DOI:10.1021/jf1032153

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Fractional Isolation and Chemical Structure of Hemicellulosic Polymers Obtained from *Bambusa rigida* Species

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Water and aqueous alkali sequential treatments of delignified bamboo particles were performed to extract hemicelluloses with a high yield and weight-average molecular mass (M_w). The sequential treatment together dissolved 42% of hemicelluloses based on dry holocellulose. GPC results showed that the alkali-extractable hemicelluloses have higher M_w (35000 and 44450 g mol^{-1}) than water-extractable ones (20100−28100 g mol^{-1}). Structural determination based on FT-IR and ^{1}H, ^{13}C, and 2D-HSQC NMR analyses showed that both the water- and alkali-extractable hemicelluloses shared the structure composed of the (1→4)-linked β-D-xylopyranosyl backbone with 4-*O*-methyl-α-D-glucuronic acid attached to *O*-2 of the xylose residues and L-arabinose attached to *O*-3 of the xylose residues. Moreover, it revealed that the water-extractable hemicelluloses retained original structure without cleaving chemical linkages. Furthermore, it was also found that the hemicelluloses with the highest yield and M_w were obtained by the aqueous alkali treatment from the delignified bamboo. A small amount of other minor hemicelluloses (β-glucans) including xylans in the water-extractable hemicelluloses could be identified by NMR and other approaches.

KEYWORDS: *Bambusa rigida*; hemicelluloses; water-extractable; alkali-extractable; GPC; NMR; HSQC; CP/MAS

INTRODUCTION

Lignocellulose feedstock is considered to be an attractive raw material not only for liquid fuel but also for the production of chemicals and materials, that is, the development of fuels, chemicals, and carbohydrate-based materials. *Bambusa rigida* species that belong to Bambusoideae of Gramineae are widely distributed in Szechwan, China. This kind of bamboo has strong and abundant woody stems. Therefore, it can be used for many purposes. For instance, it is mainly used for building materials and load-bearing materials in the rural areas of China. However, these utilizations are still of low value, which restricts the local economic development. Therefore, applications of biomass residues in pulping processes, alcohol production, and hemicellulose-based materials not only provide alternative substrates but also help boost local farmers' income.

According to a research conducted by Scurlocka et al., the cellulose of bamboo is about 40−48% on a dried basis, compared to the cellulose content reported in softwoods (40−52%) and hardwoods (38−56%) (*1*). Such a high content of cellulose can be used as commendable material for the paper manufacturing and bioethanol production industries. It was also reported that the content of hemicelluloses in bamboo is 22−35% (*1*). As compared to the content of hemicelluloses reported for nonwoody biomass (33.5%), such as sugar cane bagasse (SCB), the hemicelluloses in bamboo are relatively abundant (*2*). It was reported that hemicelluloses from plant cell wall have a very wide variety of direct food and nonfood applications. They can be converted to chemicals as feed, such as furfural, erythritol, xylitol, ethanol, or lactic acid (*3*). They can also be used as intermediates for hemicellulose-based materials in the film, coating, pharmaceutical, food, papermaking, and other industries (*4*).

Hemicelluloses, the second most abundant constituent of lignocellulosic biomass, are not chemically well-defined compounds but rather a family of polysaccharides, composed of different five- and six-carbon monosaccharide units (*5*). In the plant cell wall, hemicelluloses are bound to cellulose and lignin, and detailed isolation procedures are required to separate these components from plant raw material (*6*). Removal of hemicelluloses in a pure form from plant cell wall involves hydrolysis of ester and ether bonds, which link the hemicelluloses to lignin. A number of methods are used to isolate hemicelluloses from plant sources, including extraction with alkali, dimethyl sulfoxide (DMSO), or methanol/water, as well as steam or microwave treatment. The composition of the extracted hemicelluloses can be highly dependent on the isolation process and, for example, deacetylation, degradation, and contamination with lignin can occur (*6*). In particular, the isolation of hemicelluloses from straw by aqueous alkali has been widely accepted. One approach is to isolate the hemicelluloses from holocellulose by extraction using aqueous alkali. The hemicelluloses isolated by this method have a light brown color and contain a minor amount of bound lignin. This method was developed for characterization purposes, but it could also be used as a preparative method (*7*). Besides, hot water could be used as

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 Published on Web 10/13/2010

a tool to separate water-soluble hemicelluloses from plant cell wall (*8*, *9*). Unfortunately, only a few papers about the comparison of hemicellulosic fractions obtained by hot water and alkaline solution have been published (*2*, *10*).

The main advantages of the water and alkali extractions are the fact that they are simple to perform and cost-effective. To investigate the effect of different solvents on the chemical structure, it is feasible to successively extract hemicelluloses with different solvents. Besides, delignification with $NaClO_2$ was also implemented to obtain holocellulose, and then the sequential extractions were performed; the residue is mainly cellulose with a high purity. Therefore, the delignified biomass could be used for preparation of hemicelluloses and cellulose. To date, although bamboo (a renewable source for the production of xylose and xylooligosaccharides) is considered to be abundant (*11*, *12*), the physicochemical features and structural characteristic of hemicelluloses from *B. rigida* species have been poorly characterized (*13*).

The present study discusses the relationship between the structure and properties of the hemicelluloses from delignified *B. rigida* species. In addition, to assess the possibility of producing xylitol and other chemicals based on hemicelluloses extracted from the delignified *B. rigida* species, it is particularly important to elucidate the physicochemical features and structural characterization in this study.

MATERIALS AND METHODS

Materials. *B. rigida* was obtained from Szechwan province, China. It was dried in an oven at 50 °C and then cut into small pieces; the cut *B. rigida* was ground and screened to prepare 20−40 mesh size particles (450−900 μm). The dried bamboo particles were first extracted with toluene/ethanol (2:1, v/v) in a Soxhlet extractor for 6 h, and the dewaxed meal was allowed to dry in an oven at 60 °C for 16 h. All standard chemicals, such as monosaccharide and chromatographic reagents, were purchased from Sigma Chemical Co. (Beijing, China).

Isolation and Purification of Hemicelluloses. To study the structural differences of the hemicelluloses present in *B. rigida*, hemicellulosic fractions were obtained by sequential extractions and fractionation according to the scheme in **Figure 1**. The dewaxed particles (15 g) were delignified with sodium chlorite in acidic solution (pH 3.8−4.0, adjusted by 10% acetic acid) at 75 °C for 2 h. After treatment, the residue was filtered off with a nylon cloth, washed thoroughly with distilled water, and further dried in a cabinet oven with air circulation for 16 h at 50 °C, and then 9.65 g of holocellulose was recovered. Hemicelluloses were obtained from the holocellulose by successive extractions with water at 50, 65, 80, and 95 °C, 1 M KOH, and 1 M LiOH for 3 h with a solid to liquor ratio of 1:25 (g mL^{-1}). The filtrate was neutralized with 6 M hydrochloric acid or acetic acid solution to pH 5.5 (the four water-extractable solutions do not need to be neutralized to pH 5.5 because the water-extractable solution showed weak acidity) and then concentrated under reduced pressure to about 50 mL. Three volumes of ethanol was added to each concentrated solution with continuous stirring, and then the flocculent precipitate appeared. After filtration with filter paper on a Buchner funnel, the isolated hemicelluloses were purified by thorough washing with 70% ethanol and then freeze-dried. Note that hemicellulosic preparations (H_1, H_2, H_3, and H_4) represent the water-extractable hemicellulosic preparations isolated sequentially with water at 50, 65, 80, and 95 °C for 3 h from the holocellulose and that hemicellulosic preparations H_5 and H_6 represent the alkali-extractable hemicellulosic preparations extracted sequentially with 1 M KOH and LiOH from the water-extracted residue, respectively. The relative standard deviation, determined by dividing the standard deviation by the mean value, was $< 3\%$.

Determination of Chemical Composition. To determine the composition of the isolated hemicelluloses, the neutral sugars and uronic acids in the isolated hemicellulosic fractions were determined by high-performance anion exchange chromatography (HPAEC). The neutral sugars and uronic acids in the hemicellulosic preparations were obtained by hydrolyzing 5 mg of sample with 10% H_2SO_4 for 2.5 h at 105 °C. After hydrolysis, the hydrolysate was diluted 50-fold, filtered, and injected into the HPAEC system (Dionex ICS3000) with pulsed amperometric detector and an ion exchange Carbopac PA-1 column (4 × 250 mm). The neutral sugars were separated in 18 mM NaOH (carbonate free and purged with nitrogen) with postcolumn addition of 0.3 M NaOH at a rate of 0.5 mL/min. Run time was 45 min, followed by a 10 min elution with 0.2 M NaOH to wash the column and then a 15 min elution with 18 mM NaOH to reequilibrate the column. Calibration was performed with a standard solution of L-rhamnose, L-arabinose, L-glucose, L-galactose, D-mannose, D-xylose, glucuronic acid, and galacturonic acids. Measurements were conducted with two parallels, and reproducibility of the values was found within the range of 5%.

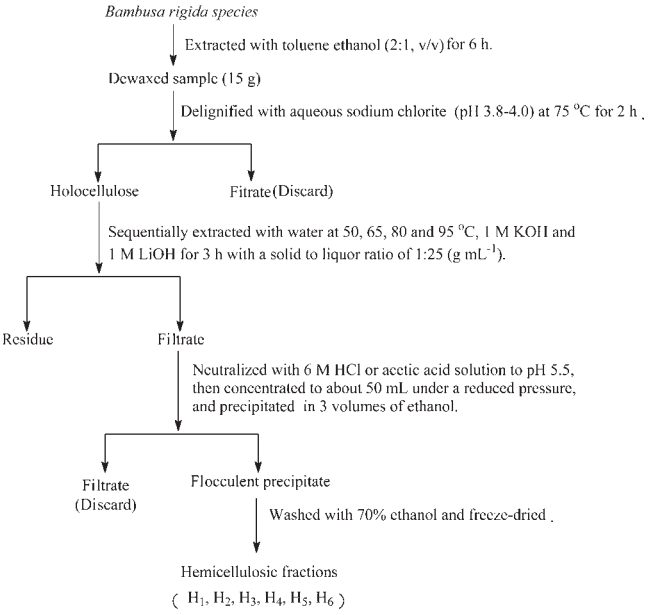

Figure 1. Scheme for extraction of water- and alkali-extractable hemicelluloses from *Bambusa rigida* species.

Determination of Molecular Weight. The molecular weights of the hemicellulosic preparations were determined by gel permeation chromatography (GPC) on a PL aquagel-OH 50 column (300 × 7.7 mm, Polymer Laboratories Ltd.), calibrated with PL pullulan polysaccharide standards (peak average molecular weights of 783, 12200, 100 000, 1 600 000 g mol^{-1}, Polymer Laboratories Ltd.). A flow rate of 0.5 mL/min was maintained. The eluent was 0.02 M NaCl in 0.005 M sodium phosphate buffer, pH 7.5. Detection was achieved with a Knauer differential refractive index detector (RID). The column oven was kept at 30 °C. Hemicelluloses were dissolved with 0.02 M NaCl in 0.005 M sodium phosphate buffer, pH 7.5, at a concentration of 0.1%.

Spectroscopic Characterization. FT-IR spectra of hemicellulosic preparations were obtained on an FT-IR spectrophotometer (Bruker Tensor 27) using a KBr disk containing 1% finely ground samples. Thirty-two scans were taken for each sample recorded from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1} in the transmission mode.

The solution-state ^1H NMR spectra were recorded on a Bruker AV III NMR spectrometer at 400.13 MHz using 15 mg of hemicelluloses in 1.0 mL of D_2O. In addition, to increase solubility of alkali-extractable hemicelluloses H_5 in D_2O, a few drops of sodium deuteroxide (7.5 M NaOD) were added. The chemical shifts were calibrated relative to the signals from D_2O, used as an internal standard, at 4.70 ppm for the ^1H NMR spectra. The acquisition time (AQ) was 3.9 s, and the relaxation time was 1.0 s. ^{13}C NMR spectra were obtained on a Bruker spectrometer at 100.6 MHz. The sample (80 mg) was dissolved in 1 mL of D_2O (99.8%D) overnight at room temperature. The ^{13}C NMR spectra were recorded at 25 °C (298 K) after 30000 scans. A 30° pulse flipping angle, a 9.2 μs pulse width, a 1.89 s delay time, and a 1.36 s AQ between scans were used.

A semiquantitative analysis of the HSQC cross-signal intensities was performed. The spectral widths for the HSQC were 5000 and 20000 Hz for the ^1H and ^{13}C dimensions, respectively. The number of collected complex points was 1024 for the ^1H dimension with a recycle delay of 5 s. The number of transients for the HSQC spectra was 128, and 256 time increments were always recorded in the ^{13}C dimension. The $^1J_{C-H}$ used was 146 Hz.

Table 1. Yield and Relative Content of Sugars and Uronic Acids of the Isolated Hemicellulosic Preparations and the Residue

	H_1[a]	H_2[a]	H_3[a]	H_4[a]	H_5[a]	H_6[a]	residue
yield	5.53[b]	3.57[b]	3.94[b]	4.23[b]	19.1[b]	5.58[b]	58.0[b]
arabinose	5.25	4.07	4.00	3.85	5.52	4.07	0.40
galactose	1.55	1.37	1.12	1.61	1.10	1.07	0.20
glucose	21.3	16.3	28.8	31.2	2.03	4.39	86.5
xylose	69.7	74.7	64.8	61.5	85.6	88.5	11.4
uronic acid	2.22	3.58	2.19	1.91	5.80	2.03	1.57

[a] Note that H_1, H_2, H_3, and H_4 represent the water-extractable hemicellulosic preparations isolated sequentially with water at 50, 65, 80, and 95 °C, respectively, for 3 h from the holocellulose; H_5 and H_6 represent the alkali-extractable hemicellulosic preparations extracted sequentially with 1 M aqueous KOH or LiOH, respectively, from the water-extracted residue. [b] The yields of the hemicellulosic preparations and residue (cellulose) are expressed on the basis of dry holocellulose.

Prior to Fourier transformation, the data matrices were zero filled to 1024 points in the ^{13}C dimension. Data processing was performed using standard Bruker Topspin-NMR software. Because the cross-signal intensity depended on the particular $^1J_{C-H}$ value, as well as on the t_2 relaxation time, a direct analysis of the intensities was indeed impossible. Therefore, the integration of the cross-signals was performed separately for the different regions of the HSQC spectra, which contain signals that correspond to chemically analogous carbon−proton pairs (in similar samples). For these signals, the $^1J_{C-H}$ coupling value was relatively similar and was used semiquantitatively to estimate the relative abundance of the different constituents.

Solid-state cross-polarization/magic angle spinning (CP/MAS) ^{13}C NMR spectra of samples were obtained at 100.6 MHz using a Bruker AV-III 400 M spectrometer (Germany). Dry sample of the cellulose-rich fraction was packed in a 4 mm zirconia (ZrO_2) rotor, and measurement was performed using a CP pulse program with a 1 ms match time and a 2 s delay between transients. Spinning rate was 5 kHz. Calibration was done externally to the carbonyl carbon of glycine at 176 ppm.

RESULTS AND DISCUSSION

Yield of Water- and Alkali-Extractable Hemicelluloses. The hemicelluloses are a mixture of a number of different polysaccharides, and their yield and the composition of the polymer can vary depending on the method of isolation (*14*). The isolation and purification of hemicelluloses from bamboo are presented schematically in **Figure 1**. To isolate the pure hemicelluloses, a pretreatment by extraction with organic solvents is required to remove the non-cell wall components such as wax and chlorophyll. It was found that pretreatment with toluene/ethanol (2:1, v/v) under the condition used (100 °C for 6 h) removed most of the chlorophyll, wax, and other extractives with a combined yield of 5.3% dry matter. The delignification with sodium chlorite ($NaClO_2$) was then performed at 75 °C for 2 h under an acidic condition (pH 3.8−4.0). In this case, the loss of the dewaxed dry matter was 5.35 g (36% based on the dewaxed dry matter) including most of the lignin, residual protein, ash, and starch, as well as minor quantities of polysaccharides, and yielded a dry holocellulose (9.65 g), which corresponded to 64% of the dried dewaxed bamboo (15 g), then the holocellulose was sequentially extracted with water at 50, 65, 80, and 95 °C and 1 M KOH and 1 M LiOH at 50 °C for 3 h, which released 5.53, 3.57, 3.94, 4.23, 19.1, and 5.58% of dry holocellulose (shown in **Table 1**), respectively. Taken together, the total yield of the hemicellulosic preparations was nearly 42% based on the dry holocellulose. Moreover, it could be seen that most hemicelluloses were removed in the early part of the alkaline extraction procedure (19.1% based on dry holocellulose, 1 M KOH). The reason for this maximum yield was probably that the hydrogen bonds between hemicelluloses and cellulose were weakened under the aqueous alkaline treatment. However, some substance of nonpolysaccharides as minor constituents in the water-extractable hemicelluloses probably led to the unreasonable yield. In contrast to H_2, the first water-extractable sample (H_1) presented a slightly higher yield than that of H_2, which is probably due to the coprecipitation of a glucose-rich substance. However, from H_2 to H_4, the yield showed an increasing tendency with the increment of the temperature (from 65 to 95 °C), probably suggesting that increasing temperature could enhance the dissolving hemicelluloses. In addition, it was found that extraction with hot water (H_2−H_4) resulted in a slightly acidic medium (pH 5.1), which may have a degradation effect, thereby increasing the solubility of the hemicelluloses in hot water (65−95 °C), and then resulted in increasing yield. By contrast, the yield of alkali-extractable hemicelluloses showed a decreasing trend from aqueous 1 M KOH to 1 M LiOH, which was consistent with the result obtained by Peng et al. (*15*) when they extracted hemicelluloses with aqueous alkalis from delignified *Populus gansuensis*.

Content of Neutral Sugars and Uronic Acids. The neutral sugar and uronic acid analysis is regarded as a reliable approach to determine the monosaccharide in hemicellulose hydrolysate. In this case, the neutral sugars and uronic acids of the six hemicellulosic preparations, expressed as a relative percentage of total sugars and uronic acids, are listed in **Table 1**. The sugar analysis of the four water-extractable hemicelluloses showed that xylose (61.5−74.7%) was a dominant component, whereas glucuronic acid (Glc*p*A) or 4-*O*-methyl-glucuronic acid (4-*O*-Me-α-D-Glc*p*A) (1.91−3.58%) and arabinose (3.85−5.25%) were observed in minor amounts. The predominance of xylose and the lesser amounts of arabinose and uronic acids indicated that the water-extractable hemicelluloses of *B. rigida* probably consisted mainly of arabinoglucuronoxylans. However, the water-extractable hemicellulosic preparations (between H_1 and H_4) obtained from delignified bamboo powder were also rich in glucose (16.3−31.2%). The higher content of glucose in these samples probably resulted from the released β-glucans under the condition of increasing temperature. The existence of β-glucans in bamboo was supported by Wilkie et al. (*11*), in which the authors stated that the hemicelluloses from delignified bamboo contained not only xylans but also glucose-rich fractions. These subsequent enzymic studies on hemicelluloses supported the view expressed above that the glucose residues were apparently all in heterolinked β-D-glucans. Coincidentally, Sun et al. also stated that D-glucose may be present as β-D-glucans, which are widely distributed in the cell walls of various monocotyledons (*16*). It also should be noted that the water-extractable hemicelluloses contained more glucose than the alkali-extractable hemicelluloses. Furthermore, in this study, the increased glucose was potentially regarded as the direct consequence of the favorable effect of reaction temperature on the diffusion of the hemicelluloses. However, further evidence to support this hypothesis awaits additional experiments.

In comparison, the two alkali-extractable hemicellulosic preparations contained xylose with an extremely predominant ratio (85.6 and 88.5%), suggesting that the alkaline treatment dissolved the hemicelluloses with even more linear molecular structure. Besides, the majority of xylose and the small amount of glucuronic acid as well as arabinose indicated that the alkali-extractable hemicelluloses from bamboo also probably consisted of arabinoglucuronoxylans. Moreover, it should be noted that an increasing glucose content in the LiOH fraction (H_6, 4.4%) suggested that treatment of the delignified bamboo particles with 1 M LiOH probably resulted in a slight degradation of cellulose fragments.

Molecular Weight Distribution. In general, the molecular weight values (M_w) are considerably dependent on the method of isolation performed. In this study, the apparent values of molecular weights of the hemicellulosic polymer were analyzed by GPC. The weight-average (M_w) and number-average (M_n) molecular weights as well as polydispersity (M_w/M_n) are given in **Table 2**. As expected,

Table 2. Weight-Average (M_w) and Number-Average (M_n) Molecular Weights (g mol^{-1}) and Polydispersity (M_w/M_n) of the Hemicellulosic Fractions

	hemicellulosic fractions[a]					
	H_1	H_2	H_3	H_4	H_5	H_6
M_w	22130	28170	20100	28000	44450	35200
M_n	11930	12300	9140	13670	21600	24260
M_w/M_n	1.85	2.29	2.20	2.05	2.06	1.45

[a] Corresponding to the hemicellulosic fractions in **Table 1**.

the average molecular weights of water-extractable hemicellulosic preparations with M_w values of 20100−28000 g mol^{-1} were lower than those of the two alkali-extractable hemicellulosic preparations, ranging from 35200 to 44450 g mol^{-1}. This suggested that the treatments with water released only hemicellulosic polymers with low molecular weights, whereas alkaline extraction could result in dissolution of large hemicellulosic molecules. It was also observed that first alkaline extraction with 1 M KOH resulted in the highest molecular weight among the six hemicellulosic fractions. However, it should be noted that the hemicellulosic polymers could be partially degraded under the conditions given (1 M LiOH for 3 h). In other words, the hemicelluloses with low molecular weights in the present case are due to partial depolymerization during treatment with a relatively strong alkalinity of alkali substances. By contrast, the four water-extractable hemicellulosic polymers seemingly presented molecular weights without huge differences at different temperatures.

Molecular weight distribution curves (shown in Figure S1 of the Supporting Information) could also vividly illustrate the structural features of the hemicelluloses obtained by various methods to a certain degree. The distinguishing molecular weight distribution curves might derive from the polymers differing in primary structures and/or in different composition of polymers with the same primary structure. It was observed that all of the water-extractable hemicellulosic polymers exhibited bimodal molecular weight distribution curves. On the basis of the existing literature about bamboo hemicelluloses (*11*, *12*), these hemicelluloses differ in primary structures, such as xylan or β-glucans. In other words, the molecular weight distribution curves probably revealed the presence of two kinds of hemicelluloses that are not chemically linked to each other in the water-extractable fractions. By contrast, the alkaline hemicelluloses displayed unimodal molecular weight distribution curves. This might imply that the alkali-extractable hemicelluloses gave a homogeneous structure, mainly xylan-based hemicelluloses, as revealed by sugar analysis results.

FT-IR Spectra. Infrared spectroscopy (FT-IR) has been proved to be useful for studying functional groups of polysaccharides in plant materials. In addition, it could be applied to explore structural features when combined with chemical methods as well as other spectrum approaches, such as NMR spectroscopy. The FT-IR spectra of water-extractable hemicelluloses (H_1−H_4) and alkali-extractable hemicelluloses (H_5 and H_6) are shown in **Figure 2**, panels **a** and **b**, respectively. Most of the absorption peaks were assigned according to data presented in previous papers (*17*, *18*).

As shown in **Figure 2a**, the four water-extractable hemicelluloses displayed very similar spectra. The signal observed at 3392 cm^{-1} is assigned to the −OH stretching vibrations of the hemicelluloses, and the band at 2904 cm^{-1} relates to C−H stretching. In addition, the intense absorption band at 1641 cm^{-1} corresponds to the bending mode of the absorbed water, because the hemicelluloses usually have a strong affinity for water, and in the solid state these macromolecules may have disordered structures, which can easily be hydrated (*19*). A major absorbance at 1046 cm^{-1} originates from the C−O−C stretching of glycosidic linkages, which is typical of xylans. Moreover, a small peak at about 900 cm^{-1} in the spectra is characteristic of β-glycosidic linkages between the xylose units in the hemicelluloses. By comparison of panels **a** and **b** of **Figure 2**, it was found that most of the absorption peaks are similar; however, the two alkaline hemicelluloses presented a more intense absorbance at 899 cm^{-1}, indicating that β-glycosidic linkage is the main linkage between units in the alkaline hemicelluloses. Moreover, in the water-extractable hemicellulosic fractions H_1−H_4, the small but sharp peak at 1732 cm^{-1} is due to the C=O stretching of acetyl groups and esterified *p*-coumaric acid in the hemicellulosic polymers. However, the disappearance of this absorbance in the two alkaline hemicellulosic fractions (H_5 and H_6) indicated that the ester groups in the hemicelluloses isolated by alkaline are substantially saponified under the conditions given. In summary, the FT-IR spectra of the hemicelluloses isolated with hot water showed more original hemicelluloses than those isolated with aqueous alkalis.

1D and 2D NMR Spectra. To further elucidate the structural characteristics of the polymers extracted with different media, the hemicellulosic preparations H_3 and H_5 were investigated using 1D and 2D NMR spectroscopy. NMR spectra are supposed to assay and identify the polymer backbone and the type of side-chain branching along the backbone. The ^{1}H and ^{13}C NMR and HSQC spectra of hemicelluloses fractions H_3 and H_5 are shown in **Figures 3**, **4**, and **5**, respectively. The signals for ^{1}H and ^{13}C NMR were assigned on the basis of the HSQC spectra and previous literature (*10*, *20*, *21*). The assignment data of ^{1}H and ^{13}C NMR spectra are given in **Table 3**.

As can be seen from **Figure 3**, the two hemicellulosic preparations showed analogous ^{1}H NMR spectra, indicating a similar structure of hemicelluloses. The anomeric signals in the ^{1}H NMR spectra of H_3 and H_5 were assigned according to sugar analysis and the literature data (*22*), and they were found in the spectral region of 4.3−5.6 ppm. The relevant signals occurred in two regions, namely, the anomeric region (δ 5.6−4.9 for α-anomers and δ 4.9−4.3 for β-anomers) and the ring proton region (δ 4.5−3.0). The anomeric protons were well distinguished at δ 4.28 and 4.30, which are assigned as (1→4)-β-D-Xyl*p* of H_3 and H_5, respectively. This confirmed that (1→4)-β-D-Xyl*p* is linked β-glycosidically, which is consistent with the presence of the small sharp peak at 899 cm^{-1} in the IR spectra of H_3 and H_5.

The ^{13}C NMR spectrum (**Figure 4b**) of H_5, extracted with 1 M KOH, exhibits five major signals corresponding to those of (1→4)-linked-β-D-xylan. The signal at 102.4 ppm corresponds to the anomeric region in a β-configuration, as confirmed by the ^{1}H NMR spectra, whereas the signals at 76.0, 75.2, 73.6, and 63.2 ppm corresponded to C-4, C-3, C-2, and C-5 of (1→4)-linked-β-D-Xyl*p* units, respectively. The signal at 97.5 ppm originated from the anomeric carbons of 4-*O*-methy-α-D-glucuronic acid (4-*O*-Me-α-D-GlcpA), and the signals at 72.4, 71.7, and 82.9 ppm arise from C-3, C-2, and C-4 of 4-*O*-Me-α-D-GlcpA, respectively. Among other weak signals observed at 177.1 and 59.4 ppm were characteristic signals of C-6 and the methoxyl group of 4-*O*-methyl-D-glucuronic acid residue in the H_5, respectively.

From HSQC spectra of H_3 (**Figure 5a**), the dominant five cross-peaks could be expressly identified at 102.7/4.30, 76.0/3.63, 75.2/3.36, 73.3/3.15, 63.3/3.94 + 3.24 ppm (**Table 3**), which are assigned to C_1−H_1, C_4−H_4, C_3−H_3, C_2−H_2, and C_5−H_5 of the (1→4)-linked-β-D-Xyl*p* units, respectively. In addition, the presence of the methyl group (OCH_3) of 4-*O*-methyl-D-glucuronic acid was confirmed by a corresponding small but distinguishable cross-peak at 60.7/3.33 ppm. Furthermore, some weak cross-peaks, which represented 4-*O*-methyl-D-glucuronic acid and L-arabinose, were also observed. However, the weak signals for them are

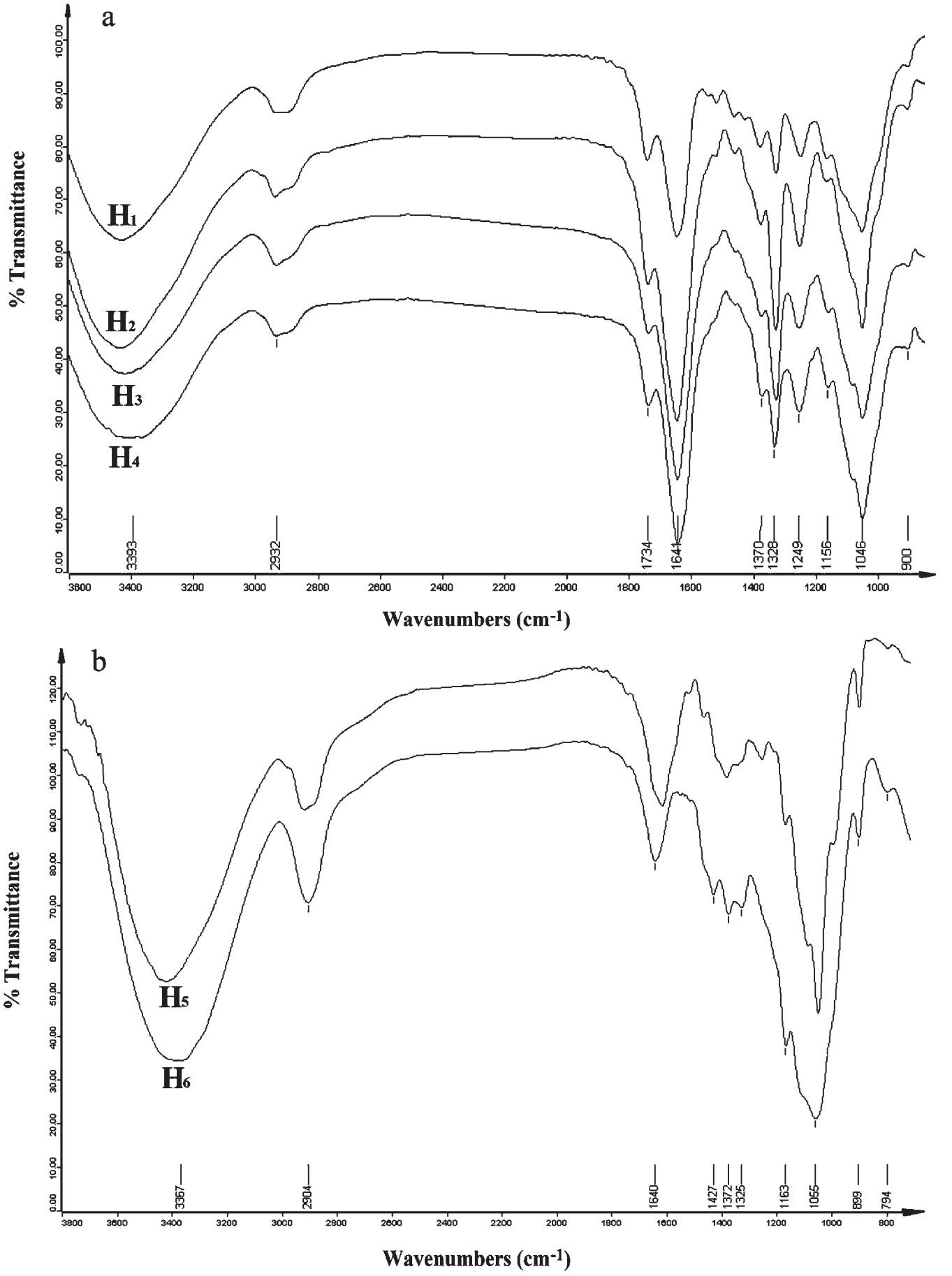

Figure 2. FT-IR spectra of the hemicelluloses extracted with (**a**) water at 50 (H_1), 65 (H_2), 80 (H_3), and 95 °C (H_4) and (**b**) aqueous alkali (1 M KOH (H_5), 1 M LiOH (H_6)).

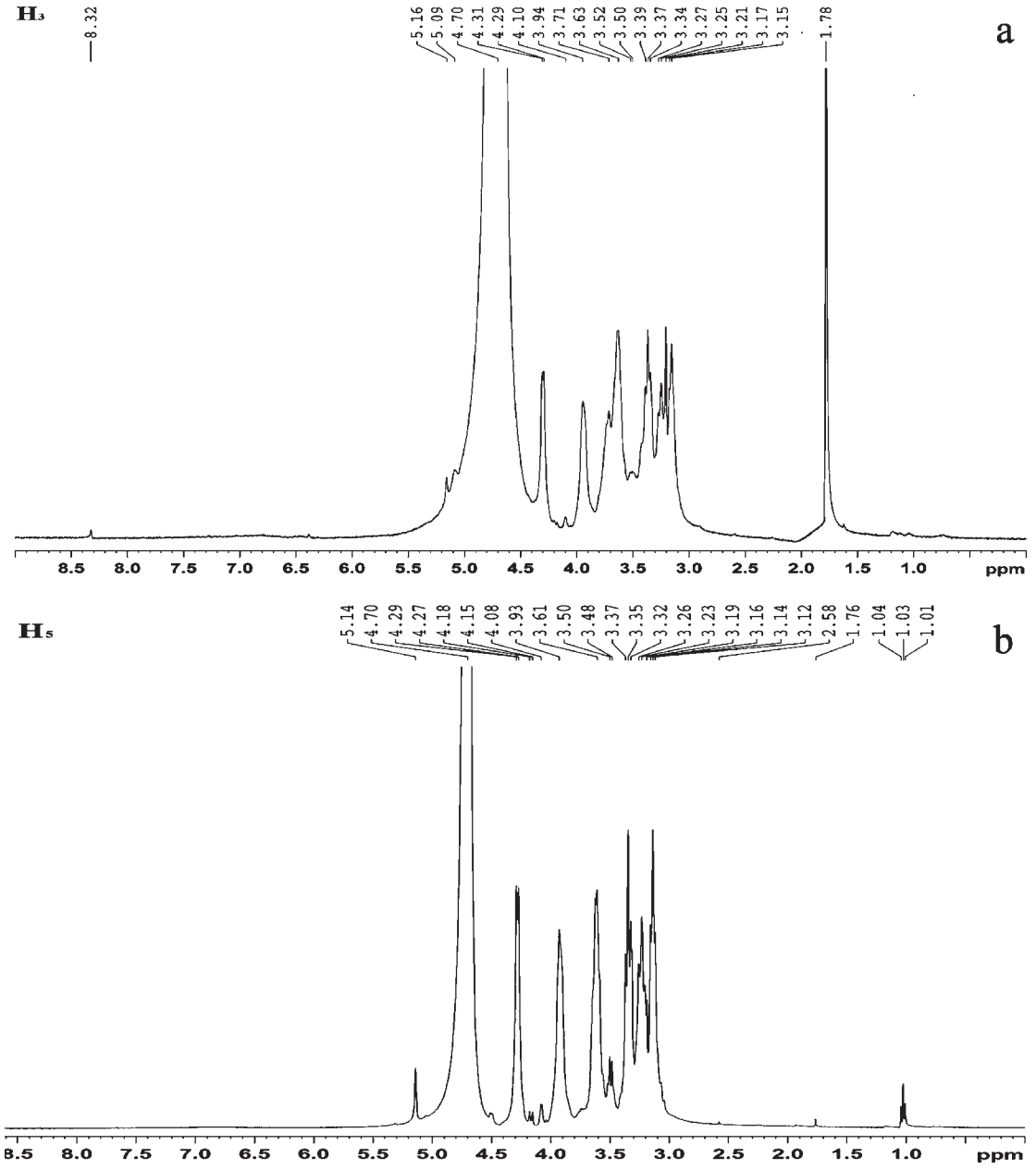

Figure 3. ^{1}H NMR spectra of hemicellulosic fractions H_3 (water-extractable) and H_5 (alkali-extractable).

probably due to the low abundance of these components. As a summary, all of the 2D-HSQC assignments of these hemicelluloses are listed in **Table 3**.

It should be noted that the signal at 173 ppm in H_3, which is assigned to carbonyl groups in the acetyl groups, is more intense than the corresponding signal in H_5. Similarly, as shown in the spectrum in **Figure 4a**, the corresponding methyl of the acetyl group gives a signal at 23.5 in H_3; however, it almost disappeared in H_5 (**Figure 4b**). This phenomenon indicated that the ester bonds in the acetyl ester of the hemicelluloses were significantly cleaved under the alkaline condition (1 M KOH at 50 °C for 3 h). It is widely believed that a significant portion of the xylose in plant cell walls was found to be acetylated, mainly on C-2 but also on C-3. However, it is not known if the acetyl substituent is attached to the xylosyl backbone randomly or at regular repeating sequences (*23*). One point that should not be ignored is an obvious signal appearing in H_5 at 17.2 ppm (**Figure 4b**), which is probably due to the residual ethanol in the isolated hemicellulosic preparation. Besides, the weak signal appearing at 168.4 ppm in H_3 suggests that some esterified *p*-coumaric acid also existed in the water-extractable hemicellulosic polymers. This implied that these water-extractable hemicelluloses contained minor esterified *p*-coumaric acid. A similar result was reported in a previous paper (*24*), in which the authors revealed that a small amount of *p*-coumaric acids is esterified to arabinose residues of the hemicelluloses in wheat straw cell wall. Besides, the hemicelluloses' bonding with *p*-coumaric acid, obtained from the cell wall of maize bran, suggests the possibility that hemicelluloses from the Gramineae plant are possibly also cross-linked via *p*-coumarates to other hemicelluloses, lignin, and proteins (*25*). However, in comparison with H_3, there are no signals represented for *p*-coumarates in H_5, suggesting that ester-linked *p*-coumaric acid was saponified during the treatment with aqueous alkalis. Moreover, it should be noted that the ^{13}C NMR spectrum of H_3 showed resonances at 181 ppm (probably due to the C-6 of dissociative glucuronic acid) in the low-field region, whereas the spectrum of H_5 presented a signal at 177 ppm (C-6 of glucuronic acid attached to hemicelluloses). The difference may be attributed to the different chemical environments

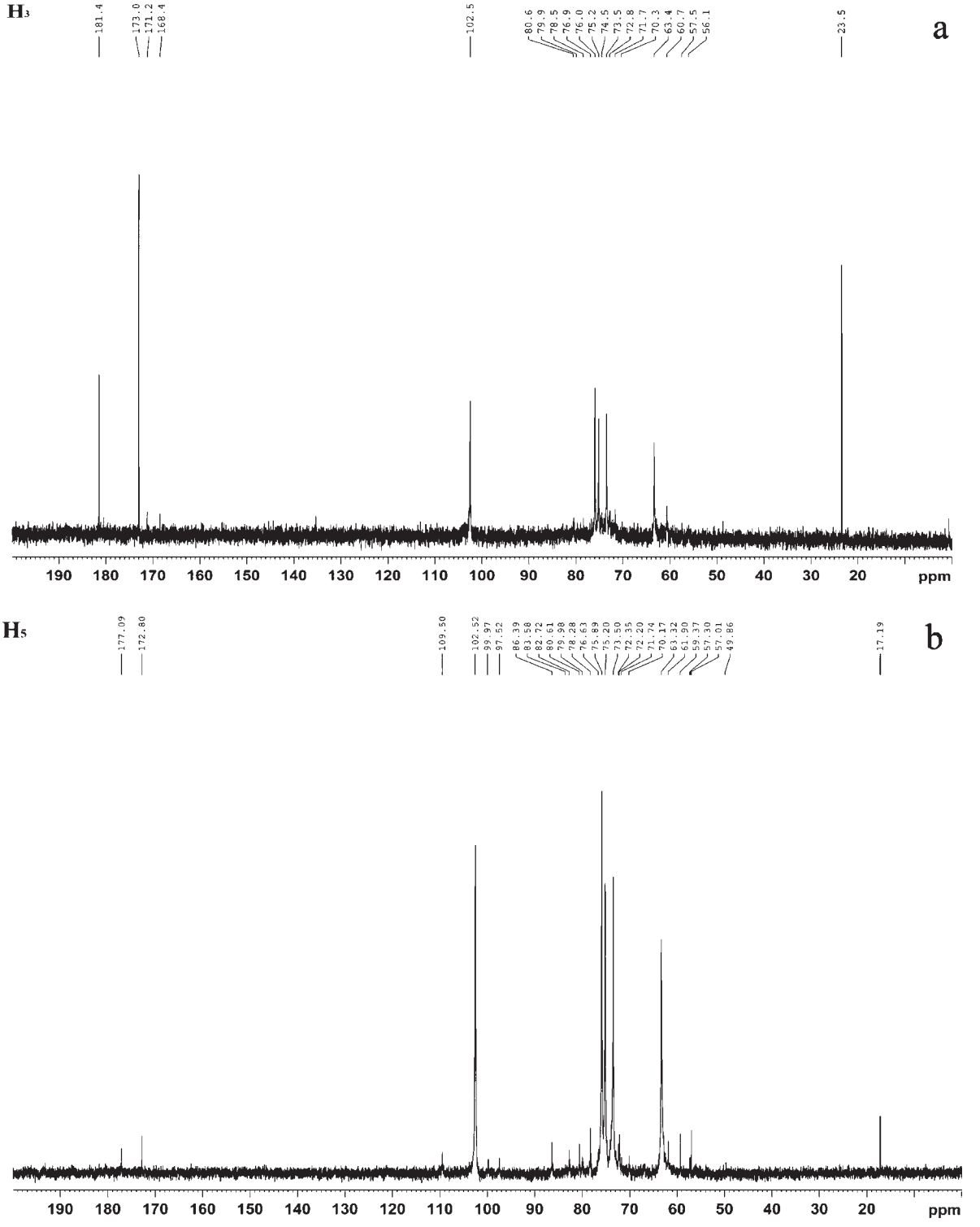

Figure 4. ^{13}C NMR spectra (in D_2O) of hemicellulosic fractions H_3 (water-extractable) and H_5 (alkali-extractable).

of the C-6 in glucuronic acid. It is possible that the dissociated glucuronic acid was obtained by autohydrolysis in the acidic conditions (pH 5.1), due to the cleavage of the acetyl group in the extraction with water at a high temperature (80 °C). Furthermore, some distinguishable signals in the spectrum of H_3 probably represent β-glucans, such as 79.9 ppm (C-3) and 60.7 ppm (C-6) (*26*). However, some signals of β-glucans are overlapped with those of xylan-type hemicelluloses. In addition, the reason for these low intense signals of β-glucans was probably because of the low solubility of β-glucans in D_2O at ambient temperature (298 K). Overall, the data reported here almost elucidate the heterogeneous structure of the bamboo hemicelluloses isolated with different solvents and methods.

HSQC contour volume integral is a promising method to estimate the relative quantity of components in hemicelluloses. By applying a semiquantitative HSQC, the relative ratios of compositions in these hemicellulosic preparations could be estimated by integrating contour volumes corresponding to carbohydrates' anomeric C_1-H_1 correlations. The relative ratio of X/A (xylose/arabinose) in H_3 was determined on the basis of integration of the corresponding anomeric cross-peaks, and the ratio of X/A was subsequently calculated. The integral result gave an approximate

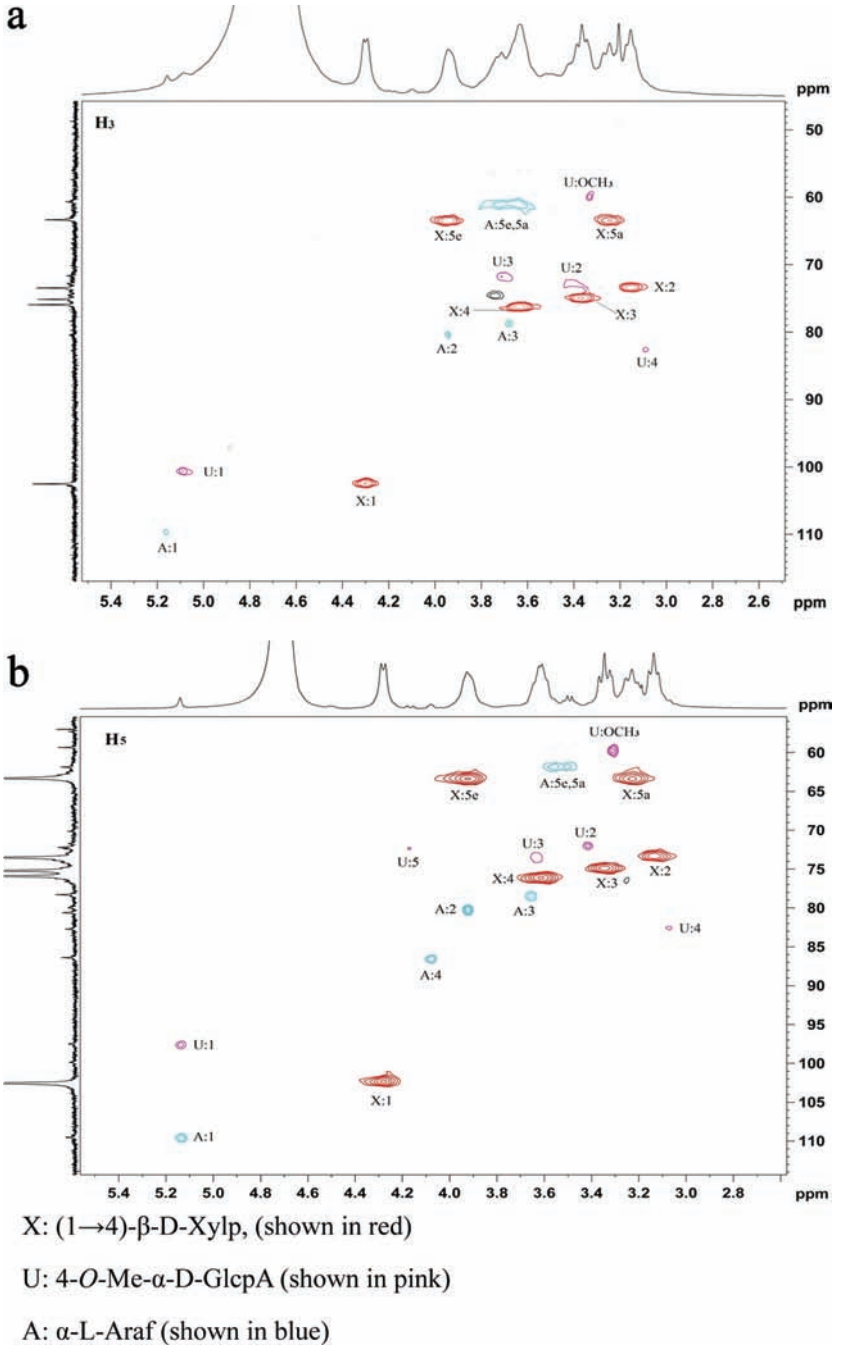

Figure 5. $^{1}H/^{13}C$ spectra (HSQC) of hemicellulosic fractions H_3 and H_5.

value of 12, and this value obtained is analogous to the result obtained by sugar analysis, in which the X/A molar ratio is approximately 16. However, the relatively lower X/A ratio obtained by HSQC integration is probably because some low content of hemicelluloses cannot be detected in HSQC at the present S/N ratio in **Figure 5**. In comparison with H_3, the H_5 fraction extracted with 1 M KOH presented a similar molar ratio of X/A (*12*) estimated by contour volume integral, whereas sugar analysis of H_5 gives a X/A molar ratio of 15. The discrepancy of ratios X/A obtained from sugar analysis and NMR contour volume integral is similarly attributed to the above-mentioned reason. Similarly, the xylose/uronic acid ratios (X/U ratio) of H_3 and H_5 were estimated to be 24 and 14, respectively. However, in sugar analysis, the X/U ratios are 30 and 15, respectively. However, compared with the relative sugar molar ratio obtained by sugar analysis, the HSQC contour volume integral could be still considered as a methodology of semiquantitative estimates. Nevertheless, as a novel and speedy approach to achieve both structural information and quantitative information, 2D-HSQC-NMR spectroscopy demonstrates powerful functionalities in qualitative and quantitative estimation of polysaccharides.

On the basis of FT-IR spectra, NMR spectra, and existing literature concerning the linkages between the monosaccharides of the bamboo species (*11*), it could be concluded that water-extractable hemicelluloses (H_3) are formed by a linear backbone of (*β*-1→4)-Xyl*p* residues and the xylose residues is substituted at C-3 by arabinose, whereas the xylose residue is substituted at C-2 by 4-*O*-Me-GlcA. In addition, the (*β*-1→4)-Xyl*p* residues in the backbone may contain substituted phenolic acids (ferulic acid and *p*-coumaric acid). On the basis of the result of sugar analysis, the molar ratio of arabinose/4-*O*-MeGlcA/xylose (A/U/X) in the water-extractable hemicelluloses (H_3) is 2:1:30. Moreover,

Table 3. Chemical Shifts (δ) of Signals in ^{1}H and ^{13}C NMR Spectra of Hemicellulosic Preparations H_3 and H_5

		assignments							
glycosyl		1	2	3	4	5eq[e]	5ax[f]	6	OCH_3
H_3									
X[a]	^{13}C	102.5	73.3	75.2	76.0	63.3	63.3		
	^{1}H	4.30	3.15	3.36	3.63	3.94	3.24		
U[b]	^{13}C	97.8	71.7	72.8	83.1	na[d]		181.4[g]	59.2
	^{1}H	5.09	3.50	3.68	3.08	na			3.33
A[c]	^{13}C	109.5	80.6	78.5	86.3	61.9	61.9		
	^{1}H	5.16	3.92	3.71	4.28	3.71	3.68		
H_5									
X[a]	^{13}C	102.4	73.5	75.2	76.0	63.2	63.2		
	^{1}H	4.28	3.14	3.35	3.60	3.92	3.23		
U[b]	^{13}C	97.5	71.7	72.4	82.5	72.8[h]		177.1	59.4
	^{1}H	5.16	3.48	3.73	3.10	4.31			3.30
A[c]	^{13}C	109.8	80.6	78.3	86.3	61.9	61.9		
	^{1}H	5.13	3.92	3.76	4.07	3.55	3.51		

[a] X, (1→4)-β-D-Xyl*p*. [b] U, 4-*O*-methyl-α-D-GlcpA. [c] A, α-L-Araf residues. [d] na, not assigned. [e] eq, equatorial. [f] ax, axial. [g] C-6 of dissociative glucuronic acid in the hemicelluloses (H_3). [h] C_5-H_5 in U[b] has one cross peak because only one proton is linked to C-5.

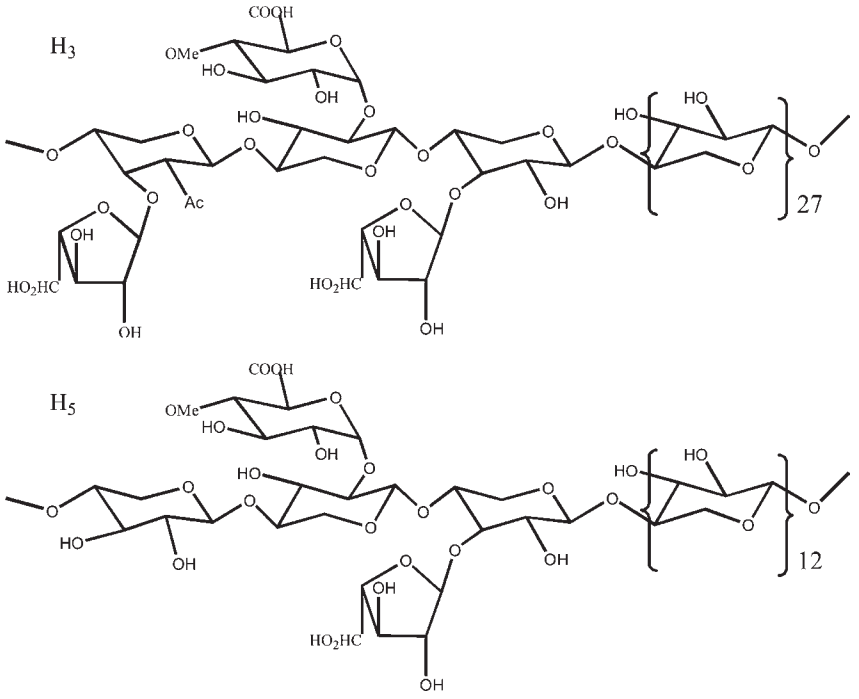

Figure 6. Potential structures of water (H_3)- and alkali-extractable (H_5) hemicellulosic fractions from *B. rigida* species.

the results from the sugar analysis, NMR, and a previous study (*12*) also potentially suggested that a certain amount of β-glucans was also found as a kind of hemicelluloses in the water-extractable hemicelluloses of this bamboo. In comparison, on the basis of the above analysis and previous studies on linkage positions of monosaccharide (*11*), it could be concluded that the alkali-extractable hemicelluloses from the cell wall of the bamboo had a structure composed of the (β-1→4)-Xyl*p* backbone with 4-*O*-Me-α-D-glucuronic acid attached to *O*-2 and L-arabinose linked to *O*-3 of the xylose residue, giving a ratio of A/U/X of 1:1:15. The potential structures of water-extractable (H_3) and alkali-extractable (H_5) hemicelluloses from *B. rigida* species are illustrated in **Figure 6**.

CP-MAS NMR Spectra. To investigate the effects of water and alkali treatments on the structural feature of cellulose, the raw material, the residue after sequential extractions and microcrystalline cellulose (MCC) were examined by CP-MAS NMR spectra. As shown in **Figure 7a**, the signals of the raw material appeared at 105 ppm (cellulose/xylan C-1), 89 ppm (crystalline cellulose C-4), 84 ppm (amorphous cellulose/xylan C-4), 72−76 ppm (cellulose C-2/3/5; xylan C-2/3), 65 ppm (crystalline cellulose C-6), and 62 ppm (amorphous cellulose C-6/xylan C-5) (*26*). In addition, 56 ppm is related to the methoxyl of lignin, and the weak and broad peaks between 110 and 160 ppm revealed that the raw material contained a certain amount of lignin fractions (*27*). However, after delignification and sequential alkaline extractions, the final residue shown in **Figure 7b** is very similar to that of microcrystalline cellulose (S_c) (**Figure 7c**). This implied that after delignification and multistep extractions, the lignin and hemicelluloses were removed significantly from bamboo cell wall. In addition, hemicelluloses are regarded as amorphous material in the raw material, which probably contributed to the amorphous area of the raw material (shown in **Figure 7a**). In the CP-MAS NMR spectrum, hemicelluloses signals are overlapped with the amorphous area of cellulose (about 84 ppm). After

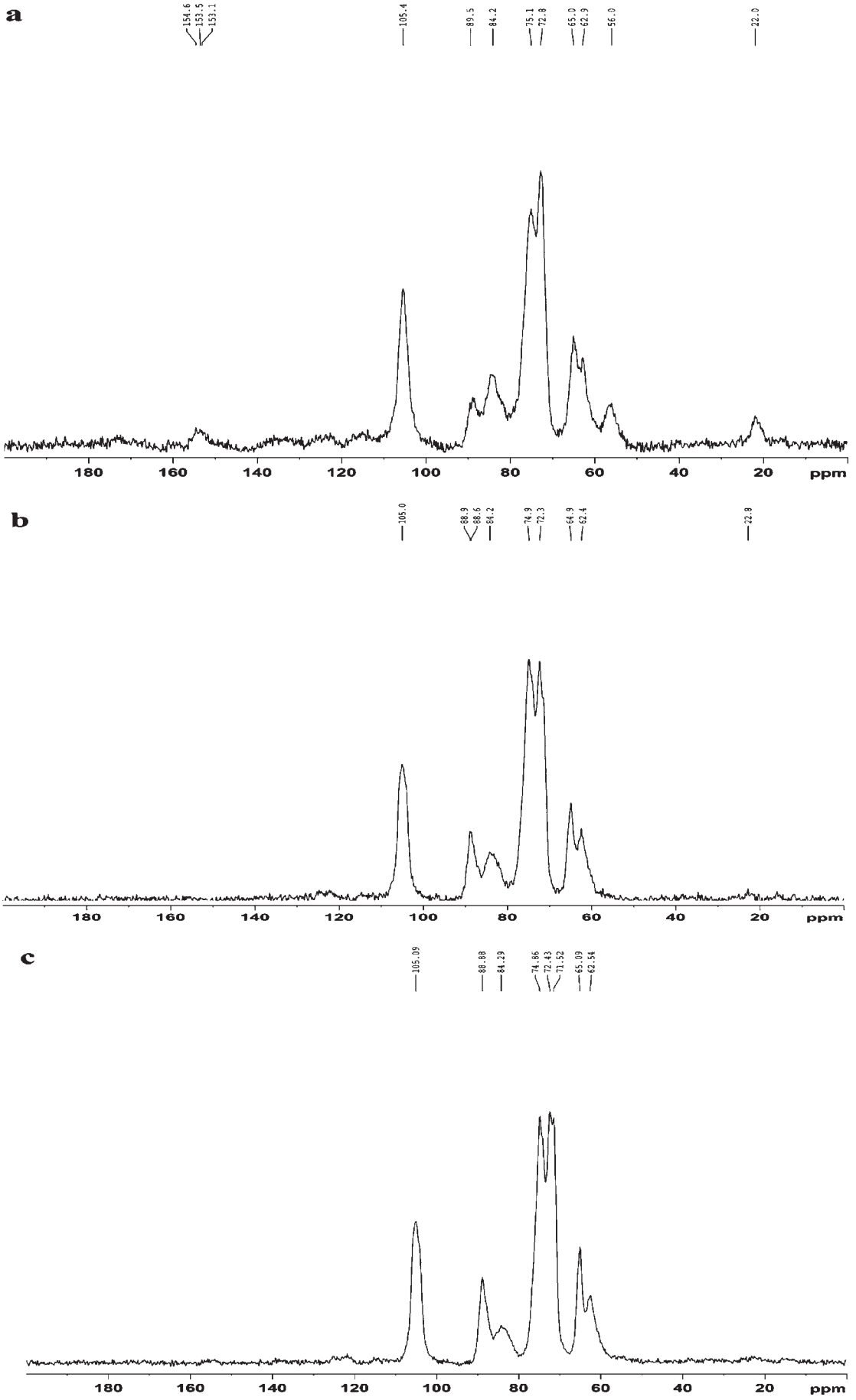

Figure 7. Solid ^{13}C CP-MAS NMR spectra of bamboo: (**a**) dewaxed bamboo powder; (**b**) residue after sequential extractions; (**c**) microcrystalline cellulose (MCC).

extraction with water and aqueous alkalis, most of the hemicelluloses were removed, and then the amorphous area of the residue decreased and the corresponding ratio of crystalline region increased. Moreover, the result of sugar analysis showed that the glucose is 86.5% and the xylose is 11.4% (relative molar %) in the residue, which suggested that the residue contained minor hemicelluloses and further revealed that cellulose and hemicelluloses linked tightly even after the chemical treatments. However, the ultimate residue after various treatments presented the celluloses with a high purity.

Furthermore, on the basis of CP-MAS NMR spectra, the crystallinity index (CrI) of the raw material, the residue after sequential extractions, and microcrystalline cellulose (MCC) could also be estimated. CrI was calculated using the intensities of the signal representing the crystalline area (89 ppm, C-4) and of the amorphous area (84 ppm, C-4), and eq 1 is (*28*)

$$\mathrm{CrI} = \frac{I_c}{I_c + I_a} \quad (1)$$

where I_c is the integral value of the signals representing the crystalline area (81−86.4 ppm) and I_a is the integral value of the signals representing the amorphous area (86.4−93 ppm).

CrI of the raw material was estimated to be 0.33, which is lower than the residue after sequential extractions (rich in cellulose) (0.52). The reason for this higher CrI of the residue was because the treatments with water and aqueous alkalis removed the majority of amorphous hemicelluloses and increased the ratio of crystalline region. Furthermore, the MCC showed a CrI of 0.58, which is approximated to that of the residue after six-step extractions (0.52). Therefore, the isolation approach probably provides a potential way to efficiently obtain hemicelluloses and cellulose for industrial application. Nevertheless, to increase reactivity of the cellulose-rich residue, there may be a need to tailor pretreatment technologies to decrease the value (0.52) of the highly crystalline structure of the residue (*29*).

Finally, we can conclude that the results obtained showed both water-extractable and alkali-extractable hemicelluloses consisting of the (β-1→4)-Xyl*p* backbone with 4-*O*-Me-α-D-glucuronic acid attached to *O*-2 and L-arabinose linked to *O*-3 of the xylose residue. In addition, a significant proportion of β-glucans were also considered to be included in the water- extractable hemicellulosic polymers. However, further work is now in progress on the fine structure to obtain a more precise insight into the granular structure. Furthermore, after delignification and multistep extractions, the cellulose-rich residue contained a small amount of hemicelluloses. Overall, the isolation method probably provides a possible approach to efficiently obtain low-branched xylans for further producing the xylose, an intermediate for the production of xylitol, and a variety of xylo-oligosaccharides. Simultaneously, the cellulose-rich residue obtained has potential use for industrial application, such as in the papermaking and bioethanol production industries.

Supporting Information Available: Figure S1: Molecular weight distribution curves of the hemicellulosic preparations H_1−H_6. This material is available free of charge via the Internet at http://pubs.acs.org.

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Received for review August 22, 2010. Revised manuscript received September 21, 2010. Accepted September 21, 2010. We are extremely grateful for financial support from the Major State Basic Research Projects of China (973-2010CB732204), the National Natural Science Foundation of China (30930073, 30710103906), the China Ministry of Education (111), and the State Forestry Administration (200804015, 2010-4-16).