SUPPORTING INFORMATION

Understanding the Chemical Transformations of Lignin during Ionic Liquid Pretreatment

Jia-Long Wen, Tong-Qi Yuan, Shao-Long Sun, Feng Xu, and Run-Cang Sun

Table S1 Assignment of main lignin ^{13}C-^{1}H cross-signals in the HSQC spectra of the lignin fractions

label	δ_C/δ_H (ppm)	assignment
C_β	53.4/3.49	C_β−H_β in phenylcoumarane substructures (C)
B_β	53.5/3.10	C_β−H_β in resinol substructures (B)
-OCH_3	55.6/3.73	C−H in methoxyls
A_γ	59.5−59.7/ 3.40−3.63	C_γ−H_γ in β-O-4′ substructures (A)
I_γ	61.3/4.10	C_γ−H_γ in p-hydroxycinnamyl alcohol end groups (I)
C_γ	62.5/3.68	C_γ−H_γ in phenylcoumaran substructures (C)
B_γ	71.1/3.83 and 4.19	C_γ−H_γ in resinol substructures (B)
A_α	71.8/4.88	C_α−H_α in β-O-4′ substructures (A)
$A_{\beta(G/H)}$	83.6/4.32	C_β−H_β in β-O-4′ substructures linked to G and H units (A)
B_α	84.8/4.69	C_α−H_α in resinol substructures (B)
$A_{\beta(S)}$	85.8/4.12	C_β−H_β in β-O-4′ substructures linked to S units (A)
C_α	86.7/5.49	C_α−H_α in phenylcoumaran substructures (C)
$S_{2,6}$	104.0/6.72	$C_{2,6}$−$H_{2,6}$ in etherified syringyl units (S)
$S'_{2,6}$	106.3/7.35	$C_{2,6}$−$H_{2,6}$ in oxidized (C_α=O) syringyl units (S′)
G_2	110.9/6.98	C_2−H_2 in guaiacyl units (G)
G_5	114.7/6.74	C_2−H_2 in guaiacyl units (G)
G_6	119.0/6.80	C_6−H_6 in guaiacyl units (G)
$H_{2,6}$	127.9/7.19	$C_{2,6}$−$H_{2,6}$ in *p*-hydroxyphenyl units (H)
$PB_{2,6}$	131.3/7.82	$C_{2,6}$−$H_{2,6}$ in free *p*-hydroxybenzonic acid (PB)

EXPERIMENTAL SECTION

1. Isolation and Purification of Alkaline Lignin

The ball-milled poplar wood (20 g) was first extracted with 1% sodium hydroxide with a solid-to-liquid ratio of 1:15 (g/mL) at 75 ^{o}C for 3 h. After filtration, the pH of the filtrate was adjusted to 5.5-6.0 by 6 M HCl. The filtrate was concentrated at reduced pressure and then precipitated in 3 volumes of 95% ethanol. A pellet rich in hemicelluloses was recovered by filtering. After evaporation of ethanol, the alkali-soluble lignin was obtained by precipitation at pH 1.5 to 2, which was adjusted by 6 M HCl. This lignin fraction was named AL and washed thoroughly with acidified water (pH 2.0) before freeze-drying.

2. Acetylation of the Lignins

10 mg of Lignin sample was dissolved in 1.5 mL of a solution of acetic anhydride: pyridine (1:1). After stirring for 24 h at room temperature under the exclusion of light, the mixture was concentrated under reduced pressure by adding ethanol for several times. The mixture was dropped slowly into 60 mL of ice water (pH = 2.0) to induce precipitation, and the precipitate was washed thoroughly with deionized water (pH = 2.0), and freeze-drying.

3. Structure Elucidation of the Lignins

GPC Analysis

The weight-average (M_w) and number-average (M_n) molecular weights of the lignins were determined by gel permeation chromatography (GPC) on a PL-gel 10 mm Mixed-B 7.5 mm ID column. A 4 mg acetylated sample was dissolved in 2 mL of tetrahydrofuran, and a 10 μL sample in solution was injected. The column was operated at ambient temperature and eluted with tetrahydrofuran at a flow rate of 1 mL/min. Monodisperse polystyrene was used as the standard for the molecular weight of lignin.

2D-HSQC NMR

About 50 mg of lignin was dissolved in 0.5 mL of DMSO-d_6 (99.8% D). For quantitative 2D-HSQC spectra, the Bruker standard pulse program hsqcetgpsi2 was used for HSQC experiments. The spectral widths were 5000 Hz and 20000 Hz for the ^{1}H- and ^{13}C-dimensions, respectively. The number of collected complex points was 1024 for ^{1}H-dimension with a recycle delay of 1.5 s. The number of transients was 64, and 256 time increments were always recorded in the ^{13}C-dimension. The $^{1}J_{CH}$ used was 145 Hz. Prior to Fourier transformation, the data matrixes were zero filled up to 1024 points in the ^{13}C-dimension. Data processing was performed using standard Bruker Topspin-NMR software.

A quantitative analysis of the intensities of the HSQC cross-signal was performed according to the following formula:

$I(C_9)$ units = $0.5I(S_{2,6}) + I(G_2)$

Where $I(S_{2,6})$ is the integration of $S_{2,6}$, including S and S', $I(G_2)$ is the integral value of G_2. $I(C_9)$ represents the integral value of the aromatic ring. According to the internal standard ($I(C_9)$), the amount of I(X)% could be obtained by the following formula:

$I(X)\% = I(X)/I(C_9) \times 100\%$

Where I(X) is the integral value of the α-position of A (β-*O*-4′), B (β-β), and C (β-5), the integration should be in the same contour level.

In the aromatic region, $C_{2,6}$-$H_{2,6}$ correlations from S units and C_2-H_2 correlation from G units were used to estimate the S/G ratio of lignin. The S/G ratio could be obtained by the following formula:

$S/G = 0.5I(S_{2/6})/I(G_2)$

Quantitative ^{13}C NMR

For the quantitative ^{13}C NMR experiments, 100 mg of lignin was dissolved in 0.35 mL of DMSO-d_6 (99.8% D), and 20 μL of chromium(III) acetylacetonate (0.01 M) was added as a relaxation agent to reduce the relaxation delay. The quantitative ^{13}C NMR spectra were recorded in the FT mode at 100.6 MHz. The inverse gated decoupling sequence (C13IG sequence from Bruker Standard Library), which allows quantitative analysis and comparison of signal intensities, was used with the following parameters: 30^{o} pulse angle; 1.4 s acquisition time; 2 s relaxation delay; 64K data points, and 30000 scans.

Quantitative ^{31}P NMR

20 mg lignin was dissolved in 500 μL of anhydrous pyridine and deuterated chloroform (1.6:1, v/v) under stirring. This was followed by the addition 100 μL of cyclohexanol (10.85 mg/mL in anhydrous pyridine and deuterated chloroform 1.6:1, v/v) as an internal standard and 100 μL of Chromium (III) acetylacetonate solution (5 mg mL^{-1} in anhydrous pyridine and deuterated chloroform 1.6:1, v/v) as relaxation reagent. The mixture was reacted with 100 μL of phosphitylating reagent (2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholate, TMDP) for about 10 min and was transferred into a 5 mm NMR tube for subsequent NMR analysis.