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Comparative Study of Hemicelluloses Obtained by Graded Ethanol Precipitation from Sugarcane Bagasse

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The sequential treatment of dewaxed sugarcane bagasse with H_2O and 1 and 3% NaOH at a solid to liquid ratio of 1:25 (g mL^{-1}) at 50 °C for 3 h yielded 74.9% of the original hemicelluloses. Each of the hemicellulosic fractions was successively subfractionated by graded precipitation at ethanol concentrations of 15, 30, and 60% (v/v). Chemical composition, physicochemical properties, and structures of eight precipitated hemicellulosic fractions were elucidated by a combination of sugar analysis, nitrobenzene oxidation of bound lignin, molecular determination, Fourier transform infrared (FT-IR), ^1H and ^{13}C nuclear magnetic spectroscopies, and thermal analysis. The results showed that the sequential treatments and graded precipitations were very effective on the fractionation of hemicelluloses from bagasse. Comparison of these hemicelluloses indicated that the smaller sized and more branched hemicelluloses were extracted by the hot water treatment; they are rich in glucose, probably originating from α-glucan and pectic polysaccharides. The larger molecular size and more linear hemicelluloses were dissolved by the alkali treatment; they are rich in xylose, principally resulting from L-arabino-(4-*O*-methylglucurono)-D-xylans. In addition, noticeable differences in the chemical composition and molecular weights were observed among the graded hemicellulosic subfractions from the water-soluble and alkali-soluble hemicelluloses. The Ara/Xyl ratio increased with the increment of ethanol concentration from 15 to 60%, and the arabinoxylans with higher Ara/Xyl ratios had higher molecular weights. There were no significant differences in the structural features of the precipitated hemicellulosic subfractions, which are mainly constituted of L-arabino-(4-*O*-methyl-D-glucurono)xylan, whereas the difference may occur in the distribution of branches along the xylan backbone.

KEYWORDS: Bagasse; hemicelluloses; extraction; graded precipitation; sugars; lignin

INTRODUCTION

Among the various agricultural crop residues, sugarcane bagasse is the most abundant lignocellulosic material in tropical countries. It is a residue produced in large quantities by the sugar and alcohol industries in Brazil, India, Cuba, and China (*1*). In general, 1 ton of sugarcane generates 280 kg of bagasse, and 5.4×10^8 dry tons of sugarcane is processed annually throughout the world (*2*−*4*). About 50% of this residue is used in distillery plants as a source of energy (*5*); the remainder is stockpiled. The stockpiled bagasse is of low economic value and creates an environmental problem for sugar mills and surrounding districts (*6*). Therefore, because of the importance of sugarcane bagasse as an industrial waste, there is great interest in developing chemical methods for the biological production of fuel and chemicals that offer economic, environmental, and strategic advantages (*7*). Sugarcane bagasse has around 40−45% cellulose, 30−35% hemicelluloses, and 20−30% lignin (*8*), which cannot be easily separated into readily utilizable components due to their recalcitrant nature. In our laboratory we are interested in the isolation and chemical modification of hemicelluloses and celluloses from sugarcane bagasse as novel materials for industrial utilizations.

Hemicelluloses are the second most abundant plant renewable materials after celluloses (*9*). They are heteropolysaccharides present in large quantities in wood and annual plants, where they are interconnected together with cellulose and lignin in the cell wall. However, hemicelluloses are closely associated with cellulose by physical intermixing and hydrogen bonds and are linked to lignin by covalent bonds (mainly α-benzyl ether linkage) (*10*). They are branched polymers of low molecular weight with a degree of polymerization of 80−200. Their general formula is $(C_5H_8O_4)_n$, and they are called pentosans and hexosans, respectively (*11*). Hemicelluloses are made of a number of sugar residues. The principal ones are D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, D-glucuronic

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acid, 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid, and, to a lesser extent, L-rhamnose, L-fucose, and various O-methylated neutral sugars. The most abundant hemicellulose in annual plants is arabinoxylan. It contains a backbone of D-xylopyranosyl residues, linked together by β-(1→4)-glycosidic bonds. To these are attached (through position C-2 or C-3 or both) a number of α-L-arabinofuranose and α-D-glucuronic acid (or its 4-*O*-methyl derivative) residues as single-unit side chains (*12*). Naturally occurring xylan contains *O*-acetyl groups located at some of the hydroxyl groups in the xylan backbone.

Alkali treatment of lignocellulosic substances such as cereal straw and bagasse disrupts the cell wall by dissolving hemicelluloses, lignin, and silica, by hydrolyzing uronic and acetic esters, and by swelling cellulose, decreasing the crystallinity of cellulose (*13*). In addition, the treatment also cleaves the α-ether linkages between lignin and hemicelluloses and the ester bonds between lignin and/or hemicelluloses and hydroxycinnamic acids, such as *p*-coumaric and ferulic acids (*14*). More importantly, the alkaline treatment has been proved to be a promising process to achieve complete utilization of lignocelluloses without impact to the environment. By this process, straw and bagasse can be simply fractionated into alkali-soluble lignin and hemicelluloses and residue, which makes it easy to utilize them for more valuable products. The end residue (mainly cellulose) can be used for either paper or cellulose derivatives. The lignin can be converted to valuable products, such as carbon fiber and adhesives (*15*, *16*). From the solubilized hemicelluloses, food additives and other polymers can be produced (*17*). Recently, some important applications for hemicelluloses, such as xylans, have been discovered. The current uses of xylans on an industrial scale involve their conversion to xylose, xylitol, and furfural. Xylitol is produced by hydrolysis of xylan, crystallization of xylose, and hydrogenation. This has been tested in a variety of food products (*18*). In addition, the beneficial effect of some xylans in papermaking was confirmed in the case of ramie hemicelluloses that might be used as a beater additive (*19*). Moreover, xylans from cereals contribute to the effects of dietary fiber upon some biochemical and physical processes in human and animal organisms by lowering of blood cholesterol and decrease of postprandial glucose and insulin responses (*20*−*22*). More recently, growing interest in using hemicelluloses as a raw material for various technological application, for example, the synthesis of cationic polymers (*23*), hydrogels (*24*), long-chain ester derivatives (*24*, *25*), and thermoplastic xylan derivatives (*25*, *26*), has been developed.

The yield from alkali extraction of annual plant biomass has been reported to be around 50% of the available hemicelluloses (*27*). Sun et al. (*28*) were able to extract 91% of the available hemicelluloses from barley straw using eight sequential extraction steps with sodium hydroxide, hydrogen peroxide, and potassium hydroxide. The influence of different extraction conditions on the yield and physicochemical properties of the isolated hemicelluloses has also been investigated (*29*). The purpose of the present work is to study the structural features and physicochemical and thermal properties of hemicellulosic preparations, subfractionated by graded precipitation at the ethanol concentrations of 15, 30, and 60% (v/v) from water-soluble and alkali-soluble hemicelluloses.

MATERIALS AND METHODS

Materials. Sugarcane bagasse was obtained from a local sugar factory (Guangzhou, China). It was first dried in sunlight and then cut into small pieces (1−3 cm). The cut bagasse was ground to pass an 0.8 mm size screen and dried again in a cabinet oven with air circulation for 16 h at 60 °C. Fats, waxes, and oils were removed from the cut bagasse in a Soxhlet apparatus for 6 h with 2:1 (v/v) toluene/ethanol. The composition (%, w/w) of the bagasse was cellulose, 43.6%; hemicelluloses, 33.5%; lignin, 18.1%; ash, 2.3%; and wax, 0.8%, on a dry weight basis (*30*). All standard chemicals, such as sugars and phenolics, were of analytical grade, purchased from Sigma Chemical Co. (Beijing).

Fractionation of Hemicelluloses. To study structural differences in the hemicelluloses present in bagasse, hemicellulosic fractions were isolated by sequential extraction according to the scheme in **Figure 1**. The dewaxed sugarcane bagasse (10 g) was soaked in 250 mL of distilled water at 50 °C for 3 h under stirring. After filtration, the filtrates were concentrated to about 40 mL at the reduced pressure. Ninety-five percent ethanol was then added to the concentrated filtrates slowly at room temperature under constant stirring, until an ethanol concentration of 15% (v/v) was reached. The precipitated hemicelluloses were recovered by filtration, washed with 70% ethanol, and freeze-dried. The filtrates were further concentrated to about 30 mL at the reduced pressure, and the hemicelluloses were precipitated in the 30% ethanol according to the same method above. Then the filtrates were concentrated to 20 mL, and the residual hemicelluloses were precipitated in the 60% ethanol solution. The solid residue (Rsc) of water treatment was successively treated with 1 and 3% NaOH aqueous solutions at a solid to liquid ratio of 1:25 ($g\,mL^{-1}$) at 50 °C for 3 h. After the indicated period of treatment, the insoluble residue was collected by filtration, washed with distilled water until the pH of the filtrate was neutral, and then dried at 60 °C. The pH of each of the filtrates was adjusted to 5.5 with 6 M HCl, and then the filtrates were concentrated to about 50 mL under reduced pressure and sequentially precipitated in 15, 30, and 60% ethanol soulution according to the same method mentioned above, respectively. The six hemicellulosic subfractions were thoroughly washed with 70% ethanol and then freeze-dried. All experiments were performed at least in duplicate. Note that the three hemicellulosic subfractions precipitated with 15, 30, and 60% ethanol solution from the hemicellulosic fraction solubilized in the water treatment were labeled as water-soluble hemicelluloses H_1, H_2, and H_3; the precipitated hemicellulosic subfractions obtained from the hemicellulosic fraction solubilized in the 1% NaOH treatment were designated 1% NaOH-soluble hemicelluloses H_4, H_5, and H_6; and the hemicellulosic subfractions from the hemicelluloses released in the 3% NaOH treatment were considered to be 3% NaOH-soluble hemicelluloses H_7, H_8, and H_9, respectively.

Chemical Characterization. The constituent neutral sugar in the isolated hemicellulosic subfractions was determined by high-performance anion exchange chromatography (HPAEC). The neutral sugars in the hemicellulosic fractions were liberated by hydrolysis with 6% H_2SO_4 for 2.5 h at 105 °C. After hydrolysis, the sample was diluted 30-fold, filtered, and injected into the HPAEC system (Dionex ISC 3000) with an amperometric detector, an AS50 autosampler, and a Carbopac PA1 column (4 × 250 mm, Dionex). 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 0.018 M NaOH to re-equilibrate the column. The uronic acid was eluted with 0.4 M NaOH for 20 min at a rate of 1 mL/min with postcolumn addition of 0.3 M NaOH at a rate of 0.5 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 of 783, 12 200, 100 000, 1 600 000, Polymer Laboratories Ltd.). A flow rate of 0.5 mL/min was maintained. The eluent was 0.02 N NaCl in 0.005 M sodium phosphate buffer, pH 7.5. Detection was achieved with a Knauer differential refractometer. The column oven was kept at 30 °C. Polysaccharides were dissolved with 0.02 N NaCl in 0.005 M sodium phosphate buffer, pH 7.5, at a concentration of 0.1%. The chemical composition of phenolic acids and aldehydes liberated from alkaline nitrobenzene oxidation of the lignin associated with the hemicellulosic subfractions was determined by high-performance liquid chromatography (HPLC, Agilent). Identification of the individual compounds was made at 280 nm by computer comparison of the retention times and peak areas with the authentic phenolics. Klason lignin

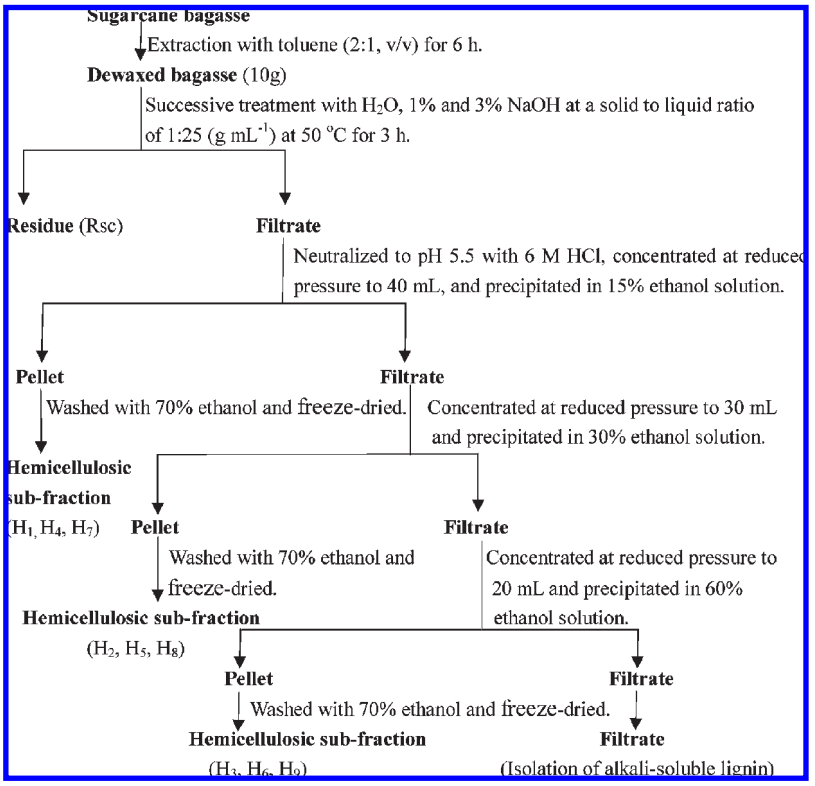

Figure 1. Scheme for fractional isolation of hemicelluloses from dewaxed bagasse.

content in the hemicellulosic samples was determined according to Tappi method T 249 cm-85. The measurements were conducted with two parallels, and the reproducibility of the values was kept within the range of 6%.

Spectroscopic and Thermal 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 spectrum was recorded on a Bruker MSL300 spectrometer at 300 MHz using 15 mg of hemicelluloses in 1.0 mL of D_2O. A ^{13}C NMR spectrum was obtained on a Bruker MSL300 spectrometer at 74.5 MHz. The sample (80 mg) was dissolved in 1 mL of D_2O (99.8% D) with overnight stirring at room temperature. The spectrum was recorded at 25 °C after 30 000 scans. Chemical shifts (δ) are expressed relative to the resonance of Me_4Si (δ 0). A 60° pulse flipping angle, a 3.9 μs pulse width, and an 0.85 s delay time between scans were used. Thermal behavior of the hemicelluloses was performed using thermogravimetric analysis (TGA) and differential thermal analysis (DTA) on a simultaneous thermal analyzer (DTG-60, Shimadzu). The apparatus was continually flushed with a nitrogen flow of 30 mL/min. The sample weighed between 9 and 11 mg and was heated from room temperature to 550 °C at a rate of 10 °C/min.

RESULTS AND DISCUSSION

Yield of Hemicelluloses. The hemicellulosic polymer is a mixture of a number of different polysaccharides, and the yield and composition of the polymer can vary depending on the method of isolation (*31*). However, the liberation of the hemicellulosic component from the plant cell walls is restricted by the lignin−hemicellulose linkages. In addition, extensive hydrogen bonding between the individual polysaccharide and other components may impede isolation of hemicelluloses (*18*). High concentrations of hydroxide result in higher yields of extraction when performed at room temperature, indicating a disruption of stronger linkages, such as ferulic acid bridges between hemicelluloses and lignin (*32*). In the present study, the dewaxed bagasse was sequentially extracted at 50 °C for 3 h by distilled water and 1 and 3% NaOH aqueous solutions. The yields of the soluble hemicelluloses and lignin are shown in **Table 1**. As can be seen, sequential extractions of the bagasse with water amd 1 and 3% NaOH aqueous solutions at 50 °C for 3 h yielded 4.8, 10.9, and 9.4% hemicelluloses, corresponding to release of 14.3, 32.5, and 28.1% of the original hemicelluloses and 2.2, 31.5, and 20.4% of the original lignin removal, respectively. Taken together, the three-stage treatments yielded 25.1% polysaccharides solubilized from bagasse and accounted for 74.9% of the original hemicelluloses. These results indicated that 1 and 3% NaOH aqueous solutions under the conditions used significantly cleaved the α-ether bonds between lignin and hemicelluloses from the cell wall of bagasse, resulting in a substantial dissolution of hemicellulosic polysaccharides and lignin macromolecules. To date, alkaline extraction is one of the most important tools in the structural characterization of the cell wall polymers from the lignocellulosic materials.

Table 1. Yield of Hemicelluloses Solubilized during Successive Treatments of Dewaxed Bagasse with Distilled Water and 1 and 3% NaOH at 55 °C for 3 h

fraction	yield (% dry matter)
total solubilized hemicelluloses during successive treatments with distilled water and 1 and 3% NaOH	25.1
solubilized hemicelluloses in H_2O treatment	4.8
solubilized lignin in H_2O treatment	0.4
solubilized hemicelluloses in 1% NaOH treatment	10.9
solubilized lignin in 1% NaOH treatment	5.7
solubilized hemicelluloses in 3% NaOH treatment	9.4
solubilized lignin in 3% NaOH treatment	3.7
residue (crude cellulose)	65.1

Additionally, the three hemicellulosic fractions were subfractionated by graded precipitations at ethanol concentrations of 15, 30, and 60% (v/v). The yields of precipitated hemicellulosic subfractions are shown in **Table 2**. The results show that 1 and 2.9% hemicellulosic subfractions were precipitated in 15 and

Table 2. Yield of Precipitated Hemicelluloses in 15, 30, and 60% Ethanol Solutions from Water-Soluble, 1% NaOH-Soluble, and 3% NaOH-Soluble Hemicelluloses

yield (% dry matter)									
water-soluble hemicelluloses			1% NaOH-soluble hemicelluloses			3% NaOH-soluble hemicelluloses			
H_1[a]	H_2[b]	H_3[c]	H_4[a]	H_5[b]	H_6[c]	H_7[a]	H_8[b]	H_9[c]	total
1.0	tr[d]	2.9	3.5	1.6	4.6	2.5	1.2	4.2	21.5

[a] Represents the hemicelluloses obtained by precipitation in 15% ethanol. [b] Represents the hemicelluloses obtained by precipitation in 30% ethanol. [c] Represents the hemicelluloses obtained by precipitation in 60% ethanol. [d] tr, trace.

60% ethanol solution from the water-soluble hemicelluloses, but only a trace amount of hemicellulosic subfraction was obtained in 30% ethanol solution, and this fraction, H_2, was not used in the subsequent studies. Obviously, the major precipitation of the hemicellulosic subfractions was obtained at the ethanol concentration of 60%. At this concentration, 2.9, 4.8, and 4.2% hemicelluloses (percent dry bagasse) were precipitated, corresponding to precipitation of 60.4, 42.2, and 44.7% of the water-soluble, 1% NaOH-soluble, and 3% NaOH-soluble hemicelluloses, respectively. A similar phenomenon was also observed by Viëtor (*33*) in the study of arabinxylans from barley and malt cell wall material. Clearly, total yield of nine hemicellulosic subfractions accounted for 85.7% of the total solubilized hemicelluloses during the successive treatments with distilled water and 1% and 3% NaOH, indicating that 14.3% of hemicelluloses were not precipitated in 15, 30, and 60% ethanol solutions.

Sugar Composition. The hemicelluloses are a mixture of a number of different polysaccharides, in which the sugar composition can vary depending on the method of isolation. The neutral sugar composition and content of uronic acids of eight precipitated hemicellulosic subfractions and crude cellulose are given in **Table 3**. Obviously, glucose, xylose, galactose, and mannose are the major sugar components of the subfractions of H_1 and H_3 obtained from water-soluble hemicelluloses compared to the predominance of xylose (79.2−96.7%) in the six precipitated hemicellulosic subfractions obtained from the 1 and 3% NaOH-soluble hemicelluloses, respectively. However, the proportions of rhamnose (1.8 in H_1, 4.6 in H_3) and uronic acid (5.1 in H_1, 4.4 in H_3) of the precipitated hemicellulosic subfractions obtained from water-soluble hemicelluloses were higher than those of the six precipitated hemicellulosic subfractions obtained from the alkaline-soluble hemicelluloses. The hemicellulosic subfractions H_1 and H_3 resembled the so-called "hair regions" of pectic polysaccharides (*34*). These data suggested that the more branched hemicelluloses were easily extracted by the hot water treatment; they are rich in glucose, probably originating from α-glucan and pectic polysaccharides; the water-soluble hemicelluloses were more branched in structure than the six alkaline-soluble hemicelluloses (*35*). The presence of pectic polysaccharides in the water-soluble fraction from agriculture residues has been widely demonstrated in our previous studies on wheat straw and some shrubs (*34*, *36*).

It is known that alkali has been proved to be efficient in the extraction of most available hemicelluloses from the secondary cell wall. In this study the extraction process was carried out with 1 and 3% NaOH aqueous solutions, and the six hemicellulosic subfractions was obtained by 15, 30, and 60% ethanol precipitation, respectively. As shown in **Table 3**, xylose was the predominant sugar composition (79.2−96.7%), suggesting the presence of a high proportion of xylan. Arabinose was the second major sugar component, ranging from 2.8 to 12.1% of the total

Table 3. Contents of Neutral Sugars (Relative Percent Dry Hemicelluloses, w/w) and Uronic Acid (Percent Dry Hemicelluloses, w/w) in the Hemicellulosic Subfractions (Percent Dry Matter) Precipitated in 15, 30, and 60% Ethanol Solution and Residue

	hemicelluloses and residual fractions[a]								
sugar	H_1	H_3	H_4	H_5	H_6	H_7	H_8	H_9	Rsc[b]
rhamnose	1.83	4.56	0.15	0.11	0.13	tr[c]	nd[d]	tr	tr
arabinose	3.85	5.42	5.60	6.49	12.13	2.75	5.61	7.85	2.07
galactose	19.23	18.59	0.16	1.33	2.77	0.25	nd	1.10	0.33
glucose	38.27	46.68	0.49	1.11	5.20	0.26	1.35	3.33	64.98
mannose	9.17	17.95	0.35	0.25	0.53	0.13	tr	nd	tr
xylose	27.65	6.80	93.25	91.05	79.24	96.70	92.98	87.72	32.56
uronic acid	5.06	4.38	2.33	2.14	1.98	2.20	2.45	1.75	nd

[a] Corresponding to the hemicellulosic subfractions in **Table 2**. [b] Rsc, residue (crude cellulose). [c] tr, trace. [d] nd, not detected.

sugars. Small amounts of glucose (0.3−5.2%) and uronic acid (1.8−2.5%), mainly glucuronic acid or 4-*O*-methyl-D-glucuronic acid (MeGlcA), and minor quantities of galactose (0−2.8%), rhamnose (0−0.2%), and mannose (0−0.5%) were also identified in these subfractions. These data indicated that the six precipitated subfractions of the alkaline-soluble hemicelluloses consist mainly of glucuronoarabinoxylans or L-arabino-(4-*O*-methylglucurono)-D-xylans. Interestingly, the content of xylose (79.2−93.2%) in the hemicellulosic subfractions H_4, H_5, and H_6 obtained from 1% NaOH-soluble hemicelluloses decreased as the concentration of ethanol increased from 15 to 60%, whereas that of arabinose increased from 5.6 to 12.1%. Although arabinoxyloses from various cereal straws share the same basic chemical structure, they differ in the manner of the xylan backbone. The main differences were found in the ratio of arabinose to xylose (Ara/Xyl), in the relative proportions and sequence of various linkages between these two sugars, and in the presence of other substituents (*37*). The ratio of Ara/Xyl of three hemicellulosic subfractions obtained from 1% NaOH-soluble hemicelluloses appeared to increase from 0.06 (H_4) to 0.15 (H_6) as the concentration of ethanol increased from 15 to 60%. Similar results were observed in the three precipitated hemicellulosic subfractions H_7, H_8, and H_9 obtained from 3% NaOH-soluble hemicelluloses. This indicated that the Ara/Xyl ratio increased for the precipitated subfractions obtained with the increment of ethanol concentration, and the result is consistent with the results of other studies on wheat endosperm arabinoxylans (*38*), water-extractable wheat flour arabinose (*39*), and alkali-extracted arabinoxylans of wheat flour (*40*). However, the ratio of Ara/Xyl (0.03−0.09) of the three subfractions obtained from 3% NaOH-soluble hemicelluloses is lower than the ratio (0.06−0.15) of the three subfractions obtained from 1% NaOH-soluble hemicelluloses, indicating that the lower degree of branching of xylans is more easily soluble in alkaline solution and binds less tightly to cellulose than the higher degree of the branching polymers.

Analysis of the residue fractions showed that all of the hemicelluloses in sugarcane bagasse were not completely soluble by the three sequential treatments. The residue still contained a noticeable amount of hemicelluloses as shown by xylose (32.6%) and a minor quantity of arabinose (2.1%). This verified again that the hemicelluloses are strongly bound to the cell wall component, cellulose (*41*).

Content of Bound Lignin. The presence of lignin−hemicellulose linkages was studied in detail for straw, grass, and wood samples (*42*, *43*). It is commonly assumed that lignin is tightly linked to polysaccharides in the cell walls of plants by various linkage types, such as ether linkage of the hydroxyl group at the α-position of the lignin side chain with the alcoholic hydroxyl

Table 4. Yield (Percent Hemicellulosic Sample, w/w) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of the Associated Lignin in the Precipitated Hemicellulosic Subfractions

	hemicellulosic and residual fractions[a]								
phenolic acid or aldehyde	H_1	H_3	H_4	H_5	H_6	H_7	H_8	H_9	Rsc[b]
p-hydroxybenzoic acid	0.31	0.20	0.089	0.071	0.034	0.042	0.019	0.035	0.077
p-hydroxybenzaldehyde	0.65	0.25	0.48	0.33	0.14	0.035	0.023	0.032	0.16
vanillic acid	0.065	0.023	0.037	0.029	0.027	0.029	0.032	0.035	0.076
syringic acid	0.054	0.076	0.021	0.003	0.003	0.004	0.005	0.009	0.023
vanillin	1.26	0.72	1.08	0.88	0.58	0.63	0.55	0.48	0.55
syringaldehyde	1.58	0.97	1.27	0.94	0.65	0.69	0.57	0.52	0.79
p-coumaric acid	0.32	0.15	0.084	0.056	0.017	0.035	0.039	0.061	0.075
acetovanillone	0.20	0.13	0.043	0.033	0.011	0.012	0.010	0.006	0.021
acetosyringone	0.32	0.14	0.092	0.081	0.052	0.057	0.041	0.024	0.020
ferulic acid	0.083	0.011	0.054	0.021	0.007	0.041	0.025	0.011	0.010
total	4.84	2.67	3.25	2.44	1.52	1.61	1.31	1.21	1.80
content of Klason lignin	7.76	5.94	6.12	4.55	2.88	3.09	2.35	2.24	3.55

[a] Corresponding to the hemicellulosic subfractions in **Table 2**. [b] Rsc, residue (crude cellulose).

of sugar residue (*10*) or ester linkage of the cinnamic acid unit in lignin with the alcoholic OH of polysaccharides (*44*). Our previous studies found that the majority of lignins in cereal straw cell walls are directly linked to arabinose side chains of xylan by ether bonds (*45*). Another potential lignin−hemicellulosic linkage is an ester bond between the lignin and carboxyl (C-6) group of uronic acid residues (*46*). More importantly, it was reported that ferulic acid ether linkage to lignin formed a cross-link to hemicelluloses through an ester linkage (hemicelluloses−ester−ferulic acid−ether−lignin bridges) (*47*, *48*). To further verify the presence of the bound lignin, alkaline nitrobenzene oxidation of the residual lignin in the precipitated hemicellulosic subfractions was performed at 170 °C for 3 h. The phenolic aids and aldehydes were analyzed by HPLC, and the results are listed in **Table 4**. Interestingly, compared to the lignin content in the water-soluble hemicelluloses (5.9−7.8%), the six precipitated hemicellulosic subfractions from the alkaline-soluble hemicelluloses had a much lower content of associated lignin (2.2−6.1%). This is particularly true for the hemicelluloses obtained at a relatively higher concentration of 3% NaOH aqueous solution, indicating that the alkaline treatment can significantly break the α-ether bonds between lignin and hemicelluloses from the bagasse. In addition, an increase as the concentration of ethanol increased from 15 to 60% led to a decrease in lignin content from 6.1 to 2.9%. Similar results were observed in the five precipitated hemicellulosic subfractions obtained from water-soluble and 3% NaOH-soluble hemicelluloses, indicating that a relatively higher content of lignin in the hemicellulosic subfractions can be obtained by precipitation with a low concentration of ethanol. On the other hand, the measurable amount of residual lignin in the three-stage treated residue (Rsc) also implied that the polysaccharides in the cell walls of bagasse are tightly associated with lignin.

As can be seen in **Table 4**, the major products were identified as vanillin and syringaldehyde, which ranged between 26.0 and 41.9% and between 32.6 and 43.9% of the total phenolic monomer, respectively. This indicated that the bound lignin in hemicelluloses contained roughly equal amounts of noncondensed guaiacyl and syringyl units, which was in good agreement with the results obtained from the solubilized lignin preparations obtained by alkaline treatment of sugarcane bagasse (*32*). Additionally, a noticeable amount of *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid, and acetosyringone and traces of vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, and acetovanillone were also found to be present in the nitrobenzene oxidation mixtures. The association of *p*-coumaric acid and ferulic acid in the cells of grass (*49*), wheat straw, and oil palm fibers has been studied in detail (*50*−*52*). It has been suggested that *p*-coumaric acid was mostly esterified to lignin or hemicelluloses, whereas ferulic acid appeared almost equally in esterified bonds to arabinose in hemicelluloses and in etherified linkages with lignin (*53*). As can be seen from **Table 4**, the occurrence of traces of *p*-coumaric acid and ferulic acid in the hemicellulosic subfractions indicated that these two phenolic acids are strongly associated with hemicelluloses or lignin in the cell walls of sugarcane bagasse. This observation also indicated that treatment of bagasse with alkali can result in only partial cleavage of these esterified or etherified linkages, because a large proportion of the ferulic acid was quantitatively oxidized to vanillin and most of the *p*-coumaric acids were quantitatively oxidized to *p*-hydroxybenzaldehyde by nitrobenzene under the reaction conditions given (170 °C, 3 h).

Table 5. Weight-Average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersities (M_w/M_n) of the Hemicellulosic Subfractions

	hemicellulosic fractions[a]							
	H_1	H_3	H_4	H_5	H_6	H_7	H_8	H_9
M_w	12820	23770	40770	70080	86720	37480	57560	77140
M_n	5130	10990	15850	35630	24840	15100	30180	31370
M_w/M_n	2.50	2.16	2.57	1.96	3.49	2.48	1.91	2.46

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

Molecular Weight. The eight hemicellulosic subfractions were further analyzed by the determination of their weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n), and the GPC results are listed in **Table 5**. Obviously, the first two hemicellulosic subfractions H_1 and H_3 precipitated in 15 and 60% ethanol solutions from the water-soluble hemicelluloses showed a much lower degree of polymerization with M_w values of 12 820 and 23 766 g mol^{-1} than those six precipitated hemicellulosic subfractions from 1 and 3% NaOH-soluble hemicelluloses ranging from 37 480 to 86 722 g mol^{-1}. This phenomenon suggested that the first treatment of dewaxed bagasses with hot water solubilized only the small molecular size of hemicelluloses such as galactoarabinoaylans, pectic substances, and α-glucan (*54*), whereas the 1 and 3%

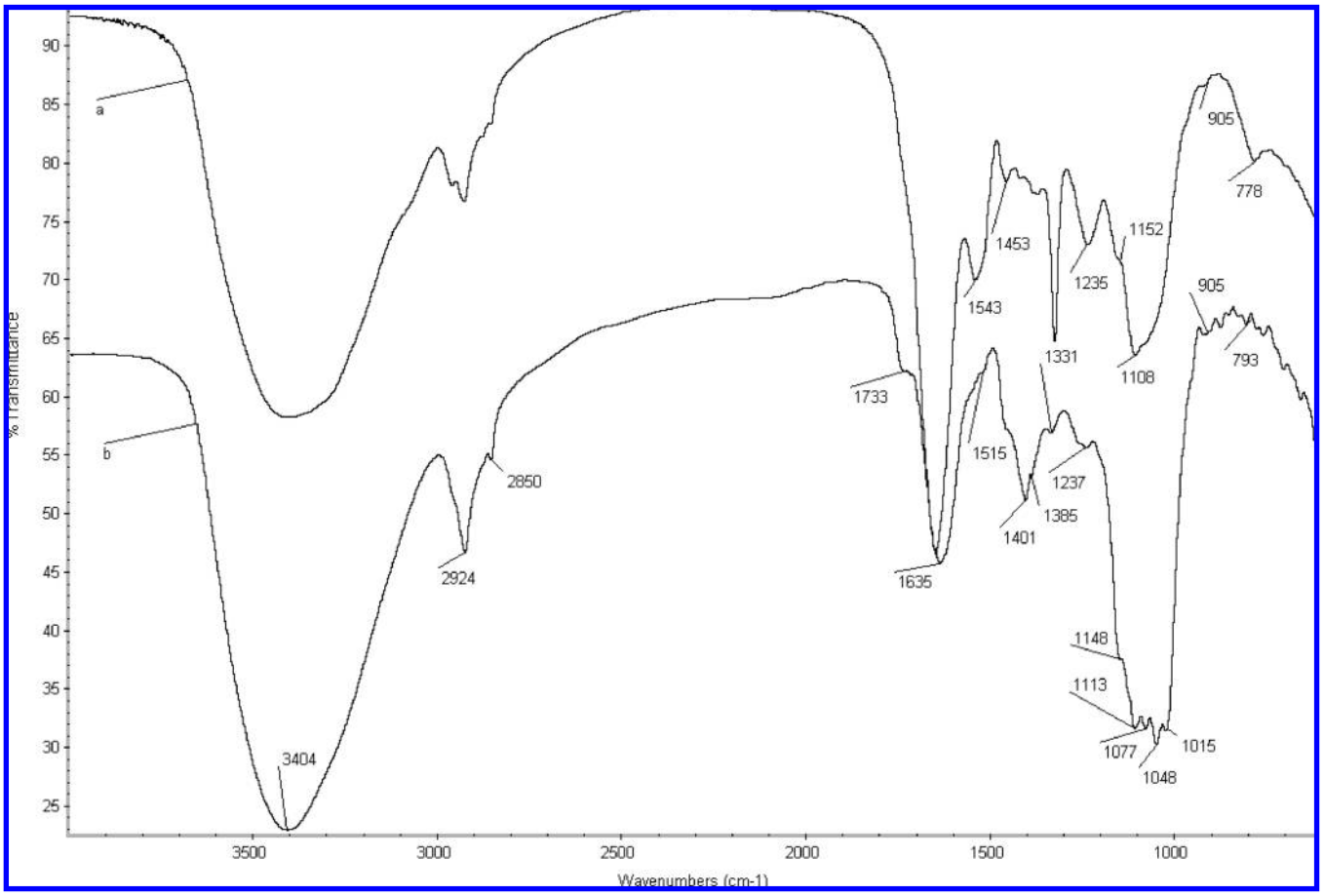

Figure 2. FT-IR spectra of the hemicellulosic subfractions H_1 (spectrum a) and H_3 (spectrum b) precipitated in 15 and 60% aqueous ethanol, respectively, from water-soluble hemicelluloses.

NaOH-soluble hemicelluloses have a high molecular weight and the hemicellulosic polymers were not substantially degraded under the alkaline extractions used. Interestingly, as the data shown in **Table 5**, an increase in precipitated ethanol concentration from 15 to 60% from the 1% NaOH-soluble hemicelluloses resulted in an increment of M_w value from 40 770 to 86 720 g mol^{-1}. A similar increasing trend was also observed in precipitated ethanol concentration from 15 to 60% in the water-soluble hemicelluloses and 3% NaOH-soluble hemicelluloses, in which the M_w increased from 12 820 in H_1 to 23 700 in H_3 and from 37 480 in H_7 to 77 140 in H_9. This suggested that the arabinoxylans with higher Ara/Xyl ratios had higher molecular weight, and the result is consistent with a previous study (*40*). It should be noted that molecular weights of polymers vary depending on the method, solvent quality, and chain aggregation for their estimation (*55*).

Additionally, the analysis showed that the two polymeric hemicelluloses, precipitated hemicellulosic subfractions in 30% ethanol from 1 and 3% NaOH-soluble hemicelluloses, gave a narrower molar mass distribution, corresponding to polydispersity indices of 1.96 for H_5 and 1.91 for H_8 as compared to those of the precipitated hemicellulosic subfractions H_4, H_6, H_7, and H_9 in 15 and 60% ethanol having polydispersity indices of 2.57, 3.49, 2.48, and 2.46, respectively. In other words, the molecular weight distribution of the precipitated hemicellulosic subfractions in 15 and 60% ethanol solutions was broader than that of the hemicellulosic subfractions obtained in 30% ethanol solution.

FT-IR Spectra. Figure 2 shows the FT-IR spectra of subfractions H_1 (spectrum a) and H_3 (spectrum b) obtained by precipitation in 15 and 60% ethanol solutions, respectively, from water-soluble hemicelluloses. The absorption at 3404 cm^{-1} is attributed to the stretching of −OH groups. The C−H stretching vibration gives signals at 2928 and 2881 cm^{-1}. The band at 1635 cm^{-1} is due to the bending mode of absorbed water. Obviously, the two hemicellulosic subfractions showed the typical signal pattern for the hemicellulosic moiety and had a specific band in the 1200−1000 cm^{-1} region, which is dominated by ring vibrations overlapped with stretching vibrations of side groups (C−OH) and the glycosidic bond vibration (C−O−C) (*56*). The high absorbance at 1331 cm^{-1} arises from the C−C and C−O skeletal vibrations. The presence of a shoulder at 1733 cm^{-1} in spectrum b implies that the hemicellulosic subfraction, solubilized during the water treatment, contains small amounts of the acetyl, uronic, and ester groups or the ester binds of the carboxylic groups of ferulic and/or *p*-coumaric acids. In the anomeric region (950−700 cm^{-1}), a small band at 905 cm^{-1}, which is due to the C-1 group frequency or ring frequency, is indicative of β-glycosidic linkages in hemicelluloses (*57*), whereas small peaks at 778 cm^{-1} in spectrum a and at 793 cm^{-1} in spectrum b are characteristic of α-anomers in side chains (*28*). The small bands at 1453, 1401, 1385, 1235, and 1237 cm^{-1} represent C−H and C−O or OH bending vibrations in hemicelluloses, respectively. Evidently, the occurrence of an intensive band at 1543 cm^{-1} in spectrum a is due to aromatic skeletal vibrations in bound lignin, whereas it becomes a rather weak in spectrum b, indicating the presence of only small amounts of bound lignin in the precipitated hemicellulosic subfraction obtained in 60% ethanol solution rom the water-soluble hemicelluloses, which corresponded to the data obtained by alkaline nitrobenzene oxidation.

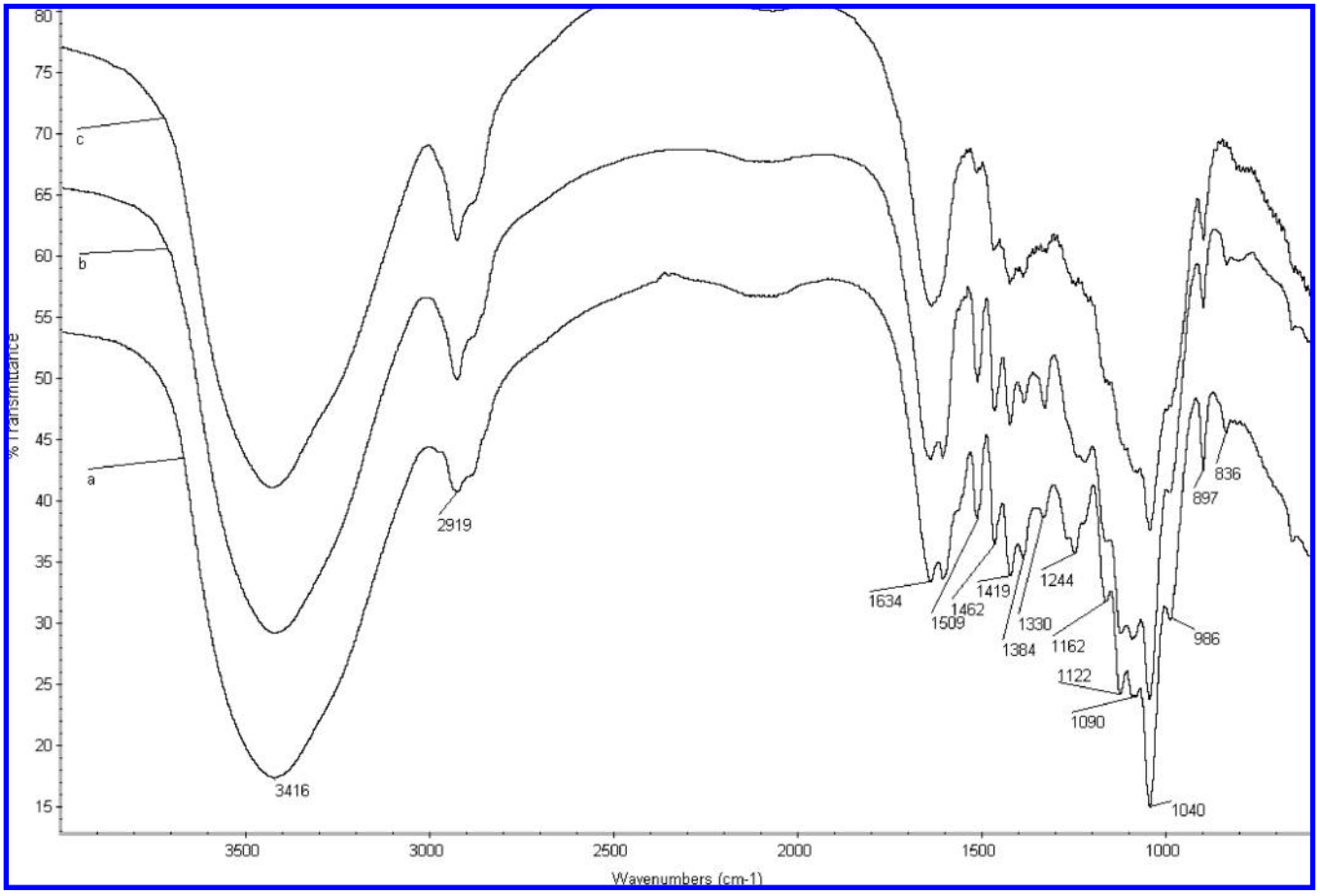

Figure 3. FT-IR spectra of the hemicellulosic subfractions H_4 (spectrum a), H_5 (spectrum b), and H_6 (spectrum c) obtained by precipitation in 15, 30, and 60% aqueous ethanol, respectively, from the 1% NaOH-soluble hemicelluloses.

The FT-IR spectra of the precipitated hemicellulosic subfractions H_4 (spectrum a), H_5 (spectrum b), and H_6 (spectrum c) in 15, 30, and 60% ethanol solutions from 1% NaOH-soluble hemicelluloses are illustrated in **Figure 3**. The absorbances at 1462, 1419, 1384, 1330, 1244, 1162, 1122, 1090, 1040, 986, and 897 cm^{-1} are associated with hemicelluloses. The spectra are dominated with stretching and bending vibrations of C−O, C−C, C−OH, and C−O−C at 1040 cm^{-1}. Bands between 1170 and 1000 cm^{-1} are typical of arabinoxylans. Evidently, the presence of the arabinosyl side chains is documented by the two low-intensity shoulders at 1162 and 985 cm^{-1}, which have been reported to be attached only at positions of the xylopyranosyl constituents (*58*). The intensity changes of these two bands can be suggested to reflect the arabinosyl substituent contribution and, therefore, used for the identification of arabinoxylan structures. That is, this band gives variation in spectral shape depending on the branches at the O-2 and O-3 positions. In comparison with the precipitated hemicellulosic subfraction in 60% ethanol solution from the water-soluble hemicelluloses in **Figure 2**, a sharp band at 897 cm^{-1} in **Figure 3** demonstrated that the 1% NaOH treatment under the conditions given did not cleave the β-glycosidic linkages between the sugar units from the backbone of hemicelluloses. As expected, the absence of a signal at 1730 cm^{-1} for carbonyl stretching in all three spectra implied that the 1% NaOH treatment under the conditions used completely cleaved this ester bond from the hemicelluloses. The occurrence of an intensive band at 1509 cm^{-1} in spectra a and b is due to the associated lignin in the hemicellulosic subfraction, but it becomes rather weak in spectrum c, indicating the presence of only a small amount of bound lignin in this hemicellulosic subfraction, precipitated in 60% ethanol solution from the 1% NaOH-soluble hemicelluloses, which corresponded to the data obtained by alkaline nitrobenzene oxidation.

FT-IR spectra of the three the precipitated hemicellulosic subfractions from 3% NaOH-soluble hemicelluloses are illustrated in **Figure 4**. As expected, the three spectral profiles and relative intensities of most bands were rather similar, indicating a similar structure of the three hemicellulosic subfractions, which corresponds to their composition. The absorbances at 3424, 2914, 1423, 1386, 1245, 1166, 1118, 1075, 1039, 985, and 894 cm^{-1} are associated with hemicelluloses, in which 1166 and 1039 cm^{-1} are typical of arabinoxylans. The lignin-related band at 1506 cm^{-1} is rather weak, which is in accordance with the content of bound lignin determined by alkaline nitrobenzene oxidation in **Table 4**.

^{1}H and ^{13}C NMR Spectra. The hemicellulosic subfractions H_7 (spectrum a) and H_9 (spectrum b) obtained by precipitation in 15 and 60% aqueous ethanol, respectively, from the 3% NaOH-soluble hemicelluloses were analyzed by ^{1}H NMR spectroscopy to characterize the structural features (**Figure 5**). As can be seen, the signals at δ 3.1−5.4 are caused by the protons of arabinose and xylose residue except for the strong signal at δ 4.7 (*59*), which is indicative of the residual solvent (HDO). Signals at δ 5.4 (spectrum a) and δ 5.1 (spectrum b) in the ^{1}H NMR spectra have been assigned to anomeric protons of a terminal α-D-arabinofuranosyl residue, indicating a significant amount of substitution at C-3 and C-2 (disubstituted) of the xylose backbone. The chemical shifts of δ 3.1−4.3 are assigned to equatorial proton and other protons of anhydroxylose units of hemicellulose. Disubstituted β-D-xylopyranose residues having α-D-arabinofuranose substituents at C-2 and C-3 are also present. Signals

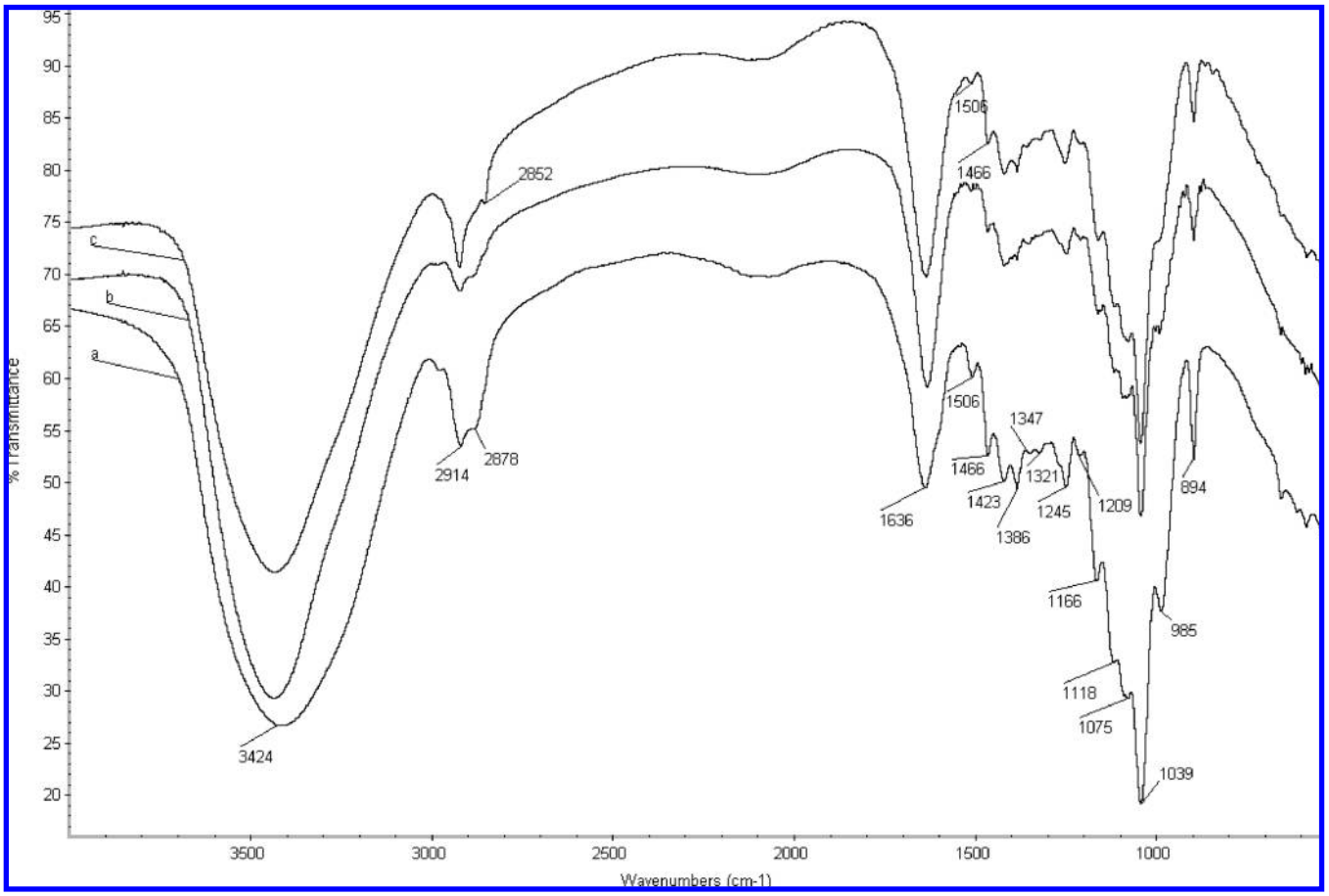

Figure 4. FT-IR spectra of the hemicellulosic subfractions H_7 (spectrum a), H_8 (spectrum b), and H_9 (spectrum c) obtained by precipitation in 15, 30, and 60% aqueous ethanol, respectively, from the 3% NaOH-soluble hemicelluloses.

at δ 4.3/4.4 are due to the anomeric protons of β-D-xylose substituted at C-3 (monosubstitued) residues, because the region δ 4.1−4.5 corresponds to the β-configuration and the region between δ 4.9 and 5.6 corresponds to the α-configuration (*60*). The methyl protons of a few amounts of 4-*O*-methyl-D-glucuronic acid give weak peaks at 1.0/1.1 ppm. Resonance signals originating from phenolic compounds (6.4 ppm) are undoubtedly due to the presence of small amounts of associated lignin in the hemicelluloses and corresponded to the results obtained by alkaline nitrobenzene oxidation (**Table 4**).

To confirm the structural features, the precipitated hemicellulosic subfractions (H_7 and H_9) obtained in 15 and 60% ethanol solutions from 3% NaOH-soluble hemicelluloses were characterized by ^{13}C NMR, respectively (**Figure 6**). The two hemicellulosic subfractions showed very similar spectra, indicating a similar structure of hemicelluloses. Most of the major resonances were assigned by references to data in the literature (*56*,*61*,*62*). The ^{13}C NMR spectrum of the precipitated hemicellulosic subfraction H_7 (**Figure 6a**) obtained in 15% ethanol solution showed five main signals at δ 102.2 (C-1), 73.2 (C-2), 74.7 (C-3), 75.9 (C-4), and 63.3 (C-5), corresponding to (1→4)-linked β-D-xylose residues. The signals at δ 109.4, 86.4, 80.2, 78.9, and 61.7 originate from C-1, C-4, C-2, C-3, and C-5 of α-L-arabinofuranosyl residues linked to β-D-xylans, respectively. Two signals at δ 72.4 and 74.5 (data not shown) represent C-4 and C-2 of the galactose residue in the xylan. The ^{13}C NMR spectrum of hemicellulosic subfraction H_9 (**Figure 6b**) precipitated in 60% ethanol was recorded in D_2O and showed a spectrum very similar to that of hemicellulosic subfraction H_7. The main (1→4)-linked β-D-xylp units are obviously characterized by the signals at δ 101.6, 76.3, 73.6, 72.7, and 62.9, which are attributed, respectively, to C-1, C-4, C-3, C-2, and C-5 of β-D-xylp units (*61*, *62*). The signals at δ 84.8, 78.6 (data not shown), 77.6, and 61.3 correspond to C-4, C-3, C-2, and C-5 of arabinofuranosyl residues linked to β-D-xylans, respectively. A weak signal at δ 59.8 originated from the *O*-methoxyl group of the glucuronic acid residue in the xylan. The signal at δ 16.8 arises from $-CH_3$ in $Ar-COCH_3$, indicating the associated lignin, which corresponded to the results obtained by alkaline nitrobenzene oxidation. In other words, these results implied that the hemicellulosic subfractions can be structurally defined as L-arabino-(4-*O*-methyl-D-glucurono)xylan together with a small amount of associated lignin. These typical signals for L-arabino-(4-*O*-methyl-D-glucurono)xylan revealed that alkaline treatment under the conditions used did not affect the overall structure of macromolecular hemicelluloses.

Thermal Analysis. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were used to study the thermal stability of the hemicelluloses isolated. Their curves of the precipitated hemicellulosic subfractions (H_7 and H_9) of 3% NaOH-soluble hemicelluloses in 15 and 60% ethanol are shown in **Figure 7**. As can be seen from the figure, the two hemicellulosic subfractions H_7 (a) and H_9 (b) began to decompose at 220 and 180 °C, respectively. The maximum rate of weight loss was observed between 260 and 305 °C for H_7 and between 240 and 300 °C for H_9. Beyond these temperatures, thermal degradation takes place. Similarly, when weight loss arrived at 50%, the temperature of the polymer samples appeared at 305 and 510 °C. The initial weight loss was probably due to generation of noncombustible gases such as CO, CO_2, formic

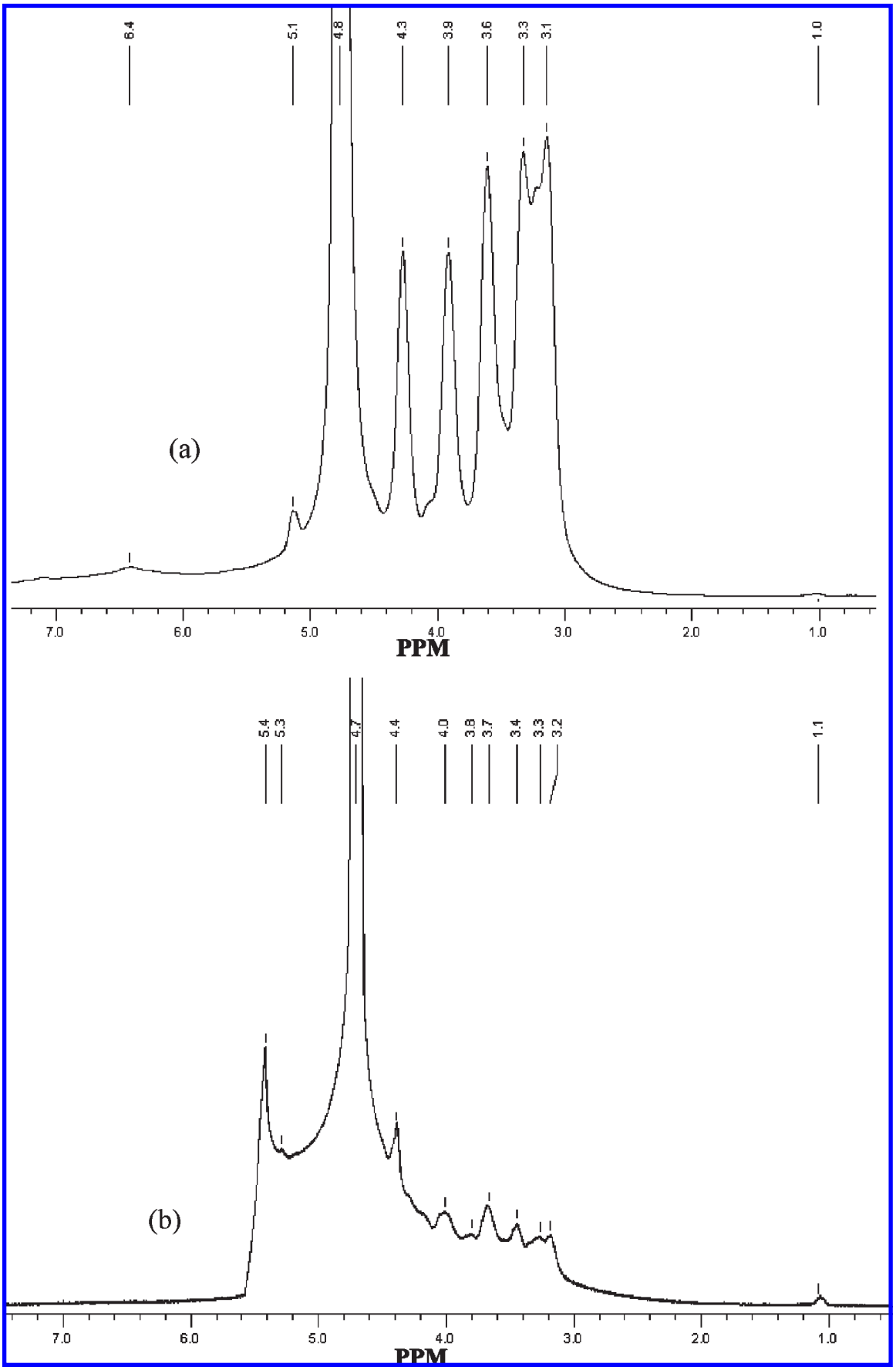

Figure 5. ^{1}H NMR spectra of hemicellulosic subfractions H_7 (spectrum a) and H_9 (spectrum b) obtained by precipitation in 15 and 60% aqueous ethanol, respectively, from the 3% NaOH-soluble hemicelluloses.

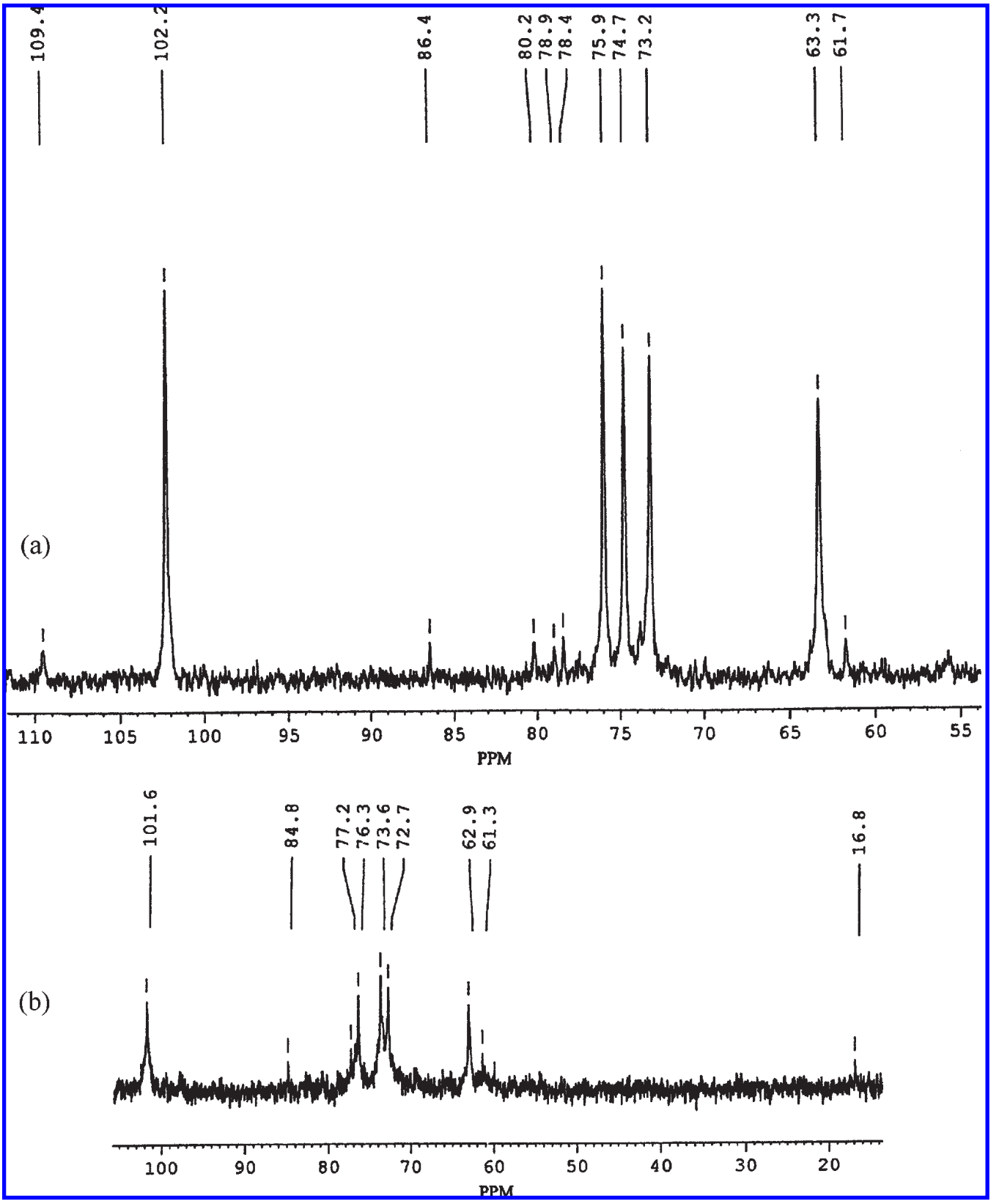

Figure 6. ^{13}C NMR spectra of hemicellulosic subfractions H_7 (spectrum a) and H_9 (spectrum b) obtained by precipitation in 15 and 60% aqueous ethanol, respectively, from the 3% NaOH-soluble hemicelluloses.

acid, and acetic acid, whereas the significant (maximum) weight loss was presumably due to the onset of pyrolysis and generation of combustible gases (*45*). These data indicate that the precipitated hemicellulosic subfractions (H_9) obtained in 60% ethanol have higher thermal stability than the precipitated hemicellulosic subfractions in 15% ethanol (H_7), corresponding to their molecular weights in **Table 5**. In short, the thermal stability of hemicelluloses increased with an increase in their molecular weight.

Conclusions. The sequential treatments of dewaxed bagasse with water and 1 and 3% NaOH aqueous solutions yielded 25.1%

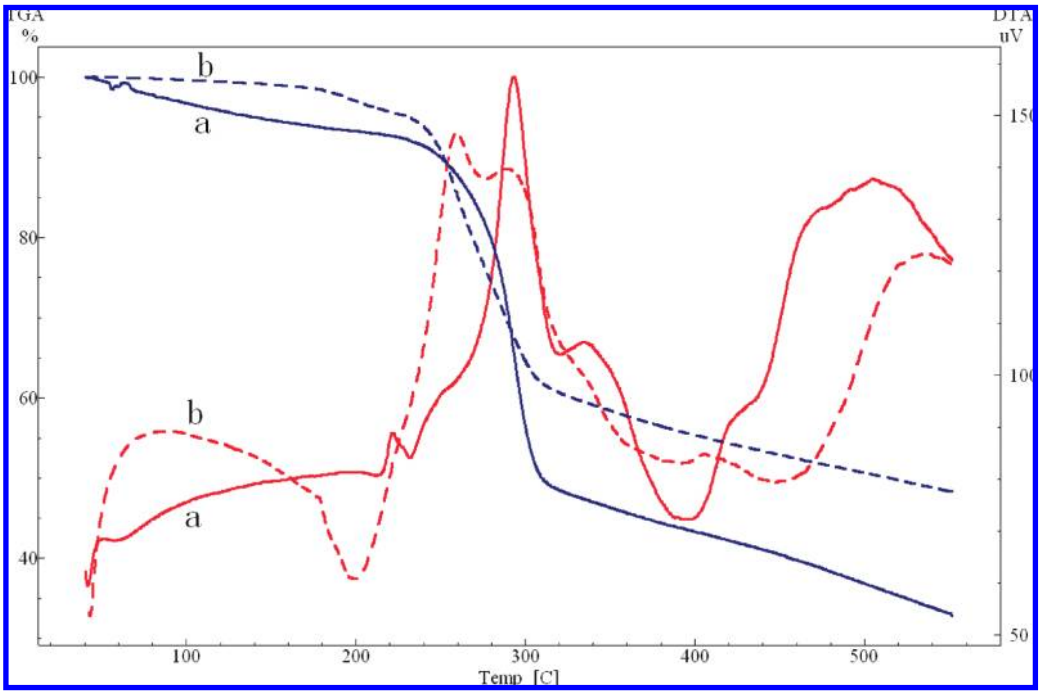

Figure 7. Thermograms of hemicellulosic subfractions H_7 (spectrum a) and H_9 (spectrum b) obtained by precipitation in 15 and 60% aqueous ethanol, respectively, from the 3% NaOH-soluble hemicelluloses.

hemicelluloses from bagasse and accounted for 74.9% of the original hemicelluloses. These results indicated that 1 and 3% NaOH aqueous solutions under the conditions used significantly cleaved the α-ether bonds between lignin and hemicelluloses from the cell wall of bagasse, resulting in a substantial dissolution of the polymers of hemicellulosic polysaccharides and lignin macromolecules. Additionally, the hemicelluloses released were subfractionated by graded precipitation at the ethanol concentrations of 15, 30, and 60% (v/v) to yield eight precipitated hemicellulosic subfractions. Comparison of these hemicelluloses indicated that the smaller molecular sized and more branched hemicelluloses were extracted by the hot water treatment; they are rich in glucose, probably originating from α-glucan and pectic polysaccharides. The larger molecular sized and more linear hemicelluloses were dissolved by the alkali treatment; they are rich in xylose, principally resulting from L-arabino-(4-*O*-methylglucurono)-D-xylans. Furthermore, noticeable differences in the chemical composition and molecular weights were observed among the graded precipitated hemicellulosic subfractions obtained from the water-soluble and alkali-soluble hemicelluloses. The Ara/Xyl ratio increased with the increment of ethanol concentration from 15 to 60%, and the arabinoxylans with higher Ara/Xyl ratios had higher molecular weights. This indicated that there is a diversity of hemicelluloses present in the cell wall of bagasse, which vary in their degree of branching and possibly in their molecular weight. The result from FT-IR and alkaline nitrobenzene oxidation analysis showed that eight precipitated hemicellulosic subfractions obtained from the water-soluble and alkali-soluble hemicelluloses contained small amounts of lignin, indicating that the hemicelluloses and lignin are more tightly linked in the cell walls of sugarcane bagasse. On the basis of the FT-IR, sugar composition, and ^{1}H and ^{13}C NMR studies, there were no significant differences in the structural features of the precipitated hemicellulosic subfractions, which mainly consisted of L-arabino-(4-*O*-methyl-D-glucurono)xylan, whereas the difference may occur in the distribution of branches along the xylan backbone. Therefore, these advantages implied that the method by first alkaline extraction and then graded precipitation could be used for the isolation of polysaccharides having different degrees of branching and molecular weights from renewable materials for industries.

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