Fractionation of Lignophenols and Structural Features of Low Molecular Weight Fractions

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For structural elucidation of native lignin, native lignins of Western hemlock (*Tsuga heterophylla*), Birch (*Betula papyrifera marsh*) and Rice straw (*Oryza sativa*) were converted to lignin-based polymers, lignophenols, through the phase-separation system. The lignophenols were divided into ether soluble (ESL) and insoluble (EIL) fractions. Their structural features were analyzed by GPC, UV-Vis, FT-IR and ¹H-NMR. The weight ratio of ESL to EIL from Rice straw (grass plant) was higher than those from Western hemlock (softwood) and Birch (hardwood), indicating the difference in the growth of primary lignin chains resulted from random radical coupling of precursors. The FT-IR spectra of ESL showed higher absorption at 815 cm⁻¹ due to C-H out of plane skeletal vibration of aromatic ring, suggesting higher frequency of grafted p-cresol, which was supported by ¹H-NMR.

Key words: native lignin, phase-separation system, lignophenols, column chromatography

1. INTRODUCTION

Lignocellulosics have attracted the attention as sustainable and recyclable resources (aliphatic and aromatic resources) instead of fossil carbon resources. However, it is very difficult to separate lignocellulosics to aliphatic (carbohydrate) and aromatic (lignin) moieties without destroying fundamental functions, because of interpenetrating polymer network structures in the cell wall and the sensitivity of lignin for environmental change. Especially it has been difficult to obtain detailed information on native lignin which is a delicate 3-dimensional natural aromatic polymer in complicated lignocellulosic composites. Lignin preparations derived by conventional methods were subjected to random modification, giving almost no information on native lignin structures.

The phase-separation system has been developed by Funaoka in 1988 for total utilization of both lignin and carbohydrate in lignocellulosics such as woody and grass materials [1-4]. Through this method, including the interface reactions between phenol derivatives and concentrated acid layers, lignocellulosics are rapidly converted and separated into water-soluble sugars and lignophenols without any heating and pressing.

The network structure of lignin is constructed through two routes. One is random radical coupling of precursors (coniferyl, sinapyl and p-coumaryl units), giving primary chain. The other includes the addition of adjacent nucleophiles, H₂O, lignin units and carbohydrates, to quinonemethides (C₆ position), giving labile oxygen containing benzyl structures [2]. When lignins contact with acid in the interface between hydrophilic and hydrophobic layers in the phase-separation treatment, benzyl carbonium ions are quickly formed, followed by stabilization by grafting of cresol (Fig. 1). This conversion proceeds selectively, and the resulting lignin derivatives (lignophenols) are composed mainly of the primary lignin chains formed by random radical coupling. In the present work, in order to elucidate basic structures of lignin polymers and obtain information for effective utilization, lignophenols are fractionated and characterized by GPC, UV-Vis, FT-IR and ¹H-NMR.

![Fig. 1 Synthesis of lignophenols through the phase-separation system.](image)

2. EXPERIMENTAL

2.1 Preparation and fractionation of lignophenols

Western hemlock (*Tsuga heterophylla*), Birch (*Betula papyrifera marsh*) and Rice straw (*Oryza sativa*) were milled to 80 mesh pass size, and were extracted with benzene-ethanol (2/1, v/v) for 48 hrs. The lignophenols were synthesized from these materials through the phase-separation system (2 step process II). p-Cresol dissolved in acetone was added to the raw materials (3 mol / C₆). After evaporating acetone, 72 % H₂SO₄ were added. The mixtures were stirred vigorously at 30°C for 60 min. The mixture was dispersed into excess amount of de-ionized water. Precipitates were collected and washed. After drying, the precipitates were extracted by...
acetone. Acetone soluble fractions (crude lignophenols containing carbohydrates) were added dropwise to excess amount of diethyl ether with vigorously stirring. The precipitates (ether insoluble fractions of lignophenols, EIL) were collected by centrifugation, and dried over P2O5 after solvent evaporation.

2.2 Removal of unreacted \( p \)-cresol by silica gel column chromatography

The mobile phase of Hexane / Ethyl acetate (10/1, v/v) was optimized by small scale pretests using thin layer chromatography (TLC). A stationary phase used in column chromatography was silica gel (silica gel 60, 0.063-0.200 mm, Merck Japan Ltd). The crude ether soluble fraction were dissolved in acetone, and applied to a silica gel column which was preconditioned with mixed solution of Hexane / Ethyl acetate (10/1, v/v). The silica gel column was eluted by gradient development with 12 mL of Hexane / Ethyl acetate (1/3, v/v), 10 mL of Hexane / Ethyl acetate (1/1, v/v) and 6500 mL of Hexane / Ethyl acetate (10/1, v/v). After free \( p \)-cresol was eluted out, the ether soluble fractions (ESL) were collected by eluting with 4000 mL of acetone.

2.3 Analyses of lignophenols

2.3.1 Gel permeation chromatography

Average molecular weight was estimated by gel permeation chromatography (GPC). GPC was carried out by LC-10 (Shimadzu Co.) with four columns (KF 801, KF 802, KF 803 and KF 804, Shodex Co.). Tetrahydrofuran (THF) was used as eluent with flow rate of 10 mL / min. \( M_w \) and \( M_n \) were determined based on standard polystyrenes.

2.3.2 FT-IR

FT-IR spectroscopy was carried out by a Spectrum GX (Perkin Elmer Co.), using the KBr pellet technique. Each spectrum was recorded in range from 4000 cm\(^{-1}\) to 400 cm\(^{-1}\).

2.3.3 UV-Vis and ionization difference spectra

UV-Vis spectroscopy was carried out by a UV-560 (JASCO Co.) Ionization difference spectrum was measured with methyl cellosolve and NaOH solutions.

2.3.4 \(^1\)H-NMR

\(^1\)H-NMR spectra was measured by JNM-A500 (JEOL Co.) in 600 \( \mu \)L of CDCl\(_3\) / C\(_2\)D\(_2\)N (3:1, v/v). The amount of grafted \( p \)-cresol units was calculated based on the signal intensity of cresolic methyl protons (1.6-2.4 ppm) against aromatic protons (7.8-8.4 ppm) of \( p \)-nitrobenzaldehyde (internal standard). The hydroxyl group contents were determined from phenolic acetoxyl proton signals (1.6-2.0 ppm) on \(^1\)H-NMR spectra of acetylated of EIL and ESL.

3. RESULTS AND DISCUSSIONS

3.1 Synthesis of lignophenols

The ether soluble fractions from Western hemlock (softwood) and Birch (hardwood) include 9% and 8% of unreacted \( p \)-cresols, respectively. That from Rice straw (grass plant) has only 0.2% of unreacted \( p \)-cresol on area percentage of GPC. The ether soluble fractions from wood were chromatographed to remove unreacted \( p \)-cresol. The yields of EILs from Western hemlock, Birch and Rice straw were 96%, 72% and 32%, respectively. The yields of ESLs from Western hemlock, Birch and Rice straw were 30%, 45% and 51%, respectively. The total of EIL and ESL is over 100%, due to the grafted cresol units and the contamination of small amount of non-lignin substances such as extractives. The theoretical yields of EIL and ESL were shown in Table I. The weight ratios of the low molecular weight fractions (ESL and water soluble fractions which were lost during the preparation of lignophenols) to the high molecular weight fractions (EIL) were 0.4, 0.6 and 4, respectively. The weight ratio got higher in order of plant evolution.

The molecular weight distribution patterns and average molecular weights of lignophenols were...
shown in Fig. 2 and Table I. EILs from western hemlock, birch and rice straw showed average molecular weights (Mw) of 6820, 4430 and 3430, respectively.

The differences in average molecular weights of EILs derived from native lignin reflect that there are differences in polymerization degree of primary lignin chain formed by radical coupling among the lignins from softwood, hardwood and grass plant.  

$^1$H-NMR spectra of lignophenols were shown in Fig.3. The amounts of $p$-cresol of ESLs were higher than EILs (Table I). This was in agreement with the correlation between the molecular weight of lignophenol and the amount of combined $p$-cresol, reported by Mikame [5]. This result suggested that low molecular weight units have higher mobility in the system, giving units with higher frequency of grafted $p$-cresol. The amounts of aliphatic hydroxyl group of ESLs were lower than that of EILs.

The differences in the balance of EIL and ESL, and the average molecular weights of EIL reflect difference in the polymerization degree of primary lignin chain formed by random radical coupling during the biosynthesis. The growth of primary lignin chains during the lignin biosynthesis in higher plants is limited, leading to flexible lignin structures composed of low molecular weight units linked through benzyl aryl ether linkages.

3.2 Structural features of lignophenols

FT-IR spectra of lignophenols were shown in Fig.4. The FT-IR spectra of EILs had the absorption around 815 cm$^{-1}$ assigned to C-H out-of-plane skeletal vibrations in aromatic ring, because of grafted $p$-cresol and had no absorption around 1650 cm$^{-1}$, assigned to conjugated or around 1700 cm$^{-1}$, assigned to unconjugated C=O. These characteristics of EIL were in agreement with the characteristics assigned to UV-Vis spectra reported by Funaoka [2]. The FT-IR spectra of ESL showed high absorption at 1700 cm$^{-1}$ unconjugated C=O. And higher absorption around 815 cm$^{-1}$ is shown in ESLs than EILs (Fig.4). These results indicated that the ESLs included components with higher amounts of grafted $p$-cresol than EILs, and these results were in agreement with the correlation between the molecular weight of lignophenol and the amount of combined $p$-cresol, reported by Mikame [5].

<table>
<thead>
<tr>
<th>Species</th>
<th>Yields$^*$ (wt % of lignin content)</th>
<th>Combined $p$-Cresol (mol / C$_9$)</th>
<th>Hydroxyl group</th>
<th>$M_w$</th>
<th>$M_n$</th>
<th>$M_w / M_n$</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>EIL</td>
<td>67.6</td>
<td>0.79</td>
<td>1.52</td>
<td>0.93</td>
<td>6820</td>
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<td></td>
<td>ESL</td>
<td>32.4</td>
<td>1.23</td>
<td>1.88</td>
<td>0.62</td>
<td>1080</td>
</tr>
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<td>Western hemlock</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EIL</td>
<td>52.6</td>
<td>0.99</td>
<td>1.69</td>
<td>0.77</td>
<td>4430</td>
</tr>
<tr>
<td></td>
<td>ESL</td>
<td>47.4</td>
<td>1.74</td>
<td>2.16</td>
<td>0.52</td>
<td>790</td>
</tr>
<tr>
<td>Birch</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>EIL</td>
<td>20.9</td>
<td>0.74</td>
<td>1.45</td>
<td>0.70</td>
<td>3430</td>
</tr>
<tr>
<td></td>
<td>ESL</td>
<td>79.1</td>
<td>1.64</td>
<td>2.31</td>
<td>0.68</td>
<td>1120</td>
</tr>
<tr>
<td>Rice straw</td>
<td></td>
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</table>

$^*$Yield of ether-insoluble fractions dose not include grafted $p$-cresol. Yields of ether-soluble fractions were calculated by subtracting amounts of ether-insoluble moieties from amounts of Klason lignin.
agreements with the characteristics from $^1$H-NMR (Table I). Both EIL and ESL from rice straw had the absorptions at 1720 cm$^{-1}$, indicating the presence of ester. This results show that there are kinds of units such as $p$-coumaric acid and ferulic acid which are linked to core lignin through ester-linkage.

The UV-Vis and ionization difference ($\Delta E_i$) spectra of lignophenols are shown in Fig. 5. These spectra of EILs from western hemlock and birch had sharp absorptions only at 280 and 300 nm and no band at longer wavelengths. These indicated selective and effective grafting of $p$-cresol to conjugated structures during phase-separation treatment [2]. The UV and $\Delta E_i$ spectra of ESLs from western hemlock and birch had the shoulder around 300-350 nm and 350-400 nm, respectively, suggesting the presence of non-lignin substances such as phenolic extractives.

The shoulders around 300-350 nm and 350-400 nm of both ESL and EIL from rice straw were higher than those of western hemlock and birch. These were in agreement with the characteristics from theses FT-IR spectra.

4. CONCLUSION

ESL obtained by the phase-separation system showed yields (wt % lignin contents) of 30 % (Western hemlock), 45 % (Birch) and 51 % (Rice straw) in whole lignophenols. As higher the evolutional states of plants, the yields of ESL were higher. This result suggested that the growth of the primary lignin chains during the biosynthesis is different with species. Compared with softwood and hardwood lignins, grass is composed mainly of small fragments linked through benzyl ether linkages, leading to flexibility of molecules and quick response for environmental change.

5. ACKNOWLEDGEMENT

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6. REFERENCES


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