Journal of Applied Polymer Science

Chemical and Structural Characterization of Alkaline-Extractable Hemicelluloses from Various Eucalyptus Species

Wen-bang Wei,[1,2] Li-na Li,[2] Liang Chang,[1] Zheng Wang[1]

[1]Research Institute of Wood Industry, Chinese Academy of Forestry, Beijing 100091, China

[2]Department of Art Design, Guangxi Eco-Engineering Vocational and Technical College, Liuzhou, Guangxi 545004, China

Correspondence to: Z. Wang (E-mail: wbweiwood@126.com)

ABSTRACT: Eucalyptus species are currently one of the main feedstock for pulping and papermaking industry in China. In the present study, alkali-extractable hemicelluloses were isolated from different eucalyptus species (*Eucalyptus camaldulensis, E. urophylla* × *grandis, and E. urophylla* × *E. tereticornis*) at mild conditions prior to pulping. Structural characterization of these hemicellulosic polymers based on monosaccharide, molecular weight, Fourier transform infrared, ^{1}H, ^{13}C, and two-dimensional heteronuclear single quantum coherence nuclear magnetic resonance analysis revealed that these alkali-extractable polysaccharides shared the common 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. The potential structures of the alkali-extractable hemicelluloses were proposed based on the comprehensive analysis. The well-characterized structures of these hemicelluloses could enlarge the industrial application of these hemicelluloses from the Eucalyptus species in a biorefinery process. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 2390–2398, 2013

KEYWORDS: spectroscopy; polysaccharides; cellulose and other wood products

Received 3 February 2013; accepted 15 April 2013; Published online 25 May 2013

DOI: 10.1002/app.39430

INTRODUCTION

Lignocellulosic materials (LCM) are a huge resource on the earth for sustainable development in the future. The biorefinery concept is based on the selective separation of the major LCM components (cellulose, hemicelluloses, and lignin), thus achieve the goal of value-added development of the separated components.[1]

Hemicelluloses, the third most abundant constituent of lignocellulosic biomass, are not a chemically well-defined compound but rather a family of polysaccharides, composed of different five- and six-carbon monosaccharide units.[2] Hemicelluloses can be utilized in their native and modified forms in various areas, including food and nonfood applications. A recent review contributing to the fractional purification and bioconversion of hemicelluloses has been published.[3] In conventional kraft pulping processes, most of the hemicelluloses from wood are degraded into oligomers or mono sugars, and dissolved in black liquor along with dissolved lignin and the pulping chemicals (inorganic substances). From the viewpoint of biorefinery, pre-exaction and isolation of hemicelluloses followed by the production of value-added products such as ethanol, sugar-based polyesters, other chemicals, and biopolymers offer a potential opportunity to realize the overall goal of biorefinery.[4]

Heretofore, there have been many methods to isolate hemicellulosic polymers from the cell wall of various LCM. The methods investigated include: alkaline extraction, alkaline peroxide extraction, liquid hot water extraction, steam explosion-based extraction, and so on.[3] However, an appropriate method has yet to be reported for selective liberation of all hemicelluloses from other components of the cell wall without degradation during the isolation process. Generally, hemicelluloses isolated with alkali at mild conditions were widely accepted. Alkaline extraction has been shown to be very effective for the removal of hemicelluloses.[5] The general mechanism of alkali treatment is including cleaving the α-ether linkages between lignin and hemicelluloses, as well as the ester bonds between lignin and/or hemicelluloses and hydroxycinnamic acids, such as *p*-coumaric and ferulic acids in the Gramineae species or grass species.[5] Abundant and significant studies have been conducted by Prof. Sun's group at Beijing Forestry University (Beijing, China) and optimized condition for extracting hemicelluloses has been developed, just to mention a few.[2,6,7] Among these studies, hemicelluloses and lignin could be extracted based on two-step process, hemicelluloses could be obtained by pouring the neutral and concentrated extractions (adjusting the pH 5.5–6.0) into ethanol solution, while remained lignin in ethanol solution could be obtained by adjusted the concentrated ethanol solution into about pH 2.0.[3] This procedure is an eco-friendly extraction process and has,

thus, been recently used in the isolation of hemicelluloses from different agricultural wastes, such as wheat straw, sugar beet pulp, barley straw, maize stems, rye straw, and rice straw.[8–10]

Fast-growing hardwood species are presently the most important source for pulp production in China, including poplar and eucalyptus species. Generally, poplar mainly grows in Northern China, while Eucalyptus is dominant in Southern China, which is a second fast-growing hardwood species after poplar in China. Even the native structural features of hemicelluloses from Eucalyptus species, such as acetylated heteroxylan from Eucalyptus globulus *Labill*, have been investigated.[11] However, structural features of hemicelluloses from different method are still uncertain, such as the structural features of hemicelluloses collected after alkaline pretreatment at mild conditions. In addition, structural determination of the isolated hemicelluloses is of prime importance prior to prepare hemicelluloses-based material.

In the present study, the chemical and structural characterization of alkali-extractable hemicelluloses from various Eucalyptus species were investigated by high-performance anion exchange chromatography (HPAEC), gel permeation chromatography (GPC), Fourier transform infrared (FT-IR) spectroscopy, ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR), and two-dimensional heteronuclear single quantum coherence (2D-HSQC) NMR spectroscopy. It is hoped that the well-characterized structures of the isolated hemicelluloses could pave avenues to convert these hemicelluloses into green and novel biomaterial from the Eucalyptus species in a biorefinery process.

EXPERIMENTAL

Materials

Eucalyptus species (*E. camaldulensis, E. urophylla* × *grandis, and E. urophylla* × *E. tereticornis*) were obtained from the experimental farm of the China Eucalypt Research Centre (Zhanjiang, Guangdong, China). They were dried in sunlight and then cut into small pieces with a plant shredder (Shanghai, China). The pieces were ground and screened to prepare 20–40 mesh size particles. The screened eucalyptus species were first extracted with 2 : 1 (v/v) toluene–ethanol in a Soxhlet apparatus for 6 h, and the dewaxed particles were allowed to dry in an oven at 60°C for 12 h. All chemicals used were of analytical or reagent grade and directly used as purchased without further purification.

Pre-Extraction of Hemicelluloses

The hemicelluloses were extracted from three Eucalyptus species (100 g, respectively) with aqueous sodium hydroxide (1*M*, 4%,

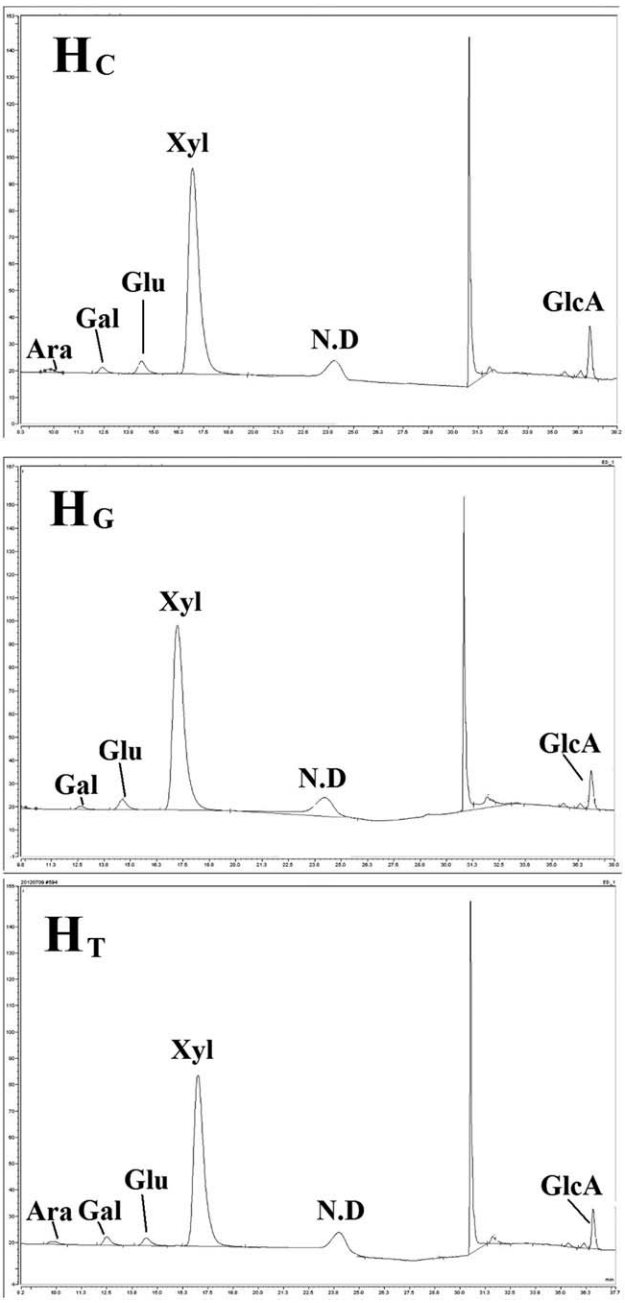

Figure 1. Sugar analysis curves of alkali-extractable hemicelluloses fractions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. Yield and the Relative Content of Neutral Monosaccharide and Uronic Acid of the Isolated Hemicelluloses from Different *Eucalyptus* Species

Eucalyptus species	Yield (%)	Ara[a]	Gal[a]	Glu[a]	Xyl[a]	UA[a]	X/U[b]
Eucalyptus camaldulensis (C)	5.10	0.92	1.49	3.84	77.54	16.18	6.2
Eucalyptus urophylla × *grandis (G)*	5.50	0.75	0.92	3.53	80.06	14.74	7.3
Eucalyptus urophylla × *E. tereticornis (T)*	6.50	1.39	2.63	3.01	77.80	15.18	6.6

[a] Ara, Arabinose; Gal, Galactose; Glu, Glucose; Xyl, Xylose; UA, Uronic acid.

[b] X/U, X = Xyl/150, U = UA/194. This means the relative molar ratios of the hemicelluloses.

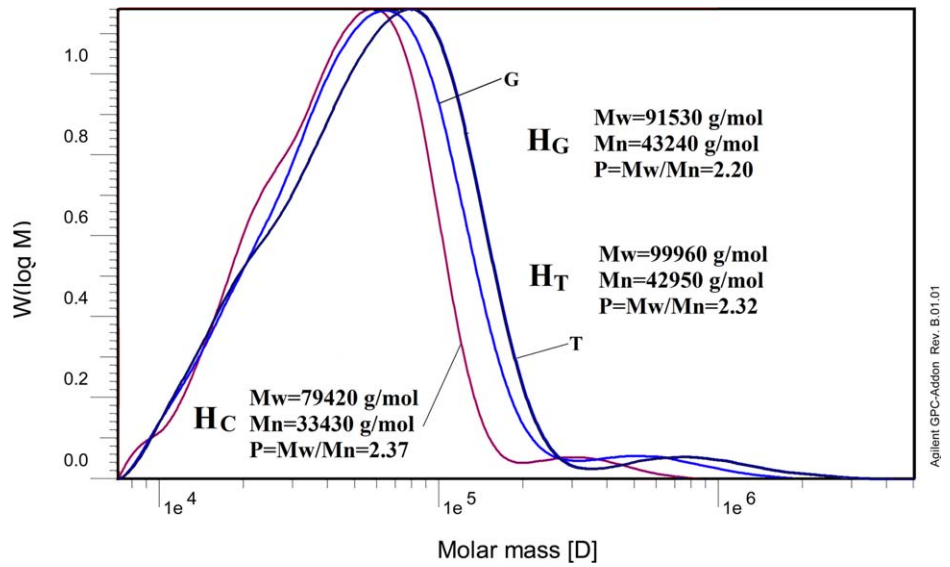

Figure 2. GPC curves of alkali-extractable hemicelluloses fractions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

solid/liquid, 1 : 20) at 75°C for 3 h. After the indicated period of time, the insoluble residue was collected by filtration through a cloth on a Buchner funnel, washed repeatedly with distilled water and 95% ethanol, and then oven-dried. The supernatant fluid was neutralized to pH 5.5 with 6*M* HCl and then concentrated under a vacuum condition. The concentrated supernatant fluid was poured into three volumes of 95% ethanol (r.t., 2 h) to precipitate the hemicelluloses. After filtration with filter paper on Buchner funnel, the isolated hemicelluloses were obtained, then redissolve the hemicelluloses in 100 mL water, the mixture was then poured into three volumes of 95% ethanol (r.t., 2 h) to get purified hemicelluloses. After filtration, the hemicelluloses were then freeze dried.

Chemical and Molecular Analysis

To determine the composition of the isolated hemicelluloses, the neutral sugars and uronic acids in the isolated hemicellulosic fractions were determined by HPAEC according to a publication.[12,13] The monosaccharide in the hemicelluloses-rich fractions was obtained by hydrolyzing 5 mg samples with 10% H_2SO_4 for 2.5 h at 105°C. The hydrolyzates were diluted to

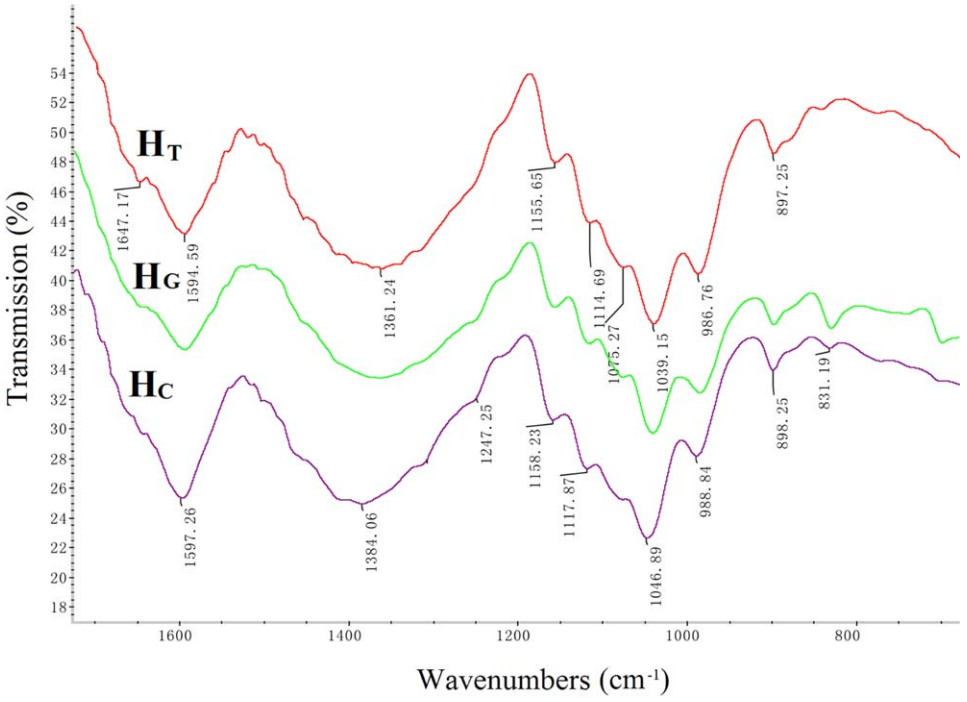

Figure 3. FT-IR spectra of alkali-extractable hemicelluloses from different *Eucalyptus* species. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

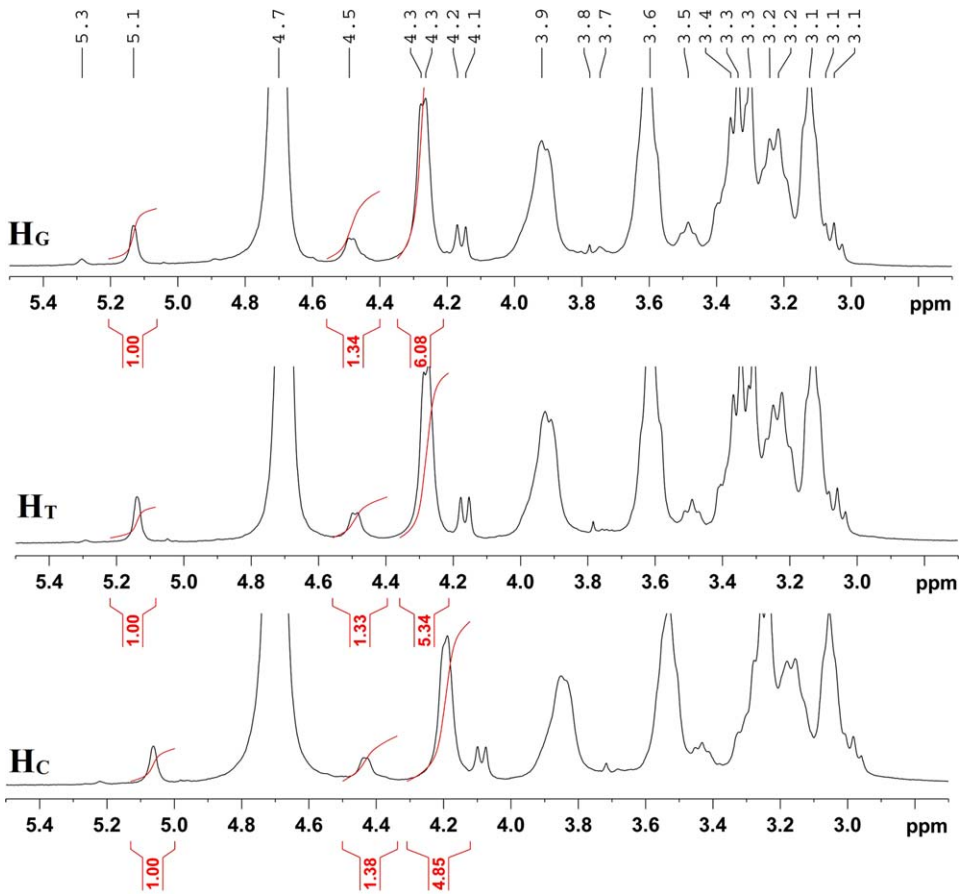

Figure 4. ^{1}H NMR spectra of alkali-extractable hemicelluloses from different *Eucalyptus* species. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

50-fold (after adjusted the pH value to 7.0), filtered, and injected into the HPAEC (Dionex ICS-3000, DIONEX, USA) with pulsed amperometric detection, a CarboPacTM PA20 column (3 × 150 mm^2), and a CarboPacTM Guard column (3 × 30 mm^2). The operations were run at 30°C for 30 min, and the flow rate during the analysis process was 0.5 mL/min. When eluting the monosaccharide, the eluent was 2 mM NaOH. However, due to the weak eluting power of the NaOH, the eluent was 100 mM NaAc and 2 mM NaOH when eluting the uronic acids. Measurements were conducted with two parallels, and error of the values was found within the range of 5%.

The molecular weights of the hemicellulosic preparations were determined by GPC on a PL aquagel-OH 50 column (300 × 7.7 mm, Polymer Laboratories) according to some previous publications,[3,10] calibrated with PL pullulan polysaccharide standards (peak average molecular weights of 783, 12,200, 100,000, 1600,000, Polymer Laboratories, Varian, USA). A flow rate of 0.5 mL/min was maintained. The eluent was 0.02*M* NaCl in 0.005*M* sodium phosphate buffer, pH 7.5. Detection was achieved with a Knauer differential refractive index detector. The column oven was kept at 30°C. Hemicelluloses were dissolved with 0.02*M* NaCl in 0.005*M* sodium phosphate buffer, pH 7.5, at a concentration of 0.1%. The experiments were analyzed in duplicate. The deviations or standard error were observed to be lower than 5%.

Spectroscopic Characterization

FT-IR spectra of hemicelluloses samples were obtained on an FT-IR spectrophotometer (Bruker Tensor 27, Bruker Corporation, Germany) 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 and ^{13}C-NMR spectra were obtained at 30°C on a Bruker MSL-600 MHz spectrometer (Bruker Corporation, Germany) with a 5 mm-PABBO probe head. The ^{1}H-NMR spectrum was recorded at 600 MHz using 20 mg of hemicelluloses in 1.0 mL of 99.8% D_2O. The chemical shifts were calibrated relative to the signals from D_2O, used as an internal standard, at 4.70 ppm for the ^{1}H-NMR spectra, and acquiring time (AQ) is 3.98 s, relaxation time is 2 s. The ^{13}C-NMR spectrum was recorded at 150 MHz and 80 mg samples were dissolved in 1.0 mL of 99.8% D_2O for the ^{13}C-NMR analysis. The ^{13}C-NMR spectrum was recorded at 25°C after 20,000 scans. A 30° pulse flipping angle, a 9.2 us pulse width, and 2.0 s delay time between scans were used, and acquired time (AQ) is 1.36 s.

The 2D-HSQC spectra were acquired by HSQCETGP experiment mode according to a previous publication,[13] a t1 spectral width of 12,000 Hz and at t2 width of 2000 Hz was adopted. The number of collected complex points was 1024 for ^{1}H-dimension with a relaxation delay of 2.0 s. The number of scans was 64, and 256 time increments were always recorded in

Applied Polymer SCIENCE

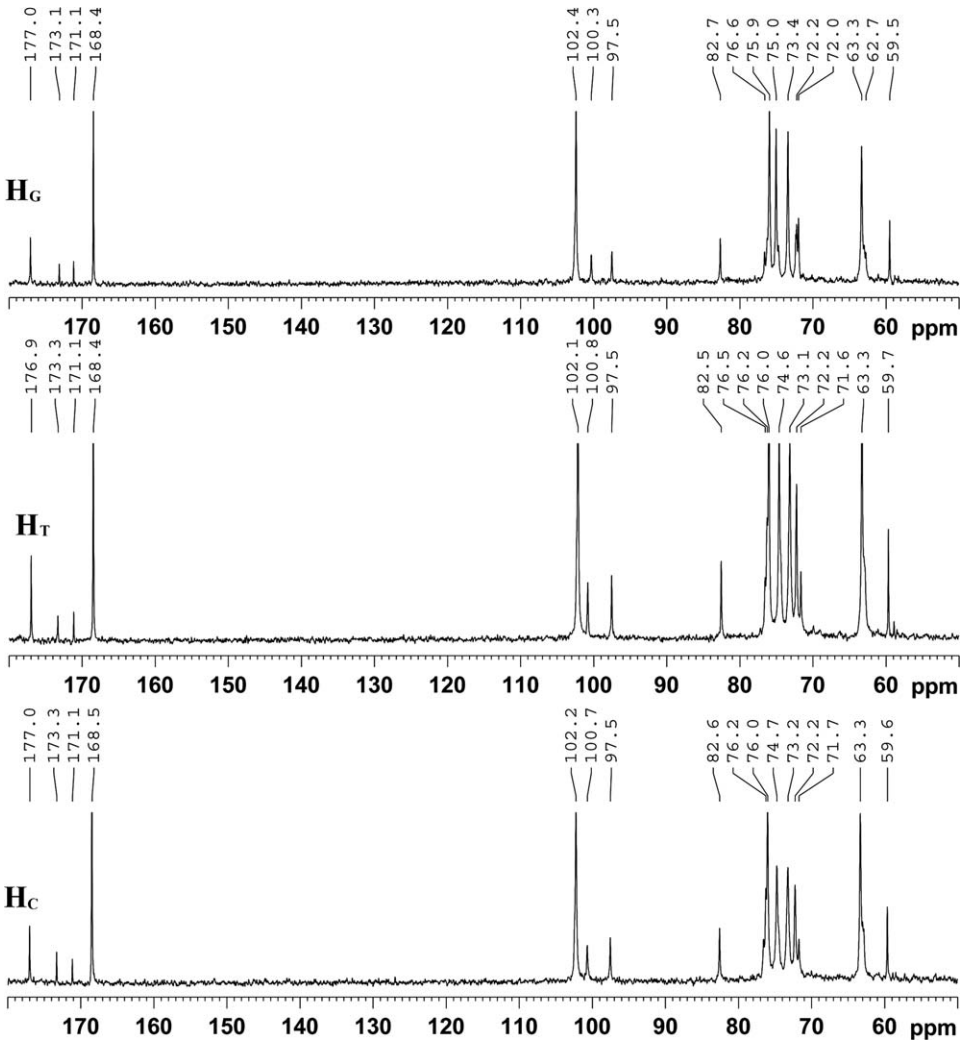

Figure 5. ^{13}C NMR spectra of alkali-extractable hemicelluloses from different *Eucalyptus* species.

^{13}C-dimension. The $^{1}J_{C\text{-}H}$ used was 146 Hz. Prior to Fourier transformation, the data matrixes were zero filled up to 1024 points in the ^{13}C-dimension. Data processing were performed using standard Bruker Topspin 2.1 software.

RESULTS AND DISCUSSION

Yield and Composition Analysis of the Alkali-Extractable Hemicelluloses

The hemicellulosic polymers are a mixture of a number of different polysaccharides, and the yield and composition of the complex can vary depending on the method of isolation.[3,12] In addition, the yield and composition is also related to the different raw LCM. In the present study, pre-extraction of dewaxed eucalyptus species with 4% aqueous NaOH at 75°C for 3 h released 5.1–6.5% of the hemicelluloses based on the dried dewaxed Eucalyptus species. This suggested that most of hemicelluloses can be collected in pre-extraction and the yields of the hemicelluloses depend on the wood species when the same method was applied. To understand the composition of the hemicelluloses fractions, sugar analysis of these hemicelluloses were conducted (Figure 1) and the results are shown in Table I. It was found that xylose (77.54–80.06%) and uronic acid (14.74–16.18%) were the predominant sugar components of the three alkali-extractable hemicelluloses, and small amounts of arabinose (0.75–1.39%), galactose (0.92–2.63%), and glucose (3.01–3.84%) were also detected. The data showed that alkali-extractable hemicelluloses were mainly composed of xylose and uronic acid, suggesting that these hemicelluloses belonged to glucuronoxylans, which are in agreement with a previous publication on the hemicelluloses of eucalyptus species.[11] The results of the sugar analysis also suggested that eucalyptus hemicelluloses comprise glucuronoxylans differing in the molar ratio of xylose to uronic acids (X/U). The X/U ratio is 6.2 (H_C), 7.3 (H_G), and 6.6 (H_T) for the hemicelluloses extracted from *E. camaldulensis (C)*, *E. urophylla* × *grandis (G)*, and *E. urophylla* × *E. tereticornis (T)*, respectively. However, on the basis of the sugar analysis alone, it is still difficult to deduce the exact branching patterns and chemical structure of the hemicelluloses.

Molecular Weight Analysis

Molecular weight distribution curves of the hemicellulosic fractions were illustrated in Figure 2. It was observed that all the

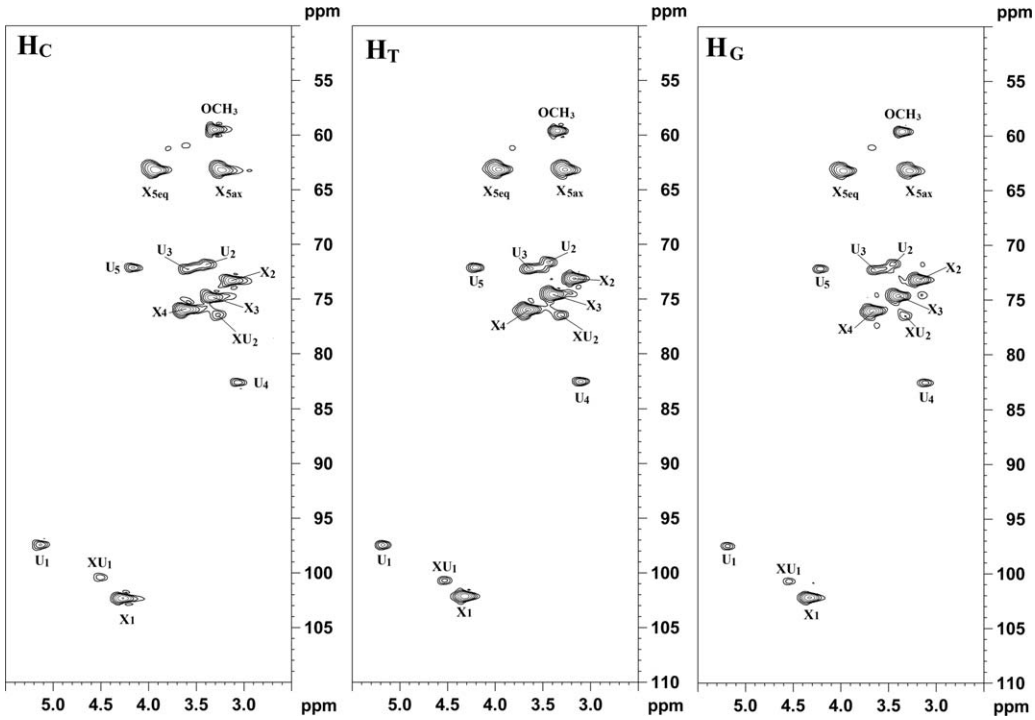

Figure 6. 2D-HSQC NMR spectra of alkali-extractable hemicelluloses from different *Eucalyptus* species.

curves showed unimodal and narrow molecular distribution, indicating the homogeneity of the hemicelluloses obtained. The weight-average (M_w) and number-average (M_n) molecular weights along with polydispersity (M_w/M_n) of all the hemicellulosic fractions were calculated based on the curves and the results are shown in Figure 2. It was found that the hemicelluloses isolated from different eucalyptus species exhibited M_w ranging between 79,420 and 99,960 g/mol, while M_n ranging between 33,430 and 43,240 g/mol. The result suggested that molecular weights of hemicelluloses vary depending on the different species when same extraction conditions used in this study. Additionally, the three alkali-extractable hemicelluloses gave narrower molecular weight distribution, corresponding to the polydispersity index of 2.12–2.37. This suggested that the extracting condition used is suitable to extract homogeneous hemicelluloses from different eucalyptus species, which will benefit subsequent chemical modification of the hemicelluloses.

FT-IR Spectra Analysis

FT-IR spectroscopy, which offers a potential for the assignment of absorbance bands to specific molecular structures, and combine with other analysis methods, can be used for approximate identification of polysaccharides in plant materials.[12] The FT-IR spectra of the alkali-extractable hemicellulosic fractions are shown in Figure 3. As can be seen, the spectral profiles and relative intensities of most bands appeared to be rather similar, indicating a similar structure of the three hemicellulosic samples. The spectra were assigned according to data presented in the literature.[14–16]

The higher wavenumber bands between 4000 and 1750 cm^{-1} are information lacking thus only the bands between 1750 and 700 cm^{-1} is presented in the spectra. Obviously, two bands at 1597 and 1411 cm^{-1} are assigned to the —COO— antisymmetric and symmetric stretching of glucuronic acid or glucuronic acid carboxylate, respectively.[12,16]

The remaining bands at 1235 and 1378 cm^{-1} represent OH in-plane and CH bending vibrations, respectively.[16] The bands at 1158 and 988 cm^{-1} are an index of arabinofuranosyl (Ara*f*) assignment, and a prominent absorption band between 1039 and 1046 cm^{-1} is assigned to the C—O—C stretching of glycosidic linkages, which is representative of xylans. Moreover, a sharp band at 898 cm^{-1} is indicative of the β-configuration of the 1→4 glycosidic bond between xylopyranose (Xyl*p*) units of the main xylan linkages.[17] Furthermore, an obvious band at 832 cm^{-1} is indicative of the presence of α-configuration glycosidic bond between sugar units. However, to obtain a deeper understanding of the structural features of the hemicelluloses, more and deeper investigations should be carried out.

NMR Analysis

The most powerful tool for polysaccharide analysis is NMR spectroscopy. To further investigate the structural features of the hemicelluloses, ^{1}H-NMR, ^{13}C-NMR, and 2D-HSQC spectrometry are conducted, and their spectra are shown in Figures 4–6.

^{1}H-NMR spectroscopy is very helpful for certain tasks, for example, determination of branching patterns and investigation of the interaction polysaccharide-solvent, because it is a fast method and the signal intensities can be used for quantification, in contrast to standard ^{13}C-NMR spectroscopy. The integral of 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, which is indicative of the residual solvent (D_2O). Signal at 8.30 ppm is

Table II. ^{1}H and ^{13}C Chemical Shifts (ppm) Assignments for Hemicelluloses from Different *Eucalyptus* Species

	Assignments (ppm)							
	1	2	3	4	5ax	5eq	6	OCH_3
H_C								
X[a]	102.3	73.2	74.8	75.9	63.3	63.3		
	4.26	3.12	3.33	3.61	3.91	3.23		
XU[b]	100.4	76.5	n.a.	n.a.	63.3	63.3		
	4.50	3.31	n.a.	n.a.				
U[c]	97.5	71.8	72.2	82.7	72.1		177.6	59.7
	5.14	3.39	3.58	3.07	4.17			3.30
H_G								
X[a]	102.1	73.1	74.6	76.0	63.2	63.2		
	4.32	3.17	3.39	3.66	3.97	3.27		
XU[b]	100.7	76.4	n.a.	n.a.	n.a.			
	4.53	3.31	n.a.	n.a.	n.a.			
U[c]	97.4	71.6	72.2	82.5	72.1		177.6	59.7
	5.17	3.44	3.64	3.10	4.20			3.35
H_T								
X[a]	102.2	73.1	74.6	76.0	63.2	63.2		
	4.32	3.19	3.40	3.66	3.97	3.27		
XU[b]	100.7	76.5	n.a.	n.a.	n.a.			
	4.54	3.27	n.a.	n.a.	n.a.			
U[c]	97.4	71.5	72.1	82.5	72.2		177.6	59.6
	5.18	3.45	3.62	3.11	4.21			3.35

X[a], (1→4)-linked *β*-D-Xylp (X).

XU[b], (1→4)-*β*-D-Xylp-2-*O*-(4-*O*-Me-α-D-GlcpA) (XU).

U[c], 4-*O*-Me-α-D-GlcpA (U).

assigned to proton of carboxy group from 4-*O*-Me-α-D-GlcA (not shown in Figure 4).[2,3,10,11] The chemical shifts of δ 3.1–4.3 are assigned to protons of anhydroxylose units of hemicelluloses.[18]

The relevant signals occurred in two regions, namely the anomeric region (δ 5.6–4.9 ppm for α-anomers and δ 4.9–4.3 ppm for *β*-anomers) and the ring proton region (δ 4.5–3.0).[19] Signals at δ 5.1 ppm in the ^{1}H-NMR spectrum has been assigned to protons at C-1 of 4-*O*-methyl-D-glucuronic acid residues. This confirmed that 4-*O*-methyl-D-glucuronic acid belonged to α-configuration, which is in agreement with the presence of small peak at 832 cm^{-1} in the FT-IR spectrum of all the hemicelluloses.

In ^{1}H-NMR spectra of the hemicelluloses, the average integration of all signals for different sugar residues (anomeric proton), demonstrate that the respective molar ratio of xylose to 4-*O*-methyl-D-glucuronic acid is 6.23 : 1, 6.67 : 1, and 7.42 : 1 for these hemicelluloses (H_C, H_T, and H_G). This result is typical of hardwood xylans as expected. Interestingly, from the sugar analysis of Table I, the corresponding ratio of xylose/uronic acids in these hemicelluloses was 6.19, 6.62, and 7.31. Another phenomenon found was that the shifting of chemical shift to high field region in the spectrum of H_C. Actually, the same amount (20 mg) of hemicelluloses was dissolved in 1.0 mL of D_2O and then investigated by NMR technique. Thus, the different chemical shift is mainly ascribed to different concentration, and this is further related to the molecular weights of the hemicelluloses samples. Combined with the molecular weights determined by GPC (Figure 2), it was observed that the H_G and H_T, which have higher molecular weights, showed slightly higher chemical shift than the corresponding signals in the spectrum of H_G. The reason for this could be explained by strong hydrogen bonding, which forms in a lower concentration of polymer and thus results in higher chemical shift.

^{13}C-NMR spectroscopy allows elucidation of the polymer backbone and can also be employed to evaluate the type of side-chain branching along the backbone.[2,12] The signals for ^{13}C were assigned on the basis of the HSQC spectrum following published NMR data for glucuronoxylans.[18–23] Indeed, the strongest signals at 102.4, 76.4, 74.7, 73.4, and 63.6 ppm are assigned to the C-1, C-4, C-3, C-2, and C-5 of the (1→4)-linked *β*-D-Xyl*p* (X) units, respectively. In addition, some signals appeared at 100.4, 76.5, and 63.1 ppm was probably attributed to the C-1, C-2, and C-5 of (1→4)-*β*-D-Xyl*p*-2-*O*-(4-*O*-Me-α-D-GlcpA) (XU), respectively. Because of substituent group at C-2 of xylan, hydrogen bonds were formed and then reduced

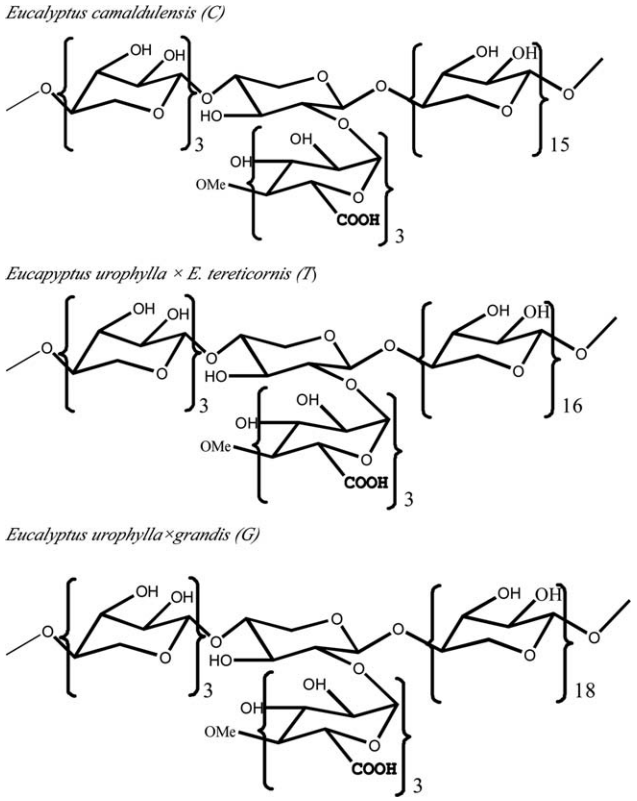

Figure 7. Potential structure of the alkali-soluble hemicelluloses from different *Eucalyptus* species.

the probability density of electron cloud around the nucleus and engendering deshielding effect, eventually leads to high δ value of (1→4)-β-D-Xyl*p* units. Moreover, some obvious signals observed at 97.9, 71.8, 72.2, 82.7, 72.1, 177.6, and 59.8 ppm are characteristic of C-1, C-2, C-3, C-4, C-5, C-6, and the methoxyl group of 4-*O*-methyl-D-glucuronic acid residues, respectively. Besides, the strong signal at 168.4 ppm was attributed to the C═O glucuronic acid carboxylate,[19] which was also in agreement with FT-IR analysis and ^{1}H-NMR spectra.

More specific information about the hemicellulosic fractions was obtained by 2D HSQC spectra. It was found that all the spectra exhibited similar patterns, suggested that the similar structures of the hemicelluloses. Obviously, xylan was observed at δ102.3/4.26 (C_1—H_1), 73.1/3.12 ppm (C_2—H_2), 74.8/3.33 ppm (C_3—H_3), 75.9/3.61 ppm (C_4—H_4), 63.6/4.06 (C_5—H_{5eq}), and 63.6/3.34 ppm (C_5—H_{5ax}), respectively. In addition, the presence of the methyl group in 4-*O*-Me-α-D-Glc*p*A was confirmed by strong correlated signals at δ59.7/3.35 ppm. The cross signals at δ97.7/5.17 ppm (C_1—H_1), 71.6/3.44 ppm (C_2—H_2), 72.2/3.64 ppm (C_3—H_3), 82.5/3.10 ppm (C_4—H_4), and 72.1/4.20 ppm (C_5-H_5) indicate that the presence of α-linked 4-*O*-Me-α-D-Glc*p*A units. Moreover, due to the linked side-chain (4-*O*-Me-α-D-Glc*p*A units), the substituted β-D-xylopyranosyl ((1→4)-β-D-Xylp-2-*O*-GlcpA, XU) was also observed at δ100.7/4.54 (C_1—H_1) and 76.5/3.27 (C_2—H_2) ppm. The results suggested their structure was very similar with a linear (1→4)-β-D-xylan backbone decorated with branches at *O*-2 of 4-*O*-methylglucuronic acid unit. In all, the assignment data of proton and carbon spectra are given in Table II, and the potential structures of the alkali-soluble hemicelluloses from different Eucalyptus species were given in Figure 7.

CONCLUSIONS

Based on the results above, it was found that alkaline extraction is an effective method to obtain hemicelluloses with an acceptable yield prior to pulping. The composition analysis, FT-IR and NMR spectroscopy show that structural features of the alkali-soluble hemicelluloses extracted under same condition from different Eucalyptus species are similar, belonged to the glucuronoxylans family. Detailedly, these alkali-soluble fractions are composed of a linear backbone of (1→4)-β-D-xylopyranosyl residues, having ramifications of 4-*O*-methyl-α-D-glucuronic acid residues monosubstituted at *O*-2. The expectation is that the enlarged knowledge of the composition of alkali-extractable hemicelluloses from different Eucalyptus species may contribute to a better industrial utilization of these isolated hemicellulosic polymers.

ACKNOWLEDGMENTS

This work was supported by the special fund for forestry-scientific Research in the Public Interest (No. 20100400410).

REFERENCES

1. Sun, R.C. *Bioresources* **2009**, *4*, 452.
2. Wen, J. L.; Xiao, L. P.; Sun, Y. C.; Sun, S. N.; Xu, F.; Sun, R. C.; Zhang, X. L. *Carbohyd. Res.* **2011**, *346*, 111.
3. Peng, F.; Peng, P.; Xu, F.; Sun, R. C. *Biotechnol. Adv.* **2012**, *30*, 879.
4. Huang, H. J.; Ramaswamy, S.; Tschirner, U. W.; Ramarao, B. V. *Sep. Purif. Technol.* **2008**, *62*, 1.
5. Sun, X. F.; Sun, R. C.; Fowler, P.; Baird, M. S. *J. Agric. Food. Chem.* **2005**, *53*, 860.
6. Sun, J. X.; Sun, X. F.; Sun, R. C.; Su, Y. Q. *Carbohydr. Polym.* **2004**, *56*, 195.
7. Xu, F.; Sun, J. X.; Geng, Z. C.; Liu, C. F.; Ren, J. L.; Sun, R. C.; Fowler, P.; Baird, M. S. *Carbohydr. Polym.* **2007**, *67*, 56.
8. Ruzene, D. S.; Silva, D. P.; Vicente, A. A.; Goncalves, A. R.; Teixeira, J. A. *Appl. Microbiol. Biotechnol.* **2008**, *147*, 85.
9. Sun, R. C.; Lawther, J. M.; Bank, W. B. *Carbohydr. Polym.* **1996**, *29*, 325.
10. Xiao, B.; Sun, X. F., Sun, R. C. *Polym. Degrad. Stab.* **2001**, *74*, 307.
11. Evtuguin, D. V.; Tomas, J. L.; Silva, A. M. S.; Neto, C. P. *Carbohydr. Res.* **2003**, *338*, 597.
12. Peng, F.; Ren, J. L.; Xu, F.; Bian, J.; Peng, P.; Sun, R. C. *J. Agric. Food. Chem.* **2010**, *58*, 5743.
13. Wen, J. L.; Sun, Y. C.; Xu, F.; Sun, R. C. *J. Agric. Food. Chem.* **2010**, *58*, 11372.
14. Sun, R. C.; Tomkinson, J. *Eur. Polym. J.* **2003**, *39*, 751.
15. Chatjigakisa, A. K.; Pappasa, C.; Proxeniab, N.; Kalantzib, O.; Rodisb, P.; Polissioua, M. *Carbohydr. Polym.* **1998**, *37*, 395.

16. Marchessault, R. H.; Liang, C. Y. *J. Polym. Sci.* **1962**, *59*, 357.

17. Gupta, S.; Madan, R.; Bansal, M. *Tappi J.* **1987**, *70*, 113.

18. Gruppen, H.; Hoffmann, R. A.; Kormelink, F. J. M.; Voragen, A. G. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.***1992**, *233*, 45.

19. Wu, Y. L.; Pan, Y. J.; Sun, C. R.; Hu, N.; Ishurd, O. *J. Nat. Prod.* **2006**, *69*, 1109.

20. Morine, C.; Krausz, P.; Chaleix, V.; Sainte-Catherine, O.; Kraemer, M.; Gloaguen, V. *J. Nat. Prod.* **2007**, *70*, 60.

21. Kardosova, A.; Matulova, M.; Malovikova, A. *Carbohydr. Res.* **1998**, *308*, 99.

22. Nabarlatz, D.; Ebringerova, A.; Montane, D. *Carbohydr. Polym.* **2007**, *69*, 20.

23. Teleman, A.; Lundqvist, J.; Tjerneld, F.; Stalbrand, H.; Dahlman, O. *Carbohydr. Res.* **2000**, *329*, 807.