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Chemical Groups and Structural Characterization of Lignin via Thiol-Mediated Demethylation

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Abstract: *A new approach to increase the reactivity of lignin by thiol-mediated demethylation was investigated in this study. Demethylated lignin was characterized by the changes in its hydroxyl and methoxyl groups, molecular weight, and other properties using titration and spectroscopy methods including FT-IR, ^{1}H NMR, UV, and GPC. The total, phenolic, and aliphatic hydroxyl contents in lignin increased while the methoxyl content decreased after demethylation reaction, which indicated the occurrence of demethylation reaction. The results from FT-IR and UV analysis also indicated that the kraft lignin in this study contains mainly guaiacyl moiety and demethylated lignin has a higher guaiacyl content than original lignin. The average molecular weight of lignin decreased after demethylation reaction due to the cleavage of ether linkages in lignin macromolecules. No severe degradation of lignin was observed during the demethylation reaction.*

Keywords Biomaterials, demethylation, odorless thiol, nucleophilic substitution, lignin reactivity

Introduction

Lignin is a type of amorphous polyphenolic biopolymer composed of three phenylpropanoid monomers (i.e., coniferyl, sinapyl, and *p*-coumaryl alcohol). They are linked by a multitude of inter-unit bonds that include several types of ether (β-O-4, α-O-4, 4-O-5) and carbon–carbon linkages.[1] Lignin represents the largest pool of phenolic compounds on earth. The greatest interest in these compounds centers on the substitution of phenol in phenol-formaldehyde (PF) resin synthesis. However, the basic phenylpropane units of lignin macromolecule are mostly methoxylated at one or both *meta* positions to the C3 side

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chain. Therefore, most bonding sites for a PF resin are not available in lignin. Direct use of lignin in PF resins as wood adhesives requires a long pressing time and high pressing temperature due to its extremely low reactivity.[1]

In recent decades, many efforts have been made to improve the reactivity of lignin.[2] The most studied methods are lignin methylolation/hydroxymethylation and phenolation, which aimed to introduce reactive functional groups to lignin. However, it is reported that methylolation reaction is slow and not capable of saturating all available *meta* positions on lignin.[3] The amount of methylol groups that can be introduced on lignin is very limited. Furthermore, no study so far has showed that phenolation treatment of lignin added new reaction sites on lignin. In addition, phenol is the most costly material in PF resin manufacturing. Therefore, practical implementation of this process could be hindered. Our approach to improve the reactivity of lignin in PF resin synthesis is by demethylation. Demethylation of lignin results in a catechol formation. Catechol, having an additional electron donating –OH, is more reactive than phenol. The catechol formation also provides additional binding sites on the lignin needed for the polymerization of PF resins.[4] Almost all the methods on lignin demethylation in the literature so far have been based on a commercial process for the production of dimethyl sulfide.[4–6] Namely, lignin reacts with sulfur in a pressure-proof reactor at 225–235°C to produce dimethyl sulfide. Demethylated lignin is the by-product of this process.

In this study, we use a thiol to demethylate kraft lignin via a nucleophilic substitution reaction. Small aliphatic thiols, such as methanethiol and ethanethiol, are excellent soft nucleophiles and have been successfully employed in a great number of demethylation reactions of aryl methyl groups.[7,8] One significant disadvantage of using small aliphatic thiols is their foul smell and toxic gases that generate during the reaction. The relative odor index of thiols generally decreases with the increase of the length of their aliphatic chains. It becomes odorless when the carbon increases to 10 in the aliphatic chain. Kraft lignin is subjected to demethylation in the presence of 1-dodecanethiol, which is commercially available and inexpensive compared to other higher-chain alkane thiols. Until now, little related research on thiol-mediated lignin demethylation has been published. Demethylated kraft lignin (DKL) was characterized by the changes in its hydroxyl and methoxyl groups, molecular weight, and other properties with titration and spectroscopy methods including Fourier transform-infrared (FT-IR), ultraviolet (UV), gel permeation chromatography (GPC), and proton nuclear magnetic resonance (^{1}H NMR).

Experimental

Materials

Kraft lignin (Indulin AT) was purchased from Mead Westvaco (Glen Allen, VA, USA). All lignin used in experiments was oven-dried at 70°C for 3 h and then kept in a sealed plastic bag in a desiccator before use. 1-Dodecanethiol was purchased from Sigma Aldrich (Milwaukee, WI, USA). Pyridine, acetic anhydride, sodium methoxide (NaOMe), and N,N-dimethylformamide anhydrous (DMF) were purchased from Aladdin (China) and used as received. Tetrahydrofurane (THF) used in the GPC experiment is of chromatographic grade.

Demethylation of Kraft Lignin

A typical thiol-mediated demethylation reaction was followed for kraft lignin.[9] In brief, 20 mL (0.08mol)1-Dodecanethiol and 50 mL DMF were added to an oven-dried, 250-mL, round-bottom flask equipped with a magnetic stirrer under nitrogen atmosphere. The flask was put into an ice-water bath and cooled to 5–10°C. When the internal temperature of the flask was below 10°C, 13 g solid NaOMe (0.24 mol) was added in one portion. The flask was then allowed to warm to 20–25°C. About 20 g kraft lignin was dissolved in 30 mL DMF and was slowly added to the flask with continual stirring. The flask was then heated in an oil bath to 130°C and refluxed under N_2 for 1 h. After reaching the desired reaction time, the reaction was quenched by adding 25 mL purified water, followed by cooling the flask in an ice-water bath. To the flask was added 1 N HCl solution dropwise to bring the pH of the solution to 1. Precipitated lignin was obtained by centrifugation and dried at ambient temperature. About 50 mL hexane was added to the dried lignin and stirred for 1 h. After filtration and drying at room temperature, DKL was obtained as a light-brown powder with high yield in 98.3% (based on original kraft lignin mass).

Analysis of General Chemical Properties

The ash contents and sugar contents of original kraft lignin (OKL) and DKL were determined according to GB/T 2677.3–93and BG/T 2677.3–94,[10,11] respectively. The OKL and DKL samples were purified before elemental analysis using a method in the literature.[12] The carbon, hydrogen, and nitrogen contents were determined on an Elementar Vario EL3 Elementarl analyzer and the oxygen content was on an Elementar Vario EL Elemental analyzer. All data were done in duplicate.

Determination of Hydroxyl Group and Methoxyl Group Contents

Determination of the hydroxyl group by titration was performed following the method described by Zakis.[13] Both OKL and DKL were first acetylated with pyridine and acetic anhydride mixture (1/1, v/v) under constant agitation at room temperature for 48 h.[14] Acetylated lignin samples were titrated to determine the total hydroxyl content. The phenolic and aliphatic hydroxyl contents were determined and calculated as in the following equations:

$$M_{HP} = M_A - M_C \tag{1}$$

$$M_{HA} = M_{HT} - M_{HP} \tag{2}$$

where M_{HP} is the phenolic hydroxyl content, M_A and M_C are the total acidic groups and carboxyl groups, respectively, determined by titration, M_{HA} is the aliphatic hydroxyl content, and M_{HT} is the total hydroxyl content.

Hydroxyl content was also determined by quantitative ^{1}H NMR (Bruker 300 MHz). P-nitrobenzaldehyde was used as the internal standard. Both lignin samples were acetylated before ^{1}H NMR analysis using the same method as for the titration. The methoxyl group (-OCH3) contents of OKL and DKL were determined in accordance with ASTM D1166–84.[15]

FT-IR and UV Spectroscopy

FT-IR analysis of OKL and DKL samples was collected on a Nicolet 550 FT-IR spectrophotometer equipped with a deuterated triglycine sulfate detector. Scans were made in a range of 4000–500cm^{-1} at resolution of 4cm^{-1} under room temperature. For UV analysis, OKL and DKL samples were dissolved in a mixed solvent of dioxane and distilled water (96/4, v/v) and the spectra were recorded on a UV-2550 (Shimadzu, Japan) PC spectrophotometer in the range from 190–380 nm at room temperature.

Gel Permeation Chromatography

DKL and acetylated OKL were dissolved in THF (0.5%,W/W) to determine the M_n and M_w by gel permeation chromatography (Waters 1550–2410 system, 1550 HPLC Pump, 2410 Refractive Index Detector, column Styragel HR1 and HR2). THF was also used as the mobile phase with a flow rate of 1 mL/min and the injection volume was 1 μL. The calibration curve was made using polystyrene standards at different molecular weights.

Results and Discussion

General Chemical Properties

Table 1 summarizes some general properties of both lignin samples. No ash was detected in DKL samples, while the ash content in OKL samples was about 3%, which is a typical value for Indulin lignin. This result was expected because the demethylation and post-reaction processes of this study were similar to a lignin purification process. Kraft lignin was first treated in an alkaline media (i.e., demethylation process) followed by precipitation in an acidic solution (i.e., post-reaction process). Almost all inorganic impurities (i.e., source of ash) in lignin samples were dissolved in the aqueous phase and separated from DKL by washing and centrifugation.

The decrease in methoxyl group content in DKL indicated the occurrence of a demethylation reaction in kraft lignin. The reaction was proposed to proceed via the *in-situ* generation of thiolate anion by NaOMe to achieve the desired product. Benzene rings in lignin containing electron-donating groups showed lower reactivities while very fast conversions were obtained for those with the electron-withdrawing groups.[9] As lignin owned lots of functional groups, such as alcohol hydroxyl, phenolic hydroxyl, aryl, carbonyl, conjugated double bond, et al., thus lignin demethylation is complicated. However, demethylation might only have occurred on part of the lignin macromolecules and/or only one methoxyl group transferred to hydroxyl group in a syringyl phenylpropane unit due to the electron-donating effect of the hydroxyl group next to both methoxyl groups.[9,16] A C9 empirical formula was deduced based on the C, H, and O content from elemental analysis and the amount of the methoxyl group in lignin samples. This value is approximate because the structures of technical lignin may have been changed during the treatment and they may also contain impurities.[17] As can be seen in Table 1, the C9 units of DKL and OKL are similar except for the lesser amount of methoxyl group in DKL.

Table 1

Element composition, methoxyl contents, and C_9-formula of OKL and DKL

Samples	Ash (%, w/w)	Sugar (%, w/w)	Element analysis (% w/w) C	H	O	OCH_3 content (% w/w)	C_9-unit
OKL	3.12	1.94	61.80	5.76	29.67	13.61	$C_9H_{8.40}O_{2.71}(OCH_3)_{0.84}$
DKL	0.00	0.38	63.76	6.07	29.39	11.68	$C_9H_{8.92}O_{2.67}(OCH_3)_{0.69}$

Table 2
Contents of hydroxyl groups in OKL and DKL

Samples	Methods	Phenolic hydroxyl (%)	Aliphatic hydroxyl (%)	Total hydroxyl (%)
OKL	titration	5.66	7.89	13.55
	^{1}H NMR	4.78	6.69	11.47
DKL	titration	6.58	9.19	15.77
	^{1}H NMR	5.34	8.64	13.98

Changes in Hydroxyl Groups

The contents of different hydroxyl groups in OKL and DKL are listed in Table 2. Although the hydroxyl contents obtained from titration are slightly higher than those determined by ^{1}H NMR, the results from these two methods are in good agreement in terms of the changes of hydroxyl groups in the lignin samples before and after demethylation.

After the demethylation reaction, the total hydroxyl content in lignin increased, including the increases in both phenolic and aliphatic hydroxyl. The mechanism of a thiol-mediated demethylation reaction is a nucleophilic S_N2 substitution reaction. In brief, the deprotonated thiol (i.e., the nucleophile) attacks the partially positive charged C in the $-OCH_3$ group and results in the leaving of the $-CH_3$ group, forming a phenlic $-OH$. However, the increase of phenolic $-OH$ was not as high as expected. It is difficult to precisely prove the formation of catechol structures in the demethylated lignin samples due to the complicated structure of lignin. A study on the reaction pathway of thiol-mediated lignin demethylation reaction using lignin model compounds is ongoing and the result of the study will be reported separately. The increase in aliphatic hydroxyl could be attributed to the cleavage of alkyl-aryl and/or alkyl-alkyl ether linkages.

There are several other methods connected to lignin demethylation. Wu et al. studied the sulfur-mediated demethylation of wheat straw soda lignin.[6] The results showed that after demethylation, the content of methoxyl groups decreased from 10.39% to 6.09%, while the content of phenolic hydroxyls increased from 2.98% to 5.51%. Nagieb et al. found that thiolignin became more reactive after oxidation reaction with potassium dichromate in the presence of acetic acid; 10.3% of methoxyl groups were also demethylated in 168 h.[18] Only 1.1% of methoxyl groups were demethylated in the time from 20 min to 3 h. Sound Pinus massoniana milled wood lignin and brown-rotted Pinus massoniana milled wood lignin were characterized in terms of functional groups' variety. Contents of methoxyl groups and phenolic hydroxyls varied from 13.14% and 1.25% to 11.43% and 2.36%, respectively.[19] Among the three methods, the first needed equipment undergoing high pressure and high temperature, the second consumed more time, and the third needed the longest time, as it took place under natural conditions. Combining the degree of methoxyl group change, the present method was suitable.

Figure 1 shows the 1H NMR spectra of OKL (a) and DKL (b), and Table 3 list the integrals of the signals in the 1H NMR spectra of acetylated OKL and DKL. The spectra of two kinds of lignin samples were similar and also very similar to those reported in the literature.[20–22] The large peak at 8.00–6.85 ppm was attributed to aromatic protons. Signal between 4.00–3.48 ppm was attributed to methoxyl protons. Peaks at 2.50–2.22 ppm and 2.22–2.00 ppm were due to acetylated phenolic and aliphatic acetates protons, respectively. Peaks at 2.00–1.21 ppm and 0.90 ppm were due to methylene and methyl protons in DKL,

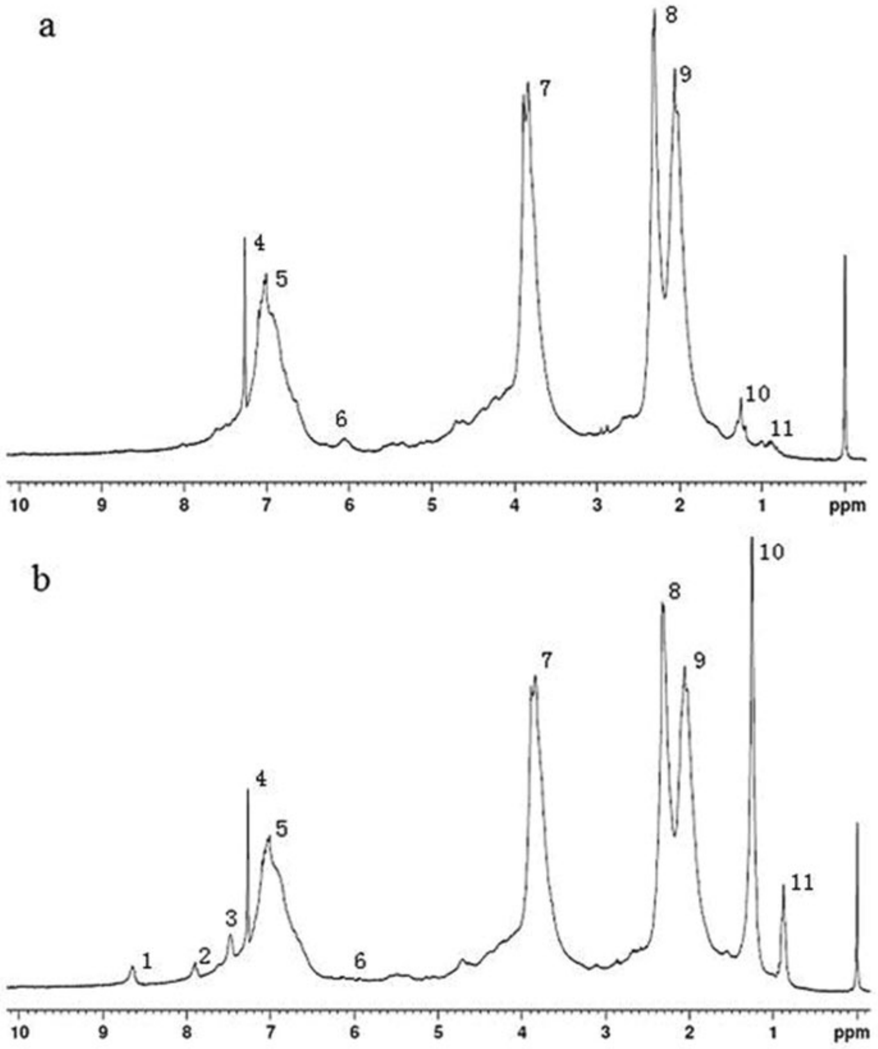

Figure 1. ^{1}H NMR spectra of OKL (a) and DKL (b).

respectively. They were dramatically higher in DKL (Figure 1b) than that in original lignin. The big change stemmed from both the release of alkyl side chains resulting from the degradation of the lignin macromolecule during demethylation reaction,[23] and the fact that 1-dodecanethiol stayed in DKL. There were several strong signals displayed in the chemical shifting region between 8.00 and 7.00 ppm in the demethylated lignin (Figure 1b), which could be aromatic ring protons adjacent to some strong polar functional groups that were generated during demethylation; for example, the signal at 8.76 ppm was due to carboxyl protons.[6] On the other hand, the weak peak at 6.03 ppm mainly caused by β-O-4 ether linkages almost disappeared after demethylation (Figure 1b), which indicated the cleavage of the ether bond.[6,19,24]

Table 3
Signal assignment for ^{1}H NMR spectrometry

Range No.	Range (10^{-6})	Maximum value (10^{-6}) OKL	Maximum value (10^{-6}) DKL	Assignment
1	8.76		8.76	H in carboxyl
2	7.92		7.92	Aromatic protons adjacent to
3	7.48		7.48	strong polar functional groups
4	7.26	7.26	7.26	$CDCl_3$
5	8.00–6.85	6.98	7.02	Aromatic protons in guaiacyl units
6	5.82–6.25	6.03		H in β-O-4
7	4.00–3.48	3.80	3.80	H in methoxyl group
8	2.50–2.22	2.28	2.28	H in aromatic acetates
9	2.22–2.00	2.10	2.10	H in aromatic acetates
10	2.00–1.21	1.23	1.23	H in methylene
11	0.84–1.00	0.92	0.90	H in methyl

FT-IR and UV Analysis

The FT-IR spectra of OKL and DKL samples are shown in Figure 2. In general, the patterns of the peaks in both spectra are similar, except for a minor shifting of some peaks and the changes in peak intensities. The assignment of the peaks and their relative values are summarized in Table 4. Peaks around 1600 and 1500 cm^{-1} corresponded to the aromatic skeletal vibration of the phenylpropane unit in lignin,[25,26] and these bands were used as

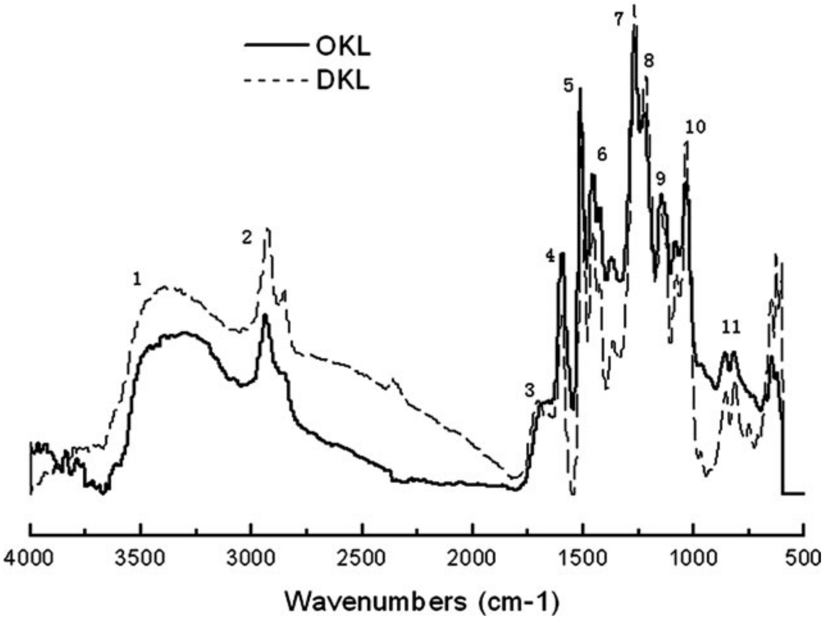

Figure 2. FT-IR spectra of OKL and DKL.

Table 4

FT-IR peak assignments and absorption values of OKL and DKL

Wn[a]	OKL			DKL			
No.	Wn (cm^{-1})	Ab[b]	Rv[c]	Wn (cm^{-1})	Ab	Rv	Assignment
1	3290.10	0.0100	0.498	3393.75	0.0129	0.759	Stretching in hydroxyl groups
2	2394.56	0.0111	0.552	2928.27	0.0165	0.971	C—H stretching in methyl and methyene groups
3	1594.47	(mean) 0.0201	1	1598.08	(mean) 0.017	1	aromatic skeletal vibration
4	1511.66			1511.53			
5	1457.58	0.0199	0.990	1458.97	0.0130	0.765	C—H deformation in methoxyl groups
6	1369.89	0.0147	0.731	1367.68	0.0095	0.559	Deformation in cellulose and hemicellulose
7	1268.94	0.0287	1.428	1268.79	0.0303	1.782	C=O stretching typical for G units
8	1221.90	0.0236	1.174	1218.21	0.0260	1.529	Stretching in phenolic hydroxyl groups
9	1145.06	0.0186	0.925	1148.14	0.0174	1.024	C—H deformation vibration in G units
10	1032.48	0.0194	0.965	1033.10	0.0220	1.294	Primary aliphatic OH and ether
11	856.14	0.0088	0.438	856.17	0.0064	0.376	C—H out-of-plane deformation vibration in G units

[a]wavenumber.
[b]absorbance.
[c]relative value.

the internal reference. In brief, the absorbance of these two bands was averaged and the relative values of other peaks were obtained by normalizing the absorbance value of the peak to the mean value of the internal reference.[19,27,28] Both lignin samples show a broad peak around 3400 cm^{-1} assigned to hydroxyl stretching. However, the relative value of this peak in the DKL sample is higher than that in OKL. This result was expected due to the demethylation reaction, which is a substitution reaction resulting in the leaving of the methyl group, forming a hydroxyl group to the original site of the methoxyl group. The same trend is observed at peaks around 1220 cm^{-1} and 1030 cm^{-1}, which are assigned to phenolic and primary aliphatic hydroxyl stretching, respectively.[6] In addition to forming a hydroxyl group at the methoxyl site, the demethylation reaction could cause the cleavage of other ether linkages in lignin and form either phenolic or aliphatic hydroxyl groups.[6] While the relative value of the hydroxyl group increased, the relative value of the methoxyl group at peak 1457 cm^{-1} decreased in the DKL sample, also indicating the occurrence of

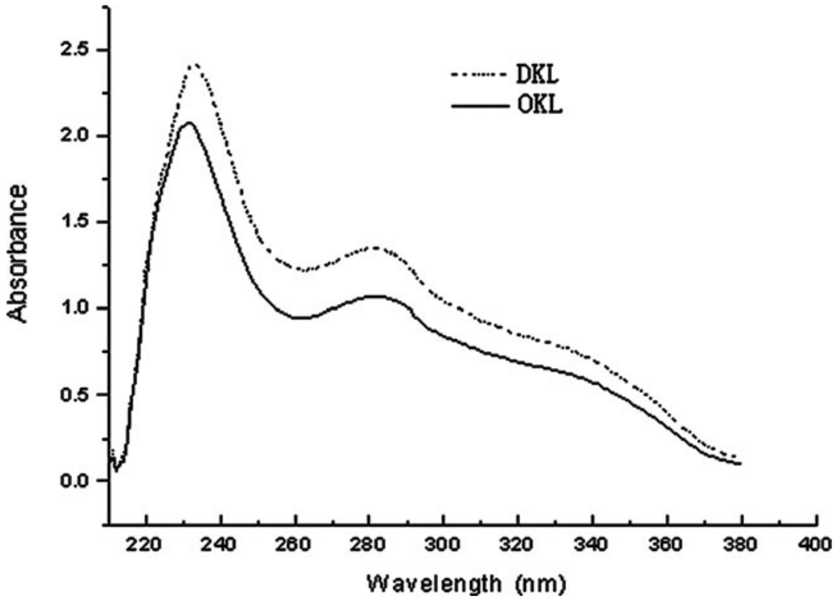

Figure 3. UV spectra of OKL and DKL.

demethylation in lignin. The display of peaks at 1268 cm^{-1}, 1145 cm^{-1}, and 856 cm^{-1} in both lignin samples implied that the Indulin lignin in this study came from softwood because the above peaks are typical bands in a G-type lignin unit and softwood lignin is mainly composed of G moieties with small contents of P unit and only traces of S unit.[29] The result from FT-IR analysis of both lignin samples is in good agreement with those from the titration method and NMR analysis.

The UV absorption spectra of both lignin samples are presented in Figure 3. Both spectra show a peak around 230 nm and a broad peak around 280 nm. The scattering at 200 to 230 nm in the dioxane/water solution is due to dioxane absorption.[21] The absorbance at 280 nm corresponds to the aromatic ring.[30,31] It has been reported that the guaiacyl structures exhibit maxima in the 280 nm and the syringyl monomer absorbs less light at the same region.[32,33] On the other hand, catechol also showed absorption maxima at 280 nm. The somewhat higher absorbance at 280 nm in DKL suggested that more free-phenolic guaiacyl units were generated in the demethylation process, which may also suggest the occurrence of demethylation and catechol formed during lignin demethylation. It should also be pointed out that the spectra of the two lignin samples are very similar, which implies that the demethylation reaction didn't alter the aromatic ring structure in lignin. Therefore, it is reasonable to use the aromatic skeleton absorbance as the internal reference in FT-IR analysis.

GPC Analysis

The average molecular weight of DKL and OKL are presented in Table 5, and GPC spectra are shown in Figure 4. Both M_n and M_w of DKL were lower than those of the OKL. As discussed in previous sections, the demethylation reaction could cause the cleavage of ether linkages in lignin and therefore reduce the size of lignin macromolecules. However,

Table 5
Average molecular weights of OKL and DKL

Samples	M_n	M_w	Polydispersity
OKL	1977	4377	2.21
DKL	1401	3264	2.33

thiol-mediated demethylation did not cause severe degradation of lignin. As indicated by their average molecular weight and molecular weight distributions (Table 6), both DKL and OKL consist of more than 50% of the molecules that weigh from 2000 to 5000, while OKL contains a higher percentage of molecules that weigh over 5000. Another indication of the lower molecular weight of DKL was that the DKL sample could be dissolved in THF

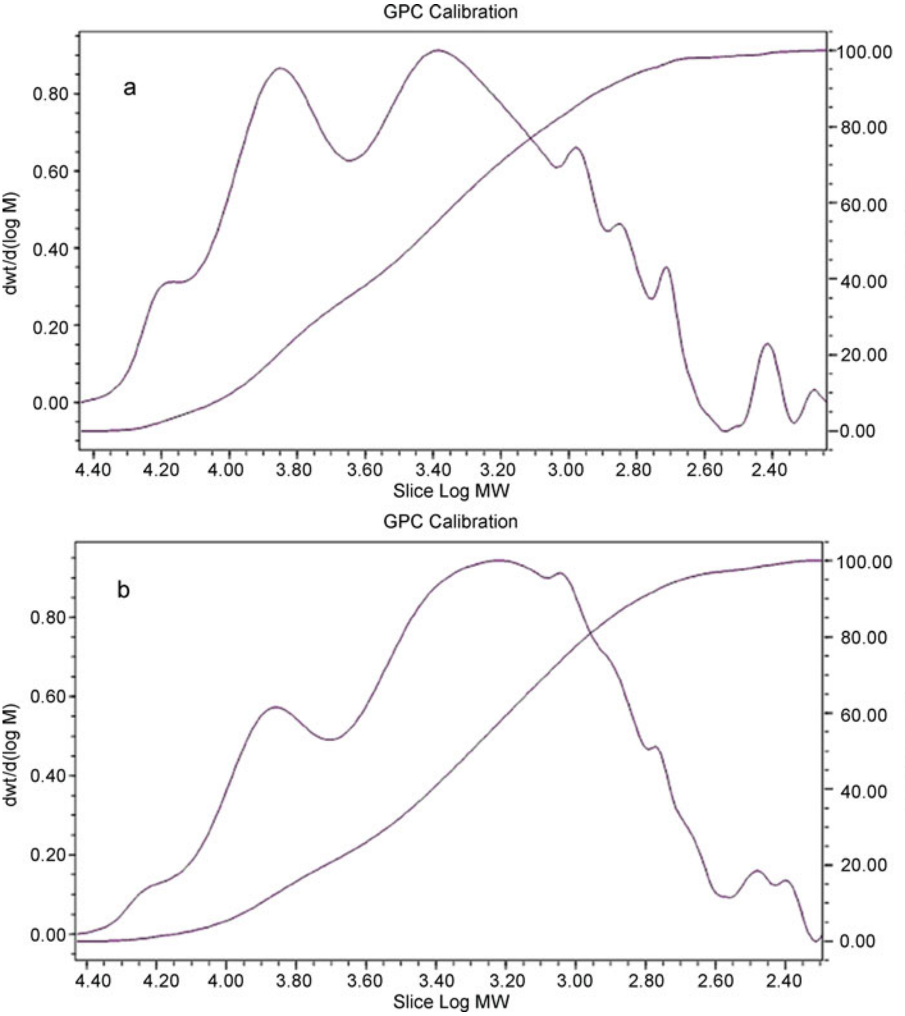

Figure 4. GPC spectra of OKL (a) and DKL (b) (color figure available online)

Table 6
Molecular weight distribution of OKL and DKL

	Samples	
Molecular weight distribution	OKL/%	DKL/%
10000	9	5
10000–5000	24	18
5000–3000	14	13
3000–2000	15	12
2000	38	52

without acetylation, whereas the OKL samples must be acetylated to be able to dissolve in THF. Although many factors could affect the dissolution of a polymer, the molecular weight of the polymer normally plays an important role.

Conclusion

It is expected that the methoxyl group on the phenylpropane unit of lignin could be replaced by a hydroxyl group through a thiol-mediated nucleophilic substitution reaction. The total, phenolic, and aliphatic hydroxyl contents in lignin increased while the methoxyl content decreased after demethylation, indicating the occurrence of the reaction. According to Table 3, the relative value of phenolic hydroxyl at 1221.90 cm^{-1} increased from 1.174 to 1.529, while the relative value of methoxyl at 1457.58 cm^{-1} decreased from 0.990 to 0.765. Demethylated lignin has more guaiacyl monomer than the original lignin does, and the near-disappearance of β-O-4 ether linkages after demethylation led to more guaiacyl monomer. The average molecular weight of lignin decreased after the demethylation reaction, which indicated that the thiol-mediated demethylation reaction could also cause the cleavage of ether linkages in lignin macromolecule.

References

1. Mansouri, N.E.; Salvadó, J. Structure characterization of technical lignins for the production of adhesives: Application to lignosulfonate, kraft, soda-anthraquinone, organosolv and ethanol process lignins. Ind. Crop. Prod. **2006**, *24*, 8–16.
2. Hu, L.H.; Pan, H.; Zhou, Y. H.; Zhang, M. Methods to improve lignin's reactivity as a phenol substitute and as replacement for other phenolic compounds: A brief review. BioResources. **2011**, *6*, 3515–3525.
3. Stephanou, A.; Pizzi, A. Rapid-curing lignin-based exterior wood adhesives. Part II. Esters acceleration mechanism and application to panel products. Holzforschung. **1993**, *47*, 501–506.
4. Schroeder, H.A.; Collins, F. Method for recovering and using lignin in adhesive resins by extracting demethylated lignin. U.S. patent 5026808, **1991**.
5. An, X.N.; Schroeder, H. A.; Thompson, G. E. Demethylated kraft lignin as a substitute for phenol in wood adhesive. Chem. Ind. For. Prod. **1995**, *15*, 36–42.
6. Wu, S.B.; Zhan, H.Y. Characteristics of demethylated wheat straw soda lignin and its utilization in lignin-based phenolic formaldehyde resins. Cell. Chem. Technol. **2001**, *35*, 253–262.
7. Nishide, K.; Ohsugi, S.; Miyamoto, T.; Kumar, K.; Node, M. Development of odorless thiols and sulfides and their applications to organic synthesis. Monatshefte für Chemie. **2004**, *135*, 189–200.

8. Node, M.; Kajimoto, T. Development of odorless organosulfur reagents and asymmetric reaction using odorless thiols. Heteroatom Chem. **2007**, *18*, 572–583.
9. Kale, B.; Shinde, A.; Sonar, S.; Shingate, B.; Kumar, S.; Ghosh, S.; Venugopal, S.; Shingare, M. 1-Decanethiol, A new reagent for the odorless deprotection of aryl methyl ethers. Tetrahedron Lett. **2010**, *51*, 3075–3078.
10. Chinese National Standard. Fibrous raw material: Determination of ash. GB/T 2677.3–93, **1993**.
11. Chinese National Standard. Fibrous raw material: Determination of pentosan. GB/T 2677.3–94, **1994**.
12. Lundquist, K.; Ohlsson, B.; Simonson, R. Isolation of lignin by means of liquid-liquid extraction. Svensk. Papperst. **1977**, *80*, 143–144.
13. Zakis, G.F. *Functional Analysis of Lignin and their Derivatives, 24, 32, 65, 89*; TAPPI Press: Atlanta, GA, **1994**.
14. Thring, R.W.; Chornet, E.; Bouchard, J.; Vidal, P.F.; Overend, R.P. Evidence for the heterogeneity of glycol lignin. Ind. Eng. Chem. Res. **1991**, *30*, 232–240.
15. American Society for Testing and Materials. Standard test method for methoxyl groups in wood and related materials. ASTM D1166–84, **2001**.
16. Zuo, L.; Yao, S.Y.; Wang, W.; Duan, W.H. An efficient method for demethylation of aryl methyl ethers. Tetrahedron Lett. **2008**, *49*, 4054–4056.
17. Cateto, C.A.; Barreiro, M.F.; Rodrigues, A.E.; Brochier-Salon, M.C.; Thielemans, W.; Belgacem, M.N. Lignins as macromonomers for polyurethane synthesis: A comparative study on hydroxyl group determination. J. Appl. Polym. Sci. **2008**, *109*, 3008–3017.
18. Nagieb, Z.A.; Egypt, C. Demethylation of thiolignin by reaction with potassium dichromate: A kinetic study. Wood Sci. Technol. **1985**, *19*, 233–242.
19. Sun, Q.N.; Qin, T.F.; Li, G.Y. Chemical groups and structural characterization of brown-rotted pinus massoniana lignin. Int. J. Polym. Anal. Ch. **2009**, *14*, 19–33.
20. Islam, A.; Sarkanen, K.V. The isolation and characterization of the lignins of jute (Corchorus capsularis). Holzforschung. **1993**, *47*, 123–132.
21. Hoareau, W.; Trindade, W.G.; Siegmund, B.; Castellan, A.; Frollini, E. Sugar cane bagasse and curaua lignins oxidized by chlorine dioxide and reacted with furfuryl alcohol: Characterization and stability. Polym. Degrad. Stabil. **2004**, *86*, 567–576.
22. Tejado, A.; Peña,C.; Labidi, J.; Echeverria, J.M.; Mondragon, I. Physico-chemical characterization of lignins from different sources for use in phenol-formaldehyde resin synthesis. Bioresour. Technol. **2007**, *98*, 1655–1663.
23. Filley, T.R.; Cody, G.D.; Goodell, B.; Jellison, J.; Noser, C.; Ostrofsky, A. Lignin demethylation and polysaccharide decomposition in spruce sapwood degraded by brown rot fungi. Org. Geochem. **2002**, *33*, 111–124.
24. Hoareau, W.; Trindade, W.G.; Siegmund, B.; Castellan, A.; Frollini, E. Sugar cane bagasse and curaua lignins oxidized by chlorine dioxide and reacted with furfuryl alcohol: Characterization and stability. Polym. Degrad. Stabil. **2004**, *86*, 567–576.
25. Björkman, A. Studies on finely divided wood. Part 1. Extraction of lignin with neutral solvents. Svensk. Papperst. **1956**, *59*, 477–485.
26. Hage, R.E.; Brosse, N.; Chrusciel, L.; Sanchez, C.; Sannigrahi, P.; Ragauskas, A. Characterization of milled wood lignin and ethanol organosolv lignin from miscanthus. Polym. Degrad. Stabil. **2009**, *94*, 1632–1638.
27. Malutan, T.; Nicu, R.; Popa, V.I. Contribution to the study of hydroxymetylation reaction of alkali lignin. Bioresources. **2008**, *3*, 13–20.
28. Xu, F.; Sun, J.X.; Sun, R.C.; Fowler, P.; Baird, M.S. Comparative study of organosolv lignin from wheat straw. Ind. Crop. Prod. **2006**, *23*, 180–193.
29. Jiang, T.D. *Lignin*; Chemical Industry Press: Beijing, China, **2009**.
30. Fang, Z.; Sato, T.; Smith, J.R.L.; Inomata, H.; Arai, K.; Kozinski, J.A. Reaction chemistry and phase behavior of lignin in high-temperature and supercritical water. Bioresour. Technol. **2008**, *99*, 3424–3430.

31. Sakakibara, A.; Sano, Y. Chemistry of lignin. In *Wood and Cellulosic Chemistry*; Hon, D.N.-S.; Shirashi N., Eds.; Marcel Dekker: New York, **2001**,; 109–168.
32. Faix, O.; Crünwald, C.; Beinhoff, O. Determination of phenolic hydroxyl groups content of milled wood lignins (MWL's) from different botanical origins using selective aminolysis, FTIR, ^{1}H NMR, and UV spectroscopy. Holzforschung. **1992**, *46*, 425–432.
33. Gärtner, A.; Geuerstedt, G.; Tamminen, T. Determination of phenolic hydroxyl groups in residual lignin using a modified UV-method. Nord. Pulp Pap. Res. J. **1999**, *14*, 163–170.