*Chemistry of Natural Compounds, Vol. 45, No. 5, 2009*

# SEPARATION AND CHARACTERISTIC ANALYSIS OF STEAM-EXPLODED LIGNIN FROM CORNSTALK RESIDUE

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UDC 547.992

*Steam-exploded lignin (SEL) was separated from cornstalk residue, which came from steam-exploded cornstalk after enzymatic hydrolysis. There are two methods to acquire SEL, the alkali solution and the organic solvent method. SEL was analyzed with respect to the elementary composition, molecular weight, IR spectrum, and $^{13}C$ NMR spectra. The $C_9$-formula of SEL was calculated from the experiment data. According to $^{13}C$ NMR, SEL can be classified as a "GSH" type of lignin, and it is composed mainly of* β-O-*4 ether bonds together with* β-*5 and* β-*1 carbon-carbon linkages between the lignin structural units.*

**Key words:** enzymatic hydrolysis of cornstalks, steam-exploded lignin (SEL), structure of SEL, separation.

Many researchers use cornstalks as raw material for making bioalcohol by transforming the saccharide components through steam explosion to enzymatic hydrolysis [1]. This is an important application for renewable natural resources. There is about 30–35% lignin in cornstalk residue [2]. Steam-exploded lignin (SEL) from cornstalk residue can be obtained by two methods, the aqueous alkali solution and the organic solvent method.

Lignin is an amorphous aromatic network polymer made up of phenylpropane ($C_6$–$C_3$) units by carbon-carbon bonds and ether linkages, in which the *β*-aryl ether linkage (48% and 60% in spruce and birch lignin, respectively) is the most dominant [3]. It is a renewable and nontoxic resource, which has potential application as polymer-based products such as lignin-phenol formaldehyde resin, lignin-isocyanate polyurethane adhesives, foam insulation, etc. [2–4]. But at present time, lignin is mainly separated from the black liquor of the pulping process, in which the original structure of lignin is destroyed. In spite of many years of development effort, industrial lignin products are limited in number. SEL is used in polymer modification because it preserves the original chemical activity perfectly. Chemical analysis as well as NMR and infrared spectroscopy were used to analyze the structure of lignin, and also to give information on the actual chemical group altered, removed, and/or added to lignin during the separation process [5]. In this paper, the structural and physical and chemical characterization of SEL was investigated by chemical analysis and infrared and NMR spectroscopy.

There are two methods for extracting SEL. An aqueous alkali solution easily reacts with lignin. Sodium hydroxide or ammonia was selected as the alkali solution. According to orthogonal analysis, the reaction temperature is set at 65–70°C. If the pH value is adjusted by $H_2SO_4$, lignin will be precipitated [6]. The second method of separating lignin is by organic solvent extraction. Some solvents such as 1,4-butanediol, glycol, tetrahydrofuran, or dimethyl sulfoxide can be chosen to dissolve the lignin, but the boiling point of tetrahydrofuran or dimethyl sulfoxide is very low, and its toxicity is unfavorable to work with. So 1,4-butanediol and glycol were chosen to dissolve the lignin. The extraction yields are listed in Table 1.

According to chemical analysis, the elemental composition and methoxyl contents of different kinds of SELs are shown in Table 1. The carbon content of SEL extracted by organic solvent is higher than that of SEL extracted by aqueous alkali solution. The $C_9$ formula, which represents the characteristic type of lignin structure, can be established by the traditional calculation method according to elemental analysis and the $OCH_3$ content. Following a proposal by Holmberg, it is now customary to express the analytical value in a $C_9$ formula to show the different structural types of lignin.

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Published in Khimiya Prirodnykh Soedinenii, No. 5, pp. 582–584, September–October, 2009. Original article submitted March 9, 2008.

TABLE 1. Elementary Composition and $C_9$-Formula of SEL

| Method | SEL | | Elementary composition, % | | | $OCH_3$, % | $C_9$-formula |
|---|---|---|---|---|---|---|---|
| | name | yield, % | C | H | O | | |
| Sodium hydroxide (3%) | SL | 35 | 57.24 | 5.83 | 34.31 | 9.36 | $C_9H_{9.83}O_{3.71}(OCH_3)_{0.61}$ |
| Ammonia (25%) | AL | 32 | 55.27 | 6.01 | 36.90 | 8.50 | $C_9H_{10.68}O_{4.22}(OCH_3)_{0.57}$ |
| 1,4-Butanediol | BL | 20 | 63.14 | 6.50 | 29.14 | 9.04 | $C_9H_{10.09}O_{2.77}(OCH_3)_{0.53}$ |
| Glycol | GL | 19 | 59.87 | 5.83 | 32.25 | 8.81 | $C_9H_{8.86}O_{3.32}(OCH_3)_{0.54}$ |

TABLE 2. Molecular Weight of SEL

| Method | SEL name | Mn | $M_P$ | Mw | Mz | Polydispersity |
|---|---|---|---|---|---|---|
| Sodium hydroxide | SL | 939 | 1047 | 1124 | 1338 | 1.20 |
| Ammonia | AL | 1044 | 1241 | 1290 | 1572 | 1.24 |
| 1,4-Butanediol | BL | 1167 | 1284 | 1544 | 1995 | 1.32 |
| Glycol | GL | 973 | 1220 | 1258 | 1597 | 1.29 |

TABLE 3. Infrared-Absorption Bands of HBS

| Position, $cm^{-1}$ | | | | Band origin |
|---|---|---|---|---|
| SL | AL | BL | GL | |
| 3332 | 3300 | 3383 | 3404 | –OH stretching |
| 2934 | 2933 | 2929 | 2930 | C–H stretching |
| – | – | 1705 | 1700 | C=O stretching nonconjugated to the aromatic ring |
| 1603 | 1610 | 1604 | 1603 | C=O stretching conjugated to the aromatic ring |
| 1514 | 1520 | 1515 | 1517 | Aromatic ring vibrations |
| 1424 | 1423 | – | 1420 | Aromatic ring vibrations, C–H deformations |
| 1328 | – | 1329 | 1342 | Aromatic ring vibrations of syringyl and guaiacyl |
| 1216 | 1201 | 1224 | 1217 | C–*O* stretching of syringyl |
| – | – | 1169 | 1161 | C–*O*–C deformation |
| 1120 | 1122 | 1125 | 1161 | Aromatic ester |
| 1030 | 1013 | 1046 | – | C–H and C–*O* deformation |
| 834 | 820 | 834 | 832 | Syringyl C–H vibrations |

The equations of Karl Freudenberg were used to calculate the $C_9$ formula [7]. The $C_9$ formula of SELs is shown in Table1. The average molecular weights of the SELs were about 1200 g.mol$^{-1}$, except for SEL extracted by 1,4-butanediol (Table 2). The dispersity in molecular weights was near 1.0, which showed that the molecular weights of the SELs were very similar.

The IR spectra of the SELs showed a strong absorbance centered about 3300–3400 cm$^{-1}$, assigned to OH groups. Other signals are listed in Table 3 [8, 9]. The IR spectra of GL and BL showed a strong absorbance centered about 1700 cm$^{-1}$, assigned to C=O in nonconjugated ketones, carbonyls, and ester groups [10], but AL and SL showed no absorbance in this band. The absorption band in 1169 cm$^{-1}$ was attributed to the C-*O*-C deformation in GL and BL, but it did not appear in AL and SL, since the ether linkage may have be destroyed by the alkali solution. Moreover, syringyl and condensed guaiacyl absorptions clearly appeared at 1328 cm$^{-1}$. Comparing the absorption of SL with BL, the bands at 1216 cm$^{-1}$ and 1328 cm$^{-1}$ were weak, which showed that the syringyl content of BL was greater than that of SL. From the absorption of SELs, the peaks in GL and BL were stronger than AL and SL, which showed that purification by the method of organic solvents preserves the basic group better than the method of alkali solution.

TABLE 4. Assignment of Signals in $^{13}$C NMR Spectra of SEL

| Signal (ppm) | | | | Assignment |
|---|---|---|---|---|
| BL | GL | SL | AL | |
| 174.7 | 174.9 | 174.3 | 174.8 | C of side chains in aliphatic acid |
| 171.6 | 171.4 | 170.7 | 171.2 | C=O in primary aliphatic acetyl groups |
| 166.8 | 166.4 | 168.2 | 168.2 | C=O in aromatic acetyl groups |
| 152.5 | 152.6 | 152.9 | 152.6 | C-3 in guaiacyl and C-$\alpha$ in cinnamaldehyde |
| 147.8 | 147.9 | 147.6 | 147.8 | C-3 in guaiacyl nonetherified<br>C-4 in guaiacyl $\beta$-$O$-4 |
| 144.7 | 145.0 | 144.5 | 144.4 | C-4 in 4-$O$-5 and $\beta$-5 units |
| 135.6 | 135.4 | 135.6 | 135.0 | C-1 in guaiacyl etherified |
| 134.5 | 134.7 | 134.6 | 134.2 | C-1 in guaiacyl and syringyl nonetherified |
| 128.9 | 128.6 | 128.0 | 128.6 | C-$\beta$ in cinnamaldehyde units<br>C-2/C-6 in $p$-hydroxybenzoate |
| 125.3 | 125.0 | 124.4 | 125.2 | Olefinic carbons, and C-6 in catechol units |
| 120.8 | 120.7 | 121.0 | 120.9 | C-1 in $p$-hydroxybenzoate |
| 118.7 | 119.0 | 119.5 | 119.1 | C-6 in guaiacyl |
| 116.3 | 115.9 | - | 116.1 | C-5 in guaiacyl |
| 111.4 | 111.3 | 111.6 | 111.4 | C-2 in guaiacyl |
| 104.6 | 104.5 | 104.4 | 104.6 | C-2/C-6 in syringyl with $\alpha$-C=O |
| 79.7 | 79.9 | 80.2 | – | C-$\beta$ in $\beta$-$O$-4 |
| 72.9 | 72.5 | 72.6 | – | C-$\gamma$ in $\beta$–$\beta$ |
| 63.9 | 63.4 | 63.8 | 63.4 | C-$\gamma$ in $\beta$-$O$-4 with $\alpha$-C=O |
| 61.1 | 61.1 | 61.0 | 61.4 | C-$\gamma$ in $\beta$-$O$-4 |
| 56.0 | 56.0 | 55.9 | 56.0 | Methoxyl |
| 53.7 | 54.0 | 54.2 | 54.5 | C-$\beta$ in $\beta$-5 and $\beta$–$\beta$ |
| 29.2 | 29.1 | 29.3 | 29.1 | -CH$_2$- |
| 21.8 | 21.9 | 21.3 | 21.9 | -CH$_3$ of acetate groups |
| 14.3 | 14.2 | 14.3 | 14.4 | -CH$_3$ of other groups |

Peak assignments of SEL $^{13}$C NMR spectra are listed in Table 4. The peaks in the $^{13}$C NMR spectra can be assigned to distinct structural units [11]. The signals of the aromatic part of the lignin could be observed in the region between 105 and 160 ppm [12, 13]. The carbonate units gave very weak signals above 160 ppm, except for SL. The results showed that the SELs were linked with guaiacyl, syringyl, and $p$-hydroxybenzoate type. A very strong signal at 55.6 ppm was assigned to the OCH$_3$ groups in syringyl and guaiacyl units. There were strong signals at 80.2 ppm, 61.1 ppm, and 63.5 ppm, which are characteristic of $C_\beta$, $C_\alpha$, $C_\gamma$ in $\beta$-$O$-4 links in the basic structure of lignin [14]. The common carbon-carbon linkages, such as $\beta$-$\beta$ ($C_\gamma$ in $\beta$-$\beta$ units, 72.9 ppm), were also present, but they were very weak.

In conclusion, SEL is a GSH type lignin with abundant OCH$_3$ groups. The $^{13}$C NMR spectra of SELs showed that the $\beta$-$O$-4 bond, $\beta$-5, and $\beta$-1 carbon-carbon linkages are the major linkages between phenylpropane units. The syringyl units were predominant in SL, but not the carbonate units. The basic lignin structure of AL was clearest in these SELs, such as $\beta$-$\beta$, $\beta$-$O$-4, $\beta$-5, and $\beta$-1 linkages. The GL was similar to BL, with just a drift in the 60–70 ppm signals of C-$\gamma$ in the $\beta$-$O$-4 link. According to spectroscopic analysis, it was found that the SELs preserve the basic structure of lignin. As a green material, SEL may have potential applications in polymer modification, such as blending with butadiene nitrile rubber to improve thermal performance [15], or reacting with aldehyde to form modified lignin resin.

## EXPERIMENTAL

**Material and Chemicals.** Cornstalk residue from enzymatic hydrolysis was provided by Prof. S. Y. Yu of Nanjing Forestry University. The cornstalk was pretreated by steam explosion at 200°C, 1.7 MPa for 6 to 10 min. The residue was separated from the steam-exploded cornstalk after trichoderma hydrolysis experiments carried out at 50±1°C, pH 4.80, and oscillation speed 80 rpm for 48 hours [1].

**Extraction by Aqueous Alkali Solution.** Weigh 5.0 g of cornstalk residue in the flask and add aqueous alkali solution. Let it react at 65°C for 1 h, then filter the residue to obtain the lignin aqueous alkali solution. Add 2% $H_2SO_4$ to adjust pH to 3, and keep the temperature at 70°C for 30 min. When the lignin floats up, stir it until it sinks, then let it stay overnight. Collect the lignin by centrifugation; wash it 3 times by water. Dissolve the solid in dioxane; then precipitate the SEL by ether and dry at 60°C.

**Extraction by Organic Solvent.** Weigh 5.0 g of cornstalk residue in the flask and add organic solvent. Extract at 70–750°C for 1 h, then filter. Add 3 times the volume of water to the filtrate to decrease the lignin solubility. Then proceed as in the method of aqueous alkali solution.

**Chemical and Spectroscopy Analysis.** Methoxyl group analysis was performed using the Zeisel procedure. Elemental analysis was carried out with a Heracus CHN-O-RAPID analyzer. Molecular weight was determined by Waters-515 gel permeation chromatography with a Waters 410 RI detector in tetrahydrofuran (THF) at 40°C in an Ultrastyragel $10^3$A column. The flow speed of THF was 1.0 mL/min, and the run time was 60 min. Infrared spectra of SEL were obtained using a PE 2000 IR spectrometer. The samples were measured on KBr discs. The $^{13}C$ NMR spectra were recorded at room temperature on a Bruker DRX-400 spectrometer in DMSO-$d_6$. Quantitative analysis and comparisons of signal intensities were carried out using the following parameters: 90° pulse angle, 3 sec pulse delay, 16 K data points, and number of scans 12000.

## ACKNOWLEDGMENT

The author thanks the Key Laboratory of Cellulose and Lignocellulosics Chemistry, Guangzhou Institute of Chemistry, Chinese Academy of Sciences for financial support (LCLC-2004-158).

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