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Fractionation of Alkali-Solubilized Hemicelluloses from Delignified *Populus gansuensis*: Structure and Properties

Feng Peng,[†] Jun-Li Ren,[†] Feng Xu,*[,‡] Jing Bian,[‡] Pai Peng,[§] and Run-Cang Sun*[,†,‡]

[†]State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, China, [‡]Institute of Biomass Chemistry and Technology, Beijing Forestry University, Beijing, China, and [§]College of Forestry, Northwest A&F University, Yangling, China

The dewaxed cell walls of *Populus gansuensis* were delignified with $NaClO_2$ and then sequentially extracted with 0.25, 0.5, and 1.0 M KOH under a solid to liquid ratio of 1: 25 (g mL^{-1}) at 25 °C for 10 h. The successive treatments together resulted in the dissolution of 83.7% of original hemicelluloses. The solubilized hemicellulosic fractions were further fractionated into six hemicellulosic subfractions by an iodine-complex precipitation technique. Their chemical and physical characteristics were determined by HPAEC, GPC, FT-IR, and ^1H and ^{13}C NMR spectroscopy. Neutral sugar composition and molecular weight analysis showed that, for each extract, the hemicellulosic subfractions that precipitated with aqueous potassium iodide−iodine had lower overall uronic acid/xylose (Uro/Xyl) ratios and higher molecular weights (M_w) than those remaining in the solution. FT-IR, ^1H, and ^{13}C NMR spectroscopy analysis indicated that the alkali-soluble hemicelluloses of *Populus gansuensis* had a 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.

KEYWORDS: ***Populus gansuensis*; hemicelluloses; fractionation; iodine-complex precipitation**

INTRODUCTION

Lignocellulosic biomass is an important renewable resource that offers the sustainable production of numerous industrial and nonfood consumer products such as fuels, chemicals, and polymeric materials. *Populus* species, a widely distributed hardwood, have been planted in the desert region of China to prevent wind erosion and control desertification (*1*). The Gansu Poplar (*Populus gansuensis* C. Wang and H. L. Yang), which are characterized by rapid growth and development, have been planted as farmland shelter-belts and cover an area of about 18,000 ha (*1*). This hardwood not only has great importance for reforestation of deserts and dry steppes but also provides wood, fuel, fodder, etc. This biomass is, therefore, a renewable feedstock for the production of value-added chemicals from its lignocellulosic constituents, such as hemicelluloses.

Hemicelluloses are generally defined as being polysaccharides that can be extracted by water or aqueous alkali from plant tissue (*2*). They are usually associated with various other cell-wall components such as cellulose, cell-wall proteins, lignin, and other phenolic compounds by covalent and hydrogen bonds, and by ionic and hydrophobic interactions (*3*). Hemicelluloses have a very wide variety of direct food and nonfood applications. Hemicelluloses can be converted to chemicals as feed, such as furfural, erythritol, xylitol, ethanol, or lactic acid (*4*). The reported industrial applications for using plant hemicelluloses also include their use as viscosity modifiers, gelling agents, tablet binders, or wet strength additives (*5*). In addition, xylans also have been studied for their possible medical use as ulcer protective (*6*), antitussive (*7*), immunostimulatory (*8*), and antitumor agents (*9*).

Isolation of hemicelluloses from wood and annual plants has been investigated for many years. Various methods have been developed to extract hemicelluloses from plant cell walls. These methods include extraction with concentrated solutions of sodium or potassium hydroxide (*10*), with alkaline hydrogen peroxide solution (*11*), or with solutions of barium or calcium hydroxides at elevated temperatures (*12*). The main advantages of the alkali extractions are the fact that they are simple to perform and cost-effective (*12*). In general, the hemicellulosic preparations consist of several hemicellulosic molecules which vary in structural characteristics. In view of the fact, several fractionation techniques, such as graded ethanol precipitation (*13*), ammonium sulfate precipitation, and anion-exchange chromatography (*14*, *15*), have been employed in an attempt to obtain more homogeneous fractions and thus explore structure−property relationships for these polymers. Furthermore, fractionation of hemicelluloses is a crucial perquisite to applications, in particular on a larger industrial scale. Subfractionation of hemicelluloses may produce low-branched xylans and further obtain the xylose, an intermediate for the production of xylitol, and a variety of xylo-oligosaccharides. Xylitol has already been used in food applications such as chewing gum or tooth paste (*16*) and could provide an alternative sweetener for diabetics (*17*). Xylo-oligosaccharides are defined as probiotics because they could enhance growth of bifidobacteria (*18*, *19*).

*To whom correspondence should be addressed. Tel: +86-10-62336972; Fax: +86-10-62336972. E-mail: rcsun3@bjfu.edu.cn.

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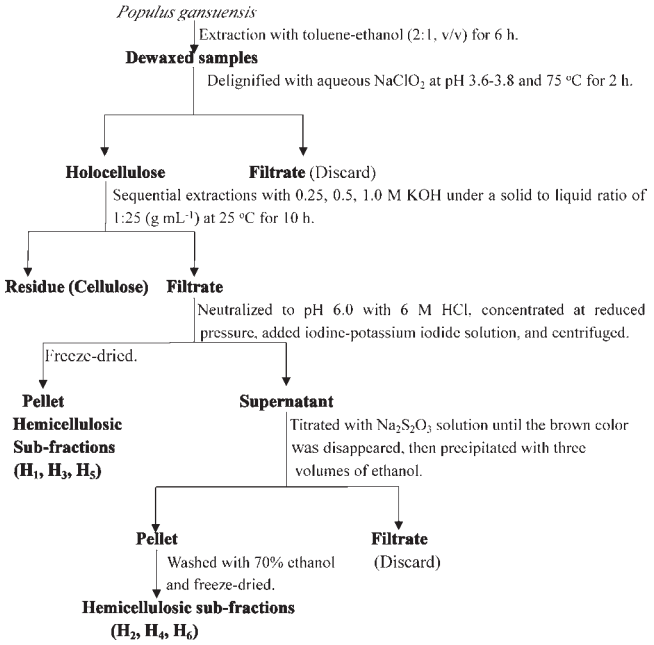

Figure 1. Scheme for fractional isolation of alkali-soluble hemicelluloses from *Populus gansuensis*.

The present paper focused on the extraction and fractionation of hemicelluloses via alkaline solubilization and the iodine-complex precipitation technique in order to obtain different substituted hemicellulosic subfractions for various industrial uses. Therefore, elucidation of the physicochemical properties and structural characteristics of the *Populus gansuensis* hemicellulosic subfractions was our particular interest.

MATERIALS AND METHODS

Materials. *Populus gansuensis* was harvested in July of 2005 in Gansu Province, China. The leaves and bark were removed, and the trunks were chipped into small pieces. The chips were dried in sunlight and then ground to pass a 0.8-mm-size screen. After being further dried at 60 °C for 16 h, the powder was dewaxed with 2:1 (v/v) toluene−ethanol in a Soxhlet apparatus for 6 h. The dewaxed sample was further dried in a cabinet oven with air circulation at 60 °C for 16 h and stored. The components (%, w/w) of the *Populus gansuensis* were 41.8% cellulose, 31.2% hemicelluloses, and 20.9% lignin on a dry weight basis, which was determined by the method for measuring the chemical composition of wheat straw described previously (*20*). All standard chemicals, such as sugars and phenolics, were of analytical grade, purchased from Sigma Chemical Company (Beijing).

Isolation and Fractionation of Hemicelluloses. In order to study structural differences of the hemicelluloses present in *Populus gansuensis*, hemicellulosic fractions were obtained by sequential extractions and fractionations according to the scheme in **Figure 1**. The dewaxed powder (15 g) was delignified with 6% sodium chlorite at pH 3.6−3.8, adjusted with 10% acetic acid, at 75 °C for 2 h (*21*). The residue, holocellulose, was subsequently washed with distilled water and ethanol, and dried at 60 °C for 16 h. Then the holocellulose was successively extracted by 0.25, 0.5, and 1.0 M KOH solution with a solid to liquid ratio of 1:25 (g mL^{-1}) at 25 °C for 10 h under stirring. After the indicated period of treatment, the insoluble residues were collected by filtration, washed with distilled water until the pH of filtrates was neutral, and then dried at 60 °C. Each of the filtrates was adjusted to pH 6.0 with 6.0 M HCl. Then the filtrate was concentrated to about 100 mL, and 10 mL of iodine solution (3 g of iodine and 4 g of KI in 100 mL of water) was added slowly under continuous stirring in the alkali-extractable solution. After stirring and standing for 12 h, the precipitate was recovered by centrifugation (3500 r.p.m, 15 min) and freeze-dried. Then, the supernatant were titrated with $Na_2S_2O_3$ solution, dialyzed, and precipitated with three volumes of ethanol. The precipitated hemicelluloses were washed with 70% ethanol and freeze-dried.

Table 1. Yield of Hemicelluloses (Percent Dry Matter, w/w) Solubilized during the Successive Treatments of Delignified *Populus gansuensis* with 0.25, 0.5, and 1.0 M KOH at 25 °C for 10 h

fractions	yield
total solubilized hemicelluloses during the successive treatments with 0.25, 0.5, and 1.0 M KOH	26.1
solubilized hemicelluloses in 0.25 M KOH treatment	17.7
solubilized hemicelluloses in 0.5 M KOH treatment	5.0
solubilized hemicelluloses in 1.0 M KOH treatment	3.4

Note that hemicellulosic subfractions (H_1, H_3, and H_5) were obtained by precipitation with iodine−potassium iodide solution, and the hemicellulosic subfractions (H_2, H_4, and H_6) remaining in the supernatant were obtained by precipitating in 3 volumes of ethanol from the 0.25, 0.5, and 1.0 M KOH-soluble hemicelluloses, respectively.

Chemical Characterization. The composition of neutral sugars and uronic acids in the isolated hemicellulosic subfractions was determined by high performance anion exchange chromatography (HPAEC). The neutral sugars and uronic acids in the hemicellulosic subfractions were liberated by hydrolysis with 10% H_2SO_4 for 2.5 h at 105 °C. After hydrolysis, the samples were diluted 50-fold, filtered, and injected into the HPAEC system (Dionex ISC 3000, USA) with an 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 0−75 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-arabinose, D-glucose, D-xylose, D-glucose, D-mannose, D-galactose, glucuronic acid, and galacturonic acids. The molecular weights of the hemicellulosic subfractions 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 783, 12 200, 100 000, and 1 600 000; Polymer Laboratories Ltd.). A flow rate of 0.5 mL/min was maintained. The eluents were 0.02 M NaCl in 0.005 M sodium phosphate buffer, at pH 7.5. Detection was achieved with a Knauer differential refractometer. The column oven was kept at 30 °C. Polysaccharides were dissolved in 0.005 M sodium phosphate buffer with 0.02 M NaCl, pH 7.5, at a concentration of 0.1%. The measurements were conducted with two parallels, and the reproducibility of the values was found within the range of 6%.

Spectroscopic Characterization. FT-IR spectra of hemicellulosic samples were obtained on an FT-IR spectrophotometer (Nicolet 510) using a KBr disk containing 1% finely ground samples. Thirty-two scans were taken of each sample recorded from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1} in the transmission mode. The solution-state ^{1}H NMR spectra were recorded on a Bruker NMR spectrometer at 400 Mz using 15 mg of hemicelluloses in 1.0 mL of D_2O. The chemical shifts reported were calibrated relative to the signals from D_2O, used as an internal standard, at 4.7 ppm for the ^{1}H NMR spectra. ^{13}C NMR spectra were obtained on a Bruker spectrometer at 100 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 after 30 000 scans. Chemical shifts (δ) are expressed relative to the resonance of Me_4Si (δ = 0). A 30° pulse flipping angle, a 3.9 μs pulse width, and a 0.85 s delay time between scans were used. The proton-detected heteronuclear single quantum (HSQC) spectra were acquired by HSQCGE experiment mode, over a t_1 spectral width of 10,000 Hz and a t_2 width of 1800 Hz, and the acquired time (AQ) was 0.1163s. The scanning time (NS) was 32. The delay between transients was 2.6 s, and the delay for polarization transfer was set to correspond to an estimated average ^{1}H−^{13}C coupling constant of 150 Hz. Data processing was performed using standard Bruker Topspin-NMR software.

RESULTS AND DISCUSSION

Yield of Hemicelluloses. The hemicellulosic complex is a mixture of a number of different polysaccharides, and the yield

Table 2. Yield of Hemicellulosic Subfractions (Percent Dry Matter, w/w) Obtained from 0.25, 0.5, and 1.0 M KOH-Soluble Hemicelluloses

	0.25 M KOH-soluble hemicelluloses		0.5 M KOH-soluble hemicelluloses		1.0 M KOH-soluble hemicelluloses		
yield/subfractions	H_1[a]	H_2[b]	H_3[a]	H_4[b]	H_5[a]	H_6[b]	total
yield	4.0	10.5	1.4	2.9	1.0	1.8	21.6

[a] Represents the hemicellulosic subfractions obtained by precipitation with iodine−potassium iodide solution. [b] Represents the hemicellulosic subfractions obtained from the remaining solution by precipitation with 3 volumes of ethanol.

Table 3. Neutral Sugars and Uronic Acids (Relative Percent Hemicellulosic Sample, w/w) and Uro/Xyl Ratios of Hemicellulosic Subfractions

subfractions[a]	Rha[b]	Ara[c]	Gal[d]	Glu[e]	Xyl[f]	uronic acid	Uro/Xyl[g]
H_1	1.12	2.43	2.51	0.43	81.73	11.61	0.14
H_2	1.31	1.80	2.61	0.25	74.54	19.48	0.26
H_3	1.23	2.93	2.92	1.18	82.65	9.09	0.11
H_4	0.75	1.35	1.39	0.20	76.57	19.74	0.26
H_5	0.58	0.75	1.37	1.28	87.61	8.41	0.10
H_6	0.56	0.53	1.10	2.71	79.41	15.67	0.21

[a] Corresponding to the hemicellulosic subfractions in **Table 2**. [b] Rha, rhamnose. [c] Ara, arabinose. [d] Xyl, xylose. [e] Gal, galactose. [f] Glc, glucose. [g] Uro/Xyl, uronic acids/xylose.

and composition of the complex can vary depending on the method of isolation. The isolation and purification of hemicelluloses from *Populus gansuensis* is presented schematically in **Figure 1**, and the yields of the soluble hemicelluloses are given in **Table 1**. Alkaline solubilization of hemicelluloses is usually ascribed to the disruption and breaking of hydrogen bonds between the cellulose and hemicelluloses. Evidently, the sequential treatments with 0.25, 0.5, and 1.0 M KOH under the conditions given solubilized 17.7, 5.0 and 3.4% of the hemicelluloses (% dry matter), corresponding to dissolution of 56.7%, 16.0%, and 10.9% of original hemicelluloses, respectively. As can be seen, the maximum yield of hemicelluloses (17.7% of dry material) was observed during the treatment with 0.25 M aqueous KOH. The reason for this maximum yield was due to the first treatment with the alkaline solution. Taken together, the total yield of hemicellulosic preparations was over 26.1% of the initial dry weight and accounts for 83.7% of the original hemicelluloses. This result indicated that the sequential extractions of the delignified *Populus gansuensis* were very effective. In addition, the extractable three hemicelluloses were further fractioned into six hemicellulosic subfractions by the iodine-complexes method. The yields of the six hemicellulosic subfractions are shown in **Table 2**. The yield of the hemicellulosic subfractions precipitated by aqueous potassium iodide−iodine was 4.0, 1.4, and 1.0%, which accounted for 22.6, 28.0, and 29.4% of 0.25, 0.5, and 1.0 M KOH-soluble hemicelluloses, respectively. This indicated that the hemicelluloses of *Populus gansuensis* could be fractionated into two hemicellulosic subfractions by the aqueous potassium iodide−iodine solution. Evidently, the major hemicellulosic subfractions were obtained by precipitation of the supernatant with 3 volumes of ethanol. Taken together, the total yield of six hemicellulosic subfractions was 21.6%, which accounted for 82.7% of total soluble hemicelluloses during the sequential fractionations, indicating that 17.3% hemicelluloses, maybe mainly degraded into small substance such as oligosaccharides, were not recovered.

Sugar Composition. The neutral sugar composition and the content of uronic acid of six hemicellulosic subfractions are listed in **Table 3**. The neutral sugar analysis of the hydrolysates showed that xylose (74.5−87.6%) was an extremely predominant component sugar in six hemicellulosic subfractions. Uronic acid (8.4−19.7%), mainly glucuronic acid (Glc*p*A) or 4-*O*-methyl-glucuronic acid (4-*O*-Me-α-D-Glc*p*A), was present as a substantial amount. Arabinose (0.5−2.9%), galactose (1.1−2.9%), glucose (0.2−2.7%), and rhamnose (0.6−1.3%) were present in smaller amounts. The predominance of xylose and the substantial amount of uronic acids indicated that the alkali-soluble hemicelluloses of *Populus gansuensis* probably consisted mainly of glucuronoxylans.

Table 4. Weight-Average (M_w) and Number-Average (*Mn*) Molecular Weights and Polydispersity (M_w/M_n) of the Hemicellulosic Subfractions

	hemicellulosic subfractions[a]					
	H_1	H_2	H_3	H_4	H_5	H_6
Uro/Xyl	0.14	0.26	0.11	0.26	0.10	0.21
M_w	267570	133280	523010	270880	90420	78660
Mn	57570	62850	78780	74280	73350	48380
M_w/M_n	4.64	2.12	6.63	3.65	1.23	1.62

[a] Corresponding to the hemicellulosic subfractions in **Table 2**.

It should be noted that in the hemicellulosic subfractions H_1 and H_2 obtained from the 0.25 M KOH-soluble hemicelluloses, the xylose decreased from 81.7% (H_1) to 74.5% (H_2), whereas uronic acid increased from 11.6% (H_1) to 19.5% (H_2). Similar results were observed in the other four hemicellulosic subfractions (H_3, H_4, H_5, and H_6) obtained from the 0.5 and 1.0 M KOH-soluble hemicelluloses. In addition, the higher ratio of Uro/Xyl (0.26) in H_2 than that of the value (0.14) in H_1 revealed that H_2 had more branched chains than H_1 since Uro/Xyl ratios were indicative of the degree of linearity or branching of hemicelluloses. From the ratio of uronic acid to xylose (0.10−0.26) in the six hemicellulosic subfractions, it can be concluded that the alkali-soluble hemicelluloses from *Populus gansuensis* consist of relatively low substituted xylans. Taken together, these data indicated that the hemicellulosic subfractions (H_1, H_3, and H_5), obtained by precipitation with aqueous potassium iodide−iodine, seemed to be more linear and have less uronic acid than those of the hemicellulosic subfractions (H_2, H_4, and H_6) remaining in the solution obtained by precipitation with 3 volumes of ethanol.

Molecular Weight Distribution. Table 4 gives the weight-average (M_w) and number-average (M_n) and polydispersity (M_w/M_n) of hemicellulosic subfractions. As shown in the data in **Table 3**, the M_w of hemicelluloses followed the order 0.5 M KOH-soluble hemicelluloses (H_3 and H_4) > 0.25 M KOH-soluble hemicelluloses (H_1 and H_2) > 1.0 M KOH-soluble hemicelluloses (H_5 and H_6). Obviously, the hemicellulosic subfractions (H_3 and H_4), obtained from 0.5 M KOH-soluble hemicelluloses, showed a relatively much higher degree of polymerization with M_w values between 523 010 and 270 880 g mol^{-1} than the other four hemicellulosic subfractions (H_1, H_2, H_5, and H_6) obtained from 0.25 and 1.0 M KOH-soluble hemicelluloses with M_w from 78 660 to 267 570 g mol^{-1}. This suggested that extraction with 0.5 M KOH can result in the dissolution of large hemicellulosic molecules from the delignified materials, whereas the hemicellulosic subfractions (H_5 and H_6) obtained from the 1.0 M KOH-soluble hemicelluloses showed relatively lower M_w of 90 420 and 78 660 g mol^{-1}, respectively, implying that a noticeable degradation occurred under the conditions used (1.0 M KOH). That is, the lower molecular weights of the hemicelluloses in the present case are due to partial deploymerization during the treatments with a

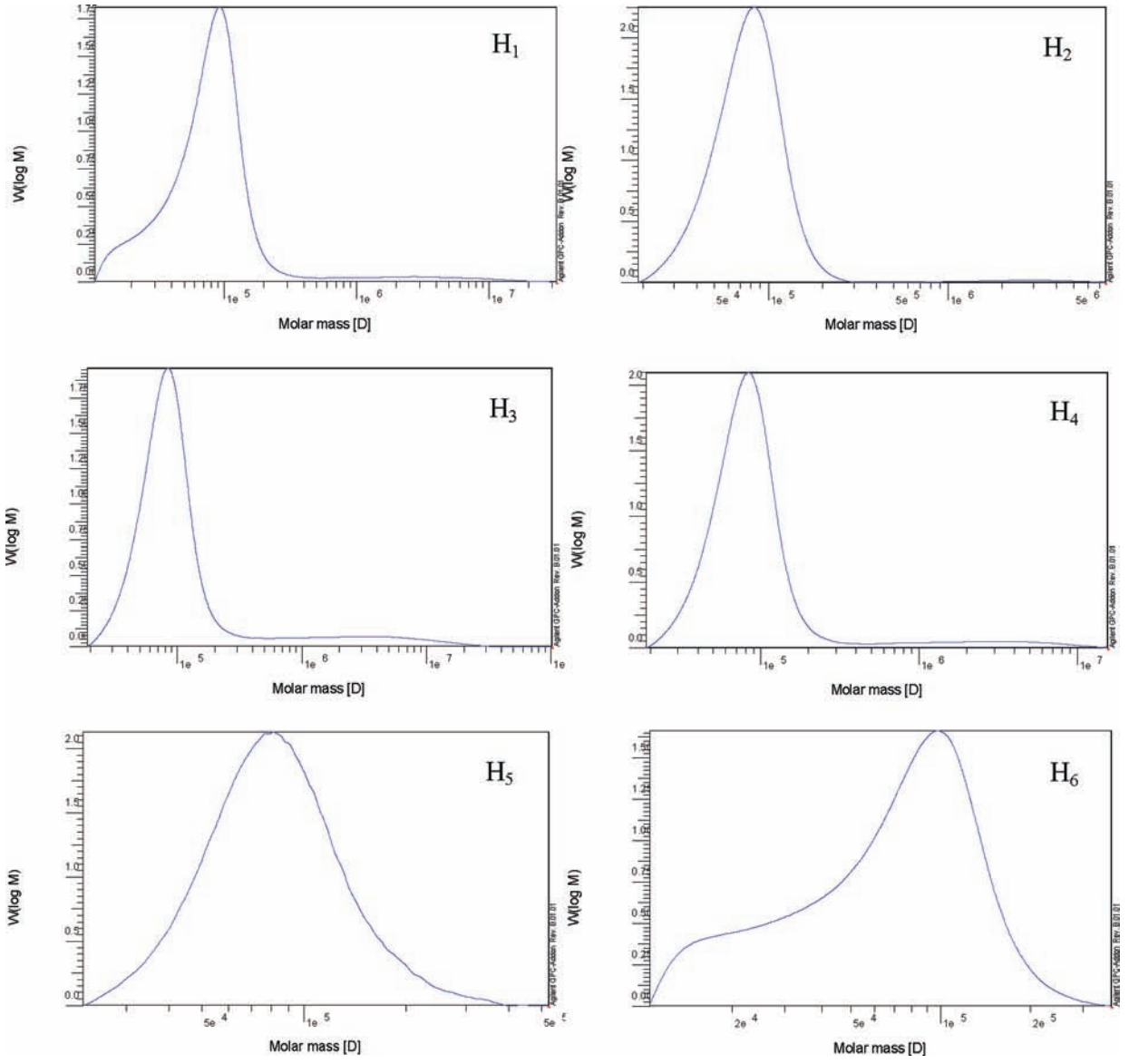

Figure 2. Molecular weight distributions of six hemicellulosic subfractions.

relatively higher concentration of alkali substances. Furthermore, by analysis of the first two hemicellulosic subfractions (H_1 and H_2) obtained from the 0.25 M KOH-soluble hemicelluloses, the H_1 obtained by precipitated with iodine−potassium iodide solution had a higher M_w of 267 570 g mol^{-1} than the H_2 (M_w = 133 280 g mol^{-1}) remaining in the solution and obtained by precipitating with 3 volumes of ethanol. The similar results were also observed in the other four hemicellulosic subfractions (H_3 and H_4, and H_5 and H_6) obtained from the 0.5 and 1.0 M KOH-soluble hemicelluloses. In addition, on the basis of this observation and the Uro/Xyl ratio, it may be concluded that the hemicellulosic subfractions with a higher Uro/Xyl ratio had lower molecular weights. In other words, the more linear hemicelluloses precipitated with iodine−potassium iodide solution had higher molecular weight than the more branched hemicelluloses remaining in the solution. However, Hoffmann et al. (*22*) and Gruppen et al. (*23*) indicated the highly branched xylan fractions to be of higher molecular weight than their less branched counterparts. These inconsistencies might reflect structural differences among hemicelluloses from different botanical origin, but they might also be due to differences in the techniques and solvent conditions employed during the measurement of the molecular weight such as solvent quality and chain aggregation events (*24*).

The molecular weight distributions of six hemicellulosic subfractions are also shown in **Figure 2**. The figure shows that the four hemicelllulosic subfractions (H_1, H_2, H_3, and H_4) obtained from the 0.25 and 0.5 M KOH-soluble hemicelluloses had a somewhat higher proportion of the high molecular weight component compared with that of the 1.0 M KOH-soluble hemicellulosic subfractions. Additionally, the 1.0 M KOH-soluble hemicellulosic subfractions gave more narrow molecular weight distribution, corresponding to the polydispersity index of 1.23 for H_5 and 1.62 for H_6 as compared to those of the 0.25 and 0.5 M KOH-soluble hemicellulosic subfractions having more broad molar weight distribution with the polydispersity indexes between 2.12 and 6.63.

FT-IR Spectra. Infrared spectroscopy has been proven to be useful for studying physicochemical and conformational properties of carbohydrates. The FT-IR spectra of hemicellulosic subfractions H_3 (spectrum a) and H_5 (spectrum c) obtained by precipitation with iodine−potassium iodide solution and H_4 (spectrum b) and H_6 (spectrum d) obtained by precipitation of the corresponding supernatant with 3 volumes of ethanol from

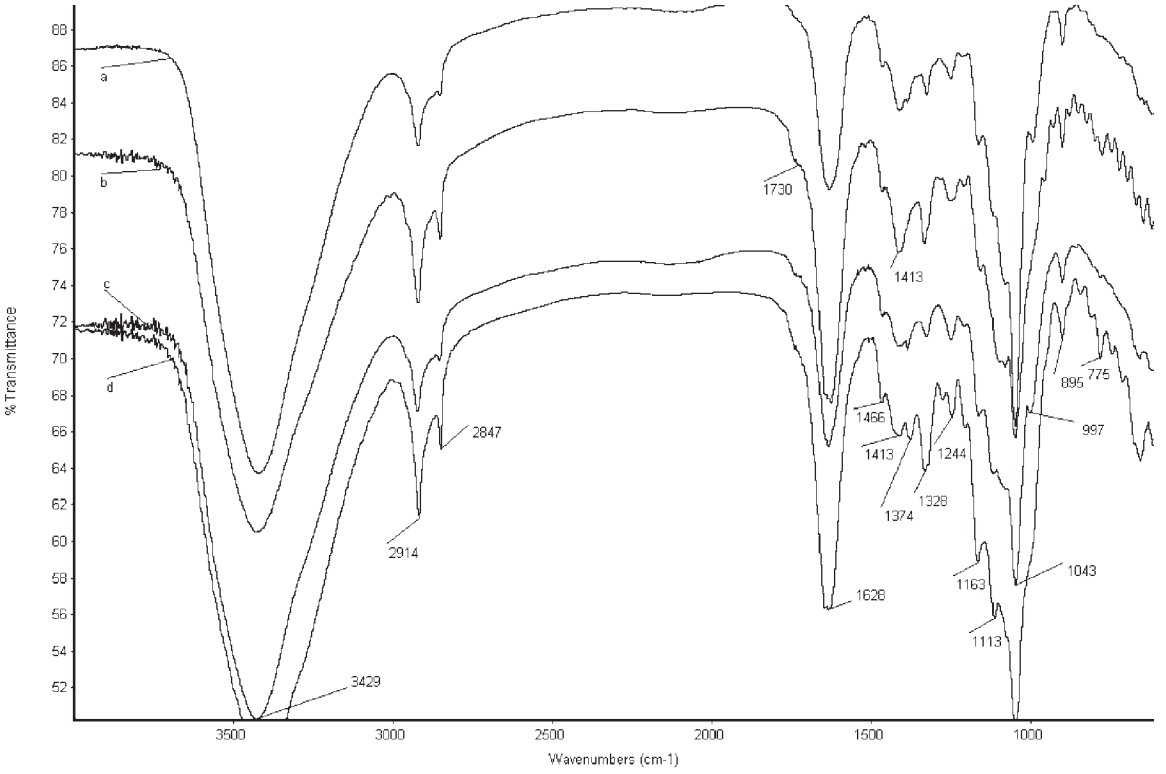

Figure 3. FT-IR spectra of the hemicellulosic subfractions H_3 (spectrum a), H_4 (spectrum b), H_5 (spectrum c), and H_6 (spectrum d).

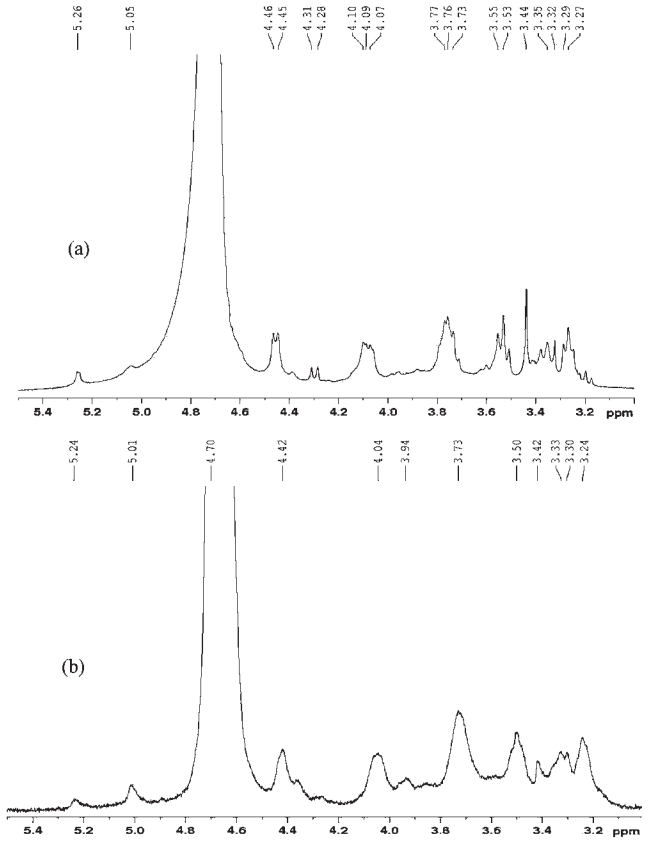

Figure 4. ^{1}H NMR spectra (in D_2O) of hemicellulosic subfractions H_1 (spectrum a) and H_5 (spectrum b).

the 0.5 and 1.0 M KOH-soluble hemicelluloses, respectively, are shown in **Figure 3**. The spectra were assigned according to data presented in the literature (*25*−*27*). The signals observed at 3429 cm^{-1} related to −OH stretching vibrations of the polysaccharide and two bands at 2919 and 2847 cm^{-1} to C−H stretching. A major absorbance at 1042 cm^{-1} was assigned to the C−O−C stretching of glycosidic linkages, which is typical of xylans (*25*). The band at 1163 cm^{-1} was assigned to the C−O−C stretching of glycosidic linkages from arabinoxylans, while the small sharp band at 895 cm^{-1} was characteristic of dominant β-glycosidic linkages between the sugar units in the hemcellulosic subfractions. In the carbonyl stretching region, an intensive signal at 1628 cm^{-1} was due to glucuronic acid or 4-*O*-methyl-glucuronic acid carboxylate (*26*,*27*), whereas the intensity increased from spectrum a to spectrum b and from spectrum c to spectrum d, corresponding to the results of uronic acid composition increasing from 9.09% (H_3) to 19.74% (H_4) and from 8.41% (H_5) to 15.67% (H_6) (**Table 3**), respectively. In addition, the presence of a small shoulder signal at 1730 $cm^{-1}$was due to the C═O stretching of esterified uronic acid, especially in the hemicellulosic subfractions H_4, corresponding to the highest content of uronic acid (19.74%) in **Table 3**. Besides these characteristic bands, another band at 1413 cm^{-1} related to the $-COO^-$ symmetric stretching of uronic acid carboxylate (*27*), and the increasing intensity was also observed from spectrum a to spectrum b and from spectrum c to spectrum d, corresponding to the increasing trend of uronic acids.

NMR Spectra. One-dimensional (^{13}C and ^{1}H) NMR spectra of hemicellulosic subfractions H_1 and H_5 (**Figures 4** and **5**) and the two-dimensional (2D HSQC) NMR spectrum of hemicellulosic subfraction H_1 (**Figure 6**) were collected in order to elucidate the structural features. The signals for ^{13}C and ^{1}H were assigned on the basis of the HSQC spectrum following published NMR data for xylooligosaccharides and glucuronoxylans (*28*−*32*), and the assignment data of proton and carbon spectra are given in **Table 5**. As can be seen from **Figures 4** and **5**, the two hemicellulosic sufractions showed very similar spectra, indicating a similar structure of hemicelluloses. The anomeric ^{1}H NMR signals of H_1 and H_5 are found in the spectral region of 4.4−5.3 ppm (**Figure 4**) (*32*). The signals of α-anomeric protons were seen in

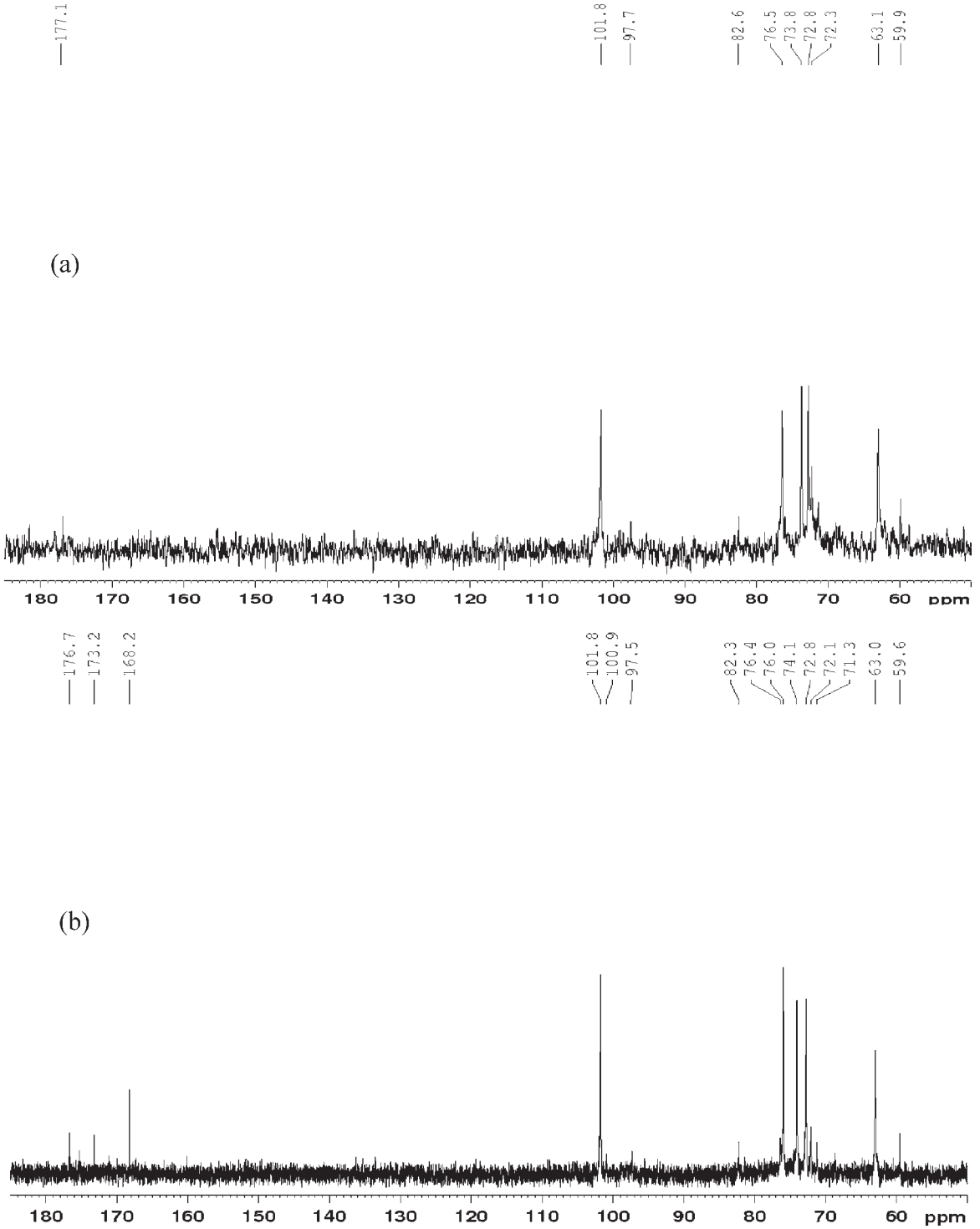

Figure 5. ^{13}C NMR spectra (in D_2O) of hemicellulosic subfractions H_1 (spectrum a) and H_5 (spectrum b).

the spectral region of 5.0−5.3 ppm and β-anomeric protons at 4.4−4.6 ppm. Examination of data relative to ^1H NMR analysis revealed three important groups of protons: the unsubstitued (1→4) linked β-D-xylopyranosyl ((1→4)-β-D-Xyl*p*, X), the substituted β-D-xylopyranosyl ((1→4)-β-D-Xyl*p*-2-*O*-Glc*p*A, XU), and (1→2) linked 4-*O*-methyl-α-D-glucopyranosyl uronic acid (4-*O*-Me-α-D-Glc*p*A, U) residues. From **Figure 6**, the dominant five signals gave HSQC $^{13}C/^1H$ cross-peaks at 101.8/4.46, 72.8/3.27, 73.8/3.53, 76.5/3.76, and 63.1/4.10 + 3.32 ppm (**Table 5**), which were attributed to C-1, C-2, C-3, C-4, and C-5 of the β-D-Xyl*p* units, respectively. In addition, the presence of the methyl group in 4-*O*-Me-α-D-Glc*p*A was confirmed by a corresponding sharp signal at 3.44. The cross-peaks at 97.7/5.26 (C-1), 71.9/3.51 (C-2), 72.3/3.73 (C-3), 82.6/3.21 (C-4), and 72.3/4.31 (C-5) ppm indicate the presence of α-linked 4-*O*-methylglucuronic acid units. A strong signal at 4.70 ppm corresponded to the residual solvent (HDO).

By comparing the 2D HSQC spectrum of the hemicellulosic subfraction (H_1) obtained by precipitation with the iodine−potassium iodide solution, the ^{13}C NMR spectrum of hemicellulosic subfraction H_5 (**Figure 5**b) also showed five main signals corresponding to that of a (1→4)-linked-β-D-xylan (*31*). The signal at 101.8 ppm corresponded to the anomeric region in a β-configuration, as confirmed by the ^1H NMR spectrum, and the signals 76.4, 74.1, 72.8, and 63.0 ppm corresponded to C-4, C-3,

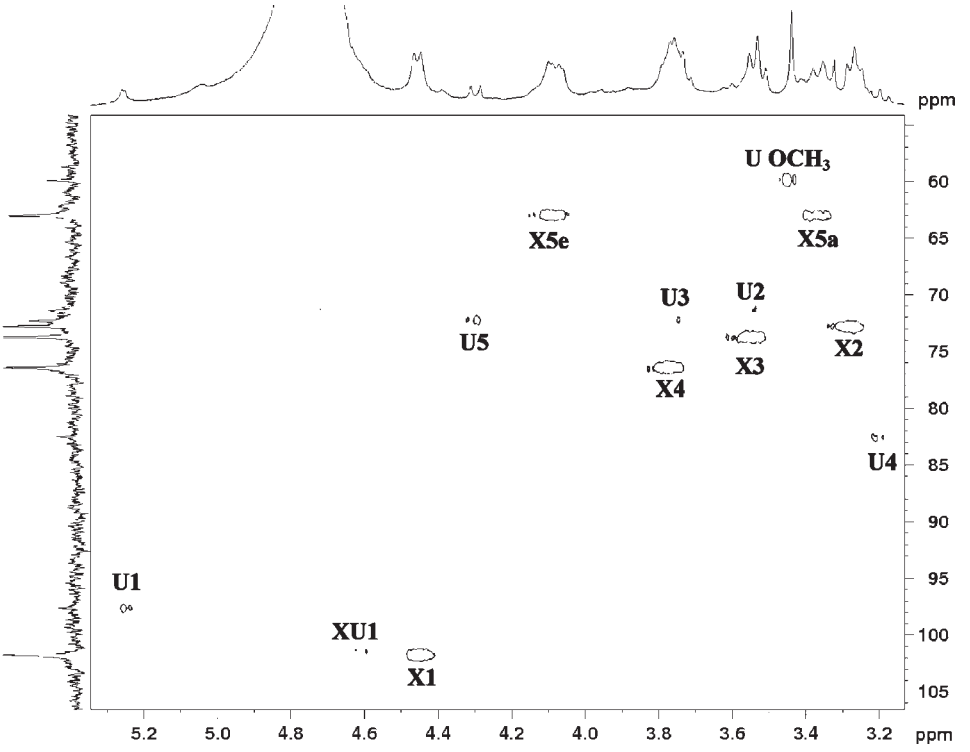

Figure 6. $^{1}H/^{13}C$ NMR (HSQC) spectrum of hemicellulosic subfraction H_1.

Table 5. ^{1}H and ^{13}C Chemical Shift (ppm) Assignments for Hemicellulosic Subfraction H_1.

	chemical shift (ppm) H/C							
sugar residues	1	2	3	4	5ax[d]	5eq[e]	6	OCH_3
X[a]	4.46	3.27	3.53	3.76	3.32	4.10		
	101.8	72.8	73.8	76.5	63.1	63.1		
XU[b]	4.59	3.41	3.55	3.77	3.35	4.07		
	101.5	76.0	na[f]	76.3	63.1	63.1		
U[c]	5.26	3.51	3.73	3.21	4.31			3.44
	97.7	71.9	72.3	82.6	72.3		177.1	59.9

[a] X, (1 → 4)-β-D-Xylp. [b] XU, (1 → 4)-β-D-Xylp-2-O-GlcpA. [c] U, 4-O-Me-α-D-GlcpA. [d] ax, axial. [e] eq, equatorial. [f] na, not assigned.

C-2, and C-5, respectively. Among the other signals observed at 176.7, 97.5, 82.3, and 59.6 ppm, respectively, were characteristic signals of C-6, C-1, C-4, and the methoxyl group of a 4-*O*-Me-Glc*p*A residue. Thus, it can be concluded that the alkali-soluble hemicelluloses from *Populus gansuensis* had a 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.

As a result of the above, the alkali-solubilized hemicelluloses from *Populus gansuensis* could form a blue complex in the aqueous potassium iodide−iodine solution. The hemicellulosic subfractions precipitated by the iodine−potassium iodide solution were more linear with more xylose (81.7−87.6%) and less uronic acids (8.4−11.6%) than the hemicellulosic subfractions remaining in the solution, which were more branched and contained a higher content of uronic acids (15.7−19.7%). In addition, the linear hemicellulosic subfractions with the low Uro/Xyl ratios (0.10−0.14) had higher molecular weights than those of the branched hemicellulosic subfractions remaining in the solution. Therefore, it appeared that the reaction of hemicelluloses with iodine−potassium iodide solution could discriminate between linear and branched polysaccharides and may be a useful method for obtaining low-branched xylans for further production of the xylose, an intermediate for the production of xylitol, and a variety of xylo-oligosaccharides.

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