Structural Features of Lignin and Lignin-Carbohydrate Complexes from Bamboo (*Phyllostachys pubescens* Mazel)

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Understanding the structural features of lignin (L) and lignin-carbohydrate complexes (LCC) in bamboo (*Phyllostachys pubescens* Mazel) is important in order to expand the use of bamboo biomass. In this study, L and LCC were isolated from four-year-old mature bamboo using an organic solvent as an extractant. The structural features of L and LCC were characterized *via* UV-vis, FT-IR, NMR, sugar composition analysis, and alkaline nitrobenzene oxidation. L and LCC contained almost the same structural units: guaiacyl (G), syringyl (S), *p*-hydroxybenzoate substructures (PB), *β*-*O*-4' substructures (A), 3-*O*-acetyl-(1→4)-linked-*β*-D-xylopyranosyl (*β*Xyl3), (1→4)-linked-*α*(*β*)-D-xylopyranosyl (*α*(*β*)Xyl), and 4-*O*-methyl-*α*-D-glucuronic acid (MeGlcA(1→4)). The contents of the units varied between L and LCC. Carbohydrates linked with lignin in L and LCC mainly consisted of xylans. The sugar units of carbohydrates linked with lignin in LCC were determined to be of xylose (76.98%), arabinose (4.08%), glucose (6.47%), mannose (6.34%), altrose (3.69%), galactose (1.75%), and ribose (0.69%). The S (75.80%) and G (24.20%) units with a high S/G ratio of 3.13 were the main structural elements of lignin associated with carbohydrates in LCC.

Keywords: Bamboo (Phyllostachys pubescens Mazel); Lignin; Lignin-carbohydrate complexes; Structural features

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INTRODUCTION

Bamboo is commonly used in various fields because of its wide distribution and broad applications in the forestry and construction industries. Bamboo is a tropical plant, and about 75 genera and 1250 species of bamboos grow in different parts of the world, especially in China (Market, Report No.11A001, 2001), Thailand (So *et al.* 1998), and India (Shanmughavel and Francis 1996). China alone has approximately 300 species in 44 genera of bamboo, occupying 33,000 km^2, or 3% of the country's total forest area (Qiu *et al.* 1992). Bamboo biomass is well known for its fast growth and versatility. Bamboo only requires a growing period of three years to reach maturity, which is extremely short compared with most wood species. Thus, bamboo is considered to be rapidly renewable and sustainable biomass material suitable for use in various fields. Several processes and products that utilize bamboo as a raw material have been reported, such as pulp and paper production, bamboo charcoal generation, and house construction (Yang *et al.* 2010; Choy *et al.* 2005; De Flander and Rovers 2009).

Bamboo (*Phyllostachys pubescens* Mazel), which has the widest distribution in China, can be used as a low-cost raw material for the industrial production of high-value products. *P. pubescens* Mazel contains about 40% to 45% cellulose, 28% to 34% hemicelluloses, and 13% to 24% Klason lignin, the amounts of which vary slightly depending on the age of the plant (Peng *et al.* 2012a, b). To better utilize the main components of *P. pubescens* Mazel, research should focus on understanding the structural characteristics of hemicelluloses, lignin, and lignin-carbohydrate complexes (LCC). In an earlier study, systems analysis was carried out on hemicelluloses isolated from *P. pubescens* Mazel at different growth stages (Peng *et al.* 2012a, b; Luo *et al.* 2012). Hemicelluloses from *P. pubescens* Mazel are mainly composed of arabinoxylans, which consist of xylose, arabinose, mannose, galactose, ribose, and uronic acid (Peng *et al.* 2012a, b; Luo *et al.* 2012). Several studies on lignins isolated from many kinds of bamboo have been reported (Yang *et al.* 2013; Sun *et al*. 2012; Suga *et al*. 2003; Wen *et al*. 2013). For example, lignin fractions from the bamboo species *Phyllostachys sulphurea* (Carr.) A. et C. Riv have been determined to be of S-G-H type (Yang *et al*. 2013). Alkali lignin fractions from the bamboo species *Neosinocalamus affinis* primarily consist of *β-O*-4' linkages combined with small amounts of *β-β*', *β*-5', *β*-1' linkages, and *p*-hydroxycinnamyl alcohol end groups (I) (Sun *et al*. 2012). Two bis-lignans, including phyllostadimers A and B, have been isolated from the stems of *Phyllostachys edulis*, and the two lignan units in these two bis-lignans were directly linked by a C–C bond (Suga *et al*. 2003). However, knowledge of the structural features of lignin and lignin-carbohydrate complexes (LCC) obtained from *P. pubescens* Mazel is still lacking. Therefore, studies should focus on investigating L and LCC from *P. pubescens* Mazel to maximize the use of this industrially important biomass.

In this study, the lignin (L) and LCC fractions from four-year-old mature *P. pubescens* Mazel were obtained after organosolvent treatment in mild conditions. The structural features of the prepared samples of L and LCC were characterized *via* UV-vis absorption spectra, Fourier transform infrared spectroscopy (FT-IR), and nuclear magnetic resonance spectroscopy (NMR). In addition, the chemical composition of LCC was analyzed *via* destructive methods, such as acid hydrolysis and nitrobenzene oxidation. This study provides additional information to a previous systematic study of the chemical characteristics of the *P. pubescens* Mazel cell wall.

EXPERIMENTAL

Materials

Four-year-old bamboo (*P. pubescens* Mazel) was obtained from a local farm (Guanxi Village, Meiling Town, Nanchang City, China). The bamboo stem was cut, dried in sunlight, and subsequently cut into smaller pieces. The small bamboo pieces were then ground through a 40-mesh screen, followed by a 100-mesh screen, and further dried in an oven at 50 °C for 12 h prior to use. The raw material was chemically characterized *via* standard Chinese methods (GB 2677.10-95, GB/T 2677.8-94, GB/T 2677.3-93, GB/T 2677.6-94). The composition (%, w/w) of the bamboo was determined to be 24.48% *α*-cellulose, 52.36% hollocellulose, 26.11% Klason lignin, 2.06% ash, and 5.49% benzene-ethanol extractives based on the weight of oven-dried bamboo powder. Distilled and deionized water (Milli-Q) was used as the solvent for the preparation of all reagent

solutions. Trimethylchlorosilane (TMS) (> 99.9%) and hexamethyldisilane (HMDS) (> 98.0%) were obtained from Sigma. All other reagents employed were of analytical grade.

Methods

Isolation of lignin (L) and lignin-carbohydrate complexes (LCC) from bamboo

The method of L and LCC fractionation from bamboo was adapted from the literature (Balakshin *et al*. 2007; Björkman 1956, 1957). The detailed isolation process is shown in Fig. 1.

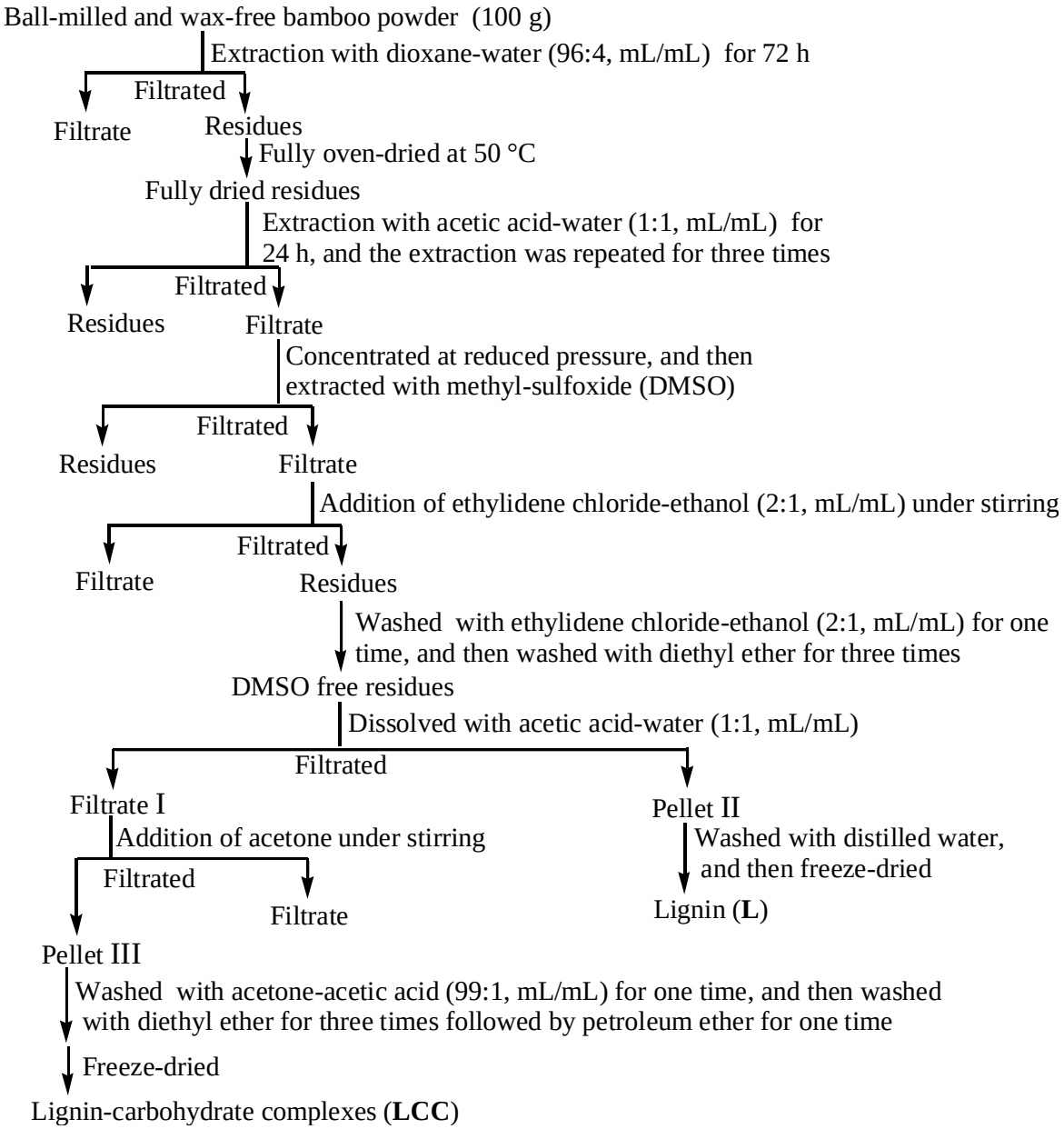

Fig. 1. Scheme for the isolation of lignin (L) and lignin-carbohydrate complexes (LCC) from bamboo

The dried bamboo powder was first dewaxed with a mixture of toluene-ethanol (2:1, mL/mL) for 6 h in a Soxhlet extractor. A total of 5.49% extractives were removed. The completely dried, dewaxed bamboo powder was then subjected to ball milling in a vibration ball mill (HDJ-5, Shanghai Tianhe Pharmaceutical Machinery Co. Ltd., China) for 64 h with a rotation rate of 60 r/min. Milling was carried out continuously at room temperature, and the diameter of the ball was 3.34 cm. The volume ratio of bamboo powder to ball was about 1:5. The ball-milled bamboo powder (100 g) was extracted using dioxane-water (96:4, mL/mL) for 72 h in a Soxhlet extractor. After complete oven drying at 50 °C, the residues were treated with acetic acid-water (1:1, mL/mL) for 24 h and then filtered. The above treatment with acetic acid-water was repeated three times, after which the combined filtrate was concentrated at a reduced pressure and then extracted using methyl-sulfoxide (DMSO). After filtration, the mixture of ethylidene chloride and ethanol (2:1, mL/mL) was added to the filtrate with stirring and filtered again. The collected residues were washed once with ethylidene chloride-ethanol (2:1, mL/mL) and then three times with diethyl ether to completely remove DMSO. The obtained DMSO-free residues were further dissolved in acetic acid-water (1:1, mL/mL) and precipitated. The filtrate I and pellet II were obtained, respectively. Acetone was added to the filtrate I with stirring. The formed pellet III was washed once with acetone and acetic acid (99:1, mL/mL), washed three times with ethyl ether, and subsequently washed once with petroleum ether. The final LCC fraction was freeze-dried. The pellet II was removed *via* filtration, repeatedly washed with distilled water, and centrifuged until the pH of the supernatant reached 7.0. The pellet II was then lyophilized and designated the L fraction.

Characterization of L and LCC

For FT-IR measurements, the L and LCC samples were blended with high-purity KBr to form pellets, and the spectra were obtained using a Nicolet 7500 FT-IR spectrophotometer (Thermo Nicolet Corporation, USA) between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1}.

UV-vis absorption spectra were obtained using a LabTech 300563 UV-visible spectrophotometer within the range of 200 nm to 800 nm. Before UV-vis measurements, the samples were dissolved in a mixture of methanol and water (1:1, mL/mL).

The solution-state 2D NMR spectra for the heteronuclear single quantum correlation (HSQC) were obtained at 30 °C using a Bruker MSL-600 MHz spectrometer (Bruker Corporation, Germany) with a 5-mm PABBO probe head. The samples (80 mg) were dissolved in 1.0 mL of DMSO-d_6 for NMR analysis, and tetramethylsilane was used as the internal standard (δ = 0 ppm). In 2D NMR spectroscopy, a spectral width of 8417.509 Hz was used in both dimensions. The number of scans was 30, and the acquired time per scan (AQ) was 0.1217012 s. All experiments were acquired with standard pulse sequences supplied with the software.

The method of determining the neutral sugar composition of the carbohydrates associated with lignin in the LCC fraction has been described in a previous paper (Peng *et al*. 2012a, b). Twenty milligrams of the sample was hydrolyzed using 15 mL of 3.0 M trifluoro acetic acid (TFA) at 120 °C for 3 h in a 30-mL pressure vessel. The hydrolysate was then diluted with anhydrous ethanol, and TFA was removed using a rotary vacuum evaporator at 45 °C. Anhydrous ethanol was added to the solids, followed by re-evaporation. This procedure was repeated several times until the obtained hydrolysates

were neutral. The dried hydrolysate solids were finally converted into their TMS derivatives and analyzed *via* gas chromatography-mass spectrometry (GC-MS). The analyses were performed using an Agilent 5973 GC-MS device equipped with a HP-5MS capillary column (30.0 m × 0.25 mm × 0.25 μm) and an integrated compliance information system (ICIS). The carrier gas was He, and the gas flow rate was 1.0 mL/min. Mass spectra were obtained *via* electron impact ionization at 70 eV. The oven temperature was programmed for 2 min at 60 °C, increased at 10 °C/min to 280 °C, and held for 5 min at 280 °C.

Nitrobenzene oxidation of the isolated LCC was performed based on methods reported in the literature (Zhang *et al*. 2010) with slight modifications. About 14 mL of 2.0 M sodium hydroxide and 0.8 mL of nitrobenzene were added to the LCC sample (20 mg) in a 30-mL pressure vessel. After full blending, the vessel was heated in an oil bath for 2.5 h at 170 °C. After filtration of the resulting mixture over a 0.22-μm microporous cellulose membrane filter, the filtrate was extracted three times with 60 mL of chloroform. The upper liquor was adjusted to pH 1.0 with 20% hydrochloric acid, and extracted three times with 60 mL of chloroform. After complete dehydration of chloroform extracts using anhydrous Na_2SO_4 as the dehydrant, the final chloroform extracts were concentrated using a rotary evaporator at a reduced pressure to obtain solid oxidation products. The chemical composition of phenolics liberated from the alkaline nitrobenzene oxidation of lignin associated with carbohydrates in the LCC sample was determined from their tetramethylsilane derivatives *via* GC-MS. The GC-MS analysis conditions were the same as those previously mentioned.

RESULTS AND DISCUSSION

FT-IR Spectra Analysis

The FT-IR spectra of the L and LCC fractions obtained from the ball-milled bamboo are shown in Fig. 2. Peaks were assigned according to the literature (Buranov and Mazza 2012; Ebringerová *et al*. 1992; Gupta *et al*. 1987; Hu *et al*. 2013; Lawther *et al*. 1996; Peng *et al*. 2012a; Singh *et al*. 2005; Vázquez *et al*. 1997; Villaverde *et al*. 2009; Wen *et al*. 2013). The spectra displayed typical lignin patterns, which included the bands of aromatic ring skeletal vibrations at 1602 and 1510 cm^{-1}. The similar spectra implied that the core of the lignin structure in L and LCC fractions was similar. A strong absorption band at about 3420 cm^{-1} was attributed to O–H stretching vibration in the aromatic and aliphatic hydroxyl groups. The peak at 2925 cm^{-1} (asymmetrical stretching) and the shoulder at 2829 cm^{-1} (symmetrical stretching) correspond to the C–H vibrations in the methyl and methylene groups (Buranov and Mazza 2012). The band at 1710 cm^{-1} to 1733 cm^{-1} was assigned to C=O stretching because the carbohydrates were linked with lignin and the conjugated carbonyls with the aromatic ring (Singh *et al*. 2005; Villaverde *et al*. 2009). The bands at 1458 and 1425 cm^{-1} correspond to C–H deformation (asymmetric in $–CH_3$ and $–CH_2$) and aromatic skeletal vibrations combined with C–H in-plane deformation, respectively. The aliphatic C–H stretch in $–CH_3$ from acetyl groups or phenolic –OH stretch was observed at 1374 cm^{-1}. The C–O stretching of syringyl (S) and condensed guaiacyl (G ring substituted at position 5) units were observed at 1318 and 1249 cm^{-1}, respectively (Lawther *et al*. 1996). The band at 1165 cm^{-1} indicates C–O, C–O–C stretching and C–OH bending (Sun *et al*. 1998). The band at about 1113 cm^{-1}

corresponds to aromatic C–H in-plane deformation in S units and C–O deformation in secondary alcohols. The band at 1044 cm^{-1} was attributed to aromatic in-plane bending in G units and C–O deformation in primary alcohols (Peng *et al*. 2012a; Vάzquez *et al*. 1997). Compared with the FT-IR spectrum of LCC, the peak absorbance at 1044 cm^{-1} in the FT-IR spectrum of L decreased, which indicates that polysaccharides in hemicelluloses were removed during isolation of the L fraction (Hu *et al*. 2013). A weak band for the LCC fraction was detected at 987 cm^{-1}, which suggests that the arabinosyl side chains that are attached at the O-3 position of the xylopyransyl constituents are linked to lignin (Ebringerová *et al*. 1992). A weak band at 894 cm^{-1} corresponds to the C-1 group frequency or ring frequency, which is characteristic of *β*-glycosidic linkages between the sugar units of carbohydrates linked to lignin (Gupta *et al*. 1987). The band at approximately 829 cm^{-1} corresponds to C–H out-of-plane deformation at positions 2 and 6 in S units (Singh *et al*. 2005). Based on the FT-IR spectra, both the L and LCC fractions possibly contained syringyl units, guaiacyl units, and xylans.

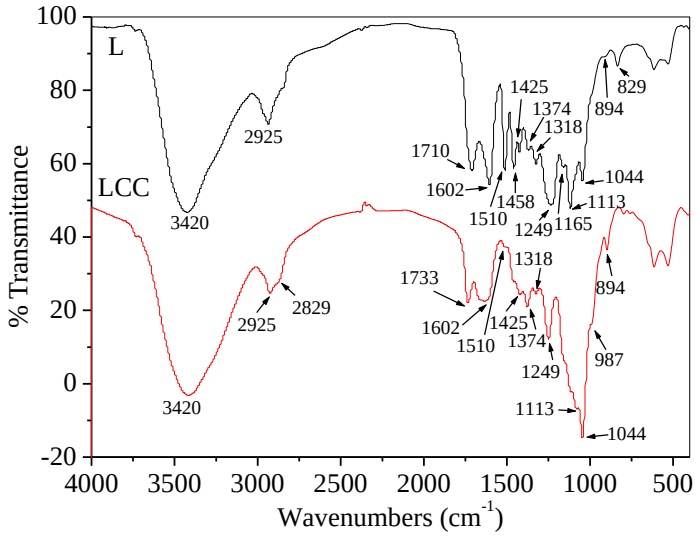

Fig. 2. FT-IR spectra of L and LCC from bamboo

UV-Vis Spectra Analysis

The UV-vis spectra of L and LCC from bamboo are shown in Fig. 3. The similarity between the two UV-vis spectra suggests no obvious differences between the chemical structures of lignin obtained from ball-milled bamboo powders. It can also be seen that the absorption maximum of LCC fraction was higher than that of L fraction, suggesting that the lignin content of LCC fraction was relatively high. The spectra showed a maximum at 208 nm originating from the $\pi \rightarrow \pi^*$ transition in the aromatic ring (Li *et al*. 2012). The peak at about 278 nm was attributed to the $\pi \rightarrow \pi^*$ electronic transition in the aromatic ring of the unconjugated phenolic units, which indicates that free and etherified OH groups formed by the relatively high proportion of guaiacyl units in lignin were detected. The peak observed at about 278 nm implied a relatively higher content of syringyl units in L and LCC fractions since syringyl units exhibit the bands at somewhat shorter wavelengths (Sun *et al*. 2001). The weak shoulder at 312 nm was attributed to the $n \rightarrow \pi^*$ transition in lignin units containing C_α=O groups and $\pi \rightarrow \pi^*$ transition in lignin units with C_α=C_β linkages conjugated to the aromatic ring (Li *et al*. 2010; Li *et al*. 2012; Yang *et al*. 2013).

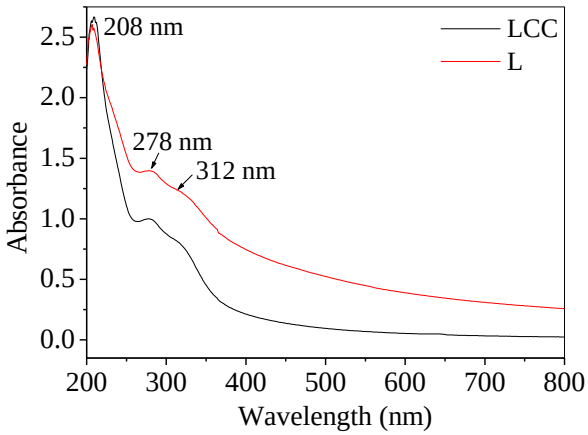

Fig. 3. UV-vis spectra of L and LCC from bamboo

2D NMR Analysis

Figure 4 presents the heteronuclear single quantum coherence (HSQC) spectra of L and LCC from mature bamboo (*P. pubescens* Mazel). The corresponding assignments are listed in Table 1. The main structural units identified *via* HSQC are shown in Fig. 5. For L and LCC, most of the signals were the same in both HSQC spectra, which implies that their structural units are similar. The weak signal observed at δ_C/δ_H 131.9/7.66 corresponds to the $C_{2,6}$-$H_{2,6}$ correlations of *p*-hydroxybenzoate substructures ($PB_{2,6}$) (Wen *et al*. 2013). And these substructures were also found in the alkali lignin from bamboo (*Bambusa rigida* sp.) from Sichuan, China (Wen *et al*. 2013). However, *p*-hydroxybenzoate substructures have not been found in the lignin from the bamboo (*Phyllostachys sulphurea* (Carr.) A. et C. Riv) (Yang *et al*. 2013). The signals observed at δ_C/δ_H 59.8–60.5/3.36–3.69 correspond to the C_γ-H_γ correlations in *β*-O-4' substructures (A_γ). Carbohydrates were also detected in this study because of the strong signals of C-H correlations in the HSQC spectra. The corresponding anomeric correlations of *β*-D-xylopyranosyl residues (Xyl_1) were observed at δ_C/δ_H 103.7/4.15, whereas the minor anomeric correlations of *β*-D-glucopyranosyl units (Glc1) might overlap in this region (Yuan *et al*. 2011). The peak at δ_C/δ_H 102.2/4.25 corresponds to the *β* anomeric C_1-H_1 of the (1→4)-linked-*β*-D-xylopyranosyl units (βXyl_1) (Peng *et al*. 2012b). Other carbohydrate signals in the HSQC spectra were δ_C/δ_H 73.1/3.03, 74.4/3.24, 75.8/3.49, and 63.6/3.16/3.87 for C_2-H_2 (βXyl_2), C_3-H_3 (βXyl_3), C_4-H_4 (βXyl_4), and C_5-H_{5ax}/H_{5eq} (βXyl_5) of (1→4)-linked-*β*-D-xylopyranosyl residues, respectively. The signals at δ_C/δ_H 98.0/4.22 and 92.8/4.84 correspond to the anomeric correlations from the reducing end of (1→4)-linked-*β*-D-xylopyranosyl ($\beta Xyl_{1(R)}$) and (1→4)-linked-α-D-xylopyranosyl residues ($\alpha Xyl_{1(R)}$), respectively. The signal at δ_C/δ_H 75.4/4.80 corresponds to C_3-H_3 in (1→4)-linked-*β*-D-xylopyranosyl units acetylated at C-3 ($\beta Xyl3_3$). The 4-*O*-methyl-*α*-D-glucuronic acid attached to xylans at O-2 was observed at δ_C/δ_H 76.7/3.09 (C_5-H_5) (Yang *et al*. 2013; Sun *et al*. 2005a). A strong cross-signal corresponding to methoxyl substituents was observed at δ_C/δ_H 56.2/3.71.

Although there were similar NMR signals, several differences were observed between the HSQC spectra of L (Fig. 4a) and LCC (Fig. 4b). In the HSQC spectrum of the L fraction, the S units showed a weak signal for the $C_{2,6}$-$H_{2,6}$ correlations at δ_C/δ_H 103.9/6.59, whereas the G units showed two correlations for C_5-H_5 (δ_C/δ_H 115.9/6.73) and C_6-H_6 (δ_C/δ_H 116.1/6.76). Additionally, *p*-hydroxyphenyl units (H) of lignin were

detected at δ_C/δ_H 128.4/7.20 ($H_{2,6}$) (Hussin *et al*. 2013; Martínez *et al*. 2008). In addition to the signals of the S, G, and H units, the L sample showed an important peak at δ_C/δ_H 130.7/7.45, which corresponds to $C_{2,6}$-$H_{2,6}$ in *p*-coumaric acid (*p*-$CA_{2,6}$) (Wen *et al*. 2013). The anomeric correlations of C_1-H_1 of 3-*O*-acetyl-(1→4)-linked-*β*-D-xylopyranosyl residues ($\beta Xyl3_1$) in the HSQC spectrum of L were observed at δ_C/δ_H 102.0/4.38 (Yuan *et al*. 2011), whereas the signal at δ_C/δ_H 74.0/4.50 corresponds to C_2-H_2 in 2-*O*-acetyl-(1→4)-linked-*β*-D-xylopyranosyl units ($\beta Xyl2_2$).

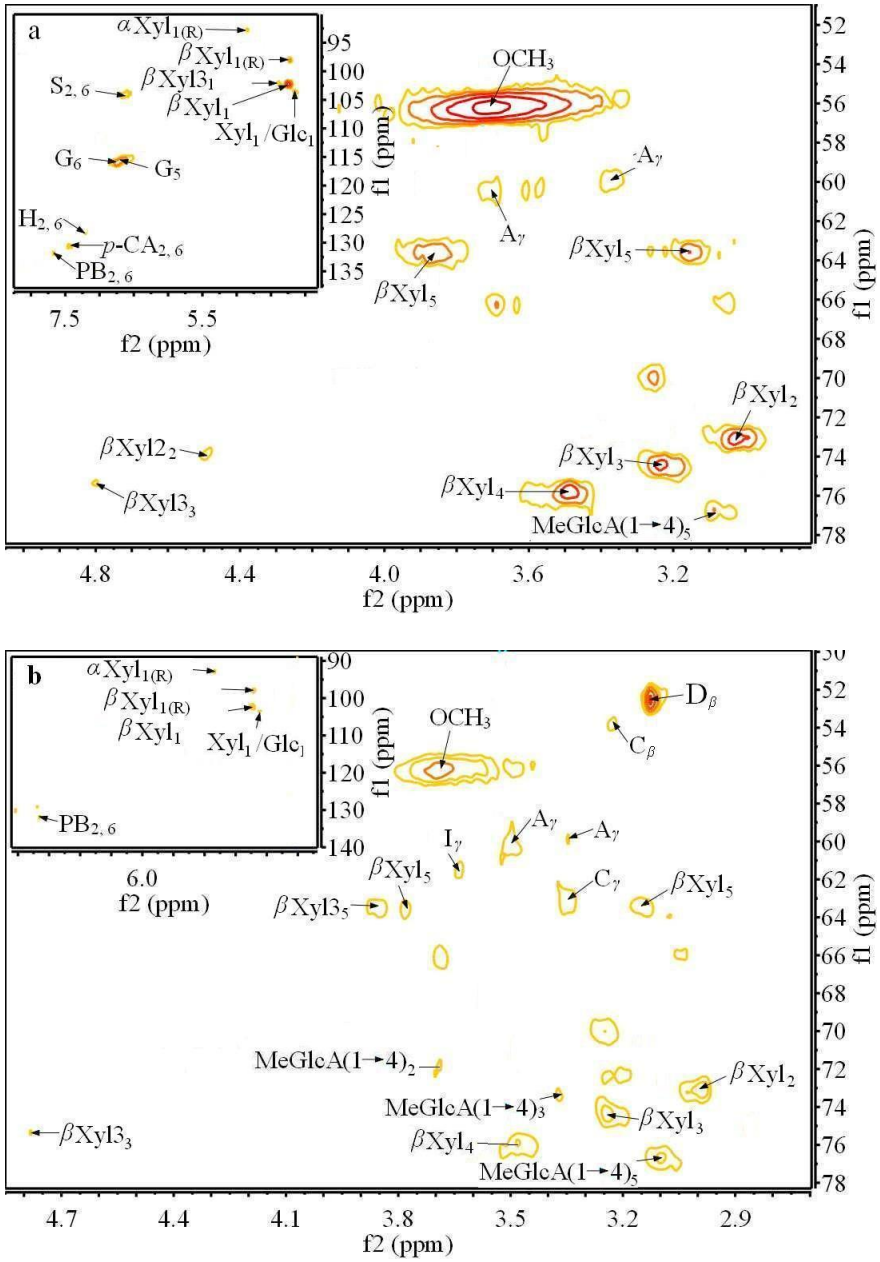

Fig. 4. HSQC spectra of (a) L and (b) LCC in DMSO-d_6 solvent

Table 1. Assignment of Signals in the HSQC Spectra of the Lignin Fraction (L) and Lignin-Carbohydrate Complexes (LCC)

δ_C/δ_H	Label	Assignment	Structural units
131.9/7.67	$PB_{2,6}$	$C_{2,6}$-$H_{2,6}$	*p*-hydroxybenzoate substructures (PB)
130.7/7.45	*p*-$CA_{2,6}$	$C_{2,6}$-$H_{2,6}$	*p*-coumaric acid
128.4/7.20	$H_{2,6}$	$C_{2,6}$-$H_{2,6}$	*p-hydroxyphenyl* units (H)
116.1/6.76	G_6	C_6-H_6	guaiacyl units (G)
115.9/6.73	G_5	C_5-H_5	guaiacyl units (G)
103.9/6.59	$S_{2,6}$	$C_{2,6}$-$H_{2,6}$	syringyl units (S)
103.7/4.15	Xyl_1/Glc_1	C_1-H_1	*β*-D-xylopyranosyl/*β*-D-glucopyranosyl
102.2/4.25	*β*Xyl_1	C_1-H_1	(1→4)-linked-*β*-D-xylopyranosyl
102.0/4.38	*β*$Xyl3_1$	C_1-H_1	3-*O*-acetyl-(1→4)-linked-*β*-D-xylopyranosyl
98.0/4.22	*β*$Xyl_{1\ (R)}$	C_1-H_1	(1→4)-linked-*β*-D-xylopyranosyl at non-reducing end (R)
92.8/4.84	*α*$Xyl_{1\ (R)}$	C_1-H_1	(1→4)-linked-*α*-D-xylopyranosyl at non-reducing end (R)
76.7/3.09	MeGlcA(1→4)$_5$	C_5-H_5	4-*O*-methyl-*α*-D-glucuronic acid attached to xylans at *O*-2
75.8/3.49	*β*Xyl_4	C_4-H_4	(1→4)-linked-*β*-D-xylopyranosyl
75.4/4.80	*β*$Xyl3_3$	C_3-H_3	3-*O*-acetyl-(1→4)-linked-*β*-D-xylopyranosyl
74.4/3.24	*β*Xyl_3	C_3-H_3	(1→4)-linked-*β*-D-xylopyranosyl
74.0/4.50	*β*$Xyl2_2$	C_2-H_2	2-*O*-acetyl-(1→4)-linked-*β*-D-xylopyranosyl
73.4/3.37	MeGlcA(1→4)$_3$	C_3-H_3	4-*O*-methyl-*α*-D-glucuronic acid attached to xylans at *O*-2
73.1/3.03	*β*Xyl_2	C_2-H_2	(1→4)-linked-*β*-D-xylopyranosyl
71.8/3.70	MeGlcA(1→4)$_2$	C_2-H_2	4-*O*-methyl-*α*-D-glucuronic acid attached to xylans at *O*-2
63.7/3. 87	*β*Xyl_5	C_5-H_{5eq}[a]	(1→4)-linked-*β*-D-xylopyranosyl
63.4/3.85	*β*$Xyl3_5$	C_5-H_5	3-*O*-acetyl-(1→4)-linked-*β*-D-xylopyranosyl
63.6/3.16	*β*Xyl_5	C_5-H_{5ax}[b]	(1→4)-linked-*β*-D-xylopyranosyl
63.2/3.35	C_γ	C_γ- H_γ	phenylcoumaran substructures (C)
61.5/3.64	I_γ	C_γ- H_γ	*p*-hydroxycinnamyl alcohol end groups (I)
60.5/3.69	A_γ	C_γ- H_γ	*β*-*O*-4' substructures (A)
59.8/3.36	A_γ	C_γ- H_γ	*β*-*O*-4' substructures (A)
56.2/3.71	OCH_3	C-H	methoxyls
53.9/3.23	C_β	C_β-H_β	phenylcoumaran substructures (C)
52.5/3.13	D_β	C_β-H_β	*β*-1' (spirodienone) substructures (D)

[a] $H\text{-}5_{eq}$, the equatorial proton linked at C-5 of (1→4)-linked-*β*-D-xylopyranosyl
[b] $H\text{-}5_{ax}$, the axial proton linked at C-5 of (1→4)-linked-*β*-D-xylopyranosyl

In the HSQC spectrum of the LCC fraction, the signals at δ_C/δ_H 63.2/3.35, 53.9/3.23, and 52.5/3.13 were characteristic of C_γ-H_γ, C_β-H_β in phenylcoumaran (*β*-5' linkages) substructures (C), and C_β-H_β in spirodienone (*β*-1' linkages) substructures (D), respectively (Villaverde *et al*. 2009; Yang *et al*. 2013). The presence of these phenylcoumaran substructures is directly related to the existence of G units in lignin (Villaverde *et al*. 2009). These substructures were also observed in lignin from bamboo (*Bambusa rigida* sp.) obtained from Sichuan, China (Wen *et al*. 2013). The signal observed at δ_C/δ_H 61.5/3.64 corresponds to the C_γ-H_γ correlations of *p*-hydroxycinnamyl alcohol end groups (I_γ) in the side-chain region of the LCC fraction. The signal at δ_C/δ_H 63.4/3.85 corresponds to C_5-H_5 in (1→4)-linked-*β*-D-xylopyranosyl units acetylated at *O*-3 (*β*$Xyl3_3$). The presence of 4-*O*-methyl-*α*-D-glucuronic acid attached to xylans at *O*-2 was further confirmed by the signals at δ_C/δ_H 71.8/3.70 (C_2-H_2) and 73.4/3.37 (C_3-H_3)

(Yang *et al*. 2013; Sun *et al*. 2005a). Additionally, in the HSQC spectrum of LCC, the signals at δ_C/δ_H 130.2/8.04 and 129.2/7.69 were not determined.

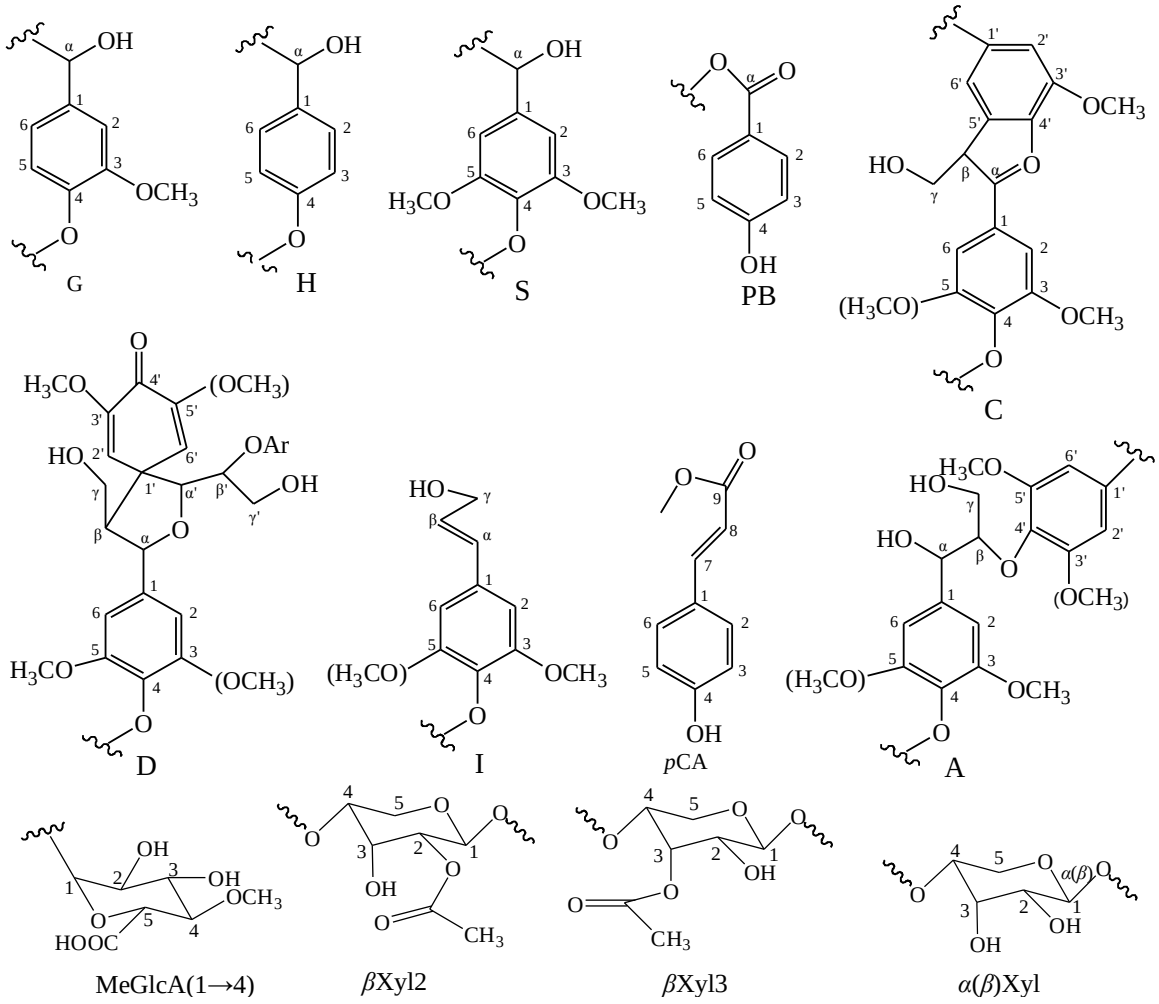

Fig. 5. Primary structural units identified by HSQC: (G) guaiacyl units; (H) *p*-hydroxyphenyl units; (S) syringyl units; (PB) *p*-hydroxybenzoate substructures; (I) *p*-hydroxycinnamyl alcohol end groups; (*p*-CA) *p*-coumaric acid; (A) *β*-*O*-4' substructures; (D) *β*-1' (spirodienone) substructures; (C) phenylcoumaran substructures; (*α*(*β*)Xyl) (1→4)-linked-*α*(*β*)-D-xylopyranosyl; (MeGlcA(1→4)) 4-*O*-methyl-*α*-D-glucuronic acid; (*β*Xyl2) 2-*O*-acetyl-(1→4)-linked-*β*-D-xylopyranosyl; (*β*Xyl3) 3-*O*-acetyl-(1→4)-linked-*β*-D-xylopyranosyl

In the HSQC spectra of the L sample and LCC fraction, several signals at δ_C/δ_H 70.0/3.25, 66.2/3.70, and 66.2/3.05 were not identified. Based on the 2D NMR analysis results, some important information was obtained. First, the carbohydrates linked with lignin in the L and LCC fractions were possibly composed of xylans with acetyl groups and 4-*O*-methyl-*α*-D-glucuronic acid as side chains. Additionally, the lignin residues in both L and LCC obviously contained *β*-*O*-4' substructures and *p*-hydroxybenzoate substructures. Finally, H units, G units, S units, and *p*-CA were detected in the L fraction, whereas I groups, phenylcoumaran substructures, and *β*-1' (spirodienone) substructures were detected in LCC. No obvious NMR signals characterizing G and S units in the HSQC spectrum of LCC were observed, probably because the lignin residues were degraded to a certain extent during the isolation process of LCC.

Composition of Neutral Sugars, Phenolic Acids, and Aldehydes in LCC

Table 2 shows the neutral sugar composition of the LCC fraction. As shown, the isolated lignin-carbohydrate complexes mainly consist of xylose (76.98%), arabinose (4.08%), glucose (6.47%), mannose (6.34%), and altrose (3.69%), as well as small amounts of galactose (1.75%) and ribose (0.69%). Xylose was the predominant sugar component, which implies the presence of a high proportion of xylans in LCC. The xyl/ara ratio was about 18.87, which was considerably higher than that from bamboo (*P. pubescens* Mazel) hemicelluloses reported in a previous paper (Peng *et al*. 2012a, b). It is very likely that the side chains of the hemicelluloses in LCC obtained in the sequential treatments were greatly cleaved or degraded. Neutral sugar analysis results suggested that the carbohydrates associated with lignin in the LCC sample obtained from mature bamboo (*P. pubescens* Mazel) were primarily composed of xylans. This result is in agreement with the conclusion drawn from 2D NMR analysis.

The phenolic acids and aldehydes resulting from the alkaline nitrobenzene oxidation of the linked lignin in the LCC fraction are also listed in Table 2. The major products obtained from nitrobenzene oxidation were identified as vanillin (18.55%), syringaldehyde (46.77%), vanillic acid (5.65%), and syringic acid (29.03%), which resulted from the degradation of non-condensed guaiacyl and syringyl units. The total content of vanillin and syringaldehyde of the total phenolic monomers was 65.32%. This value reveals that the associated lignin in the LCC sample was primarily composed of non-condensed guaiacyl (G) and syringyl (S) units (Sun *et al*. 2005b). The total content of syringaldehyde and syringic acid was 75.80%, and the content of vanillin and vanillic acid released from LCC was 24.20%. Therefore, the ratio of S to G units was 3.13. The high S/G ratio suggested that the lignin residues in LCC contained a relatively high proportion of methoxyl groups. Also, this S/G ratio was much higher than those of lignin samples obtained from bamboo (*Bambusa rigida* sp.) from Sichuan, China (Wen *et al*. 2013). Furthermore, *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid, ferulic acid, and *p*-coumaric acid were not detected in the oxidation products of lignin, in agreement with 2D NMR spectra results mentioned above. The results from alkaline nitrobenzene oxidation demonstrated that the lignin residues in LCC obtained from mature bamboo (*P. pubescens* Mazel) primarily consisted of G and S units.

Table 2. Content of Neutral Sugars (Relative %) and Nitrobenzene Oxidation Products (Relative %) in the Isolated LCC Fraction

Neutral sugar composition		Phenolic acids and aldehydes	
Sugars	Content (%)	Phenolics	Content (%)
Xylose	76.98	Vanillin	18.55
Arabinose	4.08	Syringaldehyde	46.77
Glucose	6.47	Vanillic acid	5.65
Mannose	6.34	Syringic acid	29.03
Galactose	1.75		
Ribose	0.69		
Altrose	3.69		
Xyl/ara	18.87	S/G [a]	3.13

[a] S represents syringyl units, including syringaldehyde and syringic acid, and G represents guaiacyl units, including vanillin and vanillic acid

CONCLUSIONS

1. The L and LCC in bamboo (*P. pubescens* Mazel) showed similar structural units, including guaiacyl (G), syringyl (S), *p*-hydroxybenzoate substructures (PB), *β*-*O*-4' substructures (A), 3-*O*-acetyl-(1→4)-linked-*β*-D-xylopyranosyl (*β*Xyl3), (1→4)-linked-*α*(*β*)-D-xylopyranosyl (*α*(*β*)Xyl), and 4-*O*-methyl-*α*-D-glucuronic acid (MeGlcA(1→4)).
2. The lignin residues in L fraction also contained *p*-hydroxyphenyl (H) and *p*-coumaric acid (*p*-CA). Phenylcoumaran substructures (C), *p*-hydroxycinnamyl alcohol end groups (I), and *β*-1' (spirodienone) substructures (D) were found in the lignin residues in LCC fraction.
3. The lignin residues in LCC were primarily composed of G and S units. The ratio of S to G units in the LCC fraction was 3.13. The relative content of xylose in LCC was 76.98%, and the ratio of xylose to arabinose was approximately 18.87. The carbohydrates linked with lignin in the L and LCC fractions primarily consisted of xylans with acetyl groups and 4-*O*-methyl-*α*-D-glucuronic acid as side chains.
4. The lignins in L and LCC fractions were degraded to some extent during the separation process.

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