JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Extraction and Characterization of Original Lignin and Hemicelluloses from Wheat Straw

XIAO-FENG SUN,[†,§] RUNCANG SUN,*[,‡,§] PAUL FOWLER,[§] AND MARK S. BAIRD[#]

College of Forestry, North-Western Science and Technology University of Agriculture and Forestry, Yangling 712100, China; State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China; and The BioComposites Centre and Department of Chemistry, University of Wales, Bangor, Gwynedd LL57 2UW, United Kingdom

Original lignin and hemicelluloses were sequentially extracted with high yield/purity, using acidic dioxane/water solution and dimethyl sulfoxide, from ball-milled wheat straw. The acidic dioxane lignin fraction is distinguished by high β-*O*-4′ structures and by low amounts of condensed units (β-5′, 5-5′, and β-1′). Hemicelluloses contain arabinoxylans as the major polysaccharides, which are substituted by α-L-arabinofuranose, 4-*O*-methylglucuronic acid, acetyl group (DS = 0.1), and xylose at *O*-3 and/or *O*-2 of xylans. It was found that arabinoxylans form cross-links with lignins through ferulates via ether bonds, glucuronic acid via ester bonds, and arbinose/xylose via both ether and glycosidic bonds, respectively, in the cell walls of wheat straw. Diferulates are also incorporated into cross-links between lignin and hemicelluloses as well as lignification of wheat straw cell walls. The guaiacyl unit is considered to be a significant condensed structural constructor in extracted lignin and a connector between lignin and carbohydrates.

KEYWORDS: Wheat straw; lignin; hemicelluloses; NMR

INTRODUCTION

Hemicelluloses and lignin, the abundant natural polymeric materials, next to cellulose, are extremely complicated, particularly lignin, and their structures have not yet been completely elucidated (*1*, *2*). Unlike most natural polymers, such as cellulose and starch, which consist of a single monomer and intermonomeric linkage, lignin is a network polymer made up of oxidative coupling of three major C_6-C_3 (phenylpropanoid) units with many carbon-to-carbon and ether linkages. Hemicelluloses are heteroglycans, which consist of various different sugar units, arranged in different proportions and with different substituents. In particular, in contrast to wood hemicelluloses, there are a great variety of linkages and abundance of branching types in graminaceous hemicelluloses, depending on the species and the tissue within a single species, as well as on the age of the tissue (*2*).

A major problem in native lignin structure elucidation has been in trying to isolate as much of the lignin as possible while minimizing the extent of chemical modification. Traditionally, milled wood lignin (MWL) has been used as a representative source of native lignin. However, the yield of MWL varies depending on the extent of milling, ranging from 25 to 50% (*1*). When it is applied to straw and grass materials, the isolated lignin preparations contain great amounts of associated polysaccharides, because the physicochemical properties of straw lignins are known to be different from those of softwoods or hardwoods, with straw lignins possessing a characteristic alkali solubility (*3*). Isolation of relatively pure lignin from straws has led to slower progress in obtaining structural information on straw lignin than on wood lignin. To improve the yield of milled straw lignin and to study a straw lignin sample more representative of the total lignin, addition of toluene and mild acidic dioxane extraction are carried out before and after ball-milling treatment (*4*), and a high extraction temperature is used in the study. Utilization of acidic dioxane to isolate lignin was primarily targeted to reduce the level of contamination, particularly carbohydrates. The DFRC method has been applied for structural analysis of lignin; however, it suffers from the fact that it can yield monomers from only β-*O*-4′ end groups and those linked by β-*O*-4′ linkages to the lignin macromolecule through both the phenolic hydroxyl and the β-position (*5*). Therefore, here we report the results of traditional chemical methods such as nitrobenzene oxidation and modern nuclear magnetic resonance (NMR) spectroscopic techniques including ^{13}C NMR, distortionless enhancement by polarization transfer (DEPT, $\theta = 135°$) NMR, and two-dimensional heteronuclear multiple quantum coherence (HMQC) NMR to characterize the structure of "acidic dioxane" lignin (DL).

Isolation of hemicelluloses is usually accomplished by extraction with alkali such as KOH and NaOH after delignifi-

* Address correspondence to this author at The BioComposites Centre, University of Wales, Bangor, Gwynedd LL57 2UW, U.K. (telephone +44-1248-370588; fax +44-1248-370594; e-mail bcs00a@bangor.ac.uk).
† North-Western Science and Technology University of Agriculture and Forestry.
‡ State Key Laboratory of Pulp and Paper Engineering.
§ The BioComposites Centre, University of Wales.
# Department of Chemistry, University of Wales.

10.1021/jf040456q CCC: $30.25
Published on Web 02/01/2005

cation with chlorine, chlorine dioxide, or peroxyacetic acid and alkaline peroxide (*6*), as well as Organosolv treatment (*7*). However, hemicelluloses from graminaceous plants contain 1−2% of *O*-acetyl groups and a small amount of uronic acid as well as phenolic acids substituted by ester and ether linkages, which are accessible to action with dilute acids and alkali (*2*). To avoid the loss and degradation of hemicellulose substituents, material is extracted in succession with dimethyl sulfoxide (DMSO). DMSO yielded unchanged hemicelluloses, which contain many of the structural features of hemicelluloses.

Although the investigation indicates that hemicelluloses form covalent bonds (mainly *a*-benzyl ether linkages) with lignins and ester linkages with acetyl units and hydroxycinnamic acids, it is not known, in detail, how lignin is attached to carbohydrate in plant cell walls (*8*). The relationship between the phenolic acids, *p*-coumaric acid and ferulic acid, and cell wall carbohydrate (hemicelluloses) is better established. Structurally relevant fragments have been obtained from cell walls of the gramineae and shown to consist of phenolic acid ester bound to arabinoxylan through side-chain arabinose residues (*9*, *10*). However, rather less attention has been paid to the nature of linkages formed between lignin and carbohydrate and virtually none to the nature of the lignin component itself.

In this study, isolated hemicelluloses and lignin preparations were studied by sugar analysis, nitrobenzene oxidation, Fourier transform infrared (FT-IR), one- and two-dimensional NMR spectroscopy, and gel permeation chromatography (GPC) to provide significant evidence on the elucidation of the original lignin and hemicellulose structures as well as the relationship between lignin and hemicelluloses.

MATERIALS AND METHODS

Materials. Wheat straw (variety Riband) was obtained from the Silsoe Research Institute (Silsoe, Bedfordshire). All weights and calculations were made on oven-dried material (60 °C, 16 h). The composition (w/w) of wheat straw used was as follows: cellulose, 40.2%; hemicelluloses, 38.8%; lignin, 17.0%; wax, 1.2%; ash, 2.3%; and pectin, 0.5%, on a dry weight basis, obtained according to the method of Lawther et al (*11*). The deviations of these contents from their respective means were all <6%. All chemicals used were of analytical or reagent grade.

Isolation of the Hemicellulosic Fraction (H_1) and Dioxane Lignin. Wheat straw (15 g) was first dewaxed with toluene and methanol (v/v, 2:1) and then ground in a 1-gal porcelain jar using porcelain balls, after the addition of 5 mL of toluene. The jar was then placed on a rotary mill for 7 days. Ball-milled substrate was directly suspended in acidic dioxane/water (300 mL, 80:20 v/v) with a concentration of 0.05 mol/L of hydrochloric acid and refluxed at 85 °C under nitrogen for 4 h. The resulting mixture was filtered and collected. The solid residue was washed with fresh dioxane (200 mL) until the filtrate was clear.

The combined filtered solutions were then rotary-evaporated at 30 °C to ~30 mL. The solubilized lignin−carbohydrate mixture (H_1) was precipitated by pouring the concentrated supernatant fluid into 3 volumes of 95% ethanol. After filtration, the hemicellulose pellets were washed with 70% aqueous ethanol and air-dried. The solubilized DL (2.18 g) was obtained from the corresponding supernatants by precipitation at pH 1.5−2.0. The DL preparation was then washed with acidified water (pH 2.0) and then freeze-dried.

Isolation of Hemicelluloses (H_2). The residue (7.36 g), obtained after the extraction of DL, was extracted with DMSO (221 mL) at 85 °C for 5 h and then filtered. The filtrate was then evaporated nearly to dryness, and water (20 mL) was added. The hemicellulosic preparation was obtained by precipitation of the water solution in 3 volumes of 95% ethanol and washed with 70% ethanol. **Figure 1** shows the scheme for extraction of original lignins (DL) and hemicelluloses (H_1 and H_2).

Characterization of DL and Hemicellulosic Preparations. To determine the neutral sugar composition, the two hemicellulosic preparations and one DL fraction were hydrolyzed with 2 M trifluoroacetic acid at 120 °C for 2 h. The hydrolysates were reduced, acetylated, and analyzed as their alditol acetates by gas chromatography (GC) according to the method of Blakeney et al. (*12*). The chemical composition of phenolic acids and aldehydes, liberated from alkaline nitrobenzene oxidation of the lignin at 180 °C for 2.5 h, were determined on a Hichrom H5ODS HPLC column of dimensions 250 × 4.6 mm (Phenomenex Co.). The individual compounds were detected at 280 nm by computer comparison of retention times and peak areas with the authentic phenolics (*11*). The content of uronic acids was determined colorimetrically using the 3-phenylphenol reagent according to the procedure outlined in a previous paper (*11*). Measurements of the molecular weights of hemicellulosic preparations by GPC were also described in the previous paper (*11*), and that of the lignin was performed according to the method of Chen et al. (*13*).

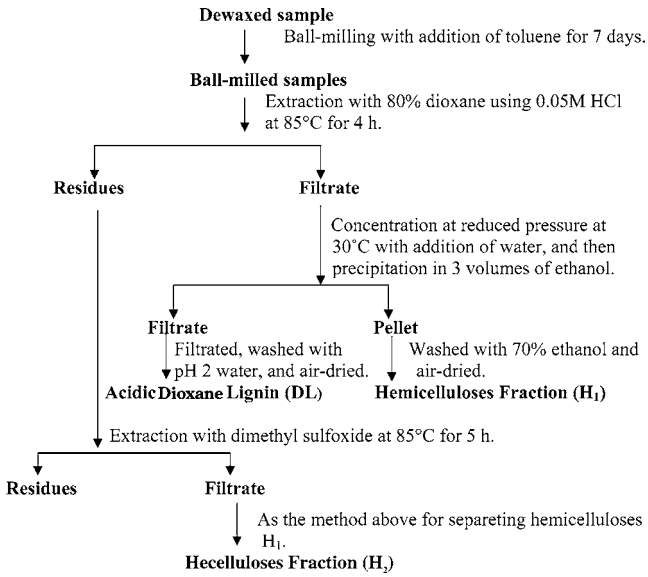

Figure 1. Scheme for extraction of original lignins and hemicelluloses.

FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet 510) using KBr disks containing 1% finely ground samples. Solution-state 1D- and 2D-NMR spectra of DL and hemicellulosic samples were recorded on a Bruker Avance 500 MHz spectrometer from 200 and 80 mg of sample dissolved in DMSO-d_6 (1.0 mL), respectively. The HMQC analysis was performed by applying a 90° pulse width, a 0.2 s acquisition time, a 1.0 s pulse delay, and $^1J_{C-H}$ of 150 Hz. The heteronuclear single quantum coherence(HSQC) spectrum was acquired by applying a 90° pulse width, a 0.2 s acquisition time, a 2.0 s pulse delay, and $^1J_{C-H}$ of 150 Hz. HSQC is a more complicated pulse sequence and thus more sensitive to calibration and tuning errors, and resolution is higher than with HMQC. However, due to the complication of HSQC, it is performed for only hemicellulose. The DEPT subspectrum was taken with a $\theta = 135°$ and a coupling constant $^1J_{C-H}$ of 150 Hz. In particular, 30000 scans were used to acquire ^{13}C NMR spectra of DL and hemicelluloses.

RESULTS AND DISCUSSION

Yield and Chemical Composition. As shown in **Table 1**, the treatment of ball-milled wheat straw with an acidic dioxane/water solution for 4 h at 85 °C solubilized 14.57% lignin and 1.67% hemicelluloses (percent dry matter), corresponding to 85.71 and 4.30% of the total lignin and hemicellulosic fractions, respectively. This indicated that acidic dioxane treatment at 85 °C led to substantial dissolution of lignin from the cell walls by breaking the linkages between lignin and hemicelluloses. More importantly, the yield of pure DL, 14.13%, corresponding to 83.13% of the total lignin, was the highest in comparison with both milled straw lignin and enzymatic lignin (*14*). On the other hand, acidic dioxane/water treatment gave only 1.52%

Table 1. Yields of Lignin and Hemicelluloses Solubilized in the Treatment of Ball-Milled Wheat Straw with 80% Dioxane in 0.05 M HCl Solution and 100% DMSO at 85 °C

fraction of lignin and polysaccharides	yield (% dry matter)	pure yield	purity (%)
DL[a]	14.57	14.13	97.00
H_1[b]	1.67	1.52	91.13
H_2[c]	5.13	5.04	98.32
H_{total}[d]	6.80	6.56	
residue[e]	48.47		
degraded substance	30.16		

[a] Lignin fraction solubilized during treatment with 80% dioxane with 0.05 M HCl for 4 h at 85 °C after wheat straw was ball-milled for 6.5 days. [b] Hemicellulosic fraction which is still solubilized in 80% dioxane with 0.05 M HCl treatment. [c] Hemicellulosic preparation solubilized during 100% dimethyl sulfoxide extraction for 5 h at 85 °C. [d] Total hemicelluloses fractions solubilzed by both acidic dioxane and dimethyl sulfoxide extractions. [e] Residue (mainly cellulose) after both acidic dioxane treatment and dimethyl sulfoxide extraction.

Table 2. Content of Neutral Sugars in Isolated Lignin and Hemicelluloses

	neutral sugars (rel % dry wt, ww) in preparation		
sugar	DL[a]	H_1[a]	H_2[a]
rhamnose	0.21	0.90	0.54
arabinose	1.31	14.84	9.37
xylose	1.24	53.58	68.34
mannose	ND[b]	0.56	ND
galactose	0.03	0.30	ND
glucose	0.21	13.45	14.42
total	3.00	83.63	92.67
uronic acids		7.50	5.65
Ara/Xyl ratio	1.06	0.28	0.14

[a] Corresponding to the fractions in **Table 1**. [b] Not detected.

pure hemicelluloses (percent dry matter), corresponding to 3.92% of the total hemicelluloses. A low yield of 5.04% pure hemicelluloses, corresponding to 13.00% of the original hemicelluloses, was obtained by the sequential extraction with 100% DMSO. Therefore, the two-stage treatments with both acidic dioxane and DMSO after ball-milling yielded 16.92% of the original hemicelluloses from wheat straw. It was found that 30.16% of the original substance, mainly hemicelluloses, disappeared, probably due to degradation and hydrolysis during both ball-milling and acidic dioxane treatments. In addition, the isolated hemicellulosic fraction (H_2) associated only 1.68% lignin as shown in **Table 3**, indicating a highly pure hemicellulosic sample.

The data on neutral sugar composition and content of uronic acid in DL and two recovered hemicellulosic fractions (H_1 and H_2) are given in **Table 2**. Obviously, xylose was a predominant sugar constituent in the two hemicellulosic preparations, comprising 53.58 and 68.34% of the total dry matter, whereas arabinose (14.84 and 9.37%) and glucose (13.45 and 14.42%) were present in smaller amounts. Galactose (0.30%), rhamnose (0.90%), and mannose (0.56%) were observed as minor constituents in H_1. The contents of uronic acid, mainly glucuronic acid or MeGlcA, were 7.50 and 5.65% in H_1 and H_2, respectively, suggesting that the acidic dioxane/water extraction favored the isolation of the hemicelluloses enriched in uronic acids. This monosaccharide analysis revealed that the two hemicellulosic fractions isolated contained arabinoxylans as the major polysaccharides. The arbinose-to-xylose ratios in two hemicellulosic fractions were 0.28 and 0.14, respectively. The higher ratio of arbinose to xylose in DL and H_1 than in H_2 revealed that arabinose plays an important role in cross-links between hemicelluloses and lignin. However, a relatively high quantity of glucose (16.08 and 15.56%) indicated not only that there are some mixed-linked glucans associated with xylans but also that the ball-milling and acidic dioxane treatments degrade small amounts of cellulose through oxidation and hydrolysis of glycosidic bonds. It is interesting to note that DL contained rather low amounts of chemically linked polysaccharides, as can be seen by the 3.00% neutral sugar content, indicating that the mild acidic dioxane/water treatment cleaves the linkages between lignin and polysaccharides in the cell walls of wheat straw significantly, such as ester bonds between ferulic acid and hemicelluloses or between *p*-coumaric acid and lignin and α-aryl ether linkages between lignin and hemicelluloses (*3*).

Table 3. Yields of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of the Isolated Lignin and Hemicelluloses

	yield (% sample, ww) in preparation		
phenolic acids and aldehydes	DL[a]	H_1[a]	H_2[a]
p-hydroxybenzoic acid	1.80	ND[b]	0.03
p-hydroxybenzaldehyde	3.09	0.17	0.08
vanillic acid	2.81	0.14	0.04
vanillin	24.50	0.82	0.27
syringic acid	4.33	0.07	0.07
syringaldehyde	31.50	0.46	0.25
acetovanillin	5.10	0.25	tr[c]
acetosyringone	3.12	0.14	0.11
G/S ratio	0.83	1.80	0.73
p-coumaric acid	0.06	ND	ND
ferulic acid	0.08	ND	ND
cinnamic acid	0.15	0.07	0.02
total	76.54	2.12	0.87
Klason lignin content (%)		8.87	1.68

[a] Corresponding to the preparations in **Table 1**. [b] Not detected. [c] Trace.

Content of Phenolic Acids and Aldehydes. Alkaline nitrobenzene oxidation has been widely used for assaying and identifying the structure of lignins and the associated lignins. According to **Table 3**, the high yield of noncondensed phenolic compounds suggested that wheat straw lignin isolated with acidic dioxane treatment had a relatively low degree of condensation (*6*). The predominant oxidation products from DL preparation were identified to be vanillin (24.50%) and syringaldehyde (31.50%), resulting from the oxidation of guaiacyl (G) and syringyl (S) units involved in the noncondensed structure of lignin, respectively (*14*). A relatively higher yield of syringyl units indicated that a large amount of the noncondensed syringyl units appeared in DL preparations and that the mild acidic dioxane treatment had more effect on the release of syringyl units, particularly *β-O*-4′ syringyl ethers. The presence of some *p*-hydroxylbenzaldehyde (3.09%) and *p*-hydroxybenzoic acid (1.80%) was considered most probably to be indicative of noncondensed *p*-hydroxyphenyl (H) units, indicating the incorporation of *p*-hydroxyphenyl alcohol in the lignin preparation. During the alkaline nitrobenzene oxidation process, a large proportion of the ferulic acid is quantitatively oxidized to vanillin, and most of the *p*-coumaric acid is quantitatively oxidized to *p*-hydroxybenzaldehyde. However, as can be seen from **Table 3**, the remaining occurrence of minor quantities of esterified or etherified *p*-coumaric (0.06%) and ferulic acid (0.08%) in the lignin preparations suggests that these two hydroxycinnamic acids are strongly linked to lignins. This observation also suggested that the acidic dioxane treatment resulted in a partial cleavage of these esterified or etherified linkages. Previously, Argyropoulos et al. observed that DL contained ~10% of ester units expressed on the basis of 100

Table 4. Weight-Average ($\bar{M}_w$) and Number-Average ($\bar{M}_n$) Molecular Masses and Polydispersity ($\bar{M}_w/\bar{M}_n$) of Lignin and Hemicelluloses from Wheat Straw

	preparation		
	DL_1[a]	H_1[a]	H_2[a]
$\bar{M}_w$[b]	3721	16789	18650
$\bar{M}_n$[b]	2139	9801	8449
$\bar{M}_w/\bar{M}_n$[b]	1.74	1.71	2.21

[a] Corresponding to the preparations in **Table 1**. [b] Determined by GPC.

phenylpropane units on the basis of quantitative ^{31}P NMR. About 77% of the carboxyl part of ester bonds present in milled straw lignin was found to be composed of *p*-coumaric acid, whereas the rest was other aromatics acids bound to lignin via intra- and/or intermolecular ester bonds. DL showed a somewhat lower number of ester bonds as compared to the milled straw lignin, and the amount of *p*-coumaric acid in DL was about half that detected in the milled straw lignin (*3*). In general, milled straw lignin contained more *p*-coumaric acid than ferulic acid (*9*), whereas the reverse was true for DL, because the esterified *p*-coumarates are more easily hydrolyzed than the ether-linked ferulic acid under acidic conditions, although *p*-coumaric and ferulic acids were shown to be present in both esterified and etherified forms by NMR in DL. In addition, cinnamic acid was identified to be present in a minor quantity, 0.15% in DL.

It is well-known that lignin is tightly linked to polysaccharides in the cell walls of plants by various linkage types, and the most commonly covalent linkage is the ether linkage of the hydroxyl group at the α-position of the lignin side chain with the alcoholic hydroxyl of sugar residues (*3*). Results concerning the composition of lignin bound to the two hemicellulosic preparations indicated that a substantial cleavage of α-ether linkage between lignin and hemicelluloses occurred as shown by the rather low amounts of bound lignin (8.87 and 1.68%) in isolated hemicellulosic fractions (H_1 and H_2). In particular, H_2 obtained by DMSO extraction, after mild acidic dioxane treatment, was very pure, containing only 1.68% bound lignin. However, associated lignin in H_1 appeared in a noticeable amount, and the hemicelluloses were more linked to noncondensed guaiacyl lignins than to noncondensed syringyl lignins, as shown by a higher G/S ratio in **Table 3** and confirmed by the NMR analysis of hemicelluloses and lignin shown below. The contents of ferulic and *p*-coumaric acids were not detected in H_1 and H_2, due to the complete oxidation during alkaline nitrobenzene oxidation at 180 °C. However, this phenomenon also suggested that most ferulate esters were cleaved during acidic dioxane treatment, and *p*-coumaric acid was seldom involved in cross-link formation between lignin and hemicelluloses. In previous studies of the contents of esterified or etherified ferulic and *p*-coumaric acids in wheat straw, we reported that wheat straw contained 0.66% *p*-coumaric acid and 1.24% ferulic acid, in which >68.3% of cell wall ferulic acid was etherified to the cell wall lignin fraction and *p*-coumaric acid (68.7%) was esterified to cell wall components, mainly to lignin (*15*, *16*).

Average Molecular Weight. The DL isolated with 80% dioxane in 0.05 M hydrochloric acid at 85 °C from ball-milled wheat straw had a weight-average ($\bar{M}_w$) molecular mass of 3720 g mol^{-1} and a number-average ($\bar{M}_n$) molecular mass of 2140 g mol^{-1} as shown in **Table 4**. The data are higher than that of milled straw lignin ($\bar{M}_w$, 2090 g mol^{-1}; $\bar{M}_n$, 1550 g mol^{-1}) (*14*). This phenomenon revealed that although mild acidic dioxane treatment significantly breaks the linkages between lignin and hemicelluloses, the isolated lignin from all cell parts is also associated with small amounts of hemicelluloses. A slight degradation of the macromolecular structure of lignin, such as cleavage of the α-*O*-4′ ether bonds between the lignin precursor, occurs only during acidic dioxane treatment because, in all cases, acidic media were found to depolymerize the lignin macromolecule. The values of the weight-average molecular mass ($\bar{M}_w$, 16790 and 18650 g mol^{-1}) and polydispersity ($\bar{M}_w/\bar{M}_n$, 1.71 and 2.21) of the solubilized hemicellulosic fractions (H_1 and H_2), calculated from the GPC chromatograms, were relatively low in comparison with the $\bar{M}_w$ (28650 g mol^{-1}) of the hemicelluloses extracted with 2% H_2O_2 at 45 °C and pH 11.6 from wheat straw and other straws such as barley ($\bar{M}_w$, 35540 g mol^{-1}) and rice ($\bar{M}_w$, 34220 g mol^{-1}) straws in our previous study (*17*−*19*). In addition, the low polydispersity ($\bar{M}_w/\bar{M}_n$) suggested that the hemicellulosic fractions isolated had a narrow molecular mass distribution, because mild acidc dioxane and DMSO treatments did not give rise to the dissociation of the small molecule and large molecular of hemicelluloses.

IR Spectra. The FT-IR spectra of hemicellulosic fractions (H_1 and H_2), illustrated in **Figure 2**, clearly showed the typical signal pattern expected for a hemicellulosic moiety. The broad band at 3430 cm^{-1} is attributed to hydroxyl groups. The two absorbances at 1733 and 1249 cm^{-1} are due to the substantial acetyl groups attached to hemicelluloses, particularly in H_2. The absorption at 1613 cm^{-1} was principally related to the absorbed water. The wavenumber characteristic for typical xylans is 1043 cm^{-1}, which is assigned to the C−O and C−C stretching and the glycosidic linkage ν(C−O−C) contributions. A sharp band at 890 cm^{-1}, corresponding to the C_1 group frequency or ring frequency, is attributed to the β-glycosidic linkages (1→4) between xylose units in hemicelluloses (*7*, *17*−*20*). The four small bands at 1461, 1414, 1362, and 1249 cm^{-1} represent the C−H and C−O bending or stretching frequencies. The presence of the arabinosyl side chains is supported by the two low-intensity shoulders at 1161 and 983 cm^{-1} in H_2 and H_1. The two rather weaker absorbances at 1507 and 831 cm^{-1} in H_2 than in H_1 originated from aromatic skeletal vibrations in associated lignin, indicating that H_2 was slightly contaminated with minimal amounts of bound lignin (*21*). This is in agreement with the results obtained from the alkaline nitrobenzene oxidation of linked lignin in isolated hemicelluloses and is also confirmed by a very weak absorbance at 4416 cm^{-1} (lignin polymer absorbance) in the near-infrared spectrum (spectrum not shown) (*22*). All evidence in IR spectra further confirmed that H_2 isolated with DMSO was more original polysaccharides.

The structural features in DL isolated by acidic dioxane treatment were also analyzed by FT-IR spectroscopy (**Figure 2**). The major peaks in the spectrum were the broad band at 3430 cm^{-1}, as attributed to hydroxyl groups in aliphatic and phenolic structures, and the band at 2933 cm^{-1}, predominantly arising from C−H stretching in the aromatic methoxyl group and methylene group. The shoulders at 1706 and 1646 cm^{-1} originated from conjugated carbonyl stretches, possibly indicating the occurrence of hydroxycinnamic acids, and are of particular interest because ball-milling treatment should result in a slight increase in this band (*1*). Aromatic skeletal vibrations gave three strong peaks at 1587, 1507, and 1421 cm^{-1}. Further bands were located at 1454 (asymmertric C−H deformations), 1355 (symmetric C−H bending), 1322 (syringyl ring breathing with C−O stretching), 1262 (guaiacyl ring breathing with C═O stretching), 1222 (aromatic ring breathing with C−O and C═O stretching), 1123 (aromatic C−H in-plane deformation, syringyl type), 1076 (C−O deformation, secondary alcohol and

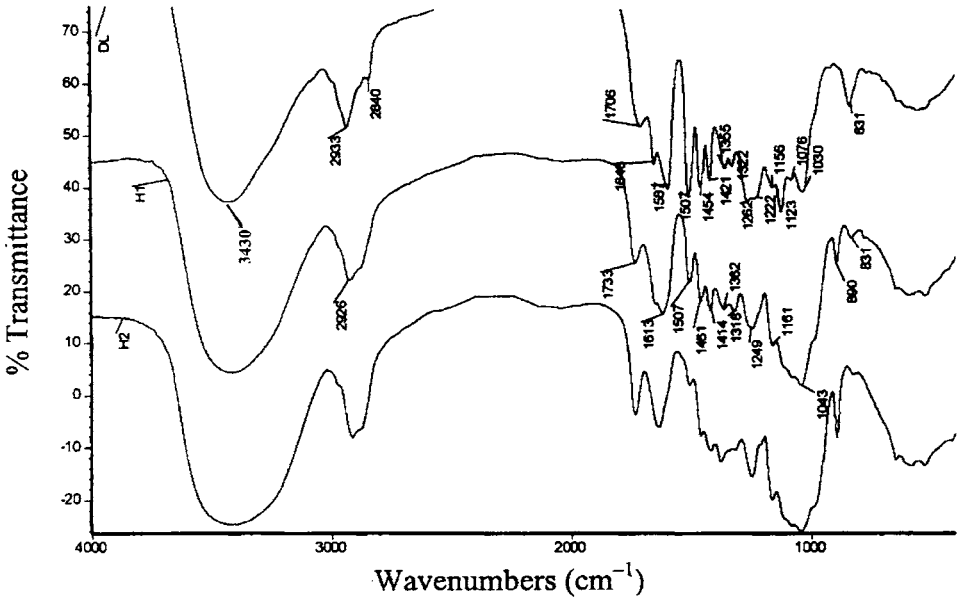

Figure 2. FT-IR spectra of DL and hemicellulosic preparations isolated with 80% dioxane in 0.05 M HCl solution at 85 °C for 4 h (H_1) and in 100% DMSO extraction at 85 °C for 5 h (H_2) from ball-milled wheat straw.

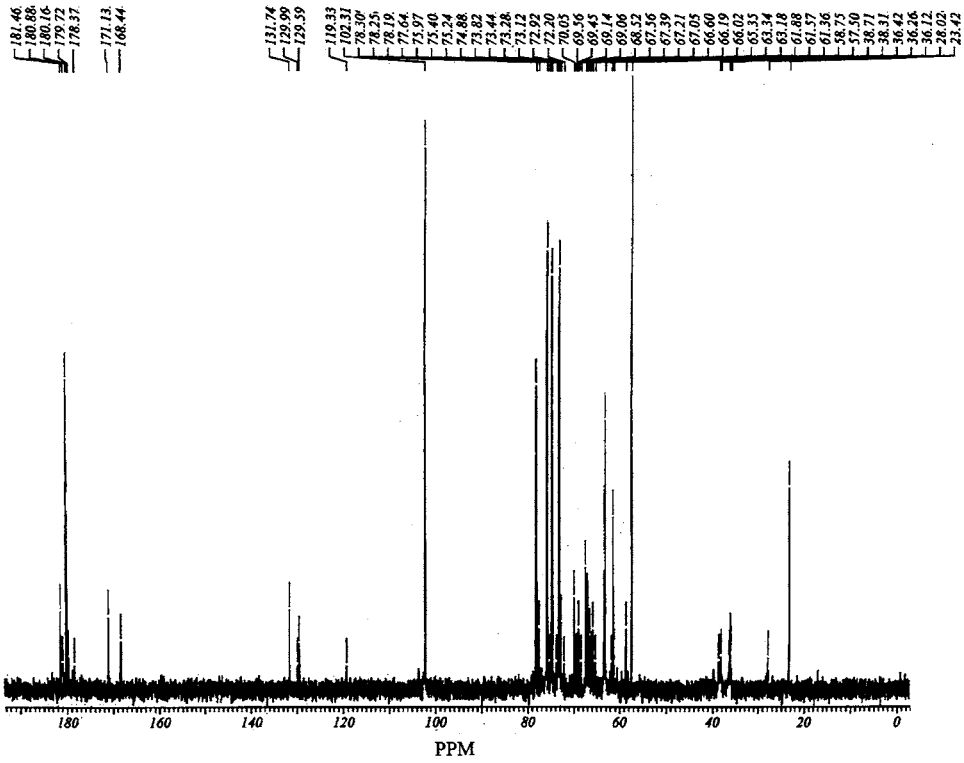

Figure 3. ^{13}C NMR spectrum (in DMSO-d_6) of the hemicellulosic preparation (H_2) isolated with 100% DMSO at 85 °C for 5 h from ball-milled wheat straw.

aliphatic ethers), 1030 (aromatic C—H in-plane deformation plus C—O in primary alcohols, guaiacyl type), 919 (C—H out of plane in aromatic rings), and 831 cm^{-1} (aromatic C—H out of plane deformation). The weak signal at 1156 cm^{-1} showed the presence of a *p*-coumaric ester group, typical for GSH lignins (*4*, *21*). In addition, it was found that the content of condensed guaiacyl units was relatively high in DL, because the signals at 1587 and 1222 cm^{-1} were strong (*21*). In short, the FT-IR spectrum of DL shows that DL was of GSH type with a small content of hydroxycinnamic acids. Analysis of the near-infrared spectroscopy of DL (spectrum not shown) also confirmed that DL was significantly pure on the basis of two strong bands at 4407 (lignin polymer absorbance) and 5964 cm^{-1} (CH in aromatic skeletal) (*22*).

^{13}C NMR Spectrum of Hemicellulosic Preparation (H_2). To further confirm the structural features of the polymers, the hemicellulosic preparation isolated with DMSO was investigated using ^{13}C NMR spectroscopy (**Figure 3**). This method allows elucidation of the polymer backbone and can also be employed to evaluate the type of side-chain branching along the backbone. The spectrum showed five strong signals at 102.3 (C-1), 73.3 (C-2), 74.9 (C-3), 76.0 (C-4), and 63.3 ppm (C-5) corresponding to (1→4)-linked β-D-Xyl residues, confirmed by a DEPT (θ = 135°) spectrum (spectrum not shown). The two signals at 78.25

Table 5. Carbon and Proton Chemical Shifts (δ) of Hemicelluloses (H2) Isolated with Dimethyl Sulfoxide

structural unit[a]	assignment				
	C-1/H-1	C-2C-2/H-2	C-3/H-3	C-4/H-4	C-5/H_2-5
β-D-Xyl[b]	102.3/4.26	73.3/3.03	74.9/3.24	76.0/3.49	63.4/3.17 and 3.87
Xyl (i)	103.6/4.49	73.8/3.31[c]	74.9/3.56[c]	77.0	66.1/3.70/3.05[b]
Xyl-3Ac	102.3	72.2	76.0	ND[d]	63.2
Xyl-2Ac	4.68[c]	74.9	72.9	77.6	63.4
Xyl-2, 3 Ac	ND	73.4	76.0	78.31	66.0
Xyl-2GlcA	ND	77.3	73.3	77.3	67.5
Xyl-3Ac-2GlcA	102.3	75.2	75.4	76.0	67.4
L-Araf (1→2)	5.3[c]	ND	78.19	ND	61.4
L-Araf (1→3)	5.4[c]	ND	78.25	ND	61.6 and 65.4[e]
α-D-Xyl (1→3)	5.2[c]	ND	72.2	77.7	60.5/3.78/3.56[b]
MeGlcA (1→2)	5.0[c]	72.9	73.1	ND	76.6/3.10[b]

[a] Abbreviations: Xyl (i), internal unsubstituted xyloses; Xyl-3Ac, xylans substituted at *O*-3 by acetyl groups; Xyl-2, 3 Ac, xylans substituted at both *O*-2 and *O*-3 by acetyl groups; Xyl-2GlcA, xylans substitued at *O*-2 by 4-*O*-methylglucuronic acid; Xyl-3Ac-2GlcA, xylans substituted at *O*-3 by acetyl group and *O*-2 by 4-*O*-methylglucuronic acid; α-L-Araf (1→3), α-L-arabinofuranose residues attached to xylans at *O*-3; MeGlcA (1→2), 4-*O*-methylglucuronic acid attached to xylans at *O*-2. [b] It belongs to signals of backbone of xylans obtained in HSQC NMR spectrum and has some solvent-induced shifts caused by the DMSO-d_6. [c] Obtained in two ^{1}H NMR spectra in DMSO-d_6 and D_2O (not shown), respectively. DMSO causes some solvent-induced shifts, and the signal is broad, however, it allows the detection of acetyl groups and lignin structure contamination in hemicelluloses. [d] Not detected. [e] Arabinofuranose esterified at *O*-5 by ferulates.

and 61.6 ppm corresponded to C-3, C-5 of α-L-arabinofuranosyl residues (1→3) linked to β-D-xylans (*20*, *23*). For the hemicellulosic fraction extracted with DMSO, the important features found in the spectrum were the two signals at 171.14 and 23.42 ppm, assigned to the acetyl group and signals of diferulates (5−5′/8-*O*-4 dehydrodiferulates) by these signals at 168.45 (C-9), 129.60 (C-1), 129.99 and 131.74 (C-1′), and 57.5 ppm (*O*Me) (*24*). The signal at 119.2 was assigned to C-6 in guaiacyl lignins (*6*). Among others, signals observed at 180.2, 73.1, 72.9, 76.6, and 58.8 ppm, respectively, are characteristic of C-6, C-3, C-2, C-5, and the methoxy group of 4-*O*-methyl-D-glucuronic acid residues (*7*, *25*). The signal at 178.38 ppm for C-6 of the glucuronic acid residue implied that some of the glucuronic acid was also esterified to lignins in the cell walls of wheat straw. As shown in **Table 5**, the complex signals suggested that the hemicellulosic preparation was substituted by α-L-arabinofuranose, glucuronic acid, acetyl group, and xylose at *O*-3 and/or *O*-2 of xylose, which is in agreement with previous studies (*20*, *25*, *26*). The signal at 65.4 for *O*-5 of α-L-arabinofuranosyl residues verified that the α-L-arabinofuranosyl side chain is esterfied to ferulic acid (168.45 ppm, C-γ) (*10*), which was possibly ether-linked to guaiacyl lignins in the cell walls of wheat straw. Previously, ferulate−guaiacyl achocol structures were detected in grass straw by Jacquet et al. (*27*). All of the data reported here clearly elucidate the complex structure of the original hemicelluloses of wheat straw. In addition, other carbonyl signals possibly arose from various uronic acids because ball-milling treatment results in substantial oxidation of carbohydrates.

HSQC and ^{1}H NMR Spectra of H_2. HSQC together with ^{1}H NMR spectra clearly showed the typical signal pattern expected for a hemicellulosic moiety (**Figure 4**) and allowed accurate quantitative analysis. The anhydroxylose units of hemicelluloses are indicated by signals at δ_C/δ_H 102.3/4.26 (C1−H), 63.4/3.87 and 3.17 (C5−H_2), 76.0/3.49 (C4−H), 74.9/3.24(C3−H), and 73.3/3.03 (C2−H) in the HSQC NMR spectra, in which the chemical shifts of 3.87 and 3.17 ppm are assigned to the equatorial and axial protons linked at C-5, respectively.

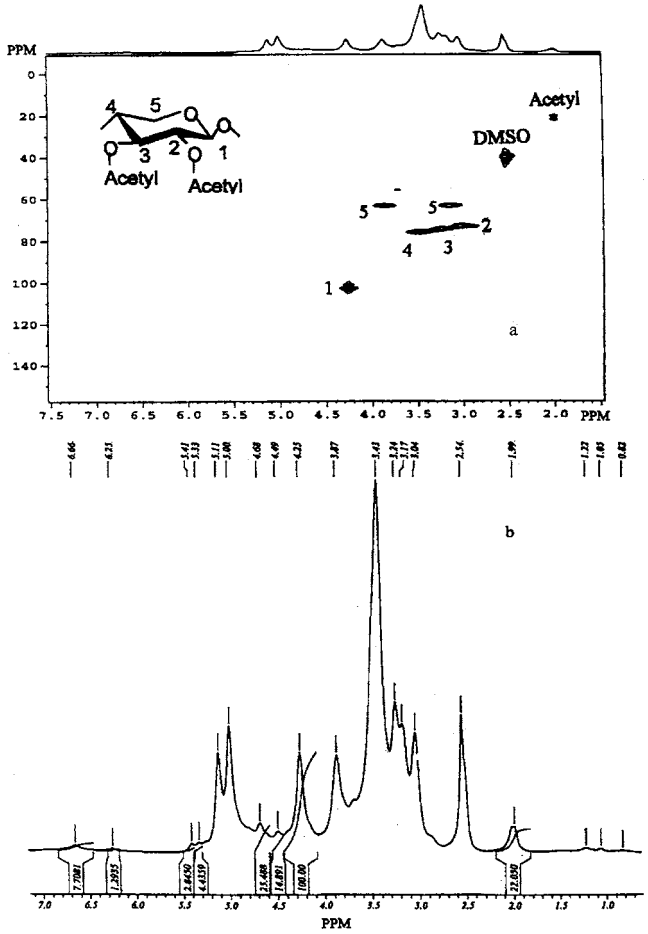

Figure 4. HSQC (**a**) and ^{1}H NMR (**b**) spectra of the hemicellulosic preparation (H_2, in DMSO-d_6) isolated with 100% DMSO at 85 °C for 5 h from ball-milled wheat straw.

Meanwhile, it was found that the signal of HDO from solvent overlapped with that of C4-H, as shown by a very strong signal at 3.43 in the ^{1}H NMR spectrum. The acetyl group attached to the hydroxyl group of hemicelluloses was seen as a signal at 1.99 ppm. The distribution of acetyl group per 100 xylose residues was calculated from the ^{1}H NMR spectrum. The degree of substitution (DS) of xylopyranose residues by the acetyl groups was found to be ~0.10. The relatively weak signal for unsubstitued internal xylose was detected at 4.49 ppm (*24*, *25*, *27*). The anomeric protons of terminal arabinofuranose linked to *O*-3 and *O*-2 of xylans were indicated by three weak resonances, respectively, at 5.41 and at both 5.33 and 5.28 ppm in the ^{1}H NMR spectrum (*27*). The two signals at 5.12 and 5.03 ppm originated from the anomeric protons of the reducing end terminal xylose and glucuronic acid as well as hydroxyl groups. 2-*O*-Acetylated nonreducing end xylose residues gave a signal at 4.68 (*26*). A very weak signal at 6.26 ppm corresponded to C-β of ferulic acid. In addition, it is very likely that the hemicellulosic fraction (H_2) formed more ether and glycosidic bonds with syringyl lignins (6.67 ppm).

^{13}C and DEPT (θ = 135°) Spectra of the Lignin Fraction. To obtain further precise knowledge of the composition and structural features of DL, the lignin preparation was investigated by both ^{13}C and DEPT (θ = 135°) NMR spectroscopy (**Figure 5**). Most of the observed signals have been previously assigned in wood and straw lignin spectra (*29*, *30*). As shown in **Table 6**, the most striking characteristics of the ^{13}C NMR spectrum were the presence of *p*-coumarate ester (C-γ, 166.4 ppm; C-4, 159.8 ppm; C-1, 125.0 ppm; C-β, 115.3 ppm) and etherified

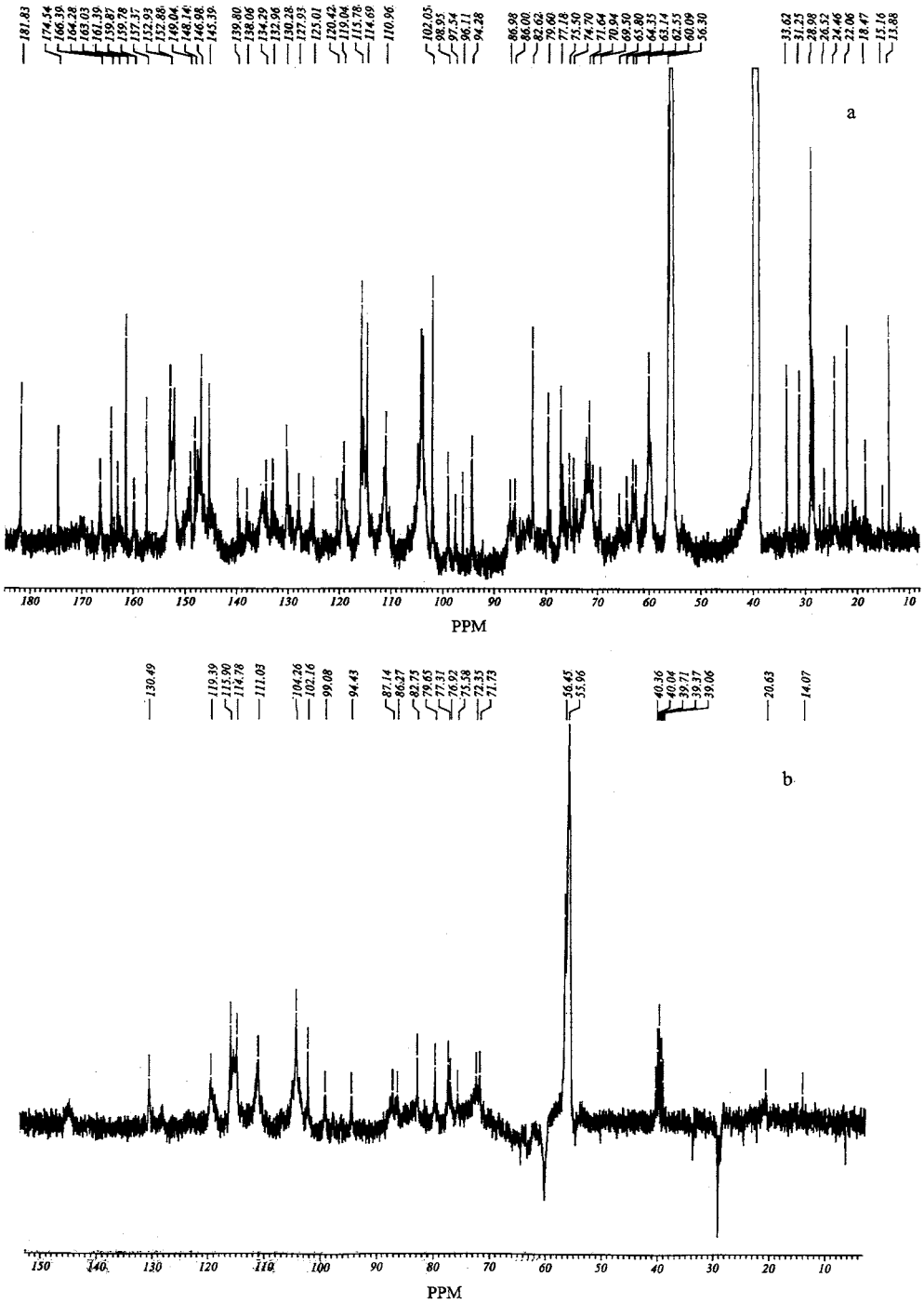

Figure 5. ^{13}C NMR spectrum (**a**) and DEPT ($\theta = 135°$) (**b**) spectrum of the lignin preparation (DL, in DMSO-d_6) isolated with 80% dioxane in 0.05 M HCl solution at 85 °C for 4 h from ball-milled wheat straw.

ferulates (C-γ, 163.0 ppm; C-6, 122.3 ppm) (*14*). A signal at 174.5 ppm arose from glucouronic acid, indicating that it is esterified to lignin. More importantly, 8-*O*-4′ diferulates and *p*-coumarate ester in β-*O*-4′ were identified by two signals at 164.3, and 161.4 ppm, respectively, assigned to C-γ and C′-4 (*24*, *30*). It is clear that *p*-coumaric acid is linked to lignin via ester bonds and a few ether bonds, whereas ferulic acid is linked to lignin by ether bonds, and its dehydrodimers are also incorporated into lignin polymer. Characteristic aromatic carbon signals of etherified and nonetherified syringyl, guaciacyl, and *p*-hydroxylphenyl residues were detected in both ^{13}C and DEPT NMR spectra, as shown in **Table 6**. It can been seen that more guaiacyl units join in the construction of the condensed structures such as β-5′, 5-5′, and β-1′, during lignification of wheat straw. In addition, the occurrence of acetyl groups showed as two signals at 170 and 22.0 ppm, implying that some monolignols were acetylated during lignification of straw, which has been studied by Lu et al. (*31*). The ^{13}C NMR spectrum of DL gave three resonances at 86.0, 72.2, and 60.1 ppm (very strong), assigned to C-β in β-*O*-4′, C-α in β-*O*-4, and C-γ in β-*O*-4′, respectively. These signals inicated that the treatment with acidic dioxane under the conditions given did not significantly attack the β-aryl ether structure, because in native wood/straw lignin, the β-*O*-4′ linkage is the predominant interunit linkage, smaller amounts of carbon−carbon and carbon−oxygen linkages being present. However, the content of β-aryl syringyl ethers decreased, possibly due to its easier cleavage as compared with β-aryl guaiacyl ethers during treatment (*32*). Interestingly,

Table 6. Carbon Chemical Shifts (δ) in DL Isolated with Acidic Dioxane/Water in ^{13}C and DEPT NMR Spectra

δ_C (intensity)[a]	assignments	δ_C (intensity)	assignments
181.8 (m)	carboxylic groups	104.2 (s)	CH-2/CH-6 in syringyl units
174.5 (w)	C-γ in glucouronic acids	102.1 (s)	CH-1 in xylans
170.0 (w)	acetyl group	98.95 (w)	CH-1 in MeGlcA
166.4 (m)	C-γ in *p*-coumarate ester, in γ-ester	97.5 (w)	C-1 in reducing xlyose, β-anomer
164.3 (w)	C-γ in 8-*O*-4′ diferulate	96.1 (w)	C-, unkown
163.0 (w)	C-γ in etherified ferulic acid	94.3 (m)	CH-1, in xylose, α-anomer
161.4 (m)	C-4 in *p*-coumarate ester, in β-*O*-4′	87.0 (m)	CH-β, in syringyl β-*O*-4′ (erythro)
159.8 (w)	C-4 in *p*-coumarate ester	86.0 (m)	CH-α, in syringyl units
157.4 (m)	C-4 in *p*-hydroxyphenyl units	82.6 (s)	CH-β, in β-*O*-4′ with α-carbonyl groups
152.9 (s)	C-3 in guaiacyl units with α-ether	79.6 (m)	CH-3 in arabinfuranose
152.1 (s)	C-3/C-5 in etherified syringyl units	77.2 (m)	CH-4 in xylans with MeGlcA
149.1 (m)	C-3 in etherified guaiacyl unit	77.0 (w)	CH-4 in xylose internal unit
148.2 (s)	C-3 in nonetherified guaiacyl unit	75.5 (w)	CH-α, in β-1′
147.2 (s)	C-3/C-5 in nonetherified syringyl unit	74.7 (w)	C-α, in β-*O*-4′
146.98 (s)	C-4 in etherified guaiacyl unit, in β-5′	72.2 (s)	CH-α, in β-*O*-4′ (erythro) guaicyl
145.4 (s)	C-4 in nonetherified guaiacyl units	71.6 (s)	CH-γ, in β-β′ units and CH-α in β-*O*-4 (threo) guaicyl
139.8 (w)	C-4 in guaiacyl with α-aryl ether	70.9 (w)	CH-γ in *p*-hydroxyphenyl units
138.1 (w)	C-4 in etherified syringyl unit	69.5 (w)	CH-4 in xylose non reducing end unit
134.5 (s)	C-1 in etherified guaiacyl unit	65.8 (w)	CH_2-5 in esterified arabinose
134.3 (w)	C-1 in etherified syringyl unit	64.4 (w)	CH_2-γ, in β-1′
132.97 (w)	C-1 in nonetherified syringyl unit	63.2 (w)	CH_2-γ, in β- 5′, C5-H_2 in xylans
130.3 (m)	CH-2/CH-6 in *p*-coumarate ester	62.6 (w)	CH_2-γ, in β-*O*-4′ with Cα = O or β-1′
127.9 (w)	CH-2/CH-6 in *p*-hydroxyphenyl units	60.1 (s)	CH_2-γ, in β-*O*-4′
125.0 (w)	C-1 in *p*-coumarate ester	56.0 (s)	OCH_3
120.4 (w)	C-6 in guaiacyl units, in 5-5′ type	54.0 (w)	CH-β, in β-β′ unit
119.1 (s)	CH-6 in guaiacyl units	53.0 (w)	CH-β, in β-5′ unit
115.8 (s)	CH-3/CH-5 in *p*-hydroxyphenyl units	34–29 (m)	CH_2-α and -β, in dihydroconiferyl alcohol
115.3 (m)	C-β in *p*-coumarate ester	22.0 (m)	acetyl- (CH_3)
114.7 (s)	CH-5 in guaiacyl unit, in β-1′ units	18.5 (s)	hexamethylbenzene $(CH_3)_6C_6$
110.96 (m)	CH-2 in guaiacyl unit	15.2/13.9 (s)	long-chain CH_2 and CH

[a] Intensity abbreviations: s, strong; m, medium; w, weak.

by comparison of the intensity of signals, the proportion of erythro-β-*O*-4′ is higher than that of threo-β-*O*-4′, thus partly giving rise to an increase in solubility for straw lignins with a relatively low weight-average molecular mass (*33, 34*). β-*O*-4′ linkages with α-carbonyl groups were detected in DL by the signal at 62.6 ppm assigned to CH_2-γ, which is an important feature in MWL and DL.

HMQC and ^{1}H Spectra of the Lignin Fraction. Two examinations of the proton and HMQC NMR spectrum of the lignin fraction (**Figure 6**) further confirmed the above results in detail. Analysis of lignin by HMQC showed (**Table 7**) the major structures of native lignin, such as guaicylglycerol-β-guaiacyl ether (β-*O*-4′), phenylcoumaran (β-5′), and pinoresinol (β-β′) moieties. So-called diarylmethane moieties of 5-CH_2-5′ type, an important condensed structure usually formed during alkaline pulping, were thought to be indicated by a signal at δ_C/δ_H 29.5/1.2 instead of 3.8 (*35*). The authors suggest that the signal is not indicative of diarylmethane moieties of 5-CH_2-5′ type. The signal at δ_C/δ_H 34.0/2.2 possibly originated from dihydroconiferyl alcohol, or β-β′ moieties (*36*). α-Esters were not detected by the absence of signals at δ_C/δ_H 73−76/5.9−6.2 in HMQC NMR spectrum of DL. However, γ-esters were detected by the signal at δ_C/δ_H 64.6/4.35 (data not shown). In general, β-esters are not present in straw lignins, and acylation normally occurs at the γ-positions (*3*). The absence of cross-peaks in the region at δ_H 5.4 − δ_C 81, indicating α-aryl ether bonds, confirmed that the α-*O*-4′ structure was present at levels lower than the detection limits of the experiment, because it is easily hydrolyzed during acidic treatment. In addition, the authors did not find a dibenzodioxocin lignin substructure, which is one of the condensed structure in softwood, and some hardwood, species.

The signal of the lignin−carbohydrate linkages of the phenyl glycoside type at δ_C/δ_H 102.5/5.0 was detected in DL, revealing that glycoside bonds are present between lignin and carbohydrates in the cell walls of wheat straw. The weak signal at δ_C/δ_H 69.8/3.3 (data not shown), also observed in the HSQC NMR spectrum of H_2, has been assigned to CH of carbohydrates linked to the α-position of lignin via a primary hydroxyl groups (*35*). Arabinoxylans were identified by several signals as shown in **Tables 6** and **7**, further suggesting that arabinoxylans form cross-links with lignins through ferulates via ether bond, through glucuronic acid via ester bond, and through xylose/arbinose via both ether and glycosidic bonds, respectively. The HMQC NMR technique, providing a high dispersion of carbohydrate signals, allows the possibility of identification of other possible linkage sites of carbohydrates bonded to lignin. However, this analysis requires precise information on the chemical shifts of the corresponding moieties and the use of equipment with higher sensitivity (*35*).

In the ^{1}H NMR spectrum, a broad peak indicating various signals centered at 3.8 ppm was assigned to the resonance of the methoxy and side-chains protons in various structures, such as β-5′, 5−5′, and β-1′ forms, and aliphatic hydroxyl groups, as well as carbohydrates, and an 8.8 H was observed. The signals from 6.6 to 7.6 ppm were attributed to the aromatic protons of the lignin, and 2.2 H observed. This indicated that DL has a relatively low degree of condensation, which is in agreement with the result of alkaline nitrobenzene oxidation. It has been observed that DL arises from all cell wall parts, not only the middle lamella, which is considered to have a more condensed lignin structure (*36*). Signals for the β-groups in β-1′ (2.7−2.9 ppm), β-5′, and β-β′ dimers were located from 3.5 to 2.3 ppm (*29*), in which the two signals at 2.5 and 3.3 ppm arose from DMSO-d_6 and HDO, respectively. The signals from 7.64 to 7.36 ppm could 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

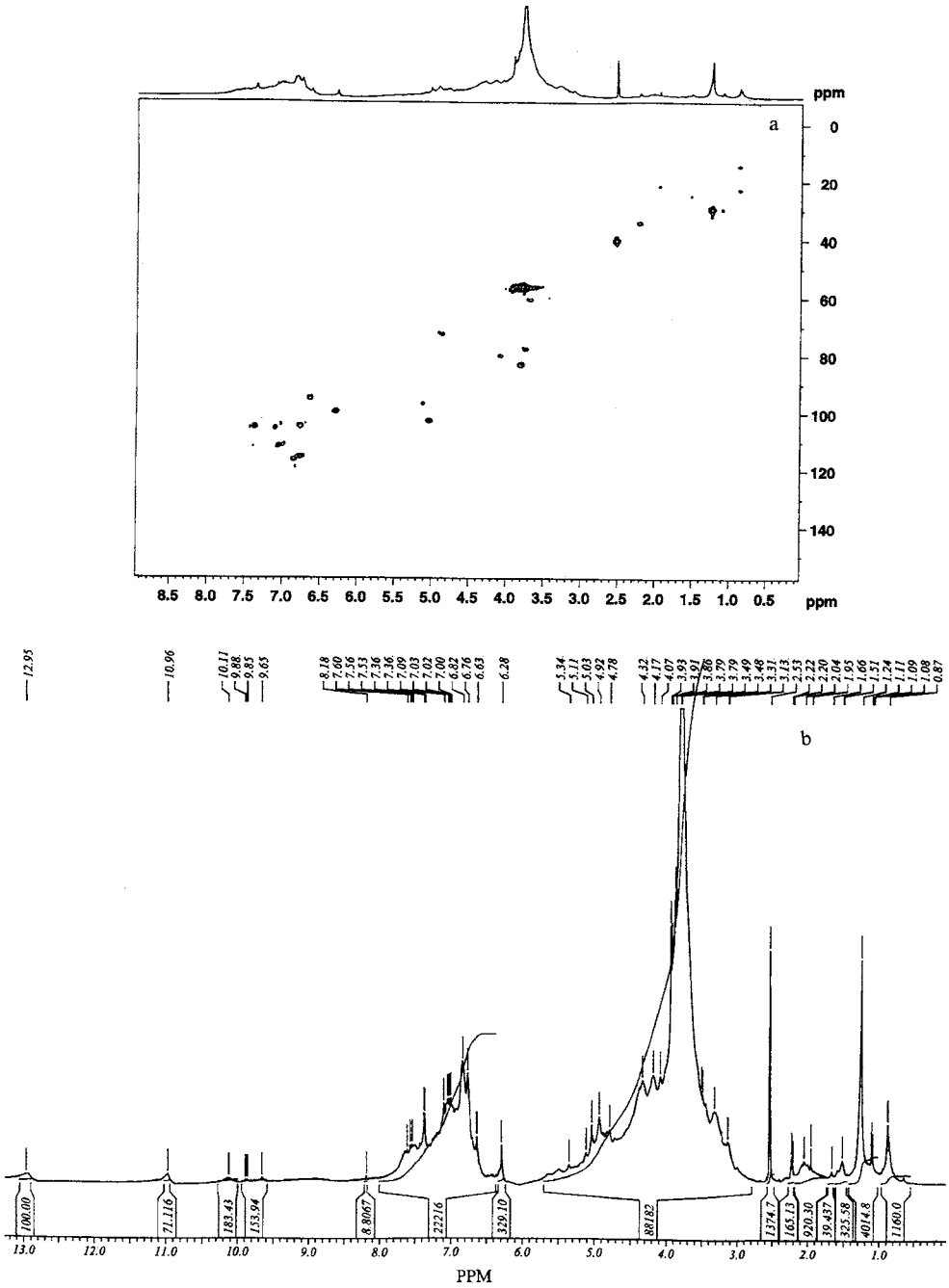

Figure 6. HMQC spectrum (**a**) and ^{1}H NMR spectrum (**b**) of the lignin preparation (DL, in DMSO-d_6) isolated with 80% dioxane in 0.05 M HCl solution at 85 °C for 4 h from ball-milled wheat straw.

bond, to the proton in $HC_\alpha{=}C_\beta$ structure, confirming the presence of *p*-coumarate-type structure and hydroxycinnamic acids in lignin (*6*). The signal at 8.18 arose from the phenolic hydroxyl groups. In addition, the contents of β-5′ (~2.00%) and 5-5′ types were low, and a trace of β-1′ and β-β′ was observed. Quantification of the β-*O*-4′, β-1′, 5-5′ types is difficult in the ^{1}H NMR spectrum, due to overlap and broad signals. However, the numbers of carboxylic acid (1.84%), free phenolic hydroxyl (3.73%), and aliphatic hydroxyl groups (3.54%) were determined by qualifying signals in the ^{1}H NMR spectrum. In fact, Gramineaene lignins are characterized by high contents of free phenolics in the β-*O*-4′ structures, implicating guaiacyl units. It has been found that 40% the guaiacyl units and only 5% of the syringyl units engaged in β-*O*-4′ structures bear free hydroxyls (*3*, *32*). Therefore, the guaiacyl lignin structures engaged in β-*O*-4′ structures were more easily degraded compared to syringyl units, although more guaiacyl units join in the construction of condensed structures, the content of which is relatively low in wheat straw lignin.

Overall, this isolation procedure, a two-stage treatment using mild acidic dioxane and sequential DMSO at 85 °C after ball-milling, gave both lignin with higher yield and hemicellulosic preparations with important features. By analysis of acidic dioxane lignin and hemicelluloses, important structure features and relationships between lignin and hemicellulose were obtained. Hemicelluloses contain arabinxylans as the major polysaccharides, which are substituted by α-L-arabinfuranose, 4-*O*-methylglucuronic acid, an acetyl group (DS = 0.1), and xylose at *O*-3 and/or *O*-2 of xylose. Ferulic acid and diferulates (5−5′/8-*O*-4′ dehydrodiferulates) are esterified to *O*-5 of α-L-arabinofuranyl residues and also ether-linked to guaiacyl lignins. 4-*O*-Methylglucuronic acid was found to be esterified to

Table 7. Chemical Shifts (δ_C/δ_H) of DL Preparation in HMQC NMR Spectrum

δ_C/δ_H	assignments	δ_C/δ_H	assignments
119.1/6.8	CH-6 in guaiacyl units	86.71/4.37	CH-β, in β-*O*-4′
116.0/6.75	CH-3/CH-5 in *p*-coumarate ester	83.04/3.78	CH-4 in arabinfuranose
111.2/7.38	CH-2 in 5-5′ diferulates	79.76/4.05	CH-3 in arabinfuranose
111.0/6.98	CH-2 in guaiacyl unit, in β-5′	77.53/3.70	CH-4 in xylans
111.3/7.02	CH-2 in guaiacyl unit	72.5/4.81	CH-α, in β-*O*-4′ (erythro)
105.2/7.05	CH-2/CH-6 in etherified S units	60.7/3.64/3.4	CH_2-γ, in β-*O*-4′
104.7/7.32	CH-2/CH-6 in syringyl units with α-carbonyl groups	55–57/3.7–3.9	OCH_3
104.5/6.7	CH-2/CH-6 in syringyl units, β-β′	102.5/5.0	CH-1 in xylans
104.0/6.99	CH-2/C-6 in syringyl units	34.0/2.0	CH in dihydroconiferyl alcohol
99.3/6.24	CH-α, possibly in β-1′	29.5/1.2/1.0	CH_2 in aliphatic lignin moieties
94.6/6.6	CH-α, unknown	22.7/0.83	CH in aliphatic lignin moieties
83.3/5.13	CH-β, in β-*O*-4′ with α-carbonyl groups	64.7/4.10/4.16	CH_2-γ, in β-β′
85.24/4.67	CH-β, in guaiacyl units, β-β′	49.2/3.17	CH-β, in β-1′
22.2/2.0/nd	acetyl group	14.3/0.84	long-chain CH

guaiacyl lignin. Acidic dioxane lignin has a relatively low degree of condensation, containing β-*O*-4′ as a predominant interunit linkage with smaller amounts of β-5′, 5-5′, β-1′, and β-β′. Guaiacyl units are thought to be a significant condensed structural constructor in acidic dioxane lignin and a connector between lignin and carbohydrates. α-Aryl ethers were significantly cleaved during the two treatments. Diferulates (mainly 5-5′) also join in the lignification of wheat straw. It was found that arabinoxylans form cross-links with lignins through ferulates via ether bond, through glucuronic acid via ester bond, and through arbinose/xylose via both ether and glycosidic bonds, respectively.

LITERATURE CITED

(1) Ikeda, T.; Holtman, K.; Kadla, J. F.; Chang, H. M.; Jameel, H. Studies on effect of ball milling on lignin structure using a modified DFRC method. *J. Agric. Food Chem.* **2002**, *50*, 129−135.

(2) Puls, J.; Schuseil, J. Chemistry of hemicelluloses: relationship between hemicellulose structure and enzymes required for hydrolysis. In *Hemicellulose and Hemicellulases*; Coughlan, M. P., Hazlewood, G. P., Eds.; Portland Press: Brookfield, VT, 1993; pp 1−27.

(3) Crestini, C.; Argyropoulos, D. S. Structural analysis of wheat straw lignin by quantitative ^{31}P and 2D NMR spectroscopy. The occurrence of ester bonds and α-O-4 substructures. *J. Agric. Food Chem.* **1997**, *45*, 1212−1219.

(4) Galkin, S.; Ammalahti, E.; Kilpelainen, I.; Brunow, G.; Hatakka, A. Characterisation of milled wood lignin from reed canary grass (*Phalaris arundinacea*). *Holzforschung* **1997**, *51*, 130−134.

(5) Jaaskelainen, A. S.; Sun, Y.; Argyropoulos, D. S.; Tamminen, T.; Hortling, B. The effect of isolation method on the chemical structure of residual lignin. *Wood Sci. Technol.* **2003**, *37*, 91−102.

(6) Sun, X. F.; Sun, R. C.; Tomkinson, J.; Baird, M. S. Isolation and characterization of lignins, hemicelluloses, and celluloses from wheat straw by alkaline peroxide treatment. *Cell. Chem. Technol.* **2003**, *37*, 283−304.

(7) Sun, X. F.; Sun, R. C.; Lu, Q.; Xu, F.; Lin, L. Fractional isolation and physico-chemical characterization of hemicelluloses by a two-stage treatment from *Haloxylon ammodendron* and *Elaeagnus angustifolia*. *J. Agric. Food Chem.* **2002**, *50*, 6400−6407.

(8) Wallace, G.; Russell, W. R.; Lomax, J. A.; Jarvis, M. C.; Lapierre, C.; Chesson, A. Extraction of phenolic-carbohydrate complexes from graminaceous cell walls. *Carbohydr. Res.* **1995**, *272*, 41−53.

(9) Iiyama, K.; Lam, T. B. T.; Stone, B. A. Phenolic-acid bridges between polysaccharides and lignin in wheat internodes. *Phytochemistry* **1990**, *29*, 733−737.

(10) Kato, A.; Azuma, J.; Koshijima, T. Isolation and identification of a new feruloylated tetrasaccharide from bagasse lignin−carbohydrate complex containing phenolic acid. *Agric. Biol. Chem.* **1987**, *51*, 1691−1693.

(11) Lawther, J. M.; Sun, R. C.; Banks, W. B. Extraction, fractionation, and characterization of structural polysaccharides from wheat-straw. *J. Agric. Food Chem.* **1995**, *43*, 667−675.

(12) Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113*, 291−299.

(13) Chen, F. G.; Li, J. Aqueous gel permeation chromatographic methods for technical lignins. *J. Wood Chem. Technol.* **2000**, *20*, 265−276.

(14) Sun, R. C.; Xiao, B.; Lawther, J. M. Fractional and structural characterization of ball-milled and enzyme lignins from wheat straw. *J. Appl. Polym. Sci.* **1998**, *68*, 1633−1641.

(15) Sun, R. C.; Sun, X. F.; Wang, S. Q.; Zhu, W.; Wang, X. Y. Ester and ether linkages between hydroxycinnamic acids and lignins from wheat, rice, rye, and barley straws, maize stems, and fast-growing poplar wood. *Ind. Crop. Prod.* **2002**, *15*, 179−188.

(16) Sun, R. C.; Sun, X. F.; Zhang, S. H. Quantitative determination of hydroxycinnamic acids in wheat, rice, rye, and barley straws, maize stems, oil palm frond fiber, and fast-growing poplar wood. *J. Agric. Food Chem.* **2001**, *49*, 5122−5129.

(17) Sun, R. C.; Tomkinson, J.; Wang, Y. X.; Xiao, B. Physicochemical and structural characterization of hemicelluloses from wheat straw by alkaline peroxide extraction. *Polymer* **2000**, *41*, 2647−2656.

(18) Sun, R. C.; Sun, X. F. Fractional and structural characterization of hemicelluloses isolated by alkali and alkaline peroxide from barley straw. *Carbohydr. Polym.* **2002**, *49*, 415−423.

(19) Sun, R. C.; Tomkinson, J.; Ma, P. L.; Liang, S. F. Comparative study of hemicelluloses from rice straw by alkali and hydrogen peroxide treatments. *Carbohydr. Polym.* **2000**, *42*, 111−122.

(20) Sun, R. C.; Lawther, J. M.; Banks, W. B. Fractionation, and structural characterization of wheat-straw hemicelluloses. *Carbohydr. Polym.* **1996**, *29*, 325−331.

(21) Vazquez, G.; Antorrena, G.; Gonzalez, J.; Freire, S. FTIR, H-1 and C-13 NMR characterization of acetosolv-solubilized pine and eucalyptus lignins. *Holzforschung* **1997**, *51*, 158−166.

(22) Workman, J. *An Introduction to Near Infrared Spectroscopy*; 2004; http://www.spectroscopynow.com/Spy/basehtml/SpyH/1,1181,3-0-0-0-0-home-0-0,00.html.

(23) Izydorczyk, M. S.; Biliaderis, C. G. Cereal arabinoxylans: advances in structure and physicochemical properties. *Carbohydr. Polym.* **1995**, *28*, 33−48.

(24) Bunzel, M.; Ralph, J.; Funk, C.; Steinhart, H. Isolation and identification of a ferulic acid dehydrotrimer from saponified maize bran insoluble fiber. *Eur. Food Res. Technol.* **2003**, *217*, 128−133.

(25) Evtuguin, D. V.; Tomás, J. L.; Silva, A. M. S.; Neto, C. P. Characterization of an acetylated heteroxylan from *Eucalyptus globulus* Labil. *Carbohydr. Res.* **2003**, *338*, 597−604.
(26) Kabel, M. A.; de Waard, P.; Schols, H. A.; Voragen, A. G. J. Location of O-acetyl substituents in xylo-oligosaccharides obtained from hydrothermally treated Eucalyptus wood. *Carbohydr. Res.* **2003**, *338*, 69−77.
(27) Jacquet, G.; Pollet, B.; Lapierre, C. New ether-linked ferulic acid-coniferyl alcohol dimers identified in grass straws. *J. Agric. Food Chem.* **1995**, *43*, 2746−2751.
(28) Cyran, M.; Courtin, C. M.; Delcour, J. A. Structural features of arabinoxylans extracted with water at different temperatures from two rye flours of diverse breadmaking quality. *J. Agric. Food Chem.* **2003**, *51*, 4404−4416.
(29) Sakakibara, A. Chemistry of lignin. In *Wood and Cellulosic Chemistry*; Hon, D. N.-S., Shiraishi, N., Eds.; Dekker: New York, 1991; pp 113−175.
(30) Ralph, S.; Ralph, J.; Landucci, L. *NMR Database of Lignin and Cell Wall Model Compounds*; 1996; http://www.dfrc.wisc.edu/software.html.
(31) Lu, F. C.; Ralph, J. Preliminary evidence for sinapyl acetate as a lignin monomer in kenaf. *Chem. Commun.* **2002**, *1*, 90−91.
(32) Billa, E.; de Choudens, C.; Monties, B. Structure and reactivity of lignins and associated phenolic acids in wheat straw TMP and CTMP pulps. *Holzforschung* **2000**, *54*, 637−640.
(33) Sugimoto, T.; Akiyama, T.; Matsumoto, Y.; Meshitsuka, G. The erythro/threo ratio of β-O-4 structures as an important structural characteristic of lignin—Part 2. Changes in erythro/threo (E/T) ratio of β-O-4 structures during delignification reactions. *Holzforschung* **2002**, *56*, 416−421.
(34) Terron, M. C.; Fidalgo, M. L.; Almendros, G.; Gonzalez, A. E. Molecular characterization of alkalilignin-1M30C: A valid alternative preparation to the Bjorkman lignin in the analytical study of wheat straw lignin. *Rapid Commun. Mass. Spectrom.* **1996**, *10*, 413−418.
(35) Balakshin, M. Y.; Capanema, E. A.; Chen, C. L.; Gracz, H. S. Elucidation of the structures of residual and dissolved pine kraft lignins using an HMQC NMR technique. *J. Agric. Food Chem.* **2003**, *51*, 6116−6127.
(36) Holtman, K. M.; Chang, H. M.; Kadla, J. F. Solution-state nuclear magnetic resonance study of the similarities between milled wood lignin and cellulolytic enzyme lignin. *J. Agric. Food. Chem.* **2004**, *52*, 720−726.

Received for review November 2, 2004. Accepted November 12, 2004. We are grateful for the financial support of this research from the LINK program of the U.K. Ministry of Agriculture, Fisheries, and Food, National Natural Science Foundation of China (No. 30271061 and 30430550), Shaanxi and Guangdong Natural Science Foundation (No. 2003k08-G18 and 013034), and Ministry of Education China for a major project.

JF040456Q