

NOTE

Selective conversion of hardwood lignin into syringyl methyl benzofuran using *p*-cresol

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INTRODUCTION

Lignins that are currently available or may become available in bulk include industrial lignins, such as kraft lignin from black liquor on pulp manufacturing; organosolv lignin on organosolv pulping using acetone, ethanol, butanol, ethylene glycol, formic acid, or acetic acid; and hydrolysis lignin obtained as residue via acid or enzymatic saccharification of woody materials. Many methods, including hydrogenolysis, pyrolysis, and supercritical water decomposition, have been used to treat such lignins in an effort to produce aromatic chemicals, such as monophenols, which are alternatives to fossil fuels. Generally, decomposition reactions that are performed at high temperatures are very fast and uncontrollable, producing innumerable types of decomposition products. Another important reason for this difficulty is a characteristic of lignin itself. Lignins undergo structural changes under isolation conditions, resulting in the formation of lignin with an unexpected and uncontrollable molecular structure.¹ Therefore, it is almost impossible to design a decomposition reaction suitable for this molecular structure. Thus, we have yet to see any successful method of obtaining significant yields of a specific aromatic chemical from lignin.

The reactive benzyl linkages in lignin, such as benzyl aryl ethers and benzyl OH (R_2 -O- C_α in Figure 1), are selectively cleaved and then subjected to phenolation with solvating *p*-cresol through a phase separation process developed by Funaoka^{2–5} utilizing wood meal, sulfuric acid, and *p*-cresol. Although cellulose and hemicellulose are swollen, partially hydrolyzed, and dissolved in sulfuric acid, lignin is extracted in the *p*-cresol layer. The rate of hydrolysis of the C_α -aryl-ether bond is much faster than that of the C_β -aryl-ether bond.⁶ Thus, in this method, the latter reaction is almost completely inhibited at atmospheric temperatures.

The derived lignin rich in 1,1-bis(aryl)propane-2-*O*-aryl ether units is known as lignocresol (Figure 1). Under alkaline conditions at ~140–170 °C, the phenoxide ions of grafted cresols readily undergo nucleophilic attack of the electron-deficient β -carbon, resulting in the selective cleavage of C_β -aryl-ether linkages (R_3 -O- C_β in Figure 1).^{7–9} In addition, lignocresol is markedly degraded because half of the native lignin linkages between the monomeric units are C_β -aryl-ether bonded.^{10,11} In principle, after cleavage of the reactive benzyl linkages and the subsequent phenolation and cleavage of C_β -aryl-ether linkages,

syringyl-, and guaiacyl-type phenolic dimers with a coumaran ring (S1 and G1 in Figure 1) could theoretically be obtained. The syringyl unit linked via two C_β -aryl-ether bonds with neighboring lignin units (R_1 : OCH₃, R_3 : Aryl, R_4 : Aryl in Figure 1) potentially produces the phenolic dimer S1. Similarly, the guaiacyl unit (R_1 : H, R_3 : Aryl, R_4 : Aryl in Figure 1) may produce the phenolic dimer G1. Previously, the phenolic dimers S1 and G1 were recovered from the acid-insoluble fraction after alkaline treatment and subsequent acidification. The yield of S1 and G1 reached 21% and 7%, respectively, when raw hardwood (birch) lignocresol was treated at 170 °C for 60 min with 1 M NaOH.¹²

Recently, we found that after acidification, the phenolic dimers are separated in the acid-soluble fraction rather than the acid-insoluble fraction, in which we succeeded in collecting all dimers into the acid-soluble fraction via repeated washing. We discovered that the total yield of dimers reached 58% of raw hardwood (eucalyptus) lignocresol.¹³ This yield is substantial and is adequate to guarantee that these dimers could constitute promising lignin-derived chemicals. Here, we describe a method of selectively converting hardwood lignin into specific benzofuran derivatives using *p*-cresol via these dimers.

MATERIALS AND METHODS

Hardwood meal from eucalyptus (*Eucalyptus globulus*) was extracted with an ethanol–benzene (1:2/v:v) solution for 48 h using a Soxhlet extractor. The lignin content of the extractive-free wood meal was 26.1%. Eucalyptus lignocresol was isolated from the wood meal through the two-step process II of the phase separation system^{3,7} as a representative of hardwood lignocresol. The yield of lignocresol in this study was 14.3% based on the wood meal, that is, the yield was 54.9% based on the lignin.

Eucalyptus lignocresol was treated with 0.5 M NaOH at 170 °C for 30 min under N₂ using small stainless steel batch-type reactors. The initial concentration of lignocresol was 50 mg ml⁻¹. After the reactor had cooled, the mixture was ultrasonically stirred, acidified, and then centrifuged, and the acid-soluble fraction was sampled. The precipitated material was again dissolved by NaOH addition. The same procedure was repeated for the alkaline solution, and the acid-soluble fractions from both NaOH treatments were combined. Washing was repeated until the peak corresponding to phenolic dimers disappeared in the GPC (gel permeation chromatography) chart for the acid-insoluble material. The final acid-soluble fraction was extracted with diethyl ether using

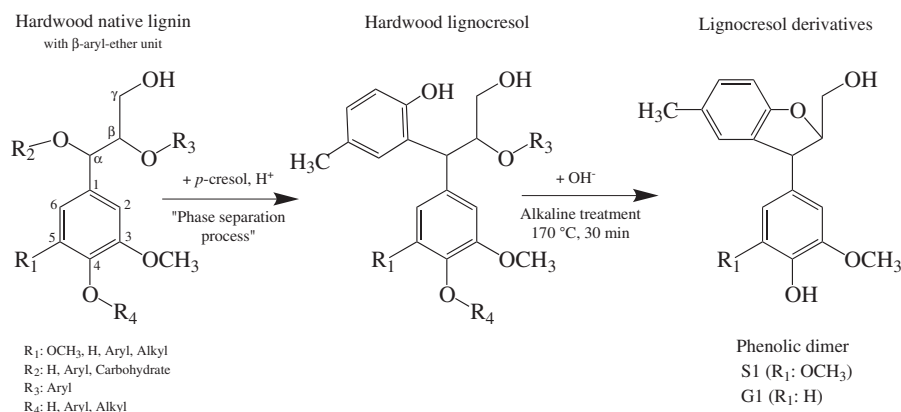


Figure 1 Selective conversion from hardwood native lignin to the phenolic dimers S1 and G1 via a 'phase separation process' and subsequent alkaline treatment.

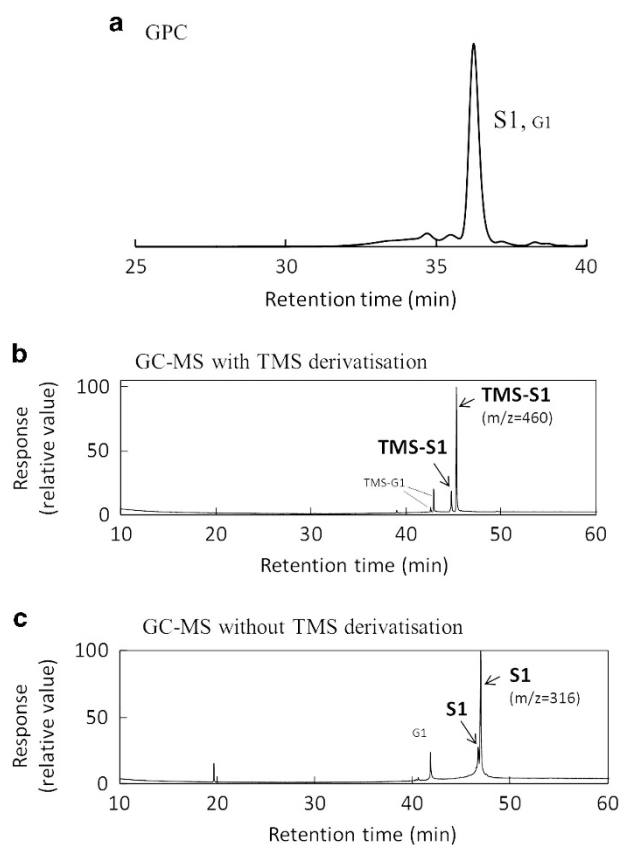


Figure 2 Analysis of the acid-soluble material derived via the alkaline treatment of eucalyptus lignocresol at 170 °C for 30 min. (a) Gel permeation chromatography,¹³ (b) GC–MS after TMS derivatization, and (c) GC–MS without TMS derivatization. GC–MS, gas chromatography–mass spectrometry.

a separating funnel and the salting-out technique. Evaporation of the diethyl ether extract gave the acid-soluble material, the yield of which reached 58% of raw lignocresol. The molecular weight distribution of the acid-soluble material was measured using a GPC with a UV detector at 280 nm (Shimadzu Class LC-20 system, Kyoto, Japan) equipped with four columns (Shodex KF804, KF803, KF802, and KF801 in series) with tetrahydrofuran as the eluent (Figure 2a).¹³ Additional details of this experimental procedure are provided in our previous publication.¹³

The acid-soluble material obtained above was treated at 170 °C for 60 min under N_2 using small stainless steel batch-type reactors. The concentration of

the acid-soluble material studied was 2 mg ml⁻¹. After cooling the reactor, the mixture was acidified and centrifuged. The acid-insoluble material was recovered, dialyzed, and freeze-dried. The materials were subjected to GPC (see above) and gas chromatography–mass spectrometry (GC–MS) analyses (Shimadzu, GC-2010+GC–MS–QP2010, Kyoto, Japan) before and after the reaction. For GC–MS analysis, both materials were dissolved in acetone, followed by trimethylsilyl (TMS) derivatization using BSA (*N,O*-bis(trimethylsilyl)acetamide). A nonpolar capillary column (Agilent, Santa Clara, CA, USA, DB-5, length: 30 m, i.d.: 0.25 mm, thickness: 1 μ m) was used with helium as the carrier gas. The column temperature was increased from 100 to 270 °C at a rate of 4 °C min⁻¹. The injector, interface, and ion source were maintained at 270, 250, and 250 °C, respectively. Nuclear magnetic resonance (NMR) spectra were measured by a JEOL JNM Alpha 500 spectrometer (Tokyo, Japan) using chloroform-*d* as the solvent.

RESULTS AND DISCUSSION

Figure 2b displays the GC–MS (total ion peak) results, including the TMS derivatization of the acid-soluble material obtained via alkaline treatment of eucalyptus lignocresol in 0.5 M NaOH at 170 °C for 30 min. TMS derivatization revealed that the one sharp peak in the GPC chart (Figure 2a) was produced by several compounds. The largest ion peak contained a molecular mass ion at $m/z=460$ with TMS derivatization or $m/z=316$ without TMS derivatization (Figure 2c), corresponding to S1. Another peak in Figure 2b was composed of the types of mass ions similar to TMS-derivatized S1 (TMS-S1). Because previous studies have reported that the levels of erythro arylglycerol- β -aryl ether structures are much higher than those of threo-type structures in hardwood lignin,^{14,15} all peaks observed here may correspond to erythro- and threo-type S1 diastereomers. The peak observed at 42.9 min is attributed to G1, with a molecular mass ion of $m/z=430$,¹⁶ which is much lower than the peaks associated with S1. The ratio of S1 to G1 was estimated to be 7.2:1 based on the peak areas of TMS-S1 and TMS-G1 in the total ion chromatogram (Figure 2b).

Our results reveal an important feature of the native lignin structure of hardwood. Because the guaiacyl units include C₅ carbon without a methoxyl group (R_1 : H in Figure 1), the ratio of linkages to neighboring units other than C β -aryl-ether bonds is increased. However, the observed difference between the quantity of S1 and G1 suggests that many more syringyl units tend to be successively linked by C β -aryl-ether bonds with neighboring lignin units than expected.

Figure 3 shows the GPC results for both acid-insoluble and acid-soluble materials obtained via further alkaline treatment of the

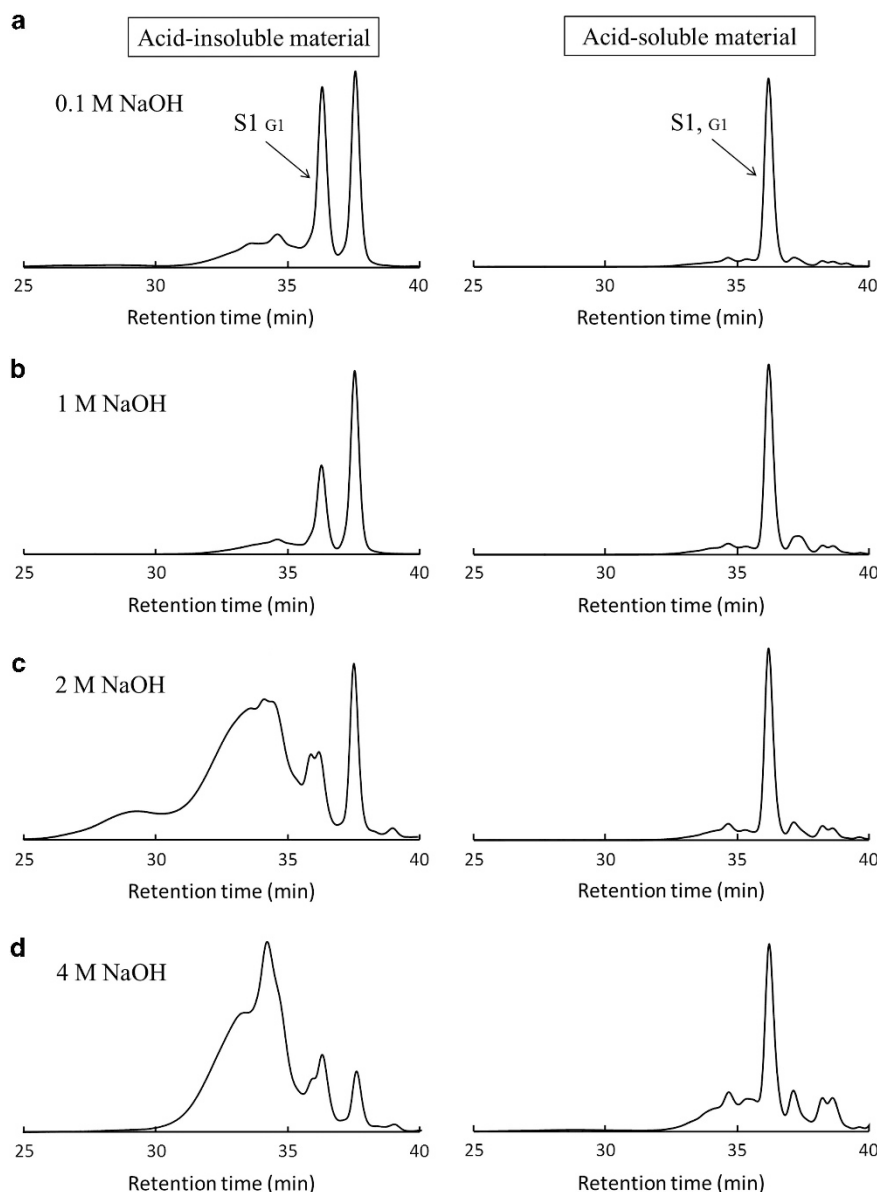


Figure 3 Gel permeation chromatography charts of the acid-insoluble and acid-soluble fractions derived by further alkaline treatment of the acid-soluble material obtained from eucalyptus lignocresol at 170 °C for 60 min in (a) 0.1, (b) 1, (c) 2, and (d) 4 M NaOH solutions.

acid-soluble material (mainly S1, Figure 2a) in 0.1–4 M NaOH at 170 °C for 60 min. The GPC data of the acid-insoluble material for 0.1 and 1 M NaOH clearly indicate that either S1 or G1 underwent partial degradation to produce new compounds with lower molecular weights than either S1 or G1. GC–MS analysis with TMS derivatization of the acid-insoluble material for 1 M NaOH detected two major peaks (Figure 4a). The mass spectrum of the largest peak (termed TMS-S2) had a molecular mass ion of 356 and a weaker ion of 73 (Figure 4c), strongly indicating that compound S2 has one hydroxyl group with a molecular weight of 284 ($=356 - 72$). The second highest peak corresponded to the TMS derivative of unreacted S1.

The alcoholic OH group partially dissociates under alkaline conditions at 170 °C. Lignin is known to suffer from the elimination of C_γ -OH from the quinone methide intermediate as formaldehyde during alkaline pulping performed under similar conditions.¹⁷ As a result, a double bond between C_α and C_β is formed to produce conjugated structures.¹⁷ It is natural to consider that the same reaction

would occur for S1, as shown in Figure 5, where the main product should be syringyl methyl benzofuran (2-syringyl 5-methyl benzofuran) with a molecular weight of 284 and one hydroxyl group. The prediction from the reaction mechanism perfectly fits our GC–MS data. The loss of one hydroxyl group leads to a decrease of hydrophilicity, which is consistent with the fact that S2 was hardly involved in the acid-soluble material (Figure 4b).

¹H-NMR spectra of both acid-insoluble and acid-soluble materials for 1 M NaOH were obtained to ensure the formation of a benzofuran-type structure (Figure 6). Because the acid-soluble material is mainly composed of S1 (Figure 4b), the NMR signals of Figure 6b could be assumed to represent S1. However, the majority of the acid-insoluble material is both S1 and S2 (Figure 4a). Therefore, by comparing the NMR spectra (Figure 6a and b), it was possible to extract the proton signals that could be attributed to S2. The shifted methyl (2.2–2.5 p.p.m.) and methoxyl (3.8–4.0 p.p.m.) protons originated from S2 were observed. Furthermore, the aromatic protons of cresol nuclei were

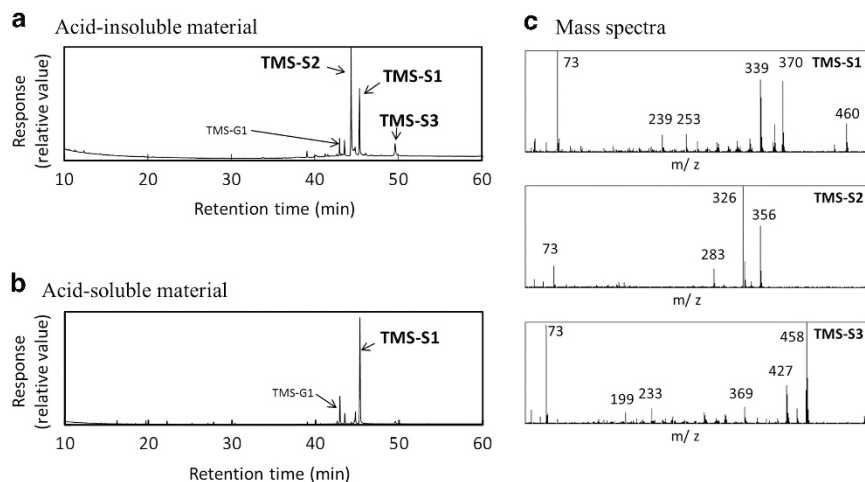


Figure 4 Gas chromatography–mass spectrometry analysis of (a) the acid-insoluble fraction and (b) the acid-soluble fraction after TMS derivatization derived by further alkaline treatment of the acid-soluble material obtained from eucalyptus lignocresol at 170 °C for 60 min in a 1 M NaOH solution (corresponding to Figure 3b). (c) Mass spectra of TMS-derivatized S1, S2, and S3.

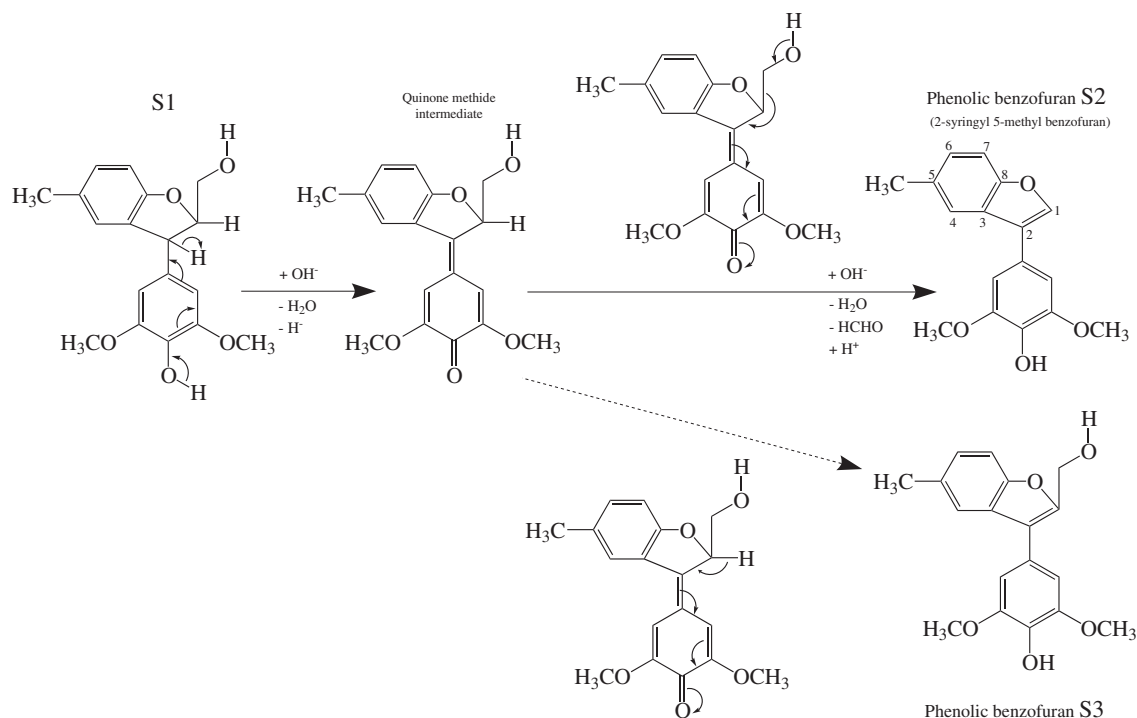


Figure 5 Mechanism of the conversion from phenolic dimer S1 with a coumaran ring to phenolic dimers S2 and S3 with a benzofuran structure via intramolecular elimination of formaldehyde or β -hydrogen under alkaline conditions, respectively.

shifted to lower field because of the formation of more conjugated structure. The signal observed at the lowest field could be attributed to the C_β proton. Every observation strongly supports the molecular structure of S2.

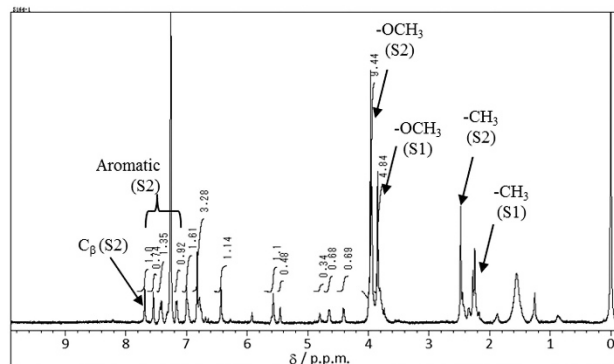
We detected a minor peak at $m/z=458$, probably corresponding to compound S3, which might have formed through intramolecular β -hydrogen elimination from the quinone methide intermediate during the second alkaline treatment step (Figure 5). Overall, the results demonstrate that during alkaline treatment, formaldehyde elimination is much more favorable than hydrogen elimination.

The most notable difference in reactivity between S1 and G1 is the rate of condensation. Figure 3 shows that condensation reactions

rarely occur during 0.1 and 1 M NaOH treatment. Guaiacyl-type dimers were easily condensed with another dimer at the methoxyl group-deficient C_5 carbon using formaldehyde;¹⁶ however, it was indicated that syringyl-type dimers, S1 or S2, do not easily condense each other because of their occupancy of the C_5 carbon by a methoxyl group. At higher concentrations of NaOH, the C_1 or C_6 carbons of syringyl nuclei and the ortho carbons of the oxygen atom of the grafted cresol were activated for the condensation reaction by formaldehyde, yielding diarylmethane-type products under alkaline pulping conditions.¹⁷

The yield of lignocresol in the present study was relatively low because of the chosen method; however, hardwood lignocresol could

a Acid-insoluble material (mainly S1 and S2, *see* Fig. 4(a))



b Acid-soluble material (almost S1, *see* Fig. 4(b))

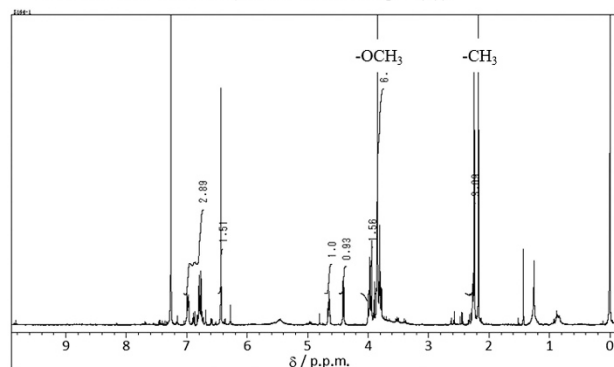


Figure 6 NMR spectra of (a) the acid-insoluble fraction and (b) the acid-soluble fraction derived by further alkaline treatment of the acid-soluble material obtained from eucalyptus lignocresol at 170 °C for 60 min in a 1 M NaOH solution (corresponding to Figure 3b).

be potentially obtained at ~110% of the yield (including grafted *p*-cresol)³ of hardwood lignin using the phase separation process developed by Funaoka. Using the subsequent alkaline treatment, phenolic dimers were obtained at 58% of the yield of hardwood lignocresol,¹³ most of which was composed of the syringyl-type phenolic dimer S1. Although we have not yet purified S2 from the reaction mixture to quantify the yield, our data successfully demonstrate that by further treating S1, its benzofuran derivative S2 was obtained via formaldehyde elimination without a condensation reaction. Moreover, we can recover syringyl nuclei from phenolic dimers¹⁸ using the nucleus exchange method developed by Funaoka.¹⁹ Our process controls the reactivity of C_α linkages, C_β-aryl-ethers, and C_γ-OH in sequence, successfully resulting in a high yield of specific aromatic chemicals from the monomeric lignin unit sandwiched between two C_β-aryl-ethers. This method is nicely designed for

hardwood native lignin using the successiveness of C_β-aryl-ethers between syringyl units.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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