

## Suggested Production of a Guaiacyl Benzofuran Derivative from Softwood *via* Lignocresol

Hiroshi Nonaka,\* Ryota Yamamoto, Hirotaka Katsuzaki, and Masamitsu Funaoka

Lignocresol was isolated from softwood with *p*-cresol using sulfuric acid and phase separation. An alkaline treatment of the lignocresol, followed by acidification, selectively yielded a guaiacyl coumaran, G1, in the acid-soluble fraction. With further alkaline treatment of G1 in 0.5 M of NaOH solution at 170 °C for 60 min, it was strongly suggested that a guaiacyl benzofuran derivative, G2, was obtained by the elimination of formaldehyde, based on analytical data of the reaction mixture. The process is very unique and well-designed based on the reactivity of C $\alpha$ -ethers, or C $\alpha$ -OH, C $\beta$ -aryl-ethers, and C $\gamma$ -OH of lignin, although condensation reactions *via* formaldehyde occurred in parallel to give condensed products with a diarylmethane structure. Because these phenolic dimers, G1 and G2, were recovered from the guaiacyl unit linked with the neighboring guaiacyl units *via* two  $\beta$ -aryl-ether bonds, they are promising lignin-derived chemicals that are obtainable in a high yield.

*Keywords:* Lignin; Lignocresol; Guaiacyl; Phenolic dimer; Benzofuran

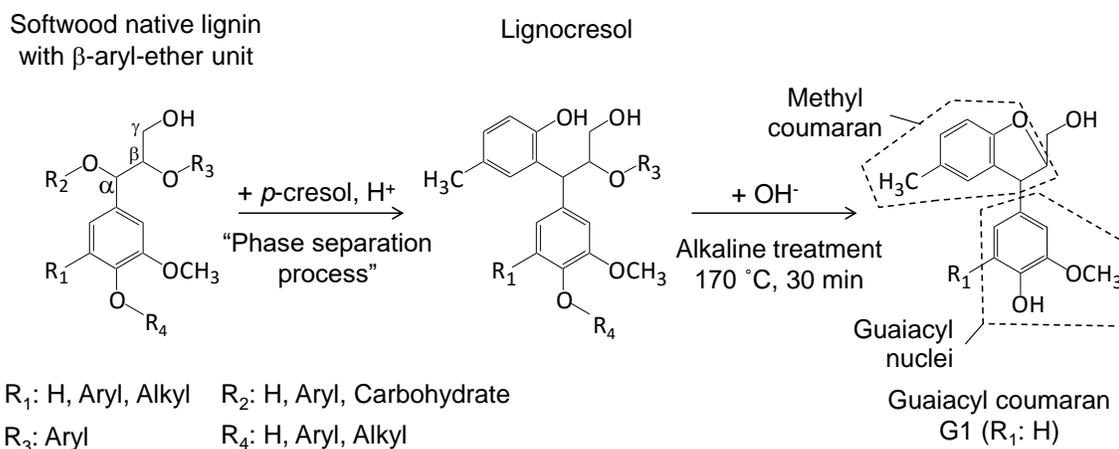
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### INTRODUCTION

Lignin forms an interpenetrating polymer network (IPN) structure with cellulose and hemicellulose in woody tissues; therefore, it is impossible to extract most lignin using only organic solvents. Tremendous effort has been made to isolate lignin from plants in order to analyze the structure of native lignin and utilize it as an alternative to fossil fuels. However, there are yet to be any successful technologies that isolate native lignin in a high yield, nor any promising lignin-based products, or lignin-derived chemicals on the market. One reason is that all types of industrial lignin, *e.g.*, kraft lignin during papermaking and hydrolysis lignin during the saccharification of woody materials, suffer from structural changes during isolation. The resultant lignin has unpredictable and uncontrollable molecular structures (Nonaka and Funaoka 2011).

The methodology described herein enables the selective and high-yield isolation of the guaiacyl methyl coumaran (G1 in Fig. 1). This methodology involves a phase separation process with sulfuric acid and *p*-cresol, followed by an alkaline treatment (Fig. 1) (Mikame and Funaoka 2008; Mikame and Funaoka 2010; Yamamoto *et al.* 2012). During phase separation (Funaoka and Abe 1989; Funaoka *et al.* 1995; Funaoka 1998; Funaoka 2013), the reactive benzyl linkages in lignin, *e.g.*, benzyl aryl ethers and benzyl alcohols (R<sub>2</sub>-O-C $\alpha$  in Fig. 1), are selectively cleaved, followed by phenolation with the added *p*-cresol. While the cellulose and hemicellulose are partially hydrolyzed and extracted to the aqueous phase, resulting in the disruption of the IPN structure, the lignin is successfully extracted to the *p*-cresol layer. As the rate of hydrolysis of the  $\alpha$ -aryl-ether bond is much higher than that of the  $\beta$ -aryl-ether bond (Johansson and Miksche 1972), the

latter process could be almost completely inhibited at room temperature for reactions lasting less than 60 min.



**Fig. 1.** Selective conversion from softwood native lignin to guaiacyl coumaran G1 by phase separation and subsequent alkaline treatment

The derived lignin, which is rich in 1,1-bis(aryl)propane-2-O-aryl ether units, is termed lignocresol (Fig. 1). Under alkaline conditions from 140 °C to 170 °C, the phenoxide groups of the grafted cresols readily carry out a nucleophilic attack on the electron-deficient  $\beta$ -carbon, resulting in the selective cleavage of  $\beta$ -aryl-ether linkages ( $R_3$ -O-C $\beta$  in Fig. 1) (Nagamatsu and Funaoka 2003; Aoyagi and Funaoka 2004; Aoyagi and Funaoka 2006).

Exactly the same mechanism as the  $\beta$ -O-4 bond cleavage of an  $\alpha$ -5 type condensation structure was first reported by Gierer and Ljunggren (1983). Consequently, lignocresol is dramatically depolymerized because half of the native lignin linkages between monomeric units are  $\beta$ -aryl-ether bonds (Erickson *et al.* 1973; Adler 1977). The guaiacyl unit, linked with the neighboring guaiacyl units *via* two  $\beta$ -aryl-ether bonds ( $R_1: H, R_3: \text{aryl}, R_4: \text{aryl}$ ), could potentially generate the phenolic dimer G1. The yield of G1 was reported to be 11% of the raw softwood (spruce) lignocresol in the acid-insoluble fraction after acidification when spruce lignocresol was treated at 170 °C for 60 min with 1 M NaOH (Mikame and Funaoka 2008).

Recently, the authors of this study found that G1 could instead be separated in the acid-soluble fraction after acidification, and they successfully isolated all of the G1 in the acid-soluble fraction by repeated washing (Yamamoto *et al.* 2012). The total yield of G1 was improved to 38% of raw softwood Hinoki (*Chamaecyparis obtusa*) lignocresol. Because the yield of softwood lignocresol is very high, typically 110% of the lignin including the grafted *p*-cresol (Funaoka 1998), this excellent yield strongly suggests that this compound could be a key lignin-derived chemical.

This study reports that a novel phenolic benzofuran derivative could be obtained selectively from G1 by further alkaline treatment. The production mechanism and a parallel occurring reaction were also demonstrated. Benzofuran derivatives are often applied to medical use, antiseptic, pesticide, fuel additive and so on.

## EXPERIMENTAL

### Preparation of Softwood Lignocresol

Softwood wood meal from Hinoki cypress (*Chamaecyparis obtusa*) was extracted with an ethanol-benzene (1:2/v:v) solution for 48 h using a Soxhlet extractor. The Hinoki lignocresol was isolated from the extractive-free wood meal using a two-step phase separation system (Funaoka 1998; Nagamatsu and Funaoka 2003). The extracted Hinoki lignocresol is representative of softwood lignophenol.

### Preparation and Analysis of the Acid-Soluble Material Rich in Guaiacyl Coumaran, G1

As described previously (Yamamoto *et al.* 2012), Hinoki lignocresol was treated with 0.5 M of NaOH at 170 °C for 30 min under N<sub>2</sub> using small stainless steel batch-type reactors. The initial concentration of lignocresol was 50 mg/mL. After cooling the reactor, the mixture was ultrasonically stirred, acidified, and centrifuged, and the acid-soluble fraction was sampled. The precipitated material was again dissolved in NaOH. The same procedure was repeated for the alkaline solution, and the acid-soluble fractions from both NaOH treatments were combined. The final acid-insoluble material was dialyzed against deionized water and freeze-dried. The acid-soluble fraction was saturated with sodium chloride and then extracted with diethyl ether using a separating funnel. The evaporation of the diethyl ether extract gave the acid-soluble material. The yield of the acid-soluble material reached 38% of the raw lignocresol. The molecular weight distribution of both materials was measured using a gel permeation chromatography (GPC) instrument (Shimadzu Class LC-20 system, UV: 280 nm; Kyoto, Japan) equipped with four columns (Shodex KF804, KF803, KF802, and KF801 in series; Toyo, Japan) with tetrahydrofuran (THF) as the eluent. Washing was repeated until the G1 peak disappeared in the GPC chart for the acid-insoluble material.

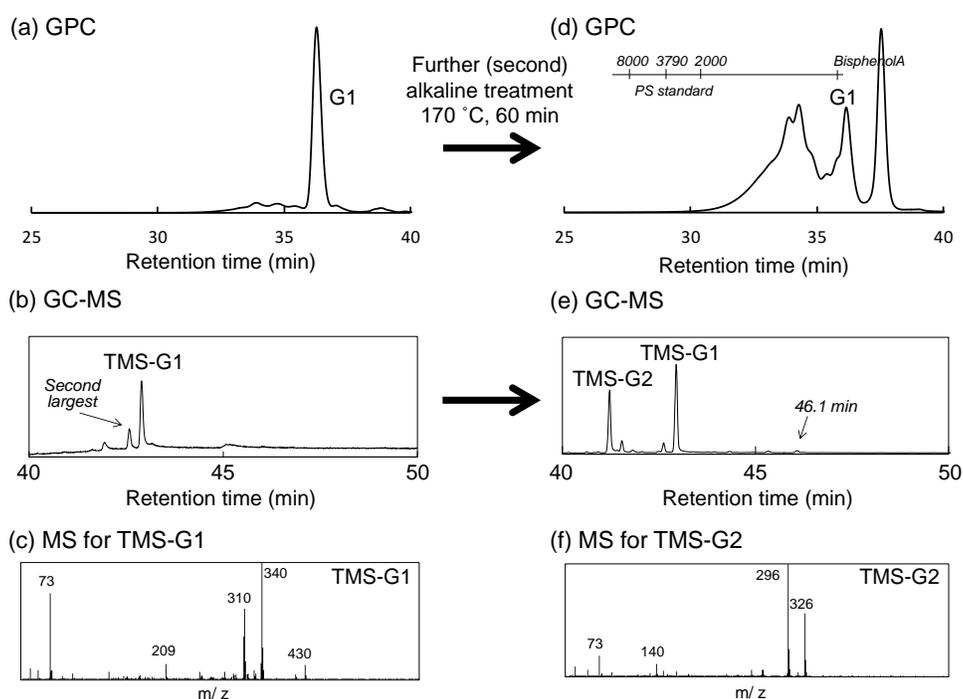
### Further Alkaline Treatment of the Acid-Soluble Material Rich in Guaiacyl Coumaran, G1

The acid-soluble material obtained above was further treated with 0.5 M NaOH at 170 °C for 60 min under N<sub>2</sub> using small stainless steel batch-type reactors. The concentration of the acid-soluble material studied was 2 mg/mL. After cooling the reactor, the mixture was acidified and centrifuged. The acid-insoluble material was recovered, dialyzed against deionized water, and freeze-dried. The materials before and after the reaction were subjected to GPC (see above) and GC-MS analyses (Shimadzu, GC-2010 + GCMS-QP2010, Kyoto, Japan). For the GC-MS analysis, both materials were dissolved in acetone followed by a trimethylsilyl (TMS)-derivatization using N,O-bis (trimethylsilyl) acetamide (BSA). A nonpolar capillary column (Agilent, DB-5, length: 30 m, I.D.: 0.25 mm, thickness: 1 µm; Santa Clara, CA, USA) was used with helium as the carrier gas. The column temperature was increased from 100 °C to 270 °C at a rate of 4 °C/min. The injector, interface, and ion source were maintained at 270 °C, 250 °C, and 250 °C, respectively. The NMR spectra were measured by a JEOL JNM Alpha 500 spectrometer (Tokyo, Japan) using chloroform-d as the solvent. *p*-Nitrobenzaldehyde (PNB) was added only for the starting sample as an internal standard to indicate representative chemical shift of the protons on a conjugated aromatic ring.

## RESULTS AND DISCUSSION

### Analysis of the Acid-Soluble Material Rich in G1

The acid-soluble material rich in G1 was used as the experimental sample of this study. Figures 2(a) and (b) show the GPC and GC-MS (total ion peak) results with a TMS derivatization for the starting sample. The TMS derivatization revealed that the one sharp peak in the GPC chart consisted of several compounds. Because the second largest ion peak ( $m/z = 73, 430, 310, 340, 327, 309, 283, 209$ ) was similar to the largest ion peak for TMS-derivatized G1 ( $m/z = 340, 73, 310, 309, 341, 311, 339, 209, 430$ , Fig. 2(c)), this peak was attributed to a diastereomer of G1. The formation of diastereomers is likely because the lignin side chains with  $\beta$ -aryl-ether bonds have two asymmetric carbons, giving four optical isomers, including erythro- and threo-type diastereomers.



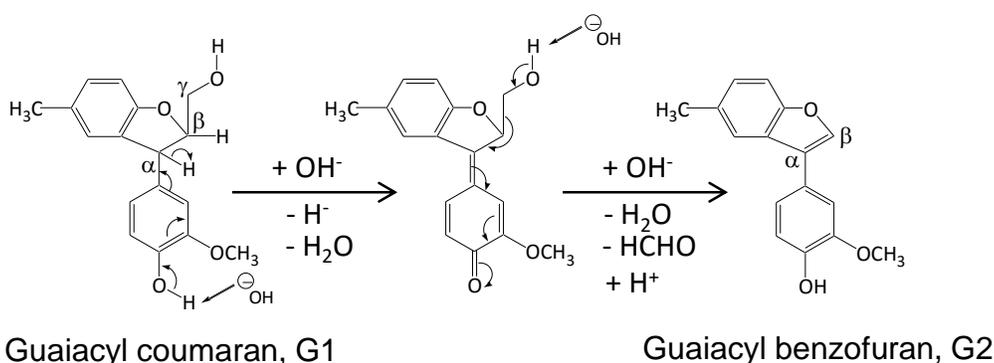
**Fig. 2.** Analysis of the starting sample: (a) GPC, (b) GC-MS after a TMS derivatization, and (c) mass spectrum for TMS derivatized G1 (TMS-G1). Analytical data of the acid-insoluble fraction derived by further alkaline treatment of the above starting sample in 0.5 M NaOH at 170 °C for 60 min are also shown on the right: (d) GPC, (e) GC-MS after a TMS derivatization, and (f) mass spectrum for TMS derivatized G2 (TMS-G2)

### Further Alkaline Treatment of the Acid-Soluble Material Rich in G1

Figures 2(d) and (e) show the GPC and GC-MS (total ion peak) results with a TMS derivatization for the acid-insoluble material, obtained by further alkaline treatment of the starting sample (mainly G1) in 0.5 M of NaOH at 170 °C for 60 min. The GPC data clearly indicated that G1 underwent partial degradation to give a new compound with lower molecular weight, as well as side condensation reactions to give various higher molecular weight compounds (Fig. 2(d)). The GC-MS analysis with a TMS derivatization detected two major peaks (Fig. 2(e)). The highest peak corresponded to the TMS derivative of the unreacted G1. The second largest peak ( $m/z = 296, 326, 297, 73, 327, 140$ ) was termed TMS-G2. The weak fragment ion of  $m/z = 73$  corresponding to TMS indicated that G1 lost

one hydroxyl group during transformation from G1 to G2. Moreover, there were only two strong ion peaks of  $m/z = 296$  and  $326$  with  $m/z = 326$  representing the molecular ion ( $M^+$ ), suggesting that the molecular structure of G2 appears to be quite stable. The major fragmentation in Fig. 2(f) appears to be the loss of two methyl groups, one from the methoxyl group and the other from the attached cresol ring. From these considerations, G2 is probably attributable to a guaiacyl benzofuran derivative. G2 could be formed by the elimination of formaldehyde from the quinone methide intermediate during the second alkaline treatment step (Fig. 3).

The alkaline treatment condition is similar to that of alkaline pulping. In his review of various chemical reactions occurred during kraft pulping, Gierer (1980) reported similar reactions that formed conjugated structures. The molecular weight decreased from 286 for G1 (un-silylated) to 254 for G2; therefore, the GPC peak that appeared after that of G1 should mainly be attributed to G2.



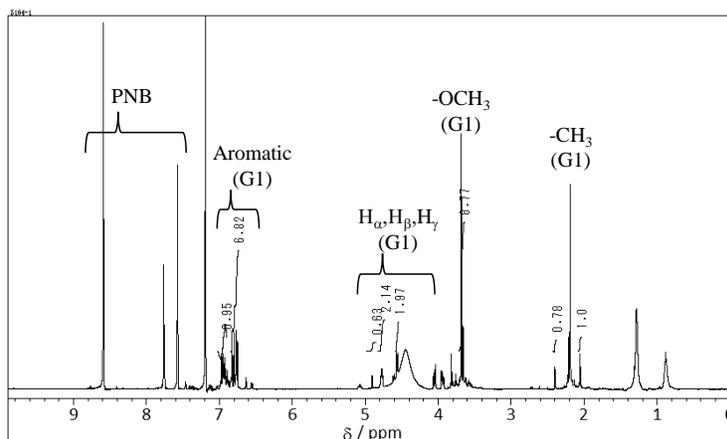
**Fig. 3.** The conversion of guaiacyl coumaran, G1, to guaiacyl benzofuran, G2, via the elimination of formaldehyde under alkaline conditions

Figures 4(a) and (b) show the NMR spectra of the starting sample and the acid-insoluble fraction after further alkaline treatment corresponding to Figs. 2(a) and 2(d), respectively. After further alkaline treatment, the methyl, methoxyl, and aromatic protons were shifted to a lower field, indicating an increase in electron density due to the formation of a new conjugated system. A significant decrease of the proton signals for  $H_\alpha$ ,  $H_\beta$ , and  $H_\gamma$  strongly suggested the loss of  $H_\alpha$  and  $H_\gamma$  during the elimination of formaldehyde. The signal that appeared at the lowest field was attributed to the  $H_\beta$  in the benzofuran structure. This data strongly supported the prediction of the molecular structure of G2.

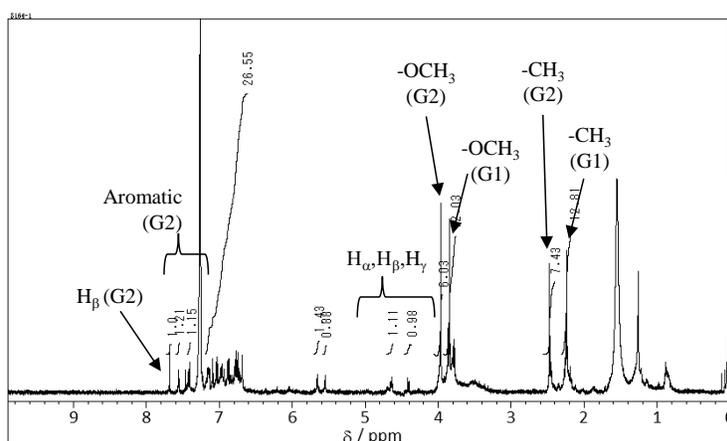
A very minor peak was found with  $m/z = 428$  at 46.1 min in the GC-MS chart (Fig. 2(e)). It was impossible to extract the proton signals that originated from this compound (Fig. 4(b)) because of the low concentration. However, it was estimated from the  $m/z$  value that this compound could be formed through intramolecular  $\beta$ -hydrogen elimination instead of formaldehyde, decreasing the molecular weight by two. Overall, the results demonstrated that formaldehyde elimination is much more favorable than the hydrogen elimination during alkaline treatment.

Condensation could be mediated *via* formaldehyde (Fig. 5), for example, because the resol resin is prepared from phenol and formaldehyde by heating it in an alkaline solution. Condensation of formaldehyde with phenolic lignin units under alkaline pulping conditions yields diarylmethane-type products (Gierer 1980).

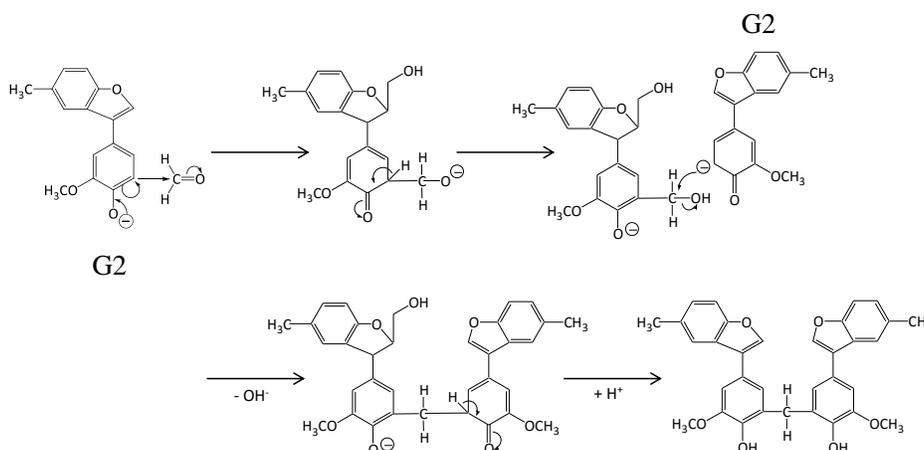
(a) Starting sample (almost G1, see Fig. 2(a)) + *p*-nitrobenzaldehyde



(b) Acid-insoluble fraction after further alkaline treatment (Fig. 2(d))



**Fig. 4.** NMR spectra of (a) the starting sample with *p*-nitrobenzaldehyde (PNB) and (b) the acid-insoluble fraction after further alkaline treatment in 0.5 M of NaOH at 170 °C for 60 min



**Fig. 5.** Condensation mechanism of two phenolic dimers (G2 + G2), mediated by formaldehyde as a cross linker under alkaline conditions; the same reaction can take place for other phenolic compounds.

Though the yield of G2 based on G1 was not determined, it is clear that G2 and the condensed materials are the main products from G1 (Figs. 2(d) and (e)). To obtain the benzofuran derivative G2 from G1 at a higher yield, parallel condensation must be inhibited. Although challenging, decreasing the reaction temperature and lowering the G1 concentration to slow the condensation rate could realize inhibition. Alternatively, parallel condensation could be inhibited by the removal of HCHO from the reaction system using an absorbent or oxidation process.

## CONCLUSIONS

1. The guaiacyl coumaran, G1, was obtained from softwood *via* lignocresol using a phase separation process followed by an alkaline treatment. G1 was further converted to its benzofuran derivative, G2, by formaldehyde elimination through a second alkaline treatment step.
2. A parallel condensation reaction *via* the formaldehyde generated polymerized products with a diarylmethane structure under the alkaline condition.
3. The process is very unique and well-designed based on the reactivity of C $\alpha$ -ethers or C $\alpha$ -OH, C $\beta$ -aryl-ethers, and the C $\gamma$ -OH of lignin. Because these phenolic dimers G1 and G2 were recovered from the guaiacyl unit linked with the neighboring guaiacyl units *via* two  $\beta$ -aryl-ether bonds, they are promising lignin-derived chemicals obtainable in a high yield.

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