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Fractional Separation and Physicochemical Characterization of Polysaccharides from Poplar Chips

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ABSTRACT

The polysaccharides in the cell walls of dewaxed poplar chips were separated into hemicellulose–lignin complexes (HLC), residual hemicelluloses, and cellulose by extraction with sodium hydroxide at various concentrations. Celluloses (43.9–44.5%), HLC (17.4–21.9%), and residual hemicelluloses (4.3–9.0%) were the major polysaccharide components. Initial extraction of the dewaxed poplar chips with 5% NaOH at 50°C for 4, 6, 8, and 12 hr, and 7.5 and 10% NaOH at 50°C for 6 hr yielded 17.4, 18.2, 18.4, 18.6, 20.3, and 21.9% HLC, respectively. Further extraction of the corresponding alkali-treated and delignified poplar chips with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 2 hr released 9.0, 8.2, 7.9, 7.2, 5.5, and 4.3% residual hemicelluloses, respectively. Xylose was found to be a predominant sugar component (84.3–90.5%) in all of the more linear, acidic, and larger molecular size HLC preparations ($\overline{M}_W$, 26,090–40,500 g·mol^{-1}), in addition to 4.0–4.8% bound lignin. These lignin molecules were found to be chemically linked to hemicelluloses mainly via syringyl units. On the other hand, all of the residual hemicellulosic fractions contained less xylose (57.3–59.1%) and less uronic acids (4.5–8.9%), but had a higher content of mannose (24.9–26.5%) and glucose (13.3–16.0%). In comparison with the HLC, the residual hemicellulosic fractions had a small molecular size ($\overline{M}_w$, 18,300–29,730 g·mol^{-1}), and had less than half the amount of bound lignin (1.5–1.7%). These lignin molecules were associated with residual hemicelluloses mainly via guaiacyl units. All of the cellulose fractions were relatively free of noncellulosic sugars (contained more than 99.6% glucose), and no considerable degradation of the cellulose was observed under the various separation conditions given.

Key Words. Poplar chips; Polysaccharides; Separation; Hemicellulose–lignin complexes; Residual hemicelluloses; Cellulose; Lignin

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INTRODUCTION

Hemicelluloses, one of the most abundant natural polysaccharides, comprise roughly one-fourth to one-third of most plant materials. On a dry weight basis, poplar chips contain approximately 27% hemicelluloses. They are heterogeneous fractions and are classically defined as the alkali-soluble material remaining after removal of the pectic substances (1). Most of the hemicellulosic preparations are soluble in water after alkaline extraction. Their isolation actually involves alkaline hydrolysis of ester linkages to liberate them from the lignocellulosic matrix followed by extraction into aqueous media. Hemicelluloses are branched polymers of low molecular weight and degree of polymerization of 80–200. Their general formulas are $(C_5H_8O_4)_n$ and $(C_6H_{10}O_5)_n$ and they are called pentosans and hexosans, respectively (2). Hemicelluloses are made of a relatively limited number of sugar residues. The principle sugars are D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid (MeGlcA), D-galacturonic acid, and to a lesser extent, L-rhamnose, L-fucose, and various *O*-methylated neutral sugars.

Hemicelluloses are potentially very useful. Properties of wheat straw hemicelluloses worth exploiting are their ability to serve as adhesives, thickeners, stabilizers, film formers, and emulsifiers (3). The hemicelluloses, isolated from hardwood or cereal straws, are a valuable source of xylose, the predominant sugar, which accounts for 70–85% of the monosaccharides present and can readily be modified to give xylitol, a sugar substitute (4). More recently, the cross-linking of hemicelluloses by oxidative coupling of ferulate-esters has attracted attention as a possible mechanism of gelation. Ferulate cross-linked polysaccharides may have applications as cloud stabilizers in beverages, and as humectants in food and nonfood products (5). For example, an arabinoxylan–ferulate polysaccharide from American corn bran has been shown to undergo gelation in the presence of peroxidase and hydrogen peroxide to form a thermostable cold-setting gel (6). With extensive investigation of the hemicelluloses from wheat straw, researchers in our laboratories found that the native hemicellulose lattices demonstrated good properties for the preparation of decorative paints, which indicated the potential use of hemicelluloses in real commercial decorative paint systems (7). In this comparative study, we investigated the hemicellulosic substances separated both from lignified poplar chips and from the delignified holocellulose using various concentrations of alkali in different extraction periods, and characterized their physicochemical properties. The emphasis is on the lignin components associated with the hemicellulosic preparations.

EXPERIMENTAL METHODS

Materials

Poplar chips were obtained from our fiber plant. The composition (%, w/w) of the chips is cellulose 43.8%, hemicelluloses 26.6%, lignin 21.3%, ash 1.5%, and wax 1.8% on a dry weight basis. After being dried at 60°C in an oven for 16 hr, the chips were ground to pass through a 0.7-mm screen and stored at 5°C until use.

Extraction and Separation of Hemicelluloses

The dried powder was first extracted with toluene–ethanol (2:1, v/v) in a Soxhlet extractor for 6 hr. The dewaxed sample was then treated with 5% NaOH (1 g sample/22 mL extractant) at 50°C for 4, 6, 8, and 12 hr, and 7.5 and 8.5% NaOH (1 g sample/22 mL extractant) at 50°C for 6 hr, respectively, under continuous agitation. The hemicellulose–lignin complexes (HLCs) were separated from the hydrolysates by precipitation of the neutralized hydrolysate with 3 volumes of ethanol. After filtration, pellets rich in the hemicellulose–lignin complexes were washed with 70% ethanol and air-dried.

After filtration on a nylon cloth, the alkali-extracted residues were washed with water and ethanol, then dried at 60°C for 16 hr. Residual lignin was removed with 1.5% $NaClO_2$ in acidic solution (pH 4.2, adjusted by 10% acetic acid) at 75°C for 2 hr. The remaining hemicelluloses were isolated from the six corresponding holocellulose samples with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 16 hr and labeled accordingly. Solubilized residual hemicelluloses were separated according to the method mentioned above for the hemicellulose–lignin complexes. The residue, corrected for ash content, was considered to consist primarily of cellulose. The scheme for extraction of HLCs and residual hemicelluloses from poplar chips is illustrated in Fig. 1.

Characterization of the Separated Hemicelluloses

The neutral sugar composition of the isolated HLCs and residual hemicellulosic preparations was determined by gas chromatography (GC) analysis of the corresponding alditol acetates, following hydrolysis of each sample with 2 M trifluoroacetic acid for 2 hr at 120°C (8). The uronic acid content was assayed colorimetrically using the 3-phenylphenol reagent according to the procedure outlined by Wedig and co-workers (9). Methods for the determination of phenolic acids and aldehydes in nitrobenzene oxidation mixtures involved high performance liquid chromatography (HPLC); measurement of the molecular weights have been described in previous papers (10–12).

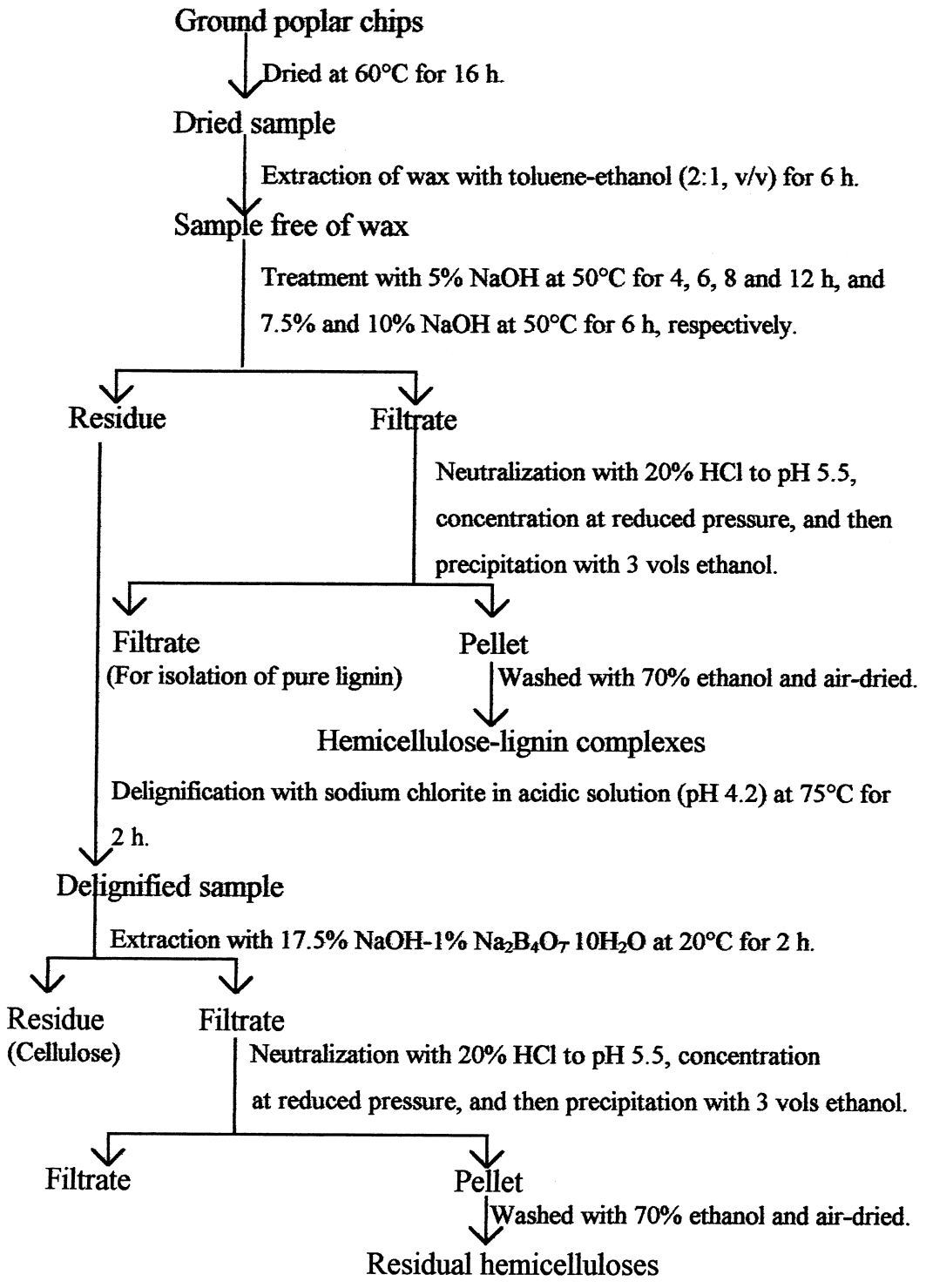

FIG. 1 Schematic procedure for isolation of HLCs from alkaline hydrolysates of poplar chips and residual hemicelluloses from the corresponding alkali-treated and delignified holocellulose.

FT-IR spectra were obtained on an FT-IR spectrophotometer using a KBr disk containing 1% finely ground samples. Solution-state ^{13}C-NMR spectra were obtained on a Bruker 250 AC spectrometer (Bangor, Wales) operating in the FT mode at 62.4 MHz under total proton-decoupled conditions. Spectra were recorded at 25°C from 200 mg of sample dissolved in 1.0 mL D_2O following 10,000 scans. A 60° pulse flipping angle, a 3.9 μs pulse width, and 0.85 sec acquisition time were used.

RESULTS AND DISCUSSION

Yield of Hemicelluloses

The yields of HLCs, residual hemicelluloses, and cellulose are listed in Table 1. As can be seen from this table, the yield of dissolved HLC increased from 17.4 to 18.6% with increased extraction time from 4 to 12 hr using 5% NaOH at 50°C, and increased from 18.2 to 20.3 and 21.9% with an increase in alkali concentration from 5 to 7.5 and 10%, respectively. Treatment of dewaxed poplar chips with 5% NaOH at 50°C for 4, 6, 8, and 12 hr, 7.5% NaOH at 50°C for 6 hr, and 10% NaOH at 50°C for 6 hr released 65.4, 68.4, 69.2, 69.9, 76.3, and 82.3% of the original hemicelluloses and 16.3, 17.2, 18.9, 19.3, 18.0, and 20.6% of the original lignin (data not shown in Table 1), respectively. This trend indicated that the release of HLC paralleled the yield of alkali-soluble lignin. However, the solubility of the former was much higher than that of the

TABLE 1
Yields of HLCs Isolated with 5%, 7.5%, and 10% NaOH at 50°C for Various Periods from Dewaxed Poplar Chips

	Alkali treatment conditions					
	5% NaOH (50°C) treatment time (hr)					
Yield (%)	4	6	8	12	7.5% NaOH 50°C, 6 hr	10% NaOH 50°C, 6 hr
HLCs[a]	17.4	18.2	18.4	18.6	20.3	21.9
Residual hemicelluloses[b]	9.0	8.2	7.9	7.2	5.5	4.3
Cellulose[c]	44.5	44.5	44.4	44.4	43.9	43.9

Residual hemicelluloses and cellulose were extracted with 17.5% NaOH–1% $Na_2B_4O_7{\cdot}10H_2O$ at 20°C for 2 hr from the corresponding alkali-treated and delignified poplar chips

[a] Extracted with 5%, 7.5%, and 10% NaOH at 50°C for various periods from dewaxed poplar chips.

[b] Extracted with 17.5% NaOH–1% $Na_2B_4O_7{\cdot}10H_2O$ at 20° for 2 hr from the corresponding alkali-treated and delignified poplar chips.

latter, indicating that the alkali treatment of the dewaxed poplar chips favored the release of HLC. This result also implied that extending the extraction time or increasing the alkali concentration under the specified conditions favored release of both HLC and lignin. The reason for the relatively higher yield of HLC and much lower yield of lignin is presumably due to partial cleavage of the linkages between hemicelluloses and lignin (e.g., α-ether bonds) under the specified conditions. In this case, alkali treatment completely or partially cleaved the ester bonds, e.g., linkages between *p*-hydroxybenzoic acid and lignin (13). Conversely, the yield of residual hemicelluloses, extracted with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 16 hr from the corresponding alkali-treated and delignified poplar chips, decreased to 9.0, 8.2, 7.9, 7.2, 5.5, and 4.3%, respectively. This trend was probably the result of increased hemicellulose solubilization during the previous prolonged extraction with 5% NaOH, or during the extractions with relatively higher NaOH concentrations of 7.5 and 10.%. The total hemicelluloses solubilized in the above-mentioned two steps were found to range between 25.8 and 26.8%. Table 1 also demonstrates that the yield of cellulose from the different alkali extraction procedures was comparatively consistent, ranging between 43.9 and 44.5%. The latter phenomenon suggested that the alkali extractions did not result in any significant degradation of the cellulose under the conditions given.

Sugar Composition and Content of Uronic Acids

The neutral sugar composition and uronic acid content of the HLCs, residual hemicelluloses, and cellulose are given in Tables 2, 3, and 4, respectively. Xylose was the predominant sugar component in all of the HLCs, comprising 84.3–90.5% of the total sugars. Uronic acids were present in smaller concentrations, ranging between 9.1 and 14.3%. Glucose, mannose, rhamnose, galactose, and arabinose were observed as minor sugar constituents in HLCs. These data suggested that the HLCs in the cell walls of poplar chips are mainly composed of xylan. This observation was in accordance with the results obtained from our previous studies on aspen and birchwood HLC (14), in which we demonstrated that xylose was the major component sugar in the HLC, obtained by water extraction of the steam-treated aspen and steam-exploded birchwood. No significant differences were found in the sugar composition of the poplar HLC, isolated by 5% NaOH at 50°C for 4–12 hr. The content of xylose decreased from 90.5 to 88.4 and 84.3%, with an increase in the alkali concentration from 5 to 7.5 and 10%, respectively. The opposite trend was observed for the mannose and glucose content, which increased from 1.4 to 5.0% and from 4.8 to 6.6%, respectively, when the alkali concentration was increased from 5 to 10%. This suggested that the solubilized HLC also contained small amounts of glucomannan, which was released at relatively higher concentrations of alkali. Similar results have been reported from other hard-

TABLE 2
The Content of Neutral Sugars (Relative Percent Dry HLCs, w/w) and Uronic Acids (Percent Dry HLCs) in HLCs Isolated with 5%, 7.5%, and 10% NaOH at 50°C for Various Periods from Dewaxed Polar Chips

Sugar/uronic acids (%)	5% NaOH (50°C) treatment time (hr)				7.5% NaOH 50°C, 6 hr[b]	10% NaOH 50°C, 6 hr[c]
	4[a]	6[a]	8[a]	12[a]		
Rhamnose	1.68	1.60	1.85	2.13	1.77	1.84
Arabinose	0.61	0.24	0.89	1.49	0.78	0.98
Xylose	88.77	90.45	90.54	89.16	88.44	84.25
Mannose	1.62	1.44	1.13	1.23	2.63	5.00
Glucose	6.14	4.82	4.41	4.59	4.91	6.58
Galactose	1.18	1.45	1.16	1.40	1.47	1.35
Uronic acids	12.50	13.38	14.25	11.50	10.00	9.12

[a] Represent HLCs extracted with 5% NaOH at 50°C for different periods from dewaxed poplar chips.
[b] Represent HLCs extracted with 7.5% NaOH at 50°C for 6 hr from dewaxed poplar chips.
[c] Represent HLCs extracted with 10% NaOH at 50°C for 6 hr from dewaxed poplar chips.

TABLE 3
The Content of Neutral Sugars (Relative Percent Dry Residual Hemicelluloses, w/w) and Uronic Acids (Percent Dry Residual Hemicelluloses, w/w) in Residual Hemicellulosic Fractions Isolated with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 2 hr from the Corresponding Alkali-Treated and Delignified Poplar Chips

Sugar/uronic acids (%)	5% NaOH (50°C) treatment time (hr)				7.5% NaOH 50°C at 6 hr[b]	10% NaOH 50°C, 6 hr[c]
	4[a]	6[a]	8[a]	12[a]		
Rhamnose	0.42	0.42	0.38	0.45	0.34	0.58
Arabinose	0.73	0.62	0.60	0.63	0.34	0.66
Xylose	58.23	58.37	59.07	57.31	57.25	58.04
Mannose	25.66	26.50	24.91	25.24	25.08	25.99
Glucose	13.95	13.34	14.04	14.38	15.96	13.32
Galactose	1.04	0.79	1.02	2.02	1.04	1.40
Uronic acids	8.87	7.13	7.00	4.75	4.75	4.50

[a] Represent residual hemicellulosic fractions extracted from the 5% NaOH-treated (at 50°C for different periods) and delignified poplar chips.
[b] Represent residual hemicelluloses extracted from the 7.5% NaOH-treated (at 50°C for 6 hr) and delignified poplar chips.
[c] Represent residual hemicelluloses extracted from the 10% NaOH-treated (at 50°C for 6 hr) and delignified poplar chips.

TABLE 4
The Content of Neutral Sugars (Relative Percent Dry Cellulose, w/w) in Cellulose-Rich Fractions Isolated with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 2 hr from the Corresponding Alkali-Treated and Delignified Poplar Chips

Sugar (%)	5% NaOH (50°C) treatment time (hr)				7.5% NaOH 50°C, 6 hr[b]	10% NaOH 50°C, 6 hr[c]
	4[a]	6[a]	8[a]	12[a]		
Xylose	0.30	0.28	0.26	0.25	0.27	0.22
Mannose	0.15	0.13	0.13	0.12	0.17	0.11
Glucose	99.55	99.59	99.61	99.63	99.56	99.67
Galactose	Tr[d]	Tr	Tr	Tr	Tr	Tr

[a] Represent cellulose fractions extracted from the 5% NaOH-treated (at 50°C for different periods) and delignified poplar chips.
[b] Represent cellulose extracted from the 7.5% NaOH-treated (at 50°C for 6 hr) and delignified poplar chips.
[c] Represent cellulose extracted from the 10% NaOH-treated (at 50°C for 6 hr) and delignified poplar chips.
[d] Tr: trace.

woods. Puls and Schuseil (15) demonstrated that xylan represented more than 90% of the hemicelluloses in aspen. In birchwood xylan, approximately every tenth xylose unit carries a single, terminal side chain, generally MeGlcA attached directly to C-2- of xylose. Seven out of 10 xylose residues contain an *O*-acetyl group at C-2 and/or C-3. In addition, hardwoods usually consist of 3–5% glucomannan, linked by β-1, 4-glycosidic bonds (15).

Data in Table 3 demonstrated no significant difference in the sugar composition between the residual hemicellulosic fractions, extracted with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 16 hr from the corresponding alkali-treated and delignified poplar chips, implying that residual hemicelluloses from both sources may have similar structures. Xylose was still the major sugar constituent and mannose and glucose were present as the second major neutral sugars, indicating that the hemicellulosic fractions consist of substantial amounts of xylan and smaller amounts of glucommanan. The higher content of mannose and glucose in the residual hemicellulsic fractions than that found in HLCs imply that extraction of the alkali-treated and delignified poplar chips with a higher concentration of alkali such as 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ favored release of hemicelluloses enriched in glucomannan. Similarly, the higher xylose and much lower mannose and glucose contents present in HLC relative to those found in the residual hemicelluloses suggest that a 5–10% NaOH solution is more effective in liberating xylose-rich hemicelluloses.

Tables 2 and 3 demonstrate that the HLC contained approximately twice the uronic acid content of the residual hemicellulosic fractions. This result indicates that the uronic acids, mainly MeGlcA, appear in side chains of xylan and may be also linked to lignin in the HLC. The existence of ester linkages between glucuronic acid or MeGlcA residues of glucuronxylan and lignin in the cell walls of poplar trees was confirmed by ^{13}C-NMR spectroscopy (16).

The six cellulose fractions, obtained by extraction of the corresponding alkali-treated and delignified poplar chips with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 16 hr, were similar in sugar composition (Table 4), and consisted of approximately 99.6% glucose and minor amounts of other sugars, such as xylose and mannose. The presence of sugars other than glucose in cellulose fractions could be the result of incomplete removal of contaminating hemicelluloses.

Content of Bound Lignin and the Composition of Phenolic Monomers

To verify the presence of associated lignin in HLC, residual hemicellulose, and cellulose fractions, nitrobenzene oxidation of bound lignin was performed at 170°C for 3 hr. This method provides an estimate of the total amount of chemically linked lignin and yields an indication of the phenolic monomer composition. The content of associated lignin; the yield of alkaline nitrobenzene oxidation; and the components of phenolic acids and aldehydes obtained from the HLC, residual hemicellulose, and cellulose fractions are given in Tables 5, 6, and 7, respectively. Obviously, the HLC contained approximately twice or four times the bound lignin of the residual hemicelluloses or cellulose, respectively.

As shown in Table 5, the major oxidation products in HLC were syringaldehyde and vanillin, which together comprised 79–84% of the total nitrobenzene oxidation products detected. Furthermore, nitrobenzene oxidation produced approximately twice as much syringaldehyde as vanillin. Thus, from these results the hemicelluloses in HLC are linked mainly to lignin via syringyl units. Small amounts of *p*-hydroxybenzaldehyde and vanillic acid, and traces of syringic acid, *p*-hydroxybenzoic acid, ferulic acid, and *p*-coumaric acid were also identified in the nitrobenzene oxidation products. No significant difference in syringaldehyde/vanillin molar ratios was found among the six HLCs. Analogously, as can be seen in Table 5, an increase in the 5% NaOH extraction period from 4 to 12 hr, or an increase in NaOH from 5 to 10% did not result in a significant decrease in lignin bound in HLC (bound lignin ranging between 4.0 and 4.8%). This result, as well as the other experiments presented here, strongly suggests that hemicelluloses in the cell walls of poplar wood are tightly linked to lignin by ether bonds at the α-position of lignin side chains and are more alkali-resistant.

TABLE 5
The Yield (Percent HLCs w/w) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of Associated Lignin in the HLCs

Phenolic acids and aldehydes	5% NaOH (50°C) treatment time (hr)				7.5% NaOH 50°C, 6 hr[b]	10% NaOH 50°C, hr[c]
	4[a]	6[a]	8[a]	12[a]		
p-Hydroxybenzoic acid	0.024	0.024	0.056	0.10	0.028	0.029
p-Hydroxybenzaldehyde	0.12	0.12	0.10	0.10	0.12	0.12
Vanillic acid	0.14	0.13	0.13	0.12	0.15	0.16
Vanillin	0.69	0.62	0.67	0.67	0.58	0.52
Syringic acid	0.058	0.054	0.051	0.068	0.068	0.078
Syringaldehyde	1.37	1.37	1.33	1.20	1.20	1.08
p-Coumaric acid	0.010	0.009	0.011	0.012	0.009	0.009
Ferulic acid	0.030	0.023	0.026	0.027	0.029	0.032
Total	2.44	2.35	2.37	2.30	2.18	2.03
Lignin content	4.8	4.6	4.6	4.5	4.3	4.0

[a,b,c] Corresponding to HLCs in Table 2.

TABLE 6
The Yield (Percent Residual Hemicelluloses, w/w) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of the Associated Lignin in the Residual Hemicellulosic Fractions

Phenolic acids and aldehydes	5% NaOH (50°C) treatment time (hr)				7.5% NaOH 50°C, 6 hr[b]	10% NaOH 50°C, 6 hr[c]
	4[a]	6[a]	8[a]	12[a]		
p-Hydroxybenzoic acid	0.086	0.088	0.084	0.088	0.047	0.090
p-Hydroxybenzaldehyde	0.12	0.12	0.12	0.13	0.12	0.10
Vanillic acid	0.13	0.14	0.14	0.14	0.12	0.11
Vanillin	0.34	0.34	0.32	0.32	0.30	0.26
Syringic acid	0.045	0.046	0.046	0.042	0.042	0.034
Syringaldehyde	0.12	0.12	0.11	0.12	0.18	0.10
p-Coumaric acid	0.010	0.011	0.010	0.012	0.011	0.009
Ferulic acid	0.031	0.026	0.028	0.024	0.043	0.034
Total	0.88	0.89	0.86	0.88	0.86	0.74
Lignin content	1.7	1.7	1.7	1.7	1.7	1.5

[a,b,c] Corresponding to residual hemicellulosic fractions in Table 3.

TABLE 7
The Yield (Percent Cellulose, w/w) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of the Associated Lignin in the Cellulosic Fractions

Phenolic acids and aldehydes	5% NaOH (50°C) treatment time (hr)				7.5% NaOH 50°C, 6 hr[b]	10% NaOH 50°C, 6 hr[c]
	4[a]	6[a]	8[a]	12[a]		
p-Hydroxybenzoic acid	0.15	0.15	0.14	0.12	0.12	0.081
p-Hydroxybenzaldehyde	0.074	0.071	0.062	0.058	0.051	0.040
Vanillic acid	0.079	0.074	0.061	0.064	0.052	0.049
Vanillin	0.085	0.097	0.16	0.072	0.069	0.052
Syringic acid	0.017	0.014	0.012	0.010	0.011	0.009
Syringaldehyde	0.062	0.057	0.059	0.047	0.038	0.042
p-Coumaric acid	0.072	0.060	0.054	0.045	0.061	0.056
Ferulic acid	0.061	0.046	0.048	0.046	0.062	0.054
Total	0.60	0.57	0.60	0.46	0.46	0.38
Lignin content	1.2	1.1	1.2	0.9	0.9	0.7

[a,b,c] Corresponding to cellulosic fractions in Table 4.

Residual hemicelluloses, extracted with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 16 hr from the corresponding alkali-treated and delignified poplar chips, yielded vanillin as the major phenolic component, as well as smaller amounts of syringaldehyde, vanillic acid, and *p*-hydroxybenaldehyde (Table 6). Minor quantities of *p*-hydroxybenzoic acid, syringic acid, *p*-coumaric acid, and ferulic acid were also identified in the nitrobenzene oxidation mixtures. This observation implied that the hemicelluloses, extracted with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ from the delignified poplar chips, are chemically linked to lignin mainly via guaiacyl units.

Results in Table 7 show the phenolic composition obtained by alkaline nitrobenzene oxidation of residual lignin derived from cellulose preparations. Compared to the hemicellulosic preparations, a much lower content of associated lignin (0.7–1.2%) indicated that these cellulose fractions are essentially lignin-free. Nevertheless, all of the nitrobenzene oxidation products detected were found to be rich in *p*-hydroxybenzoic acid, ranging between 21.3 and 26.1% of the total phenolics detected. These data were rather unexpected because *p*-hydroxybenzoic acid was found to be esterified mainly to lignin, was easily hydrolyzed by alkali, and was not easily hydrolyzed to wall polysaccharides (13). Kim et al. (17) also demonstrated that the content of the ether-linked *p*-hydroxybenzoic acid was much less than ester-linked *p*-hydroxybenzoic acid in the cell walls of fast-growing poplar. The reason for the relatively higher content of *p*-hydroxybenzoic acid in the cellulose fractions is presumably due

to the heterogeneous location of ether-linked *p*-hydroxybenzoic acid in the cell walls of poplar wood. More research is needed to clarify this hypothesis.

Molecular-Average Weight

The weight-average ($\overline{M}_w$) and number-average ($\overline{M}_n$) molecular weights, and the polydispersity ($\overline{M}_w/\overline{M}_n$) of the HLC and residual hemicellulosic fractions are presented in Tables 8 and 9, respectively. Because of the associated lignin in HLC and the much lower concentration of alkali used, the HLC preparations, extracted with 5–10% NaOH from the lignified poplar chips, clearly showed a higher degree of polymerization with molecular-average weights ranging between 26,090 and 40,500 g·mol^{-1}. This was approximately twice the value of the residual hemicellulosic fractions, extracted with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ from the 5% NaOH-treated and delignified poplar chips. These results suggest that extraction of residual hemicelluloses with higher concentration of alkali may result in a concomitant degradation of macromolecular hemicelluloses, as in the case of 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ hemicellulose extraction. No significant differences in $\overline{M}_w$ were found between the HLC, extracted with 5, 7.5, and 10% NaOH at 50°C (6 hr) from lignified poplar chips, as shown by $\overline{M}_w$ ranging between 31,190 and 32,810 g·mol^{-1}. However, a substantial difference in $\overline{M}_w$ was observed in the corresponding residual hemicellulosic fractions, as shown by $\overline{M}_w$ ranging between 19,130 and 29,730 g·mol^{-1}. In addition, Table 8 demonstrates that an increase in extraction time from 4 to 6, or 8 hr yields a corresponding increase in $\overline{M}_w$ from 26,090 to 31,190 and 40,500 g·mol^{-1}, respectively for 5% NaOH extractions of HLC. On the other hand, the 5% NaOH-soluble HLC decreased $\overline{M}_w$ from 40,500 to 31,200 g·mol^{-1} during a further extraction period of 8–12 hr, suggesting that a prolonged extraction for >8 hr may also lead to some degradation of the solubilized HLC.

TABLE 8
Weight-Average ($\overline{M}_w$) and Number-Average ($\overline{M}_n$) Molecular Weights and Polydispersity ($\overline{M}_w/\overline{M}_n$) of the HLCs Isolated with 5%, 7.5%, and 10% NaOH at 50°C for Various Periods from Dewaxed Poplar Chips

	Alkali treatment conditions					
	5% NaOH (50°C) treatment time (hr)					
	4[a]	6[a]	8[a]	12[a]	7.5% NaOH 50°C, 6 hr[b]	10% NaOH 50°C, 6 hr[c]
$\overline{M}_w$	26090	31190	40500	31200	31880	32810
$\overline{M}_n$	2900	2910	3160	4160	3250	3040
$\overline{M}_w/\overline{M}_n$	9.0	10.7	12.8	7.5	9.8	10.8

[a,b,c] Corresponding to HLCs in Table 2.

TABLE 9
Weight-Average ($\overline{M}_w$) and Number-Average ($\overline{M}_n$) Molecular Weights and Polydispersity ($\overline{M}_w/\overline{M}_n$) of the Residual Hemicellulosic Fractions Extracted with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 2 hr from the Corresponding Alkali-Treated and Delignified Poplar Chips

	Alkali treatment conditions					
	5% NaOH (50°C) treatment time (hr)					
	4[a]	6[a]	8[a]	12[a]	7.5% NaOH 50°C, 6 hr[b]	10% NaOH 50°C, 6 hr[c]
$\overline{M}_w$	18300	19130	23890	18800	29730	26860
$\overline{M}_n$	3010	2850	3580	2650	3960	3440
$\overline{M}_w/\overline{M}_n$	6.1	6.7	6.7	7.1	7.5	7.8

[a,b,c] Corresponding to residual hemicellulosic fractions in Table 3.

HPLC elution profiles of the residual hemicellulosic fraction extracted with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ from the 5% NaOH-treated (50°C, 8 hr) and delignified poplar chips yielded three peaks (Fig. 2). The molecular weight distribution ranged from >1,000,000 to <1000 $g \cdot mol^{-1}$. Peak I started to elute from a volume of 6.5 mL and had a molecular weight ≥237,140

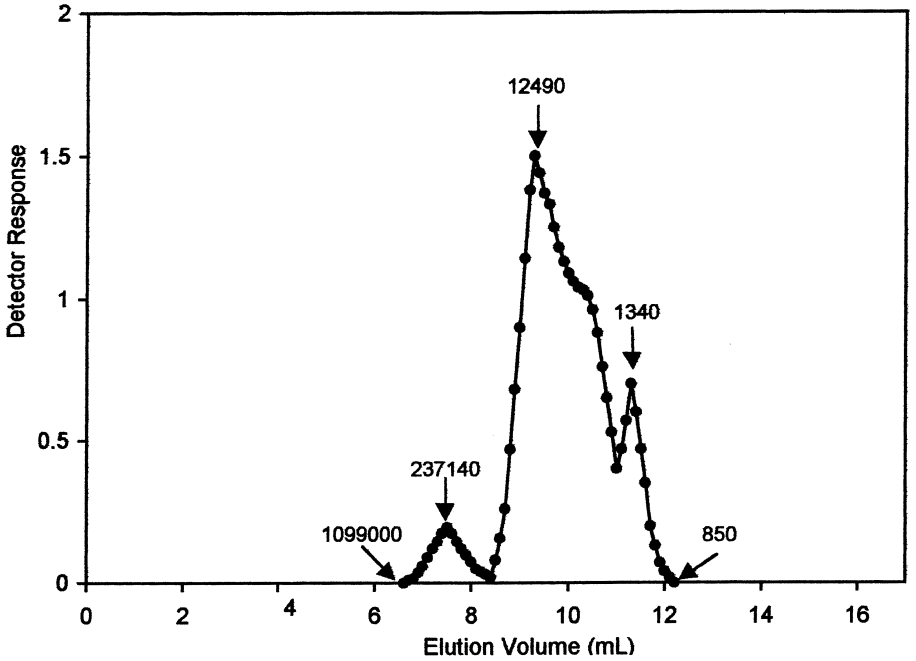

FIG. 2 Gel permeation chromatography molecular weight distribution of residual hemicellulosic fraction extracted with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10\ H_2O$ at 20°C for 2 hr from the alkali-treated (5% NaOH at 50°Cfor 8 hr) and delignified poplar chips.

$g \cdot mol^{-1}$. The major peak (peak II) had a molecular weight value around 12,490 $g \cdot mol^{-1}$. Low molecular weight material resulting from the fragmentation of residual hemicelluloses during the 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ extraction process eluted in peak III ($\overline{M}_w$, 1340 $g \cdot mol^{-1}$).

FT-IR Spectra

The FT-IR spectra in the frequencies of 800 and 4000 cm^{-1} of HLC from the dewaxed poplar chips extracted with 5% NaOH at 50°C for 4 hr (spectrum 1) and 12 hr (spectrum 2), 7.5% NaOH at 50°C for 6 hr (spectrum 3), and 10% NaOH at 50°C for 6 hr (spectrum 4) are shown in Fig. 3. The four spectral profiles and relative intensities of the bands were rather similar, confirming structural similarity between the HLC. A band at 1620 cm^{-1} is principally associated with absorbed water (18). Bands between 1125 and 1000 cm^{-1} are typical of xylans. The prominent band at 1043 cm^{-1} is attributed to the C–O stretching in hemicelluloses (19). The sharp band at 897 cm^{-1}, which corresponds to the C_1 group frequency or ring frequency, is characteristic of β-glycosidic linkages between the sugar units (20). The bands at 1467, 1428, 1381, and 1262 cm^{-1} represent C–H, O–H, or CH_2 bendings. The occurrence of very small bands at 1510 and 1332 cm^{-1} (data not shown) are assigned to the aro-

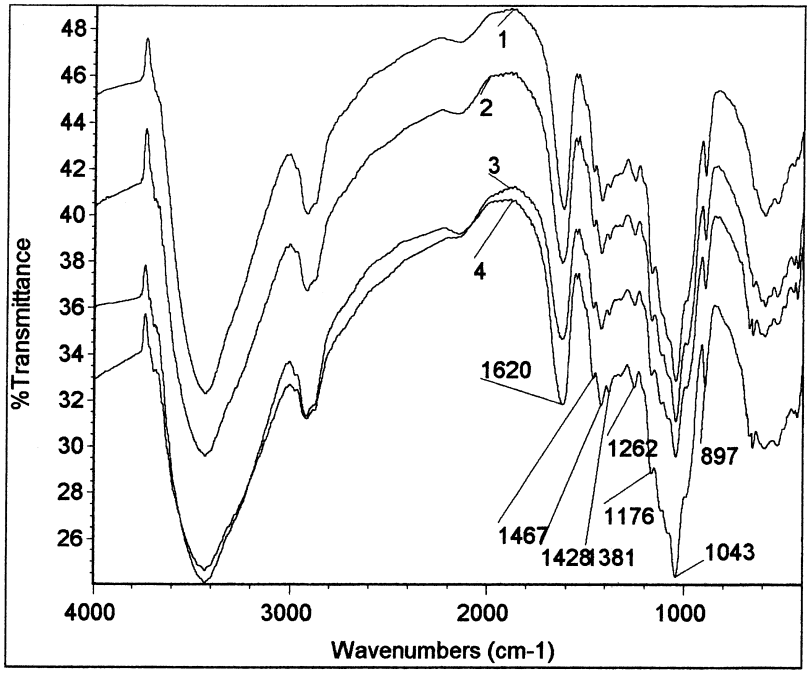

FIG. 3 FT-IR Spectra of HLCs extracted with 5% NaOH at 50°C for 4 hr (spectrum 1), 5% NaOH at 50°C for 12 hr (spectrum 2), 7.5% NaOH at 50°C for 6 hr (spectrum 3), and 10% NaOH at 50°C for 6 hr (spectrum 4) from dewaxed poplar chips.

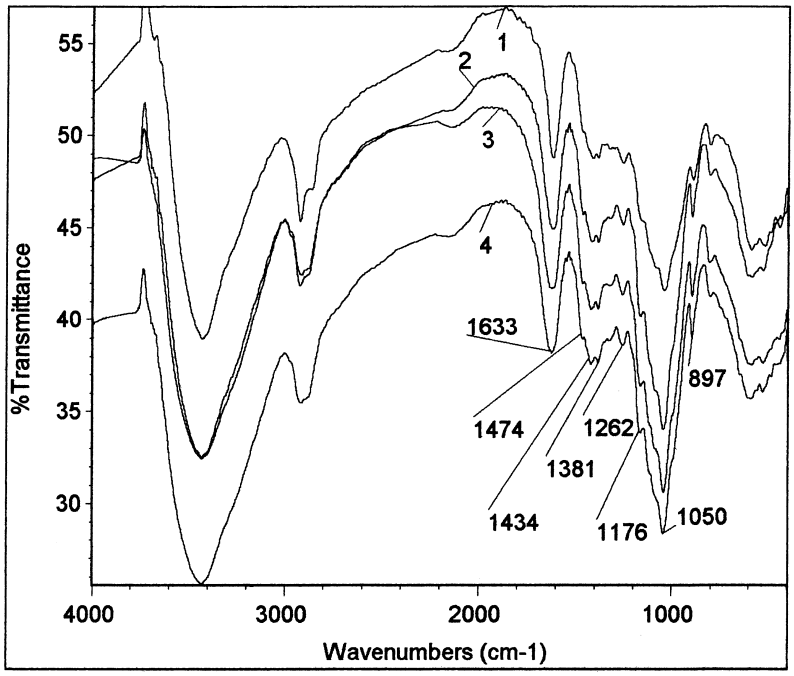

FIG. 4 FT-IR Spectra of residual hemicellulosic fractions extracted with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 2 hr from the corresponding alkali-treated and delignified poplar chips (spectrum 1, alkali treatment with 5% NaOH at 50°C for 4 hr; spectrum 2, alkali treatment with 5% NaOH at 50°C for 12 hr; spectrum 3, alkali treatment with 7.5% NaOH at 50°C for 6 hr; and spectrum 4, alkali treatment with 10% NaOH at 50°C for 6 hr).

matic skeleton vibrations and the syringyl ring breathing with CO stretching in bound lignin (16). In all of the spectra, the latter two bands are undoubtedly due to the presence of small amounts of associated lignin in the HLC, which also support results obtained by alkaline nitrobenzene oxidation.

The four FT-IR spectra of residual hemicelluloses (Fig. 4), extracted with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 2 hr after poplar chips were treated with the 5% NaOH at 50°C for 4 hr (spectrum 1) and 12 hr (spectrum 2), 7.5% NaOH at 50°C for 6 hr (spectrum 3), and 10% NaOH at 50°C for 6 hr (spectrum 4), respectively, and sequentially delignified, appear to be rather similar to the corresponding spectra for the four HLCs. The only difference lies in the vertical disappearance of resonance bands at 1510 and 1332 cm^{-1}, corresponding to associated lignin. These results also confirm the data obtained by alkaline nitrobenzene oxidation.

Figure 5 shows two analogous FT-IR spectra of cellulose fractions obtained by extraction with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 2 hr after the poplar chips were treated with the 5% NaOH at 50°C for 4 hr (spectrum 1) and 10% NaOH at 50°C for 6 hr (spectrum 2) respectively, and sequentially delignified. Absorbances at 1375, 1169, 1070, 1023, and 897 cm^{-1} in the two

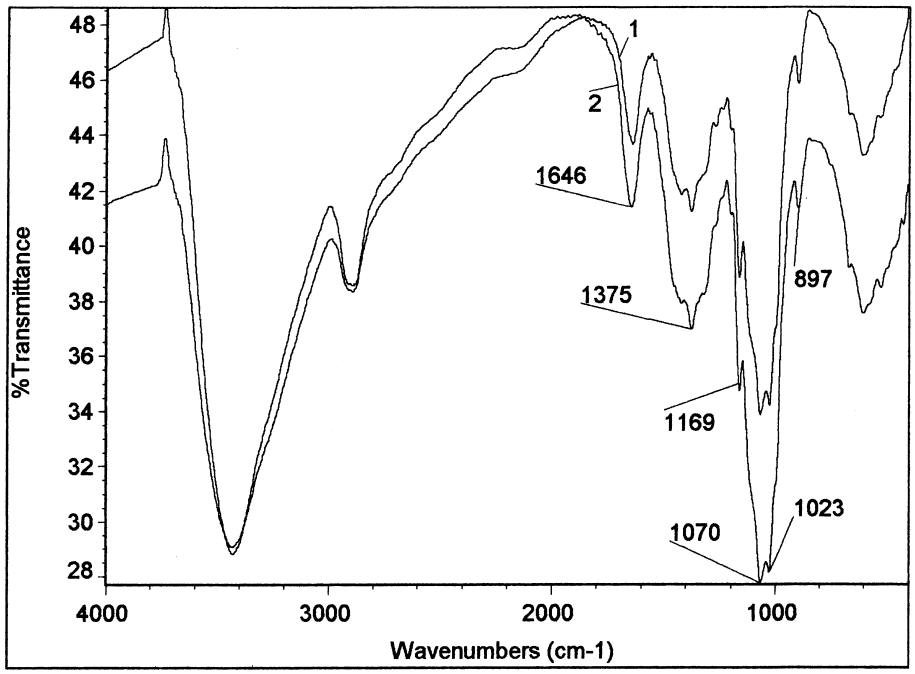

FIG. 5 FT-IR Spectra of cellulosic fractions obtained by treatment with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 2 hr from the corresponding alkali-treated (spectrum 1 derived from 5% NaOH treated at 50°C for 4 hr; spectrum 2 derived from 10% NaOH treated at 50°C for 6 hr) and delignified poplar chips.

spectra are typical of cellulose. All of the spectra have an intense absorbed water-related absorbance at 1646 cm^{-1}. The lignin-related absorbance at 1512 cm^{-1} is poorly resolved in the spectra, reflecting cellulose that is relatively free of chemically linked lignin.

^{13}C-NMR Spectrum of Residual Hemicelluloses

To obtain further information about the configuration of the glycosidic linkages in the hemicellulosic fractions, the ^{13}C-NMR spectroscopic analysis was performed. ^{13}C-NMR spectroscopy (in D_2O) of the residual hemicelluloses, extracted with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 2 hr after 5% NaOH at 50°C (4 hr) treatment of the chips, is illustrated in Fig. 6. The spectrum was interpreted on the basis of data reported for structurally defined arabinoxylan-type, glucoronoxylan-type, and L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan polysaccharides (21–23). The spectra were complicated by the relatively large molecular weights of the hemicelluloses and by the high pH of each sample. Three drops of 40% sodium deuteroxide, which was necessary to dissolve the polymer samples, caused line broadening and uneven baseline problems. The main 1,4-linked β-D-Xylp units are obviously charac-

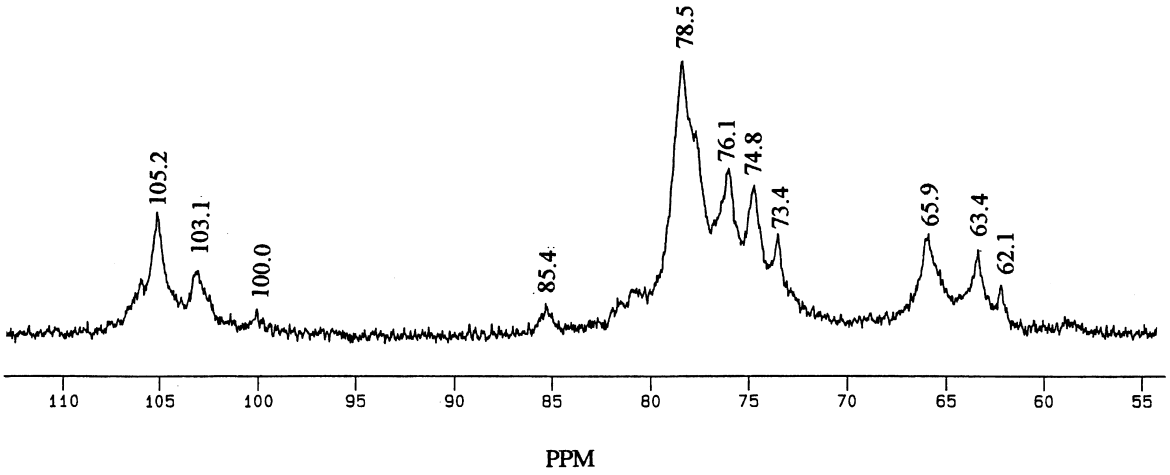

FIG. 6 ^{13}C-NMR spectrum of residual hemicellulosic fraction extracted with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 2 hr from 5.0% NaOH-treated (50°C, 6 hr) and delignified poplar chips.

terized by five strong signals at 105.2, 78.5, 76.1, 74.8, and 65.9 ppm, which are assigned respectively to C-1, C-4, C-3, C-2, and C-5 positions of the β-D-xylp units. Signals at 103.0, 73.4, and 63.4 ppm correspond to C-1, C-3, and C-6 of D-Manp residues, respectively. The signal at 62.1 ppm originates from C-6 in D-Glcp units. Signals attributed to C-1 and C-4 of MeG1A residues in the hemicelluloses appear at 100.0 and 85.4 ppm, respectively. These values indicated that the anomeric configuration of the glycosidic linkage of the D-xylopyranose residues in the main chain of the hemicelluloses is β form.

CONCLUSIONS

When combined, the results demonstrated that treatment of dewaxed poplar chips with 5% NaOH at 50°C for 4–12 hr, and 7.5% NaOH and 10% NaOH at 50°C for 6 hr solubilized 17.4–21.9% HLC, corresponding to 65.4–82.3% of the original hemicelluloses. These HLCs, which contained 4.0–4.8% bound lignin, are substantially linear and acidic as shown by the comparatively high contents of xylose and uronic acids. Extraction of the corresponding alkali-treated and delignified poplar chips with 17.5% NaOH–1% $Na_2B_4O_7 \cdot 10H_2O$ at 20°C for 2 hr yielded 4.3–9.0% residual hemicelluloses, in which the content of the associated lignin was 50% lower. The latter polymers are less acidic and may contain an appreciable amount of glucomannan, as indicated by comparatively lower uronic acid content and noticeable contents of mannose and glucose. In addition, all of the HLC were of a large molecular size, with $\overline{M}_w$ values between 26,090 and 40,500 g·mol^{-1}, whereas the residual hemicelluloses were of a relatively smaller molecular size ($\overline{M}_w$, 18,300–29,730

$g \cdot mol^{-1}$). The cellulose yield ranged between 43.9 and 44.5% of the total dry matter, and no considerable degradation of cellulose was obtained during the various extraction procedures.

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