-average ($\overline{M}_w$ = 3405–3868 g mol^{-1}) and number-average ($\overline{M}_n$ = 1411–1612 g mol^{-1}) molecular weights, and polydispersity ($\overline{M}_w/\overline{M}_n$ = 2.24–2.51).

The analysis of UV spectra indicated that the acidic dioxane treatment led to break the ester bonds between arabinose and ferulic acid, and more *p*-coumaric acid is present in the lignin of SCB than ferulic acid. NMR analysis revealed that lignin fractions isolated from MS and SCB are distinguished by high proportion of β-*O*-4 structures and low amounts of condensed units (β-β and β-1). The proportion of β-*O*-4 (threo) guaiacyl units is higher than that of β-*O*-4 (erthreo) guaiacyl units. The acidic dioxane treatment did attack the β-aryl ether structures, in particular for β-aryl syringyl ethers.

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