

NOTE

Yasuyuki Matsushita · Hitomi Sano · Masanori Imai  
Takanori Imai · Kazuhiko Fukushima

## Phenolization of hardwood sulfuric acid lignin and comparison of the behavior of the syringyl and guaiacyl units in lignin

Received: February 8, 2006 / Accepted: April 17, 2006 / Published online: July 24, 2006

**Abstract** To study the behavior of hardwood sulfuric acid lignin (SAL) during phenolization, we compared the product yield, average molecular weight, methoxy content, and reactions of simple model compounds with those of softwood SAL, focusing on the difference between syringyl and guaiacyl units. The beech SAL reacted with phenol more readily than red pine SAL and yielded a larger soluble fraction of phenolized SAL. To investigate the difference in the phenolization activity of the syringyl and guaiacyl units in beech lignin, we prepared syringyl-nucleus-rich sulfuric acid lignin (S-rich-SAL) and guaiacyl-nucleus-rich sulfuric acid lignin (G-rich-SAL) from beech, which were subjected to phenolization. The results suggest that the syringyl unit in SAL had greater phenolization activity and its phenolized products were more soluble in acidic aqueous medium and introduced less phenol than the guaiacyl unit. Using model compounds, the study also showed that the syringyl unit had higher phenolization reactivity than the guaiacyl unit.

**Key words** Lignin · Acid hydrolysis lignin · Phenolization · Syringyl nucleus · Hardwood

### Introduction

To prevent further global warming, it is necessary to reduce the consumption of fossil energy and utilize the biomass of renewable resources. One effective procedure for utilizing biomass is the acid saccharification of woody materials to produce sugar feed stock or ethanol by fermentation. However, the process also produces a large amount of less reactive acid hydrolysis lignin as a by-product. One of the keys to developing this saccharification process for practical use is to find effective ways to utilize the acid hydrolysis lignin.

Our group has been investigating the chemical conversion of acid hydrolysis lignin into functional materials. We can obtain dispersants,<sup>1,2</sup> ion-exchange resins,<sup>3,4</sup> and retention aids<sup>5,6</sup> after the phenolization of sulfuric acid lignin (SAL). However, we have used only softwood. For practical use, a study of the utilization of hardwood is needed.

Softwood SAL is characterized by condensed structures formed by intermolecular condensation between the benzylic carbons and the 6-positions of the guaiacyl nuclei.<sup>7,8</sup> In hardwood, lignin possesses a syringyl nucleus, which is involved in a more complex reaction on acid treatment. One remarkable difference between softwood and hardwood is that hardwood yields acid-soluble lignin during the acid hydrolysis process because of the presence of syringyl units.<sup>9–12</sup>

This study compared the phenolization of SAL of hardwood and softwood, focusing on the difference between the guaiacyl and syringyl units in lignin. Recently, in the course of basic studies on the chemical structures and reactivity of hardwood SAL, our group found that the syringyl unit dissolved in 72% sulfuric acid at an early stage.<sup>11,12</sup> Based on this reaction, we prepared syringyl-nucleus-rich sulfuric acid lignin (S-rich-SAL) and guaiacyl-nucleus-rich sulfuric acid lignin (G-rich-SAL) from beech.

To investigate the behavior of hardwood SAL during phenolization, we compared the reactions of S-rich-SAL and G-rich-SAL, as well as those of beech and red pine. Furthermore, we conducted an experiment using simple model compounds to confirm the phenolization behavior of hardwood SAL.

### Experimental

Preparation of sulfuric acid lignin (SAL), syringyl-nucleus-rich sulfuric acid lignin (S-rich-SAL), and guaiacyl-nucleus-rich sulfuric acid lignin (G-rich-SAL)

Beech SAL (B-SAL) was prepared from extractive-free beech (*Fagus crenata* Blume) wood meal treated with 72% sulfuric acid using the Klason method.<sup>13</sup> Red pine SAL

Y. Matsushita (✉) · H. Sano · M. Imai · T. Imai · K. Fukushima  
Graduate School of Bioagricultural Sciences, Nagoya University,  
Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan  
Tel. +81-52-789-4174; Fax +81-52-789-4163  
e-mail: ysmatsu@agr.nagoya-u.ac.jp

(R-SAL) (*Pinus densiflora* Sieb. et Zucc) was obtained in the same manner. S-rich-SAL and G-rich-SAL were prepared using the modified Klason method as follows:<sup>11</sup> extractive-free beech was treated with 72% sulfuric acid at room temperature for 30 min. The heterogeneous solution was separated with a glass filter (1G3) to give soluble and insoluble fractions. The soluble fraction was diluted with water to a 3% sulfuric acid concentration, and then boiled for 2 h. The resulting precipitate was separated by centrifugation and washed with water until neutral to obtain the S-rich-SAL (16.5% on wood meal). The insoluble fraction was retreated with fresh 72% sulfuric acid at room temperature for 3.5 h. Then, the solution was diluted with water to a 3% sulfuric acid concentration and then boiled for 2 h. G-rich-SAL was obtained by centrifugal separation followed by water washing (7.1% on wood meal).

#### Phenolization of SAL, S-rich-SAL, and G-rich-SAL

A mixture containing 0.2 g SAL and an amount of phenol (varied over the range of 0.3–1.2 g) in 3.0 ml 72% sulfuric acid was stirred at 60°C for 6 h. After quenching by dilution with water until the sulfuric acid concentration was 3%, the reaction solution was boiled for 3 h. The precipitate was separated by centrifugation and washed with water to obtain the insoluble fraction of phenolized SAL.<sup>14</sup> The soluble fraction was dialyzed using a cellulose tube with a 3500 molecular weight cutoff (Tubing TM 3500; BioDesign, Carmel, NY, USA) to remove phenol and any inorganic reagents. The reaction products were lyophilized to yield the soluble fraction of phenolized SAL.<sup>14</sup>

S-rich-SAL and G-rich-SAL were also prepared using same method with 1.2 g phenol and 3.0 ml sulfuric acid for 0.2-g samples reacted at 60°C for 6 h to obtain the insoluble and soluble phenolized products.

#### Gel filtration chromatography

The molecular weight distribution was measured using Sepharose CL-6B (51 × 1.8 cm) with 80% dimethylformamide (DMF) aqueous solution containing 1 M LiCl as the eluent and ultraviolet (UV) detection (280 nm). Sodium polystyrene sulfonates ( $M_w = 1.8 \times 10^3$  to  $1.0 \times 10^5$ ) were used as a standard.

#### Phenolization of I and II

Each 20-mg sample of compounds I and II in 0.5 ml of sulfuric acid was heated at 40°C for 0.25 h. After diluting with water and adding 5 mg of 2-naphthol (ethyl acetate solution) as an internal standard, the reaction mixture was extracted with ethyl acetate. The ethyl acetate solution was washed with water, dried over sodium sulfate, and concentrated. The reaction products were converted into trimethylsilyl derivatives by reaction with *N,O*-bis(trimethylsilyl) trifluoroacetamide and analyzed quantitatively by gas chromatography (GC-353 gas chromatograph;

GL Sciences, Tokyo, Japan) using a TC-1 capillary column (60 m × 0.25 mm i.d.; GL Sciences) at an injection temperature of 200°C, detector temperature of 230°C, and column temperature of 180° to 280°C (held at 180°C for 5 min, and raised to 280°C at 10°C/min) in N<sub>2</sub> at a flow rate of 1.1 ml/min, using a flame ionization detector (FID).

#### Determination of the methoxy group content

The methoxy group content was determined according to the literature<sup>15,16</sup> as follows. The sample (5–10 mg) and 10 ml hydroiodic acid (57% w/w) were sealed in a glass vial and heated at 130°C for 20 min. After cooling in an ice bath, 15 ml of a tetrachloromethane solution of ethyl iodide (0.1 mmol) was added, as an internal standard, through the septum. The vial was opened and the reaction mixture was extracted with 10 ml of tetrachloromethane. The tetrachloromethane solution was dried over sodium sulfate, and the methyl iodide was determined by gas chromatography (GC-353; GL Sciences) using a TC-1 capillary column (60 m × 0.25 mm i.d.; GL Sciences) at an injection temperature of 200°C, detector temperature of 230°C, and column temperature of 40° to 280°C (programmed temperature; held at 40°C for 5 min, raised to 180°C at 10°C/min) in N<sub>2</sub> carrier at a flow rate of 1.1 ml/min, using FID detection.

## Results and discussion

#### Phenolization of SAL

The average molecular weights of the phenolized products were calculated from the results of gel filtration chromatography and are shown in Table 1. The insoluble fraction of phenolized B-SAL had lower molecular weights than the corresponding R-SAL samples. The molecular weight of the soluble fraction of phenolized B-SAL was also the same as the corresponding R-SAL fraction. Independent of the

**Table 1.** Molecular weights of phenolized sulfuric acid lignins

	$M_w$ ( $\times 10^3$ )	$M_n$ ( $\times 10^3$ )
Phenolized B-SAL		
Insoluble	23.8	10.8
Soluble	18.8	13.3
Phenolized S-rich-SAL		
Insoluble	13.8	8.3
Soluble	5.2	4.4
Phenolized G-rich-SAL		
Insoluble	15.3	9.4
Soluble	6.1	5.0
Phenolized R-SAL		
Insoluble	41.0	11.6
Soluble	16.4	13.3

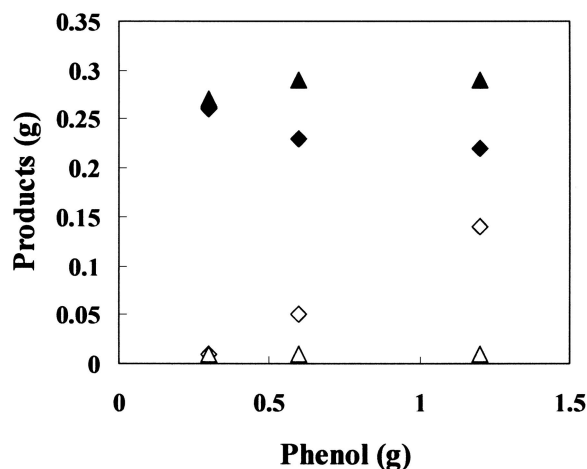
B-SAL, sulfuric acid lignin from beech; S-rich-SAL, syringyl-nucleus-rich sulfuric acid lignin from beech; G-rich-SAL, guaiacyl-nucleus-rich sulfuric acid lignin from beech; R-SAL, sulfuric acid lignin from red pine

sample, the molecular weight of the soluble fraction of phenolized SAL was lower than that of the insoluble fraction. A previous study<sup>14</sup> showed that during phenolization, the SAL of guaiacyl lignin was first subjected to depolymerization and phenol was introduced at a benzylic site. Then, the further introduction of phenol and cleavage of  $\beta$ -O-4 linkage occurred to yield low molecular weight products as the soluble fraction. In this experiment with beech, the same reactions were thought to have occurred for syringyl lignin.

Figure 1 shows the relationship between the amount of phenol and the yield of the soluble and insoluble fractions of phenolized SAL during the phenolization of SALs. Comparing beech and red pine, the phenolization products of beech included a larger soluble fraction and smaller insoluble fraction than those of red pine. The B-SAL-generated soluble fraction of SAL increased with the amount of phenol, while a constant low yield was observed for red pine. This suggests that B-SAL is more reactive with phenol because of the syringyl units. One of the reasons may be that the electron density of the syringyl unit is higher than that of the guaiacyl unit.

#### Difference in reactivity of the guaiacyl and syringyl units in B-SAL

Recently, our group found that upon treatment with 72% sulfuric acid, the syringyl unit in lignin is dissolved at an early stage. Much of this lignin is precipitated during the subsequent repeat treatment with 72% sulfuric acid and boiling in 3% sulfuric acid due to condensation reactions.<sup>11</sup> Based on this phenomenon, we obtained syringyl-nucleus-rich sulfuric acid lignin (S-rich-SAL) and guaiacyl-nucleus-rich sulfuric acid lignin (G-rich-SAL) by separating the acid-soluble lignin at an early stage from the reaction mixture produced upon treatment with 72% sulfuric



**Fig. 1.** The yields of insoluble and soluble phenolized sulfuric acid lignins (SALs). *Filled triangles*, insoluble phenolized red pine sulfuric acid lignin (R-SAL); *filled diamonds*, insoluble phenolized beech sulfuric acid lignin (B-SAL); *open triangles*, soluble phenolized R-SAL; *open diamonds*, soluble phenolized B-SAL

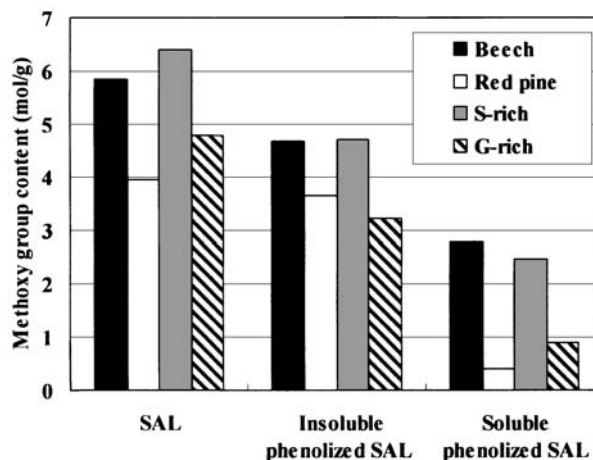
acid and then boiling the soluble and insoluble parts separately.

To investigate differences in the phenolization activity of the syringyl and guaiacyl units in B-SAL, both S-rich-SAL and G-rich-SAL were reacted with phenol. As a result, a 110-mg insoluble fraction was obtained as the phenolized product from 200mg of G-rich-SAL, while only 90mg of insoluble fraction was obtained from the S-rich-SAL. Because the insoluble fraction of phenolized S-rich-SAL was lower than that of G-rich-SAL, it appears that the syringyl unit is more reactive than the guaiacyl unit during phenolization, leading to the conversion of a large amount of the insoluble fraction into soluble material. The total of the insoluble fractions of phenolized S-rich-SAL and G-rich-SAL was lower than that of B-SAL. It is difficult to explain why the reactivity increased when the B-SAL was separated into S-rich-SAL and G-rich-SAL.

Table 1 shows the molecular weights of the phenolized products of S-rich-SAL and G-rich-SAL. The molecular weights of the insoluble and soluble fractions of phenolized S-rich-SAL were lower than those of G-rich-SAL.

#### Methoxy group content

Figure 2 shows the methoxy group content of the samples. The SAL and insoluble and soluble fractions of the phenolized products from beech had higher methoxy group contents than the corresponding red pine fractions. In addition, S-rich-SAL and its derivatives had a higher methoxy group content than the corresponding G-rich-SAL products. The methoxy group contents of the soluble fraction of phenolized R-SAL and G-rich-SAL were very low, implying that guaiacyl lignin needed to introduce more phenol to convert the insoluble fraction into the soluble fraction than the syringyl lignin because the methoxy group content decreased with increasing introduction of phenol. This may be related to the solubility of the syringyl unit with its relatively high electron density such that the syringyl lignin does not need more phenol to convert to the soluble fraction.



**Fig. 2.** The methoxy group content of SALs and phenolized SALs

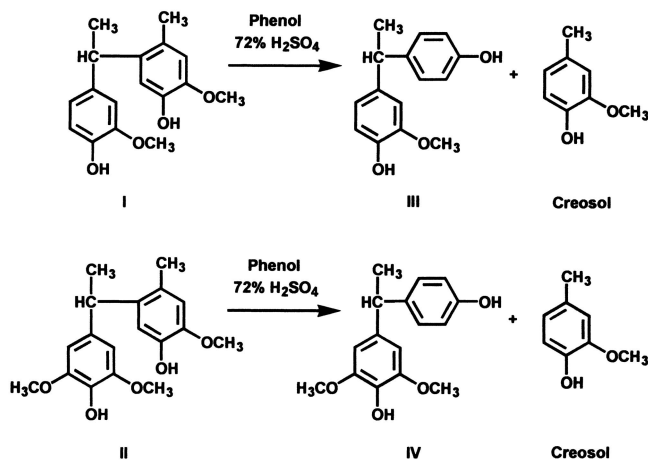


Fig. 3. Phenolization of the model compounds

Table 2. Reaction yields of model compounds

Reaction conditions		Products yield (%)		
SA <sup>a</sup>	Compound	I or II <sup>b</sup>	III or IV <sup>c</sup>	Creosol
63%	I	83.7	10.4	16.0
	II	30.9	39.4	57.7
54%	I	98.9	0.8	1.1
	II	89.2	9.9	10.8

<sup>a</sup> Sulfuric acid concentration

<sup>b</sup> Unreacted raw compound

<sup>c</sup> III and IV were yielded from I and II, respectively

### Investigating phenolization using simple model compounds

To investigate the phenolization of SAL, we conducted experiments using simple model compounds. Compounds I and II were selected as guaiacyl and syringyl nucleus models, respectively, and were subjected to phenolization (Fig. 3). The reaction compounds were analyzed using gas chromatography and the results are summarized in Table 2. When compound I reacted with phenol, it changed into compound III and creosol, while compound II yielded compound IV and creosol. Under the same conditions, the yield of compound IV was greater than that of compound III and more creosol was obtained from compound II than from compound I. This suggests that the syringyl type in SAL introduced phenol at a benzylic site and depolymerization of the condensed structure was easier than in the guaiacyl unit, which supports the hypothesis that the syringyl unit is more reactive than the guaiacyl unit in SAL.

### Conclusions

Upon phenolization, B-SAL yielded a greater soluble fraction of phenolized SAL than R-SAL, suggesting that B-SAL possessed greater phenolization activity. The investigation of the separate reactions of S-rich-SAL and G-rich-SAL from beech and the simple model compounds

showed that the syringyl unit was more reactive than the guaiacyl unit. The methoxy contents of the soluble fractions of phenolized R-SAL and G-rich-SAL were very low, while those of B-SAL and S-rich-SAL were relatively high. Therefore, the phenolized products of the syringyl unit were more soluble in acidic aqueous medium with the introduction of less phenol than the guaiacyl unit. This suggests that hardwood SAL is more readily converted into functional material under relatively simple reaction conditions.

**Acknowledgments** The authors thank Dr. Y. Matsumoto (Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan) for advice on measurement of methoxy group content. This research was conducted with the support of a Grant-in-Aid for Scientific Research (17780138) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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