Homogeneous Esterification Mechanism of Bagasse Modified with Phthalic Anhydride in Ionic Liquid, Part 3: Structural Transformation of Lignins

Huihui Wang,[a] Xueqin Zhang,[a] Yi Wei,[a] Aiping Zhang,[b] Chuanfu Liu,[a,*] and Runcang Sun [a,c]

The phthalation of bagasse was investigated comparatively with the three main isolated components in 1-allyl-3-methylidazium chloride (AmimCl) to reveal the reaction behavior of bagasse. In the first two parts, the detailed changes of cellulosic and hemicellulosic components in bagasse were elucidated during phthalation. In Part 3, the phthalation of lignins was performed in AmimCl with various ratios of phthalic anhydride/lignins from 10 to 50 mmol/g. The phthalation degree ranged from 41.1% to 68.8% for the phthalated lignins. The aliphatic hydroxyls of lignins were more easily phthalated than the phenolic hydroxyls as revealed by ^{31}P nuclear magnetic resonance (NMR) analysis. Fourier transform infrared spectroscopy (FT-IR) and two dimensional (2D) heteronuclear single quantum correlation (HSQC) confirmed the attachment of phthaloyl group onto lignins. Severe degradation of lignin macromolecules was found at high ratios of phthalic anhydride/lignins (30 to 50 mmol/g) by gel permeation chromatography (GPC) analysis. These results provide a detailed understanding of reaction behaviors of lignins during bagasse phthalation, which are beneficial to prepare composites based on phthalated lignocellulose with better properties.

Keywords: Lignins; Bagasse; Phthalic anhydride; Ionic liquid; 2D HSQC NMR; ^{31}P NMR

*Contact information: a: State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China; b: College of Materials and Energy, Guangdong Key Laboratory for Innovative Development and Utilization of Forest Plant Germplasm, South China Agricultural University, Guangzhou 510642, China; c: Beijing Key Laboratory of Lignocellulosic Chemistry, Beijing Forestry University, Beijing 100083, China; *Corresponding author: chfliu@scut.edu.cn*

INTRODUCTION

Agricultural residues represent an abundant, inexpensive, and readily available source of renewable lignocellulosic biomass for the production of environmentally friendly industrial products, and they have received increasing attention. Bagasse, an abundant agricultural lignocellulosic byproduct, is a fibrous residue of cane stalks obtained after the crushing and extraction of the juice from sugarcane. Recently, bio-products derived from bagasse have been widely applied in many industrial fields, such as coating, food-packing, and painting (Duan *et al.* 2013), paving the way to replace the fossil-based products.

However, bagasse exhibits considerable chemical and physical inertness, because of the presence of complicated chemical components, such as high crystallinity of cellulose, the complex linkages of lignin carbohydrate complexes (LCC), and the structural heterogeneity, which restricts the preparation of composites and chemicals directly from bagasse.

Chemical modification represents a common and attractive method to introduce macromolecules in a heterogeneous or homogeneous phase for the production of value-added products (Giudice *et al.* 2013; Vasiljevic *et al.* 2013). Due to the presence of considerable hydroxyls, the esterification of lignocellulose is easily accomplished, and esterified products can be used as fillers in composites (Paukszta *et al.* 2015). The common esterification reagents conclude linear anhydrides, acyl chloride, cyclic anhydrides (Chen *et al.* 2013; Yuan *et a*l. 2010), and so on. Particularly, the esterification of lignocellulose with cyclic anhydrides has received increased attention due to the several advantages, including producing no by-products (*e.g*. carboxylic acid or HCl), attaching carboxylic acid groups onto lignocellulose, providing new reaction positions, and improving hydrophilicity of products.

Moreover, compared with the heterogeneous modification of bagasse, the homogeneous modification could offset the deficiency of heterogeneous systems, including improved modification efficiency, enhanced uniformity, compatibility with thermoplastics (Giudice *et al.* 2013), and increased flexibility (Thakur and Thakur 2014). Several novel solvents or solvent systems could dissolve lignocellulosic materials (Lu and Ralph 2003; Fasching *et al.* 2008; Luan *et al.* 2013). Among these novel solvents, ionic liquids are attractive due to their recoverability, designability, extremely low vapor pressure, non-flammability, and high thermal and chemical stability (Zhu *et al.* 2006). Recently, the esterification of lignocellulose with cyclic anhydride performed in ionic liquid has rapidly developed. For example, the esterification of bagasse cellulose with phthalic anhydride in ionic liquid 1-butyl-3-methylimidazium chloride was reported, and the crystallinity of the native cellulosic polymer was completely disrupted after the homogeneous modification (Liu *et al.* 2007). It was also reported that the esterification of bagasse with maleic anhydride occurred in ionic liquid 1-butyl-3-methylimidazium chloride, and the thermal stability of maleated bagasse increased at high temperature (above 300 ^{o}C) (Chen *et al.* 2013). Moreover, plastic films directly prepared from phthalated bagasse were reported recently (Chen *et al.* 2015). However, the esterification parameters and structural uniformity were difficult to control due to the presence of the complicated linkages among different components. The goal of this study was to elucidate the detailed reaction behaviors of lignocellulose and control the substitution degree during the homogeneous esterification in ionic liquid. The recently developed "gel-state NMR method" based on ball-milling and solution-state two dimensional (2D) heteronuclear single quantum correlation (HSQC) nuclear magnetic resonance (NMR) is beneficial to clarity the structural changes of lignocellulose in detail (Hoon *et al.* 2008; Chen *et al.* 2011; del Rio *et al.* 2012).

To elucidate the structural changes and control the degree of substitution during lignocellulose homogeneous esterification, the homogeneous phthalation of bagasse was investigated comparatively with the three main isolated components under the same conditions. The reaction behaviors of cellulose and hemicelluloses were explored during bagasse phthaltion in 1-allyl-3-methylidazolium chloride (AmimCl) (Wang *et al.* 2016; Wang *et al.* 2017). Therefore, lignin isolated from bagasse was phthalated in AmimCl with different phthalic anhydride dosage (10 to 50 mmol/g) in the present study. The detailed reaction behaviors of phthalated lignin were investigated by Fourier transform infrared spectroscopy (FT-IR), 2D HSQC NMR, ^{31}P NMR, and gel permeation chromatography (GPC).

EXPERIMENTAL

Materials

Bagasse obtained from a local factory (Jiangmen, China) was dried in sunlight and cut into small pieces. The small pieces were ground and screened to prepare 40- to 60-mesh size particles (450 to 900 μm). The ground samples were extracted with a toluene-ethanol mixture (2:1, v/v) and dried in a cabinet oven with air circulation at 50 °C for 24 h. The extractive-free bagasse was finely ball-milled for 48 h in a planetary ball-mill grinder (BM4, GRINDER, Beijing, China) at 608 rpm for lignin isolation. The lignin content in the extractive-free bagasse was determined as 19.24%, according to the standard NREL methods (Sluiter *et al*. 2008).

AmimCl was purchased from Shanghai Cheng Jie Chemical Co., Ltd. (Shanghai, China) and used as received. Cellulase from *Trichoderma viride* (3 to 10 units/mg) was purchased from Sigma-Aldrich (Shanghai, China). Phthalic anhydride and other chemicals used were of analytical grade and purchased from Guangzhou Chemical Reagent Factory (Guangdong, China).

Isolation of Lignins from Bagasse

Crude lignins were isolated from the extractive-free bagasse by enzymatic treatment as the reported literature (Chang *et al.* 1975), and purified before use. For this purpose, the dried crude lignin (1 g) was dissolved in 3 mL of acetone/water mixture (9:1, v/v). The resulting solution was added dropwise into 200 mL of distilled water with agitation, and the suspension was then centrifuged. The obtained solid residues were washed with distilled water (thrice, total 90 mL) and freeze-dried. The freeze-dried solid residues were re-dissolved in 3 mL of a 1,2-dichloroethane/ethanol mixture (2:1, v/v). The resulting solution was added dropwise into 200 mL of anhydrous ether under stirring. After centrifugation, the obtained solid residues were washed with anhydrous ether (thrice, total 90 mL) and air-dried to obtain the pure lignin sample.

Phthalation of Lignins

About 0.5 g lignin was dispersed in AmimCl (10 g) at room temperature, and stirred at 90 °C for 4 h to obtain a clear solution. Phthalic anhydride was added portion-wise to the solution under various ratios of phthalic anhydride/lignin including 10 (sample L1), 20 (sample L2), 30 (sample L3), 40 (sample L4), and 50 mmol/g (sample L5). The flask was continuously purged with N_2 gas for 10 min, and the mixture was stirred for phthalation at 90 °C for 90 min. After the required time, the solution was cooled to room temperature and added into ethanol (99 wt%, 200 mL) with agitation. The suspension was further stirred for 12 h and filtered. The obtained solid residues were washed thoroughly with ethanol (four times, total 800 mL) and freeze-dried. The phthalation of bagasse was performed as well was lignin samples, as shown in our previous work (Wang *et al.* 2016).

Determination of Phthalation Degree

The hydroxyl contents of the unmodified and phthalated lignins were determined by ^{31}P NMR based on the reported method (Boeriu *et al.* 2014; Sadeghifar *et al.* 2014), and calculated according to Eq. 1. The test of each lignin sample was repeated thrice to make sure the standard deviation lower than 5.0%.

$$A = \frac{\frac{\rho \times 100 \times 10^{-6}}{100.16} \times \frac{A_2}{A_1}}{m} \times 1000 \quad (1)$$

where A (mmol/g) is the hydroxyl contents of the lignin samples, A_2 is the integral area of the resonances assigned to hydroxyl groups in the ^{31}P NMR spectra of the lignin samples, A_1 is the integral area of the resonances assigned to hydroxyl groups of cyclohexanol in the ^{31}P NMR spectra, ρ (mg/mL) is the concentration of cyclohexanol, m (g) is the dry weight of samples, 100 (μL) is the volume of cyclohexanol, and 100.16 (g/mol) is molar mass of cyclohexanol.

Phthalation degree of lignin was calculated from the substituted hydroxyl contents from the total hydroxyl contents based on Eq. 2,

$$PD = \frac{SHC}{THC} \times 100\% \quad (2)$$

where *PD* is the phthalation degree, *SHC* is the substituted hydroxyl content, and *THC* is the total hydroxyl content.

Characterization

FT-IR spectra were obtained on a spectrophotometer (Nicolet 510, ThermoFisher, Waltham, MA, USA) using a KBr disk containing approximately 1% finely ground samples. Thirty-two scans were taken for each sample with a resolution of 2 cm^{-1} in a transmittance mode in the range of 4000 to 400 cm^{-1}.

The weight-average (M_w) and number-average (M_n) molecular weights of lignin samples were determined by gel peameation chromatography (Waters 1515, Milford, MA, USA) with differential detector (Waters 2414). The columns used were Styragel HR3 N,N-dimethyl formimade (DMF) and Styragel HR4 DMF (Waters), which were calibrated with polystyrene standards. Lignin samples (4 mg) were dissolved in DMF, and the obtained solution (20 μL) was injected by automatic sampler. The column was operated at 35 °C and eluted with DMF at a flow rate of 0.6 mL/min.

The 2D HSQC NMR spectra were recorded from 40 mg samples in 0.5 mL of deuterated dimethyl sulfoxide (DMSO-d_6) on a Bruker Advance III 600 MHz spectrometer (Karlsruhe, Germany) with 5 mm MAS BBO probe. The detailed collecting and processing parameters were as follows: number of scans, 32; receiver gain, 187; relaxation delay, 1.5 s; pulse width, 11.0 s; acquisition time, 0.1420 s; spectrophotometer frequency, 600.17/150.91 MHz; and spectral width, 7211.5/24875.6 Hz.

RESULTS AND DISCUSSION

Lignin Phthalation

To elucidate the structural changes of lignin during phthalation, the phthalation parameters including substituted hydroxyl contents and phthalation degree of the phthalated lignins (sample L1-L5) were determined (Table 1). The purity of lignins isolated from bagasse was assumed as 100%, and the total hydroxyl content of unmodified lignins (sample L0) was 5.13 mmol/g, as determined by ^{31}P NMR. After phthalation in AmimCl, the free hydroxyl content of lignins decreased due to the substitution of phthaloyl groups. As shown in Table 1, the substituted hydroxyl content of lignins with the ratio of phthalic

anhydride/lignins at 10 mmol/g was 2.42 mmol/g (sample L1), corresponding to the phthalation degree 47.2%. This result suggested that lignins were easily phthalated under the selected condition. The increase in the ratio of phthalic anhydride/lignins from 10 mmol/g (sample L1) to 20 mmol/g (sample L2) resulted in a substituted hydroxyl contents increase from 2.42 mmol/g to 2.56 mmol/g, corresponding to a phthalation degree increase from 47.2% to 49.9%. These results suggested the mild effect on reaction at low ratio of phthalic anhydride/lignins (10 to 20 mmol/g) during lignin phthalation in AmimCl. However, holding the ratio of phthalic anhydride/lignins at 30 mmol/g, the substituted hydroxyl contents reached 2.11 mmol/g, corresponding to the phthalation degree 41.1%. These decreases were probably due to the acid degradation of lignins. The acidic conditions resulted from phthalic acid released from phthalic anhydride in AmimCl (Fundador *et al.* 2012; Zhang *et al.* 2015). Raising the ratio of phthalic anhydride/lignins from 30 mmol/g (sample, L3) to 40 mmol/g (sample L4) and 50 mmol/g (sample L5) resulted in an increase in the substituted hydroxyl contents from 2.11 mmol/g to 2.73 mmol/g and to 3.53 mmol/g, respectively, corresponding to an increase in the phthalation degree from 41.1% to 53.2% and 68.8%, respectively. This enhancement in phthalation was attributed to the favorable effect of high ratio of phthalic anhydride/lignins (30 to 50 mmol/g) on the reaction and the limited degradation effect during homogeneous phthalation. The favorable effect of raising phthalic anhydride ratio on the phthalation degree of lignins resulted from the greater availability of phthalic anhydride molecules in the proximity of the lignin molecules (Liu *et al.* 2007). The results in the present study were consistent with those of Liu *et al.* (2007), who studied the phthalation of cellulose in AmimCl for obtaining products with increased substitution degrees after raising the molar ratio of phthalic anhydride/anhydroglucose. However, considering the complicated structures of lignin subunits, the phthalation of lignin hydroxyls is more complicated than that of hydroxyls from hemicelluloses or cellulose; more detailed structural information of lignins during bagasse phthalation is required.

Table 1. The Phthalation Degree of the Phthalated Lignins (L1-L5)

Sample No	Temp (°C)	[a]Dosage	Solvent	Time (h)	[b]THC (mmol/g)	[c]SHC (mmol/g)	[d]PD (%)
L1	90	10	AmimCl	1.5	5.13	2.42	47.2
L2	90	20	AmimCl	1.5	5.13	2.56	49.9
L3	90	30	AmimCl	1.5	5.13	2.11	41.1
L4	90	40	AmimCl	1.5	5.13	2.73	53.2
L5	90	50	AmimCl	1.5	5.13	3.53	68.8

[a] The ratio of phthalic anhydride/lignins (mmol/g); [b] The total hydroxyl contents; [c] The substituted hydroxyl contents; [d] The phthalation degree.

The FT-IR spectra of the unmodified (sample L0) and phthalated lignins (sample L2 and L5) are shown in Fig. 1. The absorbances at 1716, 1327, and 747 cm^{-1} correspond to carbonyl group in ester, C-O stretching in carboxyl, and out-of-plane C-H bending of benzene, respectively (Ma *et al.* 2013; Liu *et al.* 2007). Compared with the unmodified lignins (L0), the intensities of these characteristic peaks in the phthalated lignins (samples L2 and L5) increased, suggesting the phthalation of lignins. Furthermore, the peaks at 1602 and 1515 cm^{-1}, assigned to the vibrations of aromatic skeleton in lignins (Chen and Shi

2015), remained prominent in the phthalated lignins, suggesting that the skeleton of lignins was not changed during homogeneous phthalation reaction.

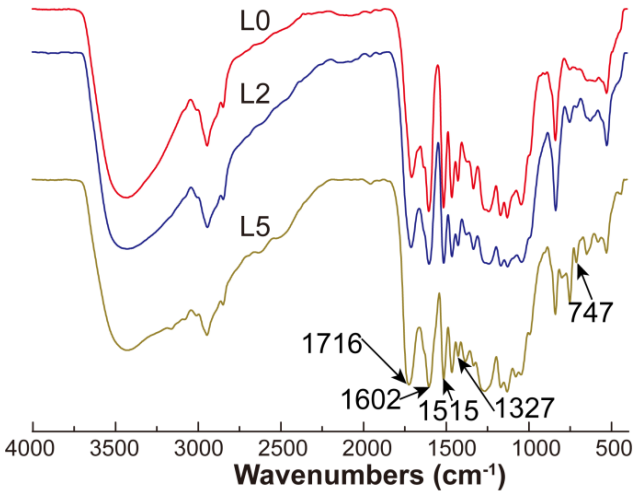

Fig. 1. FT-IR spectra of the unmodified (sample L0) and phthalated lignins (samples L2 and L5)

^{31}P NMR

^{31}P NMR spectroscopy is a very convenient method to determine and quantify the hydroxyl group contents of lignins (Boeriu *et al.* 2014; Monteil-Rivera and Paquet 2015). Besides the changes of total hydroxyl contents as discussed above, more detailed changes of the contents of hydroxyl groups from different lignin subunits were provided by ^{31}P NMR. Figure 2 illustrates the ^{31}P NMR spectra of unmodified (sample L0) and phthalated lignins (samples L3 and L5).

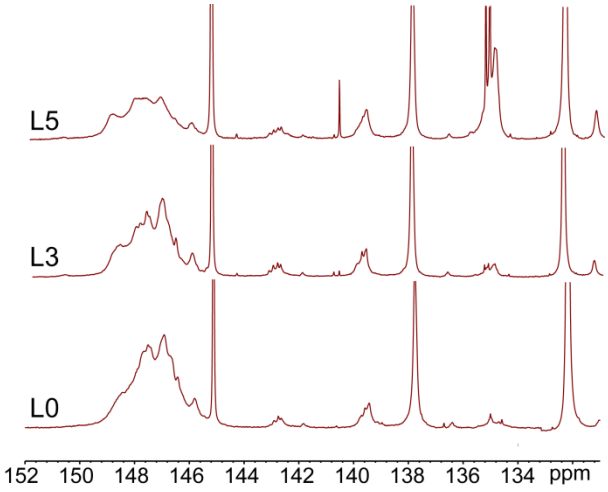

Fig. 2. Quantitative ^{31}P NMR spectra of the unmodified (sample L0) and phthalated lignins (samples L3 and L5)

According to the previous publications (Zhang *et al.* 2013; Boeriu *et al.* 2014; Sadeghifar *et al.* 2014), the signals in the ranges of 149.5 to 145.7 and 145.5 to 137.7 ppm were associated with the aliphatic and phenolic hydroxyl groups of lignins, respectively. In the phenolic hydroxyl groups region, the signals in the region of 144.5-143.3, 143.3-

142.5, 142.5-141.5, 140.2-138.8, and 138.2-137.7 ppm were assigned to condensed syringyl (CS), non-condensed syringyl (S), condensed guaiacyl (CG), non-condensed guaiacyl (G), and *p*-hydroxyphenyl (H) hydroxyls, respectively. In addition, the signals in the region of 145.7-145.5 and 135.5-134.2 ppm were assigned to the internal standard (IS) and carboxyl groups (COOH). The hydroxyl contents of the unmodified (L0) and phthalated lignins (L1-L5) were calculated from ^{31}P NMR and are listed in Table 2, and the decreased percentage of aliphatic and phenolic hydroxyl contents is depicted in Fig. 3.

Table 2. The Contents of Different Hydroxyl Groups of Unmodified (Sample L0) and Phthalated Lignins (Samples L1-L5) Calculated from ^{31}P NMR

Samples (mmol/g)	L0	L1	L2	L3	L4	L5
Aliphatic-OH	3.96	2.01	1.87	2.30	1.74	0.99
Condensed S-OH	0.02	0.03	0.03	0.03	0.03	0.03
Non-condensed S-OH	0.09	0.07	0.07	0.08	0.07	0.06
Condensed G-OH	0.04	0.03	0.03	0.03	0.03	0.03
Non-condensed G-OH	0.26	0.17	0.17	0.17	0.16	0.15
Non-condensed H-OH	0.76	0.40	0.40	0.41	0.37	0.34
COOH	0.11	0.06	0.08	0.09	0.12	0.60
S/G ratio (phenolic OH)	0.37	0.50	0.50	0.55	0.53	0.50

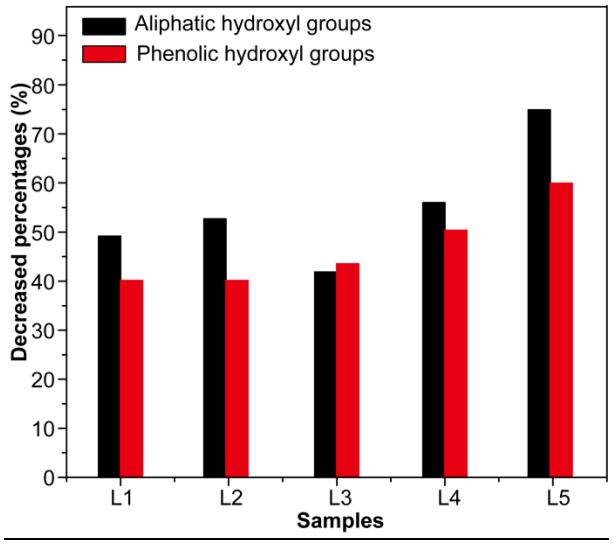

Fig. 3. The decreased percentages of lignin hydroxyl contents in the phthalated lignins (samples L1-L5): aliphatic hydroxyls (black) *vs.* phenolic hydroxyls (red)

Clearly, the content of aliphatic hydroxyls noticeably decreased from 3.96 mmol/g (sample L0) to 2.01 mmol/g (sample L1), and further decreased to 1.87 mmol/g (sample L2), indicating the phthalation of aliphatic hydroxyls. However, an increase of the aliphatic hydroxyls content from 1.87 mmol/g (sample L2) to 2.30 mmol/g (sample L3) was observed, possibly due to the increased free hydroxyls released from lignins degradation (Crestini *et al.* 1999). Raising the ratios of phthalic anhydride/lignins from 30 mmol/g (sample L3) to 40 mmol/g (sample L4), and 50 mmol/g (sample L5) led to the decrease of aliphatic hydroxyl contents from 2.30 mmol/g to 1.74 mmol/g, and 0.99 mmol/g, respectively. The same tendency for the changes of the substituted hydroxyl contents of the phthalated lignins (samples L1-L5) was also observed in Table 1. The content of non-

condensed guaiacyl hydroxyls decreased from 0.26 mmol/g (sample L0) to 0.15 mmol/g (sample L5), while the content of condensed guaiacyl hydroxyls slightly decreased from 0.04 mmol/g (sample L0) to 0.03 mmol/g (sample L5). These results suggested that the reactivity of non-condensed guaiacyl hydroxyls was much higher than condensed guaiacyl hydroxyls. Furthermore, the contents of non-condensed syringyl hydroxyls decreased from 0.09 mmol/g (sample L0) to 0.06 mmol/g (sample L5); the contents of non-condensed *p*-hydroxyphenyl hydroxyls decreased from 0.76 mmol/g (sample L0) to 0.34 mmol/g (sample L5). These results suggested that the reactivity of phenolic hydroxyls in non-condensed units followed the order of non-condensed H-OH > non-condensed G-OH > non-condensed S-OH.

Obviously, the aliphatic hydroxyls reacted much more than the phenolic ones in most samples, as illustrated in Fig. 3, suggesting that the higher reactivity of aliphatic hydroxyls. Considering the twice higher content of aliphatic hydroxyls than that of phenolic ones, the phthalation reactivity of lignins mainly depended on aliphatic hydroxyls during the homogeneous esterification. Reactions of maleic anhydride and kraft lignin in solvent-free showed that maleic anhydride reacted exclusively with aliphatic hydroxyls (Monteil-Rivera and Paquet 2015), which was consistent with the results in this study. After phthalation, the S/G ratio was improved from 0.37 (sample L0) to 0.50 (sample L5), indicating that more G-type than S-type lignins were phthalated under the selected conditions. It should also be noted that the contents of carboxyl groups continually increased from 0.06 mmol/g (sample L1) to 0.60 mmol/g (sample L5). The decreased content of aliphatic hydroxyls ranged from 1.95 to 2.97 mmol/g, much higher than the increased content of carboxyl groups in each phthalated lignin sample. According to the previous literature (Wen *et al.* 2014), the reaction conditions in the present study would not lead to the dehydration of lignin hydroxyls. The extra decrease of aliphatic hydroxyls groups was probably due to the formation of diester linkages between lignin subunits.

2D HSQC NMR

To obtain detailed structural changes of lignins during phthalation in AmimCl, the unmodified (sample L0) and phthalated lignins (sample L4) were characterized with 2D HSQC NMR spectroscopy. The lignin region in the HSQC spectrum of the phthalated bagasse (sample S5) and the HSQC spectrum of phthalated lignin obtained in the present study are comparatively illustrated in Fig. 4. The main structures of the identified lignin subunits are depicted in Fig. 5. Based on the previous publications (Hallac *et al.* 2010; Wang *et al.* 2012; Cheng *et al.* 2013; Mbotchak *et al.* 2015), the primary correlations were well assigned. The HSQC spectra of lignins were divided into two regions: aromatic region (δ_H 5.8 to 8.0 ppm, δ_C 95 to 145 ppm) and side-chain region (δ_H 2.5 to 6.0 ppm, δ_C 50 to 90 ppm).

In the aromatic region, the cross-peaks from the S, G, and H units as well as ferulate (FA) and *p*-coumarate (*p*-CA) could be recognized. An obvious signal for the S-units $C_{2,6}/H_{2,6}$ at δ_C/δ_H 104.68/6.72 ppm was distinctively identified, and the signals for the C_α-oxidized S-units (S') at δ_C/δ_H 107.15/7.34 ppm was also observed. The cross-peaks that originated from G-units were well distinguished: C_2/H_2 (δ_C/δ_H 111.69/7.00 ppm), C_5/H_5 (δ_C/δ_H 115.33/6.71 ppm), and C_6/H_6 (δ_C/δ_H 119.75/6.80 ppm). The $C_{2,6}/H_{2,6}$ correlations in H-units appeared at δ_C/δ_H 118.66/7.20 ppm. The different cross-peaks from *p*-CA were identified: $C_{2,6}/H_{2,6}$ (δ_C/δ_H 130.98/7.47 ppm), $C_{3,5}/H_{3,5}$, (δ_C/δ_H 116.38/3.77 ppm), C_7/H_7 (δ_C/δ_H 145.32/7.41 ppm), and C_8/H_8 (δ_C/δ_H 114.48/6.27 ppm). The cross-peaks for FA units C_2/H_2, C_5/H_5, and C_7/H_7 were located at δ_C/δ_H 11.84/7.34, 115.64/6.93, and 145.32/7.40

ppm, respectively. Moreover, in the HSQC spectra S5 (b), the presence of the correlations at 128.82/7.65, 129.39/7.77, 131.71/7.63, and 130.98/7.52 ppm, which correspondingly related to C-3', C-6', C-4', and C-5' positions in phthaloyl group from the esterified lignin, confirmed the attachment of phthaloyl group.

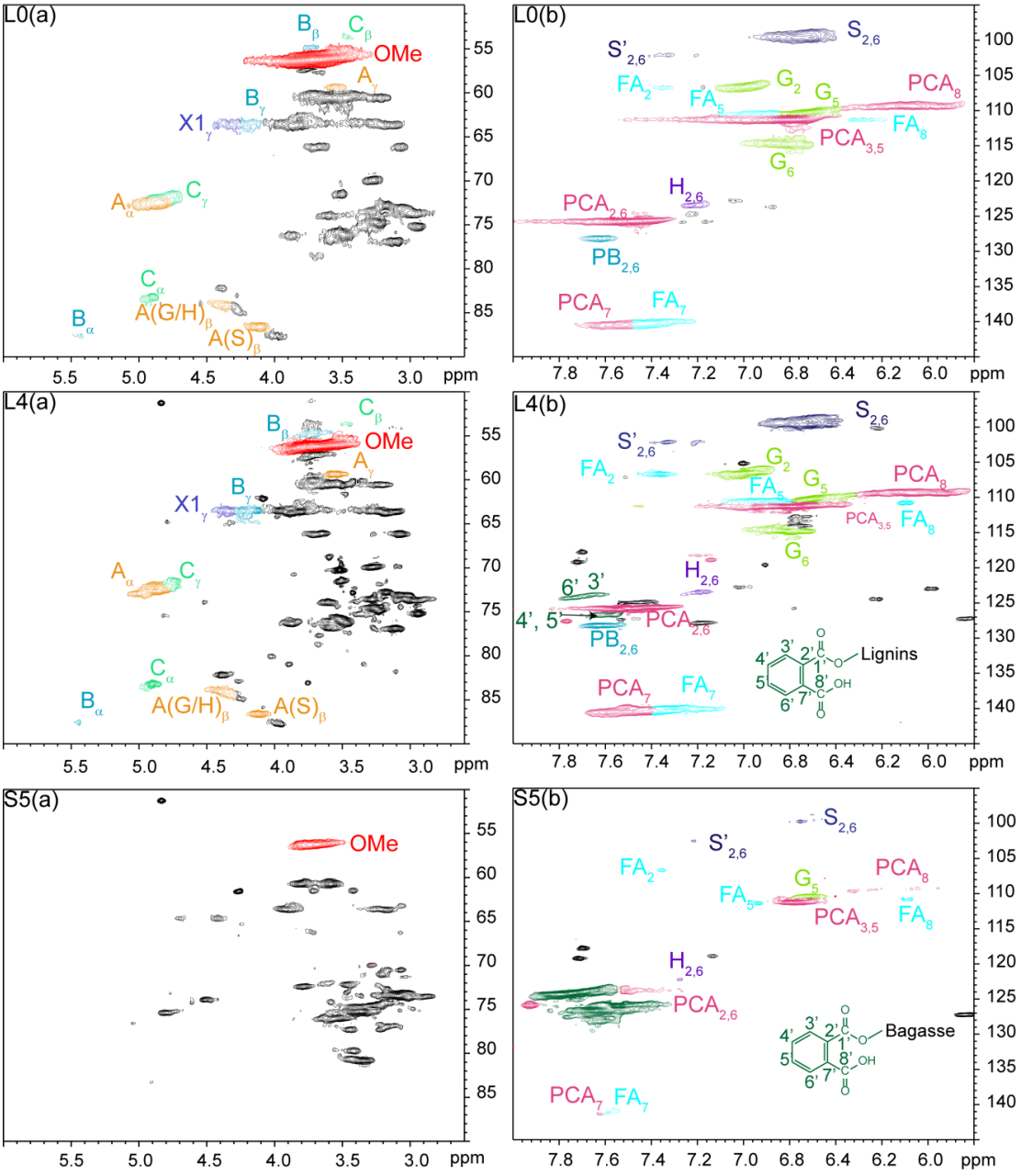

Fig. 4. Side-chain (a) and aromatic region (b) of the 2D HSQC NMR spectra of the unmodified (L0) and phthalated lignins (L4) as well as phthalated bagasse (S5)

In the side chain region, the lignin inter-unit linkages were well identified through their characteristic cross-peaks. The main substructures, including β-aryl ether (β-*O*-4', unit A), phenylcoumaran (β-5', unit B), and resinol (β-β', unit C), can be observed in the 2D HSQC spectra. The C_α/H_α correlations in β-*O*-4' linkages at δ_C/δ_H 72.43/4.84 ppm were well recognized. The cross-peaks at δ_C/δ_H 84.16/4.35 and 86.69/4.09 ppm were associated

with the C_β/H_β correlations in β-*O*-4' substructures linked to G/H and S units, respectively. The C_γ/H_γ correlations in β-*O*-4' substructures were easily observed at δ_C/δ_H 60.35/3.48 ppm, which were partially overlapped with the cross-peaks of the associated polysaccharides (Du *et al.* 2014). In addition, phenyl-coumaran (β-5', substructure B) appeared in noticeable amounts as indicated by the C_α/H_α, C_β/H_β, and C_γ/H_γ correlations at δ_C/δ_H 87.67/5.43, 55.36/3.70, and 63.50/4.21 ppm, respectively. The cross-peaks for resinol (β-β', substructure C) were also well identified: C_α/H_α, (δ_C/δ_H 83.59/4.92 ppm), C_β/H_β (δ_C/δ_H 53.73/3.45 ppm), and the double C_γ/H_γ (δ_C/δ_H 71.86/4.74 ppm). Furthermore, the cross-peak for the C_γ/H_γ correlation of *p*-hydroxycinnamyl alcohol end groups (X_1) was located at δ_C/δ_H 63.70/4.30 ppm.

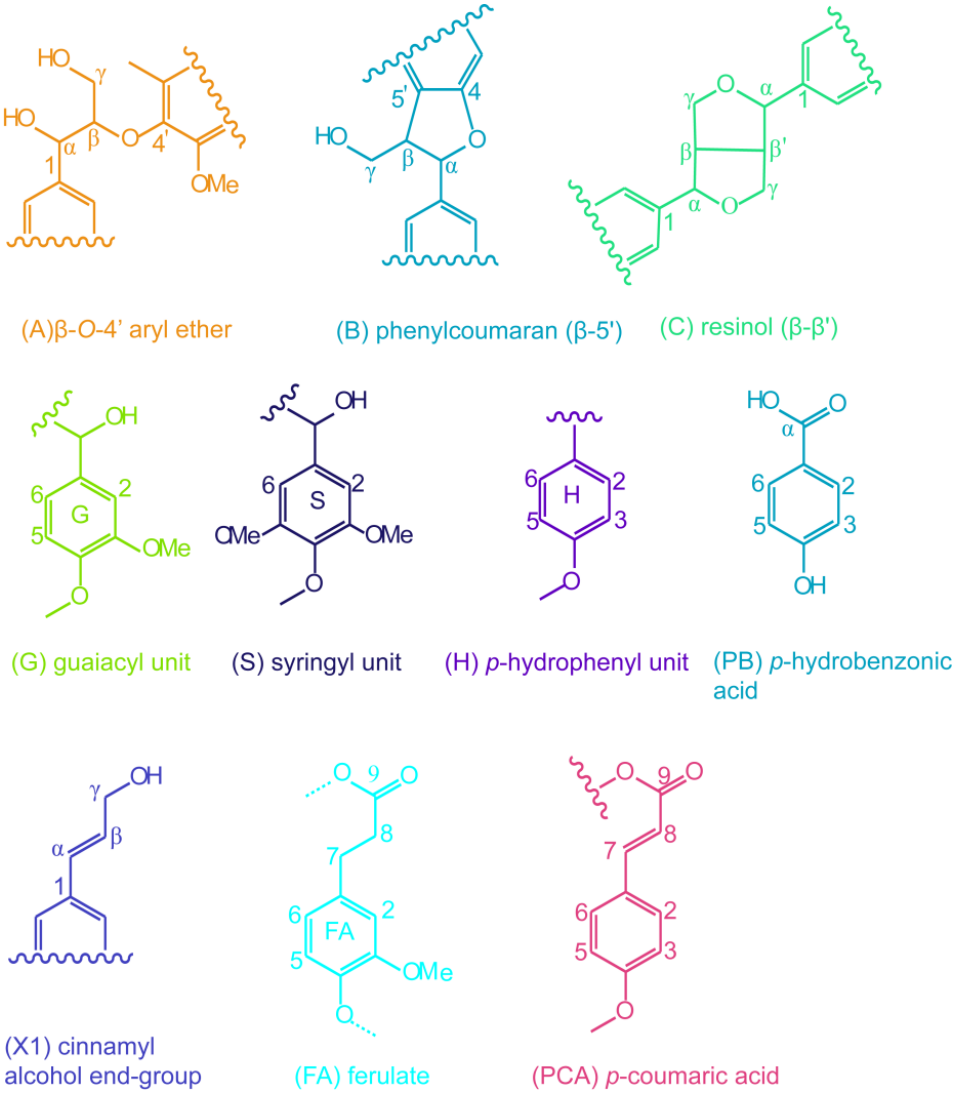

Fig. 5. Main substructures and aromatic units identified in lignins

According to the previous studies (Cheng *et al.* 2013; Wen *et al.* 2013), the relative quantities of the primary substructures could be calculated. The aromatic lignin H-, S-, and G-units were expressed as a fraction of 100%, and the relative molar quantities of aryl ether, phenylcoumaran, resinol, ferulate, and *p*-coumarate were expressed as a percentage of the

total aromatic H-, S-, and G-units. The detailed information of quantitative lignin substructures calculated from HSQC is listed in Table 3.

Table 3. Quantitative Information of Unmodified (L0) and Phthalated Lignins (L4), and Phthalated Bagasse (S5) Determined with 2D HSQC NMR

	L0	L4	S5
Aliphatic as Percentage of (S+G+H)			
Aryl ether (A)	45.6/100Ar	40.7/100Ar	N.D.
Phenylcoumaran (B)	3.5/100Ar	4.7/100Ar	N.D.
Resinol (C)	9.7/100Ar	23.3/100Ar	N.D.
Aromatic Molar percentages (S+G+H =100%)			
Guaiacyl (G)	43.9	37.2	27.3
Syringyl (S)	48.7	59.9	67.1
p-hydroxyphenyl (H)	7.5	2.9	5.6
S/G	1.1	1.6	2.5
p-hydroxycinnamates as Percentage of (S+G+H)			
p-coumarate (*p*CA)	75.0/100Ar	116.7/100Ar	101.2/100Ar
Ferulate (FA)	7.0/100Ar	10.5/100Ar	N.D.

N.D. represents not detected.

In the side-chain region of unmodified lignins, lignins displayed a predominant β-*O*-4' aryl ether linkages (45.6/100Ar), followed by resinol linkages (9.7/100Ar), and lower content of phenylcoumaran linkages (3.5/100Ar). After phthalation, the content of resinol linkages increased from 9.7/100Ar (sample L0) to 23.3/100Ar (sample L4); the content of phenylcoumaran linkages also increased from 3.5/100Ar (sample L0) to 4.7/100Ar (sample L4). These noticeable increases were probably due to the decreased intensity of $C_{2,6}/H_{2,6}$ correlations from aromatic units resulting from the degradation of lignin during the homogeneous phthalation. Comparatively, the content of β-*O*-4' aryl ether linkages obviously decreased from 45.6/100Ar (sample L0) to 40.7/100Ar (sample L4). Considering the decreased intensity of $C_{2,6}/H_{2,6}$ correlations from aromatic units, this decrease implied the cleavage of β-*O*-4' aryl ether linkages (Sannigrahi *et al.* 2008) during lignin phthalation in AmimCl.

In the aromatic region, the relative contents of S-, G-, and H-units in the unmodified lignins (sample L0) were 48.7%, 43.9%, and 7.5%, respectively. After phthalation, the relative content of G-unit decreased from 43.9% (sample L0) to 37.2% (sample L4), and a decrease in the relative contents of H-unit from 7.5% (sample L0) to 2.9% (sample L4) was observed. This result indicated that G- and H-units were degraded, which was probably due to the acidic degradation of lignin resulting from phthalic acid released from phthalic anhydride in AmimCl (Zhang *et al.* 2015). Therefore, it was reasonable that the S/G ratio increased from 1.1 (sample L0) to 1.6 (sample L4). Notably, the relative content of H-unit calculated from HSQC NMR was much lower than that of S- or G-units, which was noticeably different from ^{31}P NMR analysis. By comparing the ^{31}P NMR and HSQC analysis of unmodified lignin, the relative content of H-unit calculated from HSQC NMR was much lower than that of S- or G-units, which was noticeably different from ^{31}P NMR analyses. This discrepancy was primarily due to the different resolution of 2D HSQC NMR from ^{31}P NMR. The ^{31}P NMR records the S-, G- and H- units with 4-OH group only, while the HSQC records the total S-, G- and H- units, including the un-substituted 4-OH and substituted 4-OH (such as β-*O*-4'). In addition, the phenolic H-OH and *p*-CA-OH were overlapped in the ^{31}P NMR, while the correlations from lignin H-unit and *p*-CA were much

different and easily recognized in the HSQC. As shown in Table 3, the relative content of *p*-CA increased from 75.0/100Ar (sample L0) to 116.7/100Ar (sample L4), and an increase in the relative content of FA from 7.0/100Ar (sample L0) to 10.5/100Ar (sample L4) was also observed. These results were probably due to the degradation of lignin aromatic units, and indicated that LCCs in lignin were not significantly changed during the homogeneous esterification.

Comparatively, to identify the primary signals from the three main components, the characteristic correlations from the primary lignin substructures A-C were not detected in the phthalated bagasse (sample S5) at a relatively high contour level. The similar results were reported in the previous research (Cheng *et al.* 2013). The cross-peaks of G-, S-, and H-units were clearly observed. The relative contents of S-, G-, and H-units in the phthalated bagasse (sample S5) were 67.1%, 27.3%, and 5.6%, respectively, and the S/G ratio was 2.45. Compared with the unmodified lignin (sample L0), the relative contents of G- and H-units in the phthalated bagasse (sample S5) decreased with a correspondingly slight increase in the relative content of S-unit. These results indicated the degradation of lignin, especially G- and H-units, during bagasse phthalation in AmimCl, which was consistent with the results obtained from the phthalated lignins (sample L4). Moreover, the S/G ratio increased from 1.1 (sample L0) to 2.5 (sample S5), which further confirmed the remarkable degradation of lignin G-units during bagasse homogeneous phthalation.

Molecular Weights

To illustrate the extent of degradation of lignins during phthalation in AmimCl, the unmodified (sample L0) and phthalated lignins (samples L1-L5) were comparatively analyzed by GPC. The changes of M_w, M_n, and the polydispersities (M_w/M_n) of six lignin samples are shown in Table 4. The phthalation degree increased from 0 (L0) to 47.2% (L1), and an increase in the molecular weight from 74467 (L0) to 10243 g/mol (L1) was observed. This indicated that the phthalation resulted in the increased molecular weight. Raising the dosage of phthalic anhydride from 10 to 20 mmol/g resulted in a phthalation degree increase from 47.2% (L1) to 49.9% (L2). However, the molecular weight decreased from 102243 (L1) to 100426 g/mol (L2), and they were both higher than that of unmodified lignin (L0). The decrease in the molecular weight was probably due to the degradation of phthalated lignin samples (Yan *et al.* 2015; Wu *et al.* 2016). These results suggested that the phthalation and degradation simultaneously occurred, consistent with the 2D HSQC NMR analyses. The phthalation degree gradually increased from 41.1% (L3) to 68.8% (L5), while the molecular weight fluctuated in a range from 85722 to 68933 g/mol. These results indicated that the phthalation and degradation occurred simultaneously, leading to the molecular weight fluctuation during lignin homogenous esterification. The former resulted in an increase in the molecular weight of samples, while the latter led to a decrease in the molecular weight of samples. Besides, the polydisperisity of phthalated lignin samples gradually decreased from 2.37 (L1) to 1.85 (L5), which was possibly due to the loss of fractions with low molecular weight during homogeneous modification.

Table 4. Weight-Average (M_w) and Number-Average Molecular Weights (M_n) (g/mol), and the Polydispersities (M_w/M_n) of the Phthalated Lignins (L1-L5)

Sample	L0	L1	L2	L3	L4	L5
M_w	74467	102243	100426	77605	85722	68933
M_n	32261	43066	42758	36955	41076	37321
M_w/M_n	2.31	2.37	2.35	2.10	2.09	1.85

CONCLUSIONS

1. The phthalation degree of phthalated lignins determined by ^{31}P NMR ranged from 41.1% to 68.8%. The analyses by FT-IR and 2D HSQC NMR confirmed the attachment of phthaloyl group onto lignins.
2. ^{31}P NMR analysis suggested that the phthalation reactivity of lignins mainly depended on the reactivity of lignin aliphatic hydroxyls, and the reactivity of phenolic hydroxyls followed the order of non-condensed H-OH > non-condensed G-OH > non-condensed S-OH.
3. The phthalation and degradation simultaneously occurred during lignin homogeneous esterification, and the severe degradation at high phthalic anhydride dosage (30 to 50 mmol/g) resulted in the molecular weight fluctuation of lignin samples.

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