Isolation and Structural Exploration of Hemicelluloses from the Largest Bamboo Species: *Dendrocalamus sinicus*

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Dendrocalamus sinicus, which is the largest bamboo species in the world, has broad prospects for use in biomass-energy and biorefinery applications. In this study, five soluble hemicelluloses fractions were sequentially isolated with 80% ethanol (containing 0.025 M HCl or 0.5% NaOH), and alkaline aqueous solutions (containing 2.0, 5.0, or 8.0% NaOH) at 75 °C for 4 h from dewaxed *D. sinicus*, and their structural properties were examined. Gel permeation chromatography analysis revealed that the hemicelluloses isolated from *D. sinicus* had a wide distribution of molecular weights. The hemicelluloses isolated by ethanol had lower weight-average molecular weights (ranging from 17380 to 19620 g/mol), while the hemicelluloses isolated using alkaline aqueous solutions had higher weight-average molecular weights (ranging from 22510 to 42150 g/mol). Neutral sugar analysis indicated that the soluble hemicelluloses were mainly composed of arabinoglucuronoxylans, followed by minor amount of starch. Spectroscopic analyses suggested that the isolated arabinoglucuronoxylans from bamboo (*D. sinicus*) could be defined as a linear (1→4)-β-linked-xylopyranosyl backbone to which α-L-arabinofuranose and/or 4-*O*-methyl-glucuronic acid units were attached as single-unit side chains via α-(1→3) and/or α-(1→2) linkages.

Keywords: Bamboo; Dendrocalamus sinicus; Hemicelluloses; Isolation; Characterization

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INTRODUCTION

With the inevitable depletion of the world's fossil fuels supply, there has been an increasing worldwide interest in exploring alternative and renewable sources to support industry in the future. Bamboo consists of perennial giant tree-like grasses and includes approximately 75 genera comprising more than 1400 species, which are distributed mainly in tropical and subtropical regions (Odani 1999). Most bamboo species are fast-growing plants, and their culms can grow to their full height of 3 to 33 m within a few months due to the expansion of individual internodes already present in the buds (Metcalfe 1960; Liese 1998). Bamboo has good fiber quality for paper-making, and it shares a number of desirable fuel characteristics with certain other bioenergy feedstocks, such as low ash content and alkali index (Scurlock *et al*. 2000). Due to its easy propagation, fast growth, and high productivity, bamboo is considered as one of the most potential renewable non-woody forestry feedstock for the production of biofuel, bioenergy, and value-added biomaterials from its lignocellulosic components, such as hemicelluloses.

Hemicelluloses, a major wood component besides cellulose and lignin, are located primarily in the secondary cell walls, and together with cellulose and lignin, build up the plants in a fashion that gives the best combination of mechanical support and transport properties. Normally, hemicelluloses in various plant species differ from each other both quantitatively and qualitatively. The principal sugars of hemicelluloses are D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid, and to a lesser extent, L-rhamnose, L-fructose, and various *O*-methylated neutral sugars (Sun *et al*. 2005). The major hemicelluloses present in hardwood are the β-(1→4)-D-xylopyranose residues, which are mainly substituted by *O*-acetyl groups and α-D-glucuronic acid or 4-*O*-methyl-α-D-glucuronic acid in C-2 and/or C-3 positions (Timell 1967). The predominant softwood hemicelluloses are galactoglucomannans, which are partially acetylated (Lundqvist *et al*. 2003). Generally, α-D-galactopyranose is bonded as single-unit side chains to the main chain by (1→6)-bonds (Willför *et al*. 2003). In addition, agricultural crops residues, such as wheat straw hemicelluloses appear to be essentially β-D-(1→4)-linked xylopyranosyl units with 4-*O*-methyl-α-D-glucopyranosyluronic acid attached at position C-2, whereas L-arabino-furanosyl and D-xylopyranosyl groups are attached at position C-3 (Sun *et al*. 1998). These large amounts of hemicelluloses are worth exploiting to serve as adhesives, thickeners, stabilizers, film formers, and emulsifiers (Doner and Hicks 1997).

Conversion of lignocellulosic materials to higher value products requires fraction-ation of hemicelluloses from biomass material. However, separation of hemicelluloses from the cell wall is restricted by the presence of lignin network as well lignin-hemicel-luloses linkages (Crestini and Argyropoulos 1997). Meanwhile, extensive hydrogen bonding between the individual polysaccharide components in the cell wall may impede the isolation of the hemicellulosic components. So far, various methods have been developed to extract hemicelluloses from plant cell walls. Earlier, for large-scale isolation of hemicellulosic polysaccharides from lignocellulose materials, materials were first delignified with chlorine (Timell *et al*. 1951), chlorine dioxide (Yang and Goring 1978), or sodium chlorite (Fengel *et al*. 1989), after which the left holocellulose would then be treated with various procedures. However, such treatments aimed at delignification often oxidize some reducing-end residues of polysaccharides to aldonic acid residues and cause partial depolymerization and some loss of components (Aspinall *et al*. 1961). For these reasons, numerous methods have been developed in an attempt to extract as much of the hemicellulosic polysaccharides as possible while minimizing the extent of chemical modification. These methods include extraction with concentrated solutions of sodium or potassium hydroxide (Dupont and Selvendran 1987), alkaline hydrogen peroxide solution (Doner and Hicks. 1997), solutions of barium or calcium hydroxides at elevated temperatures (Bergmans *et al*. 1996), organic solvents (Johansson *et al*. 1987), and hot water (Sun *et al*. 2004). Among these methods, aqueous solutions of sodium and potassium hydroxide have been applied most extensively by far for extraction of hemicelluloses (Cyran and Saulnier 2007; Fares *et al*. 2001; Höije *et al*. 2005; Moine *et al*. 2007; Rao and Muralikrishna 2006). The main advantages of the alkali extractions are that they are simple to perform and cost-effective (Bergmans *et al*. 1996). In addition, organosolv treatment is another favorable method to isolate hemicelluloses from raw plant materials. In this method, the organic solvent primarily promotes the impregnation of vegetal tissue, thus making it possible to obtain hemicelluloses with only minor changes compared to its native form in the plant sources (Balogh *et al*. 1992; Gilarranz *et al*. 2000). In general, the plant hemicellulosic preparations consist of several

hemicellulosic polymers which vary in structural characteristics (Cyran and Saulnier 2007), and one step of treatment could only extract part of the hemicelluloses from the raw materials (Bergmans *et al*. 1996). In view of these facts, fractionation techniques, such as sequential extraction with acidic ethanol, alkaline ethanol, and alkaline aqueous solutions with different alkali concentrations was worth attempting to obtain more homogeneous hemicellulosic polysaccharide fractions and thus to further explore their structural properties.

The bamboo species *Dendrocalamus sinicus*, which is the world's largest bamboo species, belongs to Bambusoideae of Gramineae. It has strong woody stems (maximal diameter 30 cm, maximal height 33 m) and is mainly distributed in the southwest region of China (Ohrnberger 1999). Although *D. sinicus* is considered one of the bamboo species having the greatest potential for development, the detailed physicochemical properties of hemicelluloses present in the bamboo species have not been reported in the literature up to this point. Therefore, isolation and elucidation of the physicochemical properties, as well as structural characteristics of *D. sinicus* hemicelluloses was our particular interest. In this study, this bamboo feedstock was sequentially treated with ethanol and alkaline solutions, and the obtained hemicellulosic preparations were comparatively characterized by gel permeation chromatography (GPC), high-performance anion exchange chromatography (HPAEC), proton magnetic resonance (^{1}H NMR), carbon-13 magnetic resonance (^{13}C NMR), and 2D heteronuclear single quantum coherence magnetic resonance (2D HSQC NMR) spectroscopy.

EXPERIMENTAL

Materials

Bamboo (*D. sinicus*), 3 years old, was obtained from Yunnan Province, China. The leaves and branches were removed, and the trunks were chipped into small pieces. After drying at 60 °C for 16 h in an oven, the chips were ground and screened to obtain a 40 to 60 mesh fraction. Subsequently, the powder was subjected to extraction with toluene/ethanol (2:1, v/v) in a Soxhlet apparatus for 6 h, and the dewaxed powder was further dried in an oven with air circulation at 60 °C for 16 h. It was stored in a sealed plastic bag before use. All standard chemicals, such as monosaccharide and chromategraphic reagents, were purchased from Sigma Chemical Company (Beijing, China).

The compositions of the dewaxed bamboo are listed in Table 1, determined according to National Renewable Energy Laboratory's standard analytical method (Sluiter *et al*. 2008). Briefly, wood samples (~300 mg) were hydrolyzed with 72 % (w/w) H_2SO_4 (3.0 mL) at 30 °C for 1 h. The hydrolysates were diluted to 4 % (w/w) H_2SO_4 with deionized water, and a second hydrolysis was carried out at 121 °C for 1 h in an autoclave. Subsequently, the hydrolysis solution was cooled and filtered through a porcelain crucible, and the residue was used to determine Klason lignin content. After hydrolysis, the resulting solution was filtered and diluted 50-fold, and the sugars in the aqueous phase were quantified with high performance anion exchange chromatography system (Dionex ISC 3000, USA) with a pulsed amperometric detector, AS50 autosampler, a CarbopacTM PA-20 column (4 × 250 mm, Dionex), and a guard PA-20 column (3 × 30 mm, Dionex). Neutral sugars and uronic acids were separated in isocratic 5 mM NaOH (carbonate free and purged with nitrogen) for 20 min, followed by a 0 to 75 mM NaAc gradient in 5 mM NaOH for 15 min. Then the columns were washed with 200

mM NaOH to remove carbonate for 10 min, followed a 5 min elution with 5 mM NaOH to re-equilibrate the column before the next injection. The total analysis time was 50 min, and the flow rate was 0.4 mL/min. Calibration was performed with standard solutions of L-rhamnose (9.6 min), L-arabinose (10.1 min), D-galactose (12.8 min), D-glucose (14.8 min), D-mannose, D-xylose (17.4 min), glucuronic acid (36.4 min), and galacturonic acid (38.5 min) (Xiao *et al*. 2011, 2013). The analyses were run in duplicate, and the average value is given.

Table 1. Chemical Composition of Dewaxed Bamboo (*D. sinicus*) Material

Chemical Component	Amount (wt %)
Cellulose (as glucan)	44.5
Hemicellulosic sugars	17.6
Xylan	14.3
Arabinan	0.2
Galactan	0.4
Rhamnan	1.6
Mannan	0.1
Glucuronic acid	0.9
Galacturonic acid	0.1
Klason lignin	25.0
Acid-soluble lignin	3.6
Ash	3.5

Isolation of Hemicellulosic Fractions

The scheme for fractionation of hemicelluloses from *D. sinicus* is illustrated in Fig. 1.

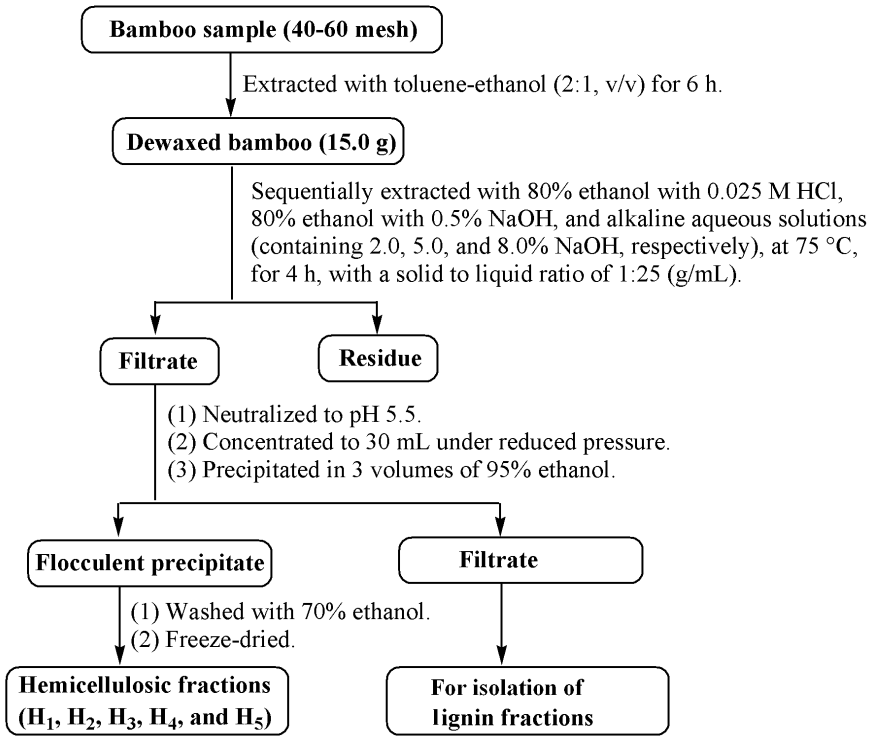

Fig. 1. Scheme for extraction of hemicellulosic fractions from bamboo (*D. sinicus*)

The dewaxed bamboo sample (15.0 g) was sequentially treated with 80% (v/v) ethanol containing 0.025 M HCl, 80% (v/v) ethanol containing 0.5% (w/w) NaOH, and alkaline aqueous solutions (containing 2.0, 5.0, and 8.0% NaOH, w/w) at 75 °C for 4 h (each individual extraction) with a solid to liquid ratio of 1:25 (g/mL). The extracted solutions were filtered with a Buchner funnel.

After filtration, the pellet residues were washed three times with 100 mL of the same solution and dried overnight at 37 °C. The supernatant was combined with washing liquor, neutralized to pH 5.5 (note that the acidic ethanol-extractable solution was neutralized with 0.025 M NaOH and the other solutions were neutralized with 6 M hydrochloric acid), and then concentrated to about 30 mL with a rotary evaporator under reduced pressure (−0.1 MPa) at 60 °C. After that, three volumes of ethanol were added to each concentrated solution with continuous stirring, and then the flocculent precipitate appeared.

The precipitated hemicellulosic fractions were centrifuged and washed with 70% (v/v) ethanol at room temperature three times. Finally, the washed hemicellulose precipitates were freeze-dried. The hemicellulosic fractions sequentially extracted with 80% ethanol containing 0.025 M HCl, 80% ethanol containing 0.5% NaOH, and alkaline aqueous solutions (containing 2.0, 5.0, and 8.0% NaOH) were labeled as H_1, H_2, H_3, H_4, and H_5, respectively.

All the experiments were performed at least in duplicate. Yields of the hemicellulosic fractions were calculated on dry weight basis related to the dewaxed bamboo samples. The relative standard deviation was observed to be lower than 4.6%.

Characterization of the Hemicellulosic Fractions

The chemical composition of the hemicellulosic fractions were determined by HPAEC, using the same procedure as was employed for the determination of raw material, as aforementioned. The apparent molar mass of the hemicellulosic preparations were determined by 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, 100000, and 1600000 g/mol, 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% (w/w).

NMR spectra were recorded with a Bruker AVIII 400 MHz spectrometer at 25 °C in D_2O according to our former published reports (Sun *et al*. 2011; Wen *et al*. 2011). The proton-detected heteronuclear single quantum (HSQC) spectra were acquired in the HSQCGE experimental mode, over a t_1 spectral width of 10000 Hz and a t_2 width of 1800 Hz. The number of collected complex points was 1024 for the ^{1}H-dimension with a relaxation delay of 1.5 s. The number of scans was 128, and 256 time increments were recorded in the ^{13}C-dimension. The $^1J_{C–H}$ used was 146 Hz. Prior to Fourier transformation, the data matrixes were zero filled up to 1024 points in the ^{13}C-dimension. Data processing was performed using standard Bruker Topspin NMR software.

RESULTS AND DISCUSSION

Fractional Yield of Hemicelluloses

The hemicellulosic preparations considered in this work were mixtures of a number of different polysaccharides, and the yield and composition of the fraction can vary depending on the method of isolation (Morrison 1974). In the present study, mild acidolysis followed by alkaline extractions were used in order to better understand the yield, purity, and chemical structure of the isolated bamboo hemicellulosic preparations. In this procedure, the mild acidic and alkaline treatments are designed to cleave the ether and ester linkages between lignin and hemicelluloses. Table 2 shows that the sequential treatments of defatted bamboo with 80% ethanol containing 0.025 M HCl, 80% ethanol containing 0.5% NaOH, and alkaline aqueous solutions (containing 2.0, 5.0, and 8.0% NaOH) at 75 °C for 4 h resulted in a dissolution of 1.4, 1.0, 3.3, 6.0, and 4.9% of the bamboo polysaccharides (percent of the dry starting matter, w/w), respectively. The total yield of the five hemicellulosic fractions was 16.6% (percent of the dry starting matter, w/w), accounting for 94.3% (w/w) of the original hemicelluloses content in the cell walls of *D. sinicus*. The results indicated that the sequential treatments with acidic ethanol and alkaline aqueous solutions were very effective to separate hemicelluloses polysaccharides from bamboo (*D. sinicus*) materials.

It should be noted that the hemicelluloses fractions H_3, H_4, and H_5, extracted with 2.0%, 5.0%, and 8.0% NaOH solutions, respectively, presented a high yield of hemicelluloses (19.0, 34.1, and 27.9% of the original hemicelluloses content in dewaxed bamboo material, respectively, w/w), suggesting that the treatment of alkaline aqueous solutions at 75 °C could significantly dissolve polysaccharides from plant cell wall. The high solubility of bamboo polysaccharides in alkaline aqueous solution resulted from the alkali function, because hydroxyl ions liberated from alkaline solution could cause swelling of cellulose, disruption of intermolecular hydrogen bonds between cellulose and hemicelluloses, hydrolysis of ester bonds which most likely play an important role in connecting the cell wall polysaccharides and lignin (Bergmans *et al*. 1996). It could be speculated that these differences in extractability of polysaccharides were the results of different structural properties of these polymers in the bamboo cell walls.

Table 2. Yield of Hemicellulosic Fractions (% Dry Matter, w/w) Solubilized during the Successive Treatments of *D. sinicus* with Ethanol and Alkaline Aqueous Solutions

Hemicellulosic Fraction (Solvent)	Yield (% dry matter, w/w)
H_1 (80% ethanol containing 0.025 M HCl)	1.4
H_2 (80% ethanol containing 0.5% NaOH)	1.0
H_3 (2.0% NaOH solution)	3.3
H_4 (5.0% NaOH solution)	6.0
H_5 (8.0% NaOH solution)	4.9
Total solubilized hemicelluloses	16.6

Contents of Neutral Sugars and Uronic Acids

To analyze the difference among these hemicelluloses fractions sequentially isolated from bamboo (*D. sinicus*), the contents of neutral sugars and uronic acids of the five hemicelluloses fractions were detected by HPAEC after hydrolysis with dilute acid, and the data are illustrated in Table 3. The neutral sugar analysis results showed that the major sugar components of the ethanol solubilized hemicelluloses fractions H_1 and H_2

were xylose (34.7-39.0%, w/w), glucose (29.7-32.3%, w/w), and arabinose (16.6-20.2%, w/w). Uronic acid (4.2-4.5%, w/w), mainly glucuronic acid (GlcpA) or 4-*O*-methyl-glucuronic acid (4-*O*-Me-D-GlcpA), was present as a minor amount. These data implied the presence of branched arabinoglucuronoxylans and starch in the ethanol soluble hemicelluloses isolated from bamboo (*D. sinicus*). On the other hand, the data in Table 3 also showed that the absolutely dominant sugar components of the three polysaccharide preparations H_3, H_4, and H_5, isolated from bamboo residue after ethanol treatments, were xylose (85.9-87.9%, w/w), followed by small amounts of arabinose (7.2-8.6%, w/w) and uronic acid (1.3-2.8%, w/w). These analysis results suggested that the alkali-soluble polysaccharide preparations (H_3, H_4, and H_5) probably contained a significant amount of arabinoglucuronoxylans, which was in agreement with those found in other bamboo species (Fengel and Shao 1984; Yoshida *et al*. 1998). Interestingly, it was found that mannose and rhamnose were not detected in any cases, suggesting the bamboo (*D. sinicus*) may not contain these sugars.

Table 3. Contents of Neutral Sugars and Uronic Acids (% Hemicelluloses Sample, w/w) in the Isolated Hemicellulosic Fractions from Bamboo (*D. sinicus*)

	Hemicellulosic Fractions				
	H_1	H_2	H_3	H_4	H_5
Arabinose	20.2	16.6	8.6	8.1	7.2
Galactose	9.0	8.0	2.4	0.5	0.7
Glucose	29.7	32.3	1.8	0.8	2.3
Xylose	34.7	39.0	85.9	87.9	87.6
GlcA	3.8	4.2	1.3	2.3	1.9
GalA	0.7	ND [a]	0.1	0.5	0.4
Uronic acid	4.5	4.2	1.3	2.8	2.3
Xylose/Arabinose	1.7	2.4	10.0	10.8	12.2
Xylose/Uronic acid	7.8	9.2	64.6	31.8	38.1
[a] ND, not detectable					

To confirm the assumption of starch being present in the isolated polysaccharide fractions from bamboo (*D. sinicus*), all five soluble polysaccharide fractions were tested with starch iodine paper. The results demonstrated that amylose starch appeared both in 80% acidic ethanol-soluble and 80% alkaline ethanol-soluble polysaccharide fractions (H_1, H_2). This result was in accordance with the reports by Toledo *et al*. (1987), in which the authors stated that the yield of starch isolated from bamboo culm *(Guadua flabellata)* with alkaline solution was 8.5% (w/w). Coincidentally, our recently published study also reported that water-soluble starch was present in the high-yielding bamboo species *Dendrocalamus brandisii* (Shi *et al*. 2011).

Although arabinoglucuronoxylans from various plants share the same basic chemical structure, they differ in the manner of substitution of the xylan backbone. The main differences were found in the ratio of arabinose to xylose, in the relative proportions and sequence of the various linkages between these two sugars, and in the presence of other substituents (Chaikumpollert *et al*. 2004).

Generally, xylose to arabinose ratios are indicative of the degree of linearity or branching of hemicelluloses (Wedig *et al*. 1987). As the data show in Table 3, higher ratios of xylose/arabinose and xylose/uronic acid were observed in the three alkaline aqueous solution soluble hemicellulosic fractions (H_3, H_4, H_5) than that of the two ethanol soluble hemicellulosic fractions (H_1, H_2).

These results indicated that the hemicellulosic polysaccharides isolated by alkaline aqueous solutions seemed to be more linear, while those of the polysaccharide fractions extracted by 80% ethanol seemed to be more branched. However, on the basis of the sugar composition alone, it is difficult to deduce the exact branching patterns and chemical structure of the hemicelluloses.

Molecular Weight Distribution

In order to investigate the molecular weights of the hemicelluloses extracted with different alkali extractions, weight-average (*Mw*) and number-average (*Mn*) molecular weights, as well as the polydispersity (*Mw/Mn*) of the isolated hemicellulosic preparations were determined by GPC, and the results are listed in Table 4. It was found that the two-step treatments of dewaxed bamboo sample with weakly acidic ethanol and weakly alkaline ethanol resulted in dissolution of hemicellulosic polysaccharides with lower average molecular weights (ranging from 17380 to 19620 g/mol), and the subsequent three-step treatments of bamboo residue with alkaline aqueous solutions containing different concentration of NaOH led to release of hemicellulosic polysaccharides with relatively higher molecular weights (ranging from 22510 to 42150 g/mol). These results suggested that the ethanol solution only released low molecular weight hemicelluloses, while the strong alkaline aqueous solution can dissolve high molecular weight hemicelluloses.

The probable mechanism for these results arises from the effect of alkaline swelling of cellulose, since strong alkaline solutions can swell the cell wall adequately and can cleave the hydrogen bonds between hemicelluloses and cellulose, as well as the ester bonds between hemicelluloses and lignin, and consequently, dissolve out more hemicelluloses with large molecular weight; by contrast, a weakly alkaline solution will have poor ability to swell the bamboo cell wall and thus can only dissolve parts of the hemicelluloses segments, and thereby show a relatively small molecular weight (Wen *et al*. 2011).

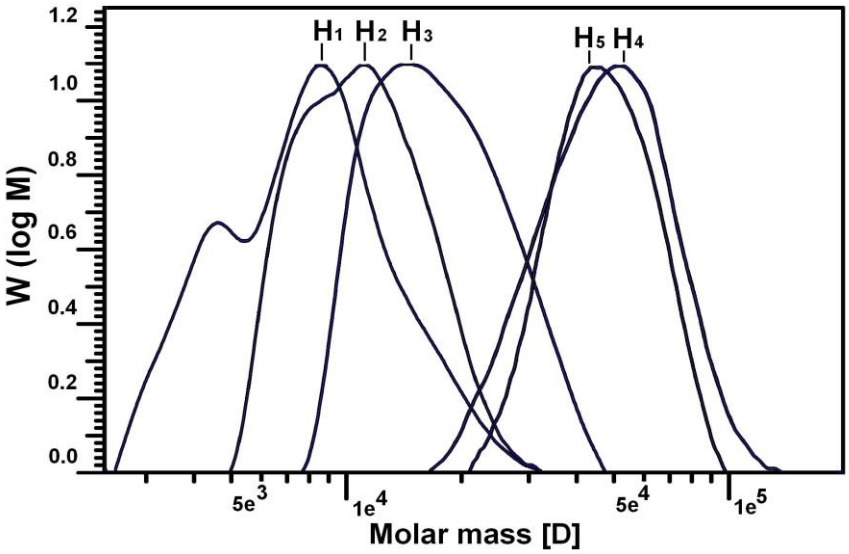

Fig. 2. Molecular weight distributions of hemicellulosic fractions isolated from bamboo (*D. sinicus*)

Table 4. Weight-average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersity (M_w/M_n) of the Hemicellulosic Fractions Isolated from Bamboo (*D. sinicus*)

	Hemicellulosic Fractions				
	H_1	H_2	H_3	H_4	H_5
M_w	17380	19620	22510	42150	41260
M_n	8670	15650	16750	25640	37320
M_w/M_n	2.0	1.3	1.3	1.6	1.1

Polydispersity is an important parameter of macromolecules relative to their applications in the chemical industry. In general, narrow polydispersity means better physicochemical stability. Therefore, it is important to obtain polymers with a relatively narrow polydispersity from plants. The molecular weight distributions of five polysaccharide fractions are shown in Fig. 2, and are in line with the results of the polydispersity calculated by M_w/M_n.

As shown by Fig. 2, the molecular weight distribution curves of H_1 exhibited two peaks in the low molar mass region. This bimodal molecular weight distribution curve probably revealed the presence of two hemicelluloses that are not chemically linked to each other in the mild acidic ethanol extractable fraction.

On the contrary, the alkaline extractable hemicelluloses fractions (H_3, H_4, H_5) displayed unimodal molecular weight distribution curves, implying that the alkaline extractable hemicelluloses have a homogeneous structure, as revealed by sugar analysis results in Table 3.

1D and 2D NMR Spectra

The most powerful tool for polysaccharide analysis is NMR spectroscopy. For the majority of these biopolymers, well-resolved ^{1}H, ^{13}C, and two-dimensional NMR spectra can be acquired from solutions of the intact polymers in DMSO-d_6, in D_2O, and in other deuterated solvents. To further elucidate the structural characteristics of the polysaccharide polymers extracted from bamboo (*D. sinicus*), the polysaccharide preparation H_4 was investigated using 1D and 2D NMR spectroscopy and its ^{1}H, ^{13}C, and 2D HSQC NMR spectra are shown in Fig. 3, 4, and 5, respectively.

Examination of the proton spectrum of 5.0% NaOH-soluble hemicelluloses fraction H_4 (Fig. 3) showed the relative simplicity of the structure. This was exhibited by major signals corresponding to non-substituted D-xylose residues, minor signals originating from L-arabinfuranosyl residues, and weak signals corresponding to 4-*O*-methyl-α-D-glucuronic acid residues.

Strong signals at 4.36, 4.00, 3.72, 3.44, 3.29, 3.20 ppm, correspond to H-1, H-5eq, H-4, H-3, H-5ax, and H-2 of non-substituted β-D-xylose residues, respectively. Minor signals at 5.22 (H-1), 4.16 (H-4), 4.00 (H-2), 3.70 (H-3), 3.66 (H-5) ppm, originate from α-L-arabinfuranosyl residues. Weak signals at 5.22, 4.22, 3.63, 3.57, and 3.13 ppm, correspond to H-1, H-5, H-3, H-2, and H-4 of 4-*O*-methyl-α-D-glucuronic acid residues, respectively (Vignon and Gey 1998).

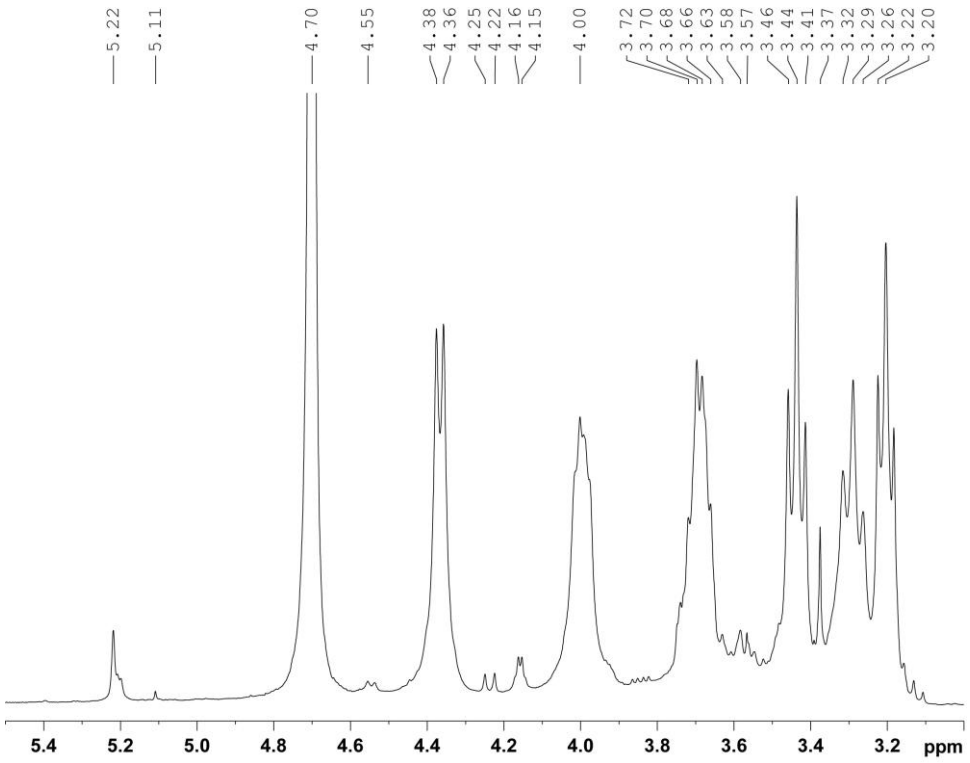

Fig. 3. ^{1}H-NMR spectrum (in D_2O) of the hemicellulosic fraction H_4 isolated from bamboo (*D. sinicus*) with 5.0% NaOH solution

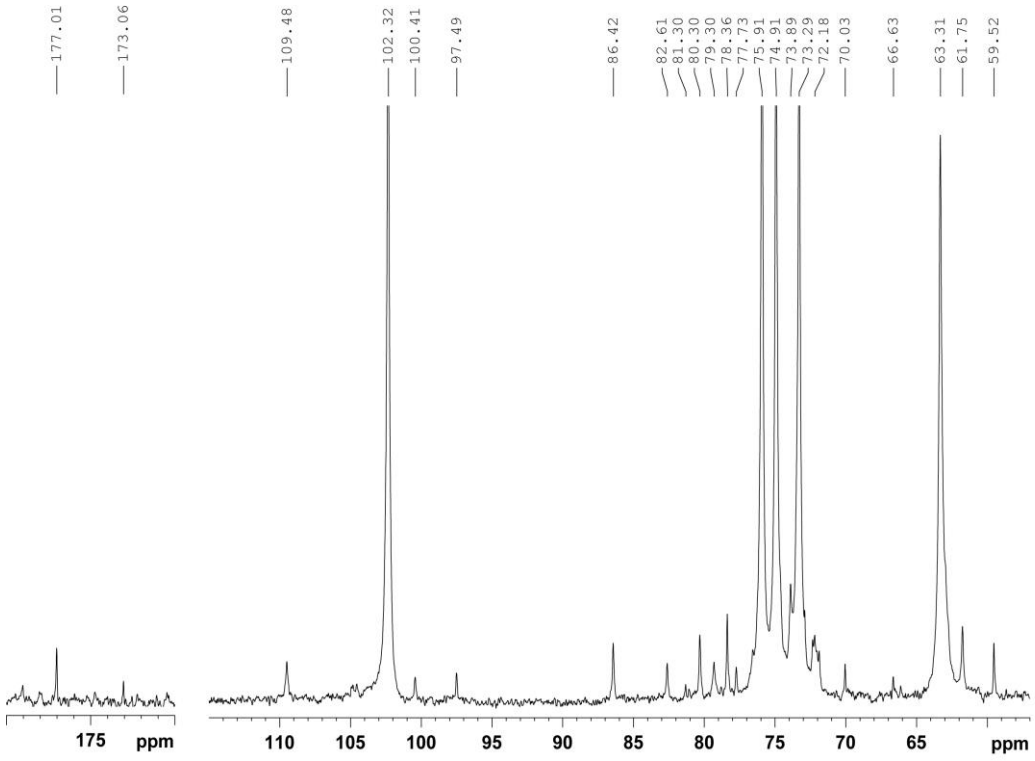

Fig. 4. ^{13}C-NMR spectrum (in D_2O) of the hemicellulosic fraction H_4 isolated from bamboo (*D. sinicus*) with 5.0% NaOH solution

The ^{13}C NMR spectrum (Fig. 4) of H_4 exhibits five major signals corresponding to those of (1→4)-linked-β-D-xylan. The signal at 102.32 ppm is attributed to the anomeric region in a β-configuration, as confirmed by the ^{1}H NMR spectra, and the signals at 75.91, 74.91, 73.29, and 63.31 ppm correspond to C-4, C-3, C-2, and C-5 of (1→4)-linked-β-D-xylopyranosyl units, respectively. Other intense signals at 109.48, 86.42, 80.30, 78.36, and 61.75 ppm are assigned to C-1, C-4, C-2, C-3, and C-5 of α-L-arabinfuranosyl residues, respectively. In addition, the signals at 177.01, 97.49, 82.61, 79.30, 72.18, and 70.03 ppm, arise from C-6, C-1, C-4, C-3, C-5, and C-2 of 4-*O*-methyl-α-D-glucuronic acid, respectively (Chaikumpollert *et al*. 2004). The minor signal at 173.06 ppm is assigned to carbonyl groups in the acetyl groups. This weak signal indicated that the ester bonds in the acetyl ester of hemicelluloses were not cleaved completely under the alkaline condition (5% NaOH at 75 °C for 4 h). (Wen *et al*. 2010) Furthermore, some distinguishable signals in the ^{13}C NMR spectrum probably represent (1→4)-β-D-xylan-3-*O*-(4-*O*-methyl-α-D-glucuronic acid), such as 100.41 ppm (C-1), 77.73 ppm (C-3), 73.89 ppm (C-2) (Wen *et al*. 2011).

In order to gain a more complete understanding of the structure of the isolated polysaccharides, the 2D HSQC technique was used in this study. From the HSQC spectrum of H_4 (Fig. 5), the dominant five cross-peaks could be expressly identified at 102.32/4.36, 75.91/3.72, 74.91/3.44, 73.29/3.20, 63.31/4.00, and 3.29 ppm, which were 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-xylopyranosyl units, respectively (Xu *et al*. 2007). The HSQC spectrum also provided additional evidence for the presence of 4-*O*-methyl-D-glucuronic acid and α-L-arabin-furanosyl residues. In summary, all of the detailed chemical shifts of the 2D HSQC spectrum of polysaccharide fraction H_4 are summarized in Table 5.

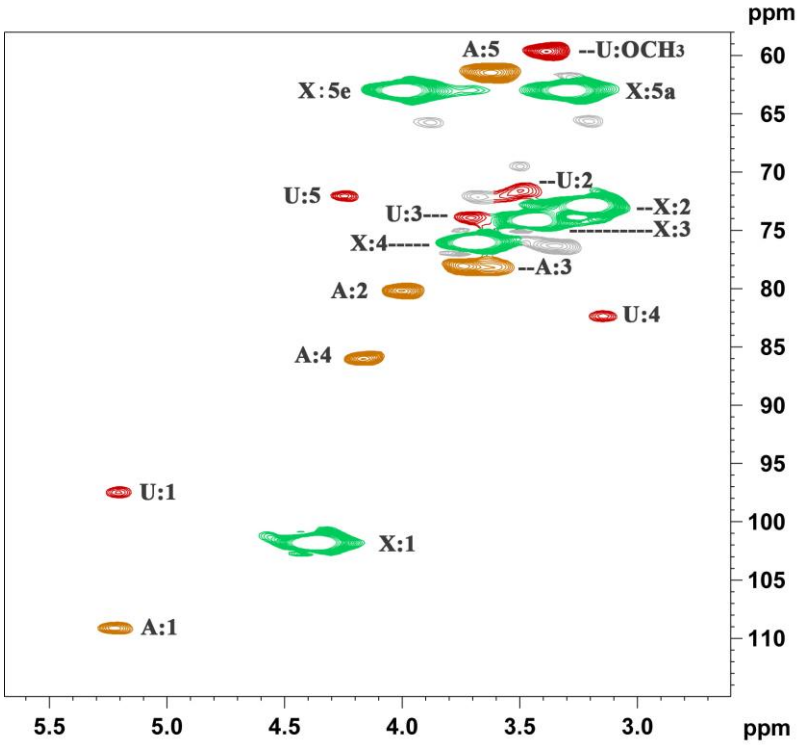

Fig. 5. $^{1}H/^{13}C$ NMR (HSQC) spectrum of the hemicellulosic fraction H_4 isolated from bamboo (*D. sinicus*) with 5.0% NaOH solution

Table 5. Assignment of $^{1}H/^{13}C$ Cross-Signals in the HSQC Spectrum of the Hemicellulosic Fraction H_4 Isolated from *D. sinicus* with 5.0% NaOH Solution

Saccharide	NMR	Chemical Shift (ppm)							
		1	2	3	4	5eq [a]	5ax [b]	6	OCH_3
X [c]	^{13}C	102.32	73.29	74.91	75.91	63.31	63.31	-	-
	^{1}H	4.36	3.20	3.44	3.72	4.00	3.29	-	-
A [d]	^{13}C	109.48	80.30	78.36	86.42	61.75	61.75	-	-
	^{1}H	5.22	4.00	3.70	4.16	3.66	-	-	-
U [e]	^{13}C	97.49	70.03	79.30	82.61	72.18	-	177.01	59.52
	^{1}H	5.22	3.57	3.63	3.13	4.22	-	8.40	3.46

[a] eq, equatorial; [b] ax, axial; [c] X, (1→4)-β-D-Xylp; [d] A, α-Araf residues; [e] U, Uronic acid

Based on the above analysis results and the existing literature pertaining to the structural properties of bamboo hemicelluloses (Wilkie and Woo 1976, 1977), it could be concluded that the polysaccharide fractions isolated from the bamboo (*D. sinicus*) were mainly composed of arabinoglucuronoxylans, together with small amounts of starch. The structure of the isolated arabinoglucuronoxylans from bamboo (*D. sinicus*) was defined as having a linear (1→4)-linked-β-xylopyranosyl backbone to which α-L-arabinofuranose and/or 4-*O*-methyl-D-glucuronic acid units were attached as single-unit side chains via α-(1→3) and/or α-(1→2) linkages, with a ratio of uronic acid/arabinose/xylose of 1:3:32. Therefore, the potential structures of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan in hemicelluloses fraction H_4 isolated from bamboo species *D. sinicus* with 5.0% alkaline aqueous solution could be illustrated as in Fig. 6.

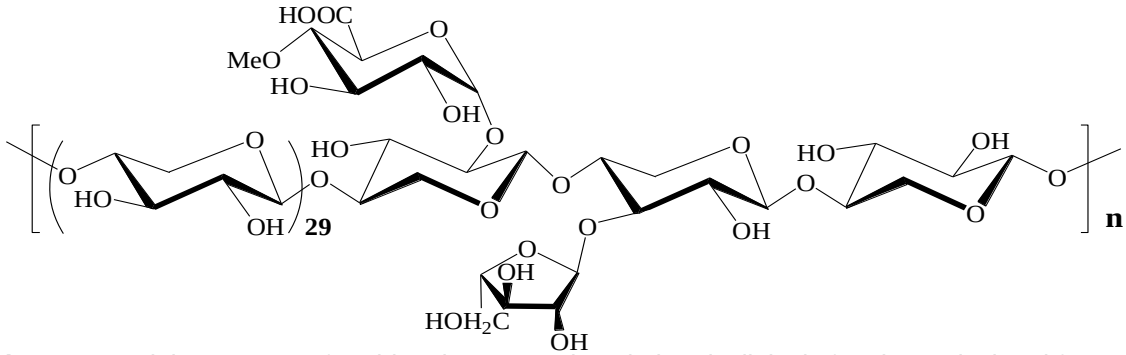

Fig. 6. Potential structures of arabinoglucuronoxylans in hemicellulosic fraction H_4 isolated from bamboo (*D. sinicus*)

CONCLUSIONS

1. The sequential treatments of dewaxed bamboo (*D. sinicus*) samples with 80% ethanol containing 0.025 M HCl, 80% ethanol containing 0.5% NaOH, and alkaline aqueous solutions (containing 2.0, 5.0, and 8.0% NaOH) at 75 °C for 4 h with a solid to liquid ratio of 1:25 (g/mL) yielded 16.6% soluble hemicelluloses, accounting for 94.3% of the original hemicelluloses content in the bamboo cell wall.
2. The dominant components of the soluble hemicelluloses from bamboo (*D. sinicus*) were arabinoglucuronoxylans, together with small amounts of starch.

3. The isolated arabinoglucuronoxylans was defined as having a linear (1→4)-β-linked-xylopyranosyl backbone to which α-L-arabinofuranose and/or 4-*O*-methyl-D-glucuronic acid units are attached as single-unit side chains via α-(1→3) and/or α-(1→2) linkages.

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