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ORIGINAL ARTICLE

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Ready chemical conversion of acid hydrolysis lignin into water-soluble lignosulfonate II: Hydroxymethylation and subsequent sulfonation of phenolized lignin model compounds*

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Abstract Highly condensed lignin can be transformed by three reactions – phenolation, hydroxymethylation, and neutral sulfonation – to water-soluble lignosulfonate. To elucidate reactivities and products in the latter two reactions, simple compounds were selected as lignin model compounds. With hydroxymethylation of creosol at 60°C, the yield of a condensed-type product with the diarylmethane structure was less than 10%. Hydroxymethylation of 1-guaiacyl-1-*p*-hydroxy-phenylethane (compound VI) as a phenolized guaiacyl lignin model compound gave four compounds. The initial reaction introduced the hydroxymethyl group mainly in the guaiacyl nucleus, and the additional reaction created two hydroxymethyl groups in the *p*-hydroxyphenyl nucleus. Contrary to our estimation, treatment of the models with ^{13}C-labeled formaldehyde ($H^{13}CHO$) did not form any diarylmethane structure. Neutral sulfite treatment of hydroxymethylated products gave corresponding sulfonates in high yields. Phenolized guaiacylglycerol-β-aryl ether (compound XVI) showed a reactivity similar to that of compound VI.

Key words Lignin · Phenolation · Hydroxymethylation · Sulfonation · Lignosulfonate

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Introduction

The development of an effective method for utilizing large quantities of by-product lignin obtainable by acid saccharification of woody materials may stimulate the wood hydrolysis industry. Such development is closely linked to characterization of acidic lignin and findings of its new reactivities. Probably its important negative characteristics are the low number of reactive sites, particularly a deficiency[1] of the alcoholic hydroxyl group at the benzylic position, in addition to highly polymerized and condensed structures.[2–8]

As reported in a previous paper[9] sulfuric acid-catalyzed phenolation[10] of the most condensed lignin, sulfuric acid lignin, with the intention of depolymerizing and introducing a reactive *p*-hydroxyphenyl group has been estimated to be effective. Experimental support[9] for its effectiveness was obtained upon quantitative induction into modified water-soluble lignosulfonate by hydroxymethylation with formaldehyde followed by neutral sulfonation under atmospheric pressure. In the phenolation experiment with model compound I, it was suggested that a condensed-type guaiacyl nucleus was substituted selectively by phenol in preference to an uncondensed guaiacyl nucleus to give compound II, as shown in Fig. 1. Hydroxymethylation and subsequent sulfonation of several phenolized lignin model compounds with a guaiacyl nucleus were investigated in the present study.

Experimental studies

All compounds gave satisfactory elemental analyses data.

Reaction of creosol with formaldehyde

A mixture of creosol in NaOH solution and 1.5 equivalent formalin (formaldehyde 37%), as shown in Table 1, was stirred at 50°–80°C for 2 h with a magnetic stirrer. After

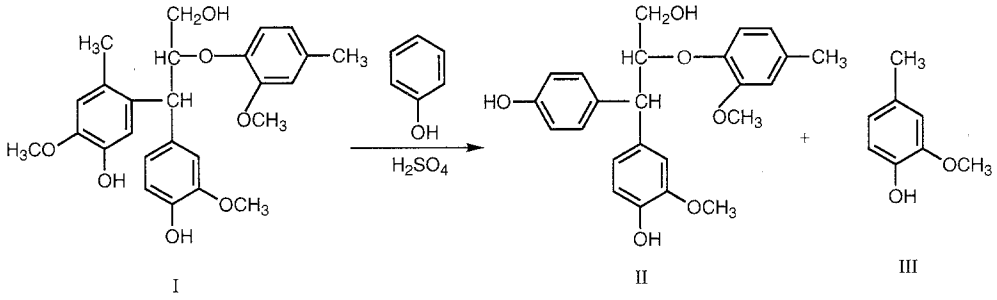


Fig. 1. Phenolation of guaiacyl sulfuric acid lignin compound I in the presence of sulfuric acid as a catalyst

Table 1. Reaction of creosol (100 mg) with formaldehyde

Treatment	Reaction conditions					Yield (%)	
	Formalin (37%) (ml)	Temp. (°C)	Time (h)	NaOH		Compound IV	Compound V
				N	ml		
1	0.5 + 0.5[a]	50	4	1	5	49.2	7.7
2	1.0 + 0.5	50	4	1	5	62.6	3.2
3	1.0 + 1.0	50	4	2	5	59.4	2.5
4	1.0 + 1.0 + 1.0 + 1.0	50	8	1	5	41.1	7.6
5	0.5 + 0.5	60	4	1	5	59.9	10.2
6	1.0 + 1.0	60	4	1	5	55.3	9.3
7	2.0 + 2.0	60	4	1	10	70.1	7.7
8	1.0 + 1.0[b]	70	2	1	5	68.2	12.2
9	1.0 + 1.0	70	4	1	5	54.3	17.0
10	1.0 + 1.0	80	4	1	5	45.3	31.5

[a] Formalin was added each 2 h (treatments 1–7, 9, 10).
[b] Formalin was added each hour.

cooling the solution was neutralized with 1N HCl and extracted with ethyl acetate. The ethyl acetate layer was dried over sodium sulfate and concentrated. Products were purified by silica gel column chromatography and eluted with *n*-hexane and acetone (4:1 v/v) to give compounds IV and V. **IV**: ^{13}C NMR (CD_3OD) δ: 21.1 (CH_3), 56.1 (OCH_3), 61.1 (CH), 110.7, 123.5, 128.8, 129.1, 142.1, 148.3. **V**[10]: ^{13}C NMR (CD_3OD) δ: 21.1 (CH_3), 29.8 (CH_2), 56.1 (OCH_3), 110.8, 123.5, 128.1, 129.3, 142.2, 148.2. Products were acetylated with acetic anhydride and pyridine in the usual manner and then analyzed by gas-liquid chromatography using a Hitachi 163 gas chromatograph under the following conditions: 2% silicone OV-1 on Chromosorb WAW DMCS (100–200 mesh) in a stainless steel column (2 m); column temperature 160°–220°C (programmed temperature 2°C/min); carrier N_2; detector FID.

Synthesis of 1-guaiacyl-1-*p*-hydroxyphenylethane (compound VI)

A mixture of 3.2 g apocynol, 16.2 g phenol, and 60 ml 36% sulfuric acid was stirred at room temperature for 30 min. After dilution with water, the reaction solution was extracted with benzene. The benzene solution was washed with water, dried over sodium sulfate, and concentrated to give products that were then purified by silica gel column chromatography eluted with *n*-hexane and acetone (4:1) to give 4.1 g (88.3% from apocynol) of VI. **VI**: mp 94.5°–96.0°C; ^{1}H NMR ($CDCl_3$) δ: 1.55 (3H, d, J = 7 Hz; CH_3), 3.78 (3H, s; OCH_3), 4.00 (1H, q, J = 7 Hz; CH), 5.41 (1H, s; OH), 5.60 (1H, s; OH), 6.65 (1H, d, J = 2 Hz; aromatic H), 6.71 (1H, q, J = 8 + 2 Hz; aromatic H), 6.73 (2H, d, J = 9 Hz; aromatic H), 6.83 (1H, d, J = 8 Hz; aromatic H), 7.02 (2H, d, J = 9 Hz; aromatic H); ^{13}C NMR ($CDCl_3$) δ: 22.3 (CH_3), 43.6 (CH), 55.9 (OCH_3), 110.4, 114.2, 115.2, 120.0, 128.6, 139.0, 143.6, 146.4, 153.7.

Hydroxymethylation of compound VI with formaldehyde

A solution 0.5 g VI in 25 ml 1N NaOH and 2.5 ml formalin was heated at 50°C under an atmosphere of nitrogen. After 2 h, 2.5 ml formalin was added again to the solution. The reaction solution was neutralized with 1N HCl and extracted with ethyl acetate. The ethyl acetate solution was dried over sodium sulfate and concentrated to give products. The products were separated by silica gel column chromatography using *n*-hexane and acetone (7:3 v/v) to isolate four compounds. **VII**: MS m/z: 274 (M^+), 259; ^{1}H NMR ($CDCl_3$) δ: 1.55 (3H, d, J = 7 Hz; CH_3), 3.81 (3H, s; OCH_3), 3.98 (1H, q, J = 7 Hz; CH), 4.77 (2H, s; CH_2), 5.76 (2H, s; 2 × OH), 6.65–7.07 (6H, m; aromatic H); ^{13}C NMR ($CDCl_3$)

δ: 22.3 (CH_3), 43.6 (CH), 55.9 (OCH_3), 64.8 (CH_2), 110.3, 114.1, 116.3, 120.0, 124.5, 126.9, 128.3, 138.4, 138.7, 143.8, 146.4, 154.2. **VIII**: MS m/z: 274 (M^+), 259; ^{1}H NMR ($CDCl_3$) δ: 1.54 (3H, d, J = 7 Hz; CH_3), 3.80 (3H, s; OCH_3), 3.98 (1H, q, J = 7 Hz; CH), 4.70 (2H, s; CH_2), 5.76 (1H, s; OH), 5.98 (1H, s; OH), 6.62–7.05 (6H, m; aromatic H); ^{13}C NMR ($CDCl_3$) δ: 22.3 (CH_3), 43.6 (CH), 56.1 (OCH_3), 62.0 (CH_2), 110.1, 115.2, 119.6, 125.7, 128.5, 138.5, 138.6, 141.9, 146.4, 154.0; ^{13}C NMR (acetone-d_6) δ: 22.7 (CH_3) , 44.6 (CH), 56.3 (OCH_3), 60.6 (CH_2), 110.4–156.3. **VIII** (acetate): ^{1}H NMR ($CDCl_3$) δ: 1.62 (3H, d, J = 7 Hz; CH_3), 2.05 (3H, s; $COCH_3$), 2.28 (3H, s; $COCH_3$), 2.30 (3H, s; $COCH_3$), 3.76 (3H, s; OCH_3), 4.13 (1H, q, J = 7 Hz; CH), 5.02 (2H, s; CH_2), 6.75 (1H, d, J = 2 Hz; aromatic H), 6.85 (1H, d, J = 2 Hz; aromatic H), 7.02 (2H, d, J = 9 Hz; aromatic H), 7.21 (2H, d, J = 9 Hz; aromatic H); ^{13}C NMR ($CDCl_3$) δ: 20.5 ($CO\underline{C}H_3$), 20.9 ($CO\underline{C}H_3$), 21.2 ($CO\underline{C}H_3$), 22.0 (CH_3), 44.1 (CH), 56.0 (OCH_3), 61.5 (CH_2), 112.1, 120.8, 121.4, 128.6, 129.0, 143.2, 144.6, 149.0, 151.2, 168.8, 169.6. **IX**: MS m/z: 304 (M^+), 289; ^{1}H NMR ($CDCl_3$) δ: 1.55 (3H, d, J = 7 Hz; CH_3), 3.82 (3H, s; OCH_3), 3.98 (1H, q, J = 7 Hz; CH), 4.68 (2H, m; CH_2), 4.74 (2H, m; CH_2), 6.63–7.07 (5H, m; aromatic H); ^{13}C NMR ($CDCl_3$) δ: 22.2 (CH_3), 43.6 (CH), 56.1 (OCH_3), 61.9 (CH_2), 64.5 (CH_2), 110.0, 116.3, 119.5, 124.7, 125.9, 127.0, 128.1, 138.1, 138.4, 141.9, 146.4, 154.2; ^{13}C NMR (acetone-d_6) δ: 22.7 (CH_3), 44.7 (CH), 56.4 (OCH_3), 60.6 (CH_2), 62.1 (CH_2), 110.5–154.2. X: MS m/z: 334 (M^+), 319; ^{1}H NMR (acetone-d_6-D_2O (9:1 v/v)) δ: 1.54 (3H, d, J = 7 Hz; CH_3), 3.81 (3H, s; OCH_3), 3.99 (1H, q, J = 7 Hz; CH), 4.64 (2H, s; CH_2), 4.70 (4H, s; 2 × CH_2), 6.77 (1H, d, J = 2 Hz; aromatic H), 6.89 (1H, d, J = 2 Hz; aromatic H), 7.08 (2H, m; aromatic H); ^{13}C NMR [acetone-d_6-D_2O (9:1)] δ: 22.6 (CH_3), 44.6 (CH), 56.3 (OCH_3), 60.1 (CH_2), 61.7 (CH_2), 110.4, 119.2, 126.1, 127.3, 128.0, 138.5, 138.6, 142.3, 147.5, 152.1. ^{13}C NMR (acetone-d_6) δ: 22.8 (CH_3), 44.8 (CH), 56.4 (OCH_3), 60.6 (CH_2), 62.4 (CH_2), 110.5–152.7.

A solution of about 1 mg of the products, obtained under various reaction conditions, in 1 ml pyridine and 50 µl trimethylsilylation reagent, *N,O*-bis(trimethylsilyl)trifluoroacetamide, was adjusted to 10 ml with pyridine and allowed to stand at room temperature for 1 h. The analysis was performed with 1 µl of the silylated sample.

The TMS derivatives were analyzed by gas chromatography-mass spectrometry (GC-MS). The mass spectra were recorded on a Jeol DX-705L double-focus spectrometer combined with a Hewlett-Packard 5890 gas chromatograph under the following conditions; column: a capillary column coated with DB-1 (15 m × 0.25 mm i.d., film thickness 0.25 mm); injection temperature 275°C; column temperature 50°–320°C (programmed temperature 50°C/min from 50° to 200°C, 5°C/min from 200° to 280°C 50°C/min from 280° to 320°C); carrier He.

Neutral sulfite solution

A solution of 4.2 g sodium bisulfite ($NaHSO_3$) in 50 ml water was adjusted to pH 8.0 with 2N NaOH. The total volume was adjusted to 88 ml with water.

Sulfonation of hydroxymethylated compounds VIII, IX, and X

Each 0.1-g sample of compounds VIII, IX, and X and 10 ml neutral sulfite solution in a stainless steel pressure tube ware heated at 110°C for 2 h. The tube was washed with water. After extraction with ethyl acetate the combined aqueous solution was adjusted to pH 2 with 0.1N H_2SO_4. The solution was concentrated to about 30 ml under reduced pressure and then adjusted to pH 8 with an aqueous solution saturated with $Ba(OH)_2$. After centrifugation the supernatant was concentrated to give a residue. The residue was extracted with methanol in a ultrasonic cleaner. Evaporation of the methanol gave almost pure corresponding sodium sulfonates of compounds XI, XIII, and XIV in 97.3%, 72.3%, and 63.3% yields, respectively. **XI**: ^{1}H NMR (D_2O) δ: 1.55 (3H, d, J = 7 Hz; CH_3), 3.80 (3H, s; OCH_3), 4.04 (1H, q, J = 7 Hz; CH), 4.18 (2H, s; CH_2), 6.81–7.22 (6H, m; aromatic H); ^{13}C NMR (D_2O) δ: 22.1 (CH_3), 44.0 (CH), 52.1 (CH_2), 57.0 (OCH_3), 112.2, 116.4, 119.5, 123.3, 129.6, 139.9, 140.1, 143.2, 148.7, 154.8. **XII**: ^{1}H NMR (D_2O) δ: 1.40 (3H, d, J = 7 Hz; CH_3), 3.63 (3H, s; OCH_3), 3.89 (1H, q, J = 7 Hz; CH), 4.02 (4H, s; 2 × CH_2), 6.67–7.14 (5H, m; aromatic H); ^{13}C NMR (D_2O) δ: 23.4 (CH_3), 45.3 (CH), 53.4 (CH_2), 53.5 (CH_2), 58.4 (OCH_3), 113.5, 118.6, 120.7, 120.8, 124.7, 130.7, 133.2, 141.1, 141.2, 144.6, 150.1, 155.1. **XIII**: ^{1}H NMR (D_2O) δ: 1.43 (3H, d, J = 7 Hz; CH_3), 3.64 (3H, s; OCH_3), 3.93 (1H, q, J = 7 Hz; CH), 4.06 (2H, s; CH_2), 4.09 (4H, s; 2 × CH_2), 6.70–7.10 (4H, m; aromatic H); ^{13}C NMR (D_2O): δ: 22.1 (CH_3), 43.9 (CH), 52.2 (CH_2), 53.1 (CH_2), 57.2 (OCH_3), 112.2, 119.6, 121.5, 123.4, 132.1, 139.1, 140.2, 149.2.

Methylation of compound XI

A 0.1-g sample of compound XI in 30 ml water was passed through a column of Amberlite 120 resin (H^+ form) and freeze-dried. The resulting residue in methanol was methylated with diazomethane in ether. After evaporation of the solvent, a methylation product was purified by silica gel column chromatography using *n*-hexane–acetone (7:3) as an eluent to isolate XIV. **XIV**: ($CDCl_3$) ^{1}H NMR (acetone-d_6) δ: 1.54 (3H, d, J = 7 Hz; CH_3), 3.72 (6H, s; OCH_3), 3.81 (3H, s; OCH_3), 3.87 (3H, s; OCH_3), 4.01 (1H, q, J = 7 Hz; CH), 4.53 (2H, s; CH_2), 6.75 (2H, d, J = 9 Hz; aromatic H), 6.85 (1H, d, J = 2 Hz; aromatic H), 6.88 (1H, d, J = 2 Hz; aromatic H), 7.08 (2H, d, J = 9 Hz; aromatic H); ^{13}C NMR (acetone-d_6) δ: 22.6 (CH_3), 44.2 (CH), 49.4 (CH_2), 56.4 (OCH_3), 57.8 (OCH_3), 112.1, 115.1, 115.8, 123.0, 129.0, 138.2, 138.9, 144.4, 148.2, 156.3.

Hydroxymethylation of compound VI with ^{13}C-labeled formaldehyde

Two mixtures of 50 mg compound VI in 3 ml 1N NaOH and 1 ml 20% ^{13}C-labeled and nonlabeled formaldehyde was heated at 60°C for 3 h under an atmosphere of nitrogen. After acidification with 1N HCl to pH 4, each solution was extracted with ethyl acetate. The ethyl acetate solution was

washed with water, dried over sodium sulfate, and then concentrated to give products.

Synthesis of phenolized guaiacylglycerol-β-aryl ether (compound XVI)

To a homogeneous mixture of 582 mg guaiacylglycerol-β-aryl ether (compound XV)[4] and 1.890 g phenol was added 10 ml 72% sulfuric acid. The contents were vigorously stirred at room temperature for 30 min. After dilution with 300 ml distilled water, it was heated at 50°C for 30 min, neutralized with 4N NaOH, and then extracted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4, and concentrated to dryness. The products were purified by silica gel column chromtography using ethyl acetate-chloroform (2:8 v/v) to separate 554 mg (77.5%) of compound XVI.[11]

Hydroxymethylation of compound XVI with formaldehyde

A solution of 50 mg compound XVI in 2.5 ml 1N NaOH and 0.15 ml formalin was heated at 50°C under an atmosphere of nitrogen. After 2 h, 0.15 ml formalin was added again to the solution. The reaction solution was neutralized with 1N HCl and extracted with ethyl acetate. The ethyl acetate solution was dried over Na_2SO_4 and concentrated to give products. The products were separated by silica gel column chromatography using *n*-hexane-acetone (3:2 v/v) to isolate monohydroxymethyl product XVII. **XVII**: MS m/z: 456 (M^+), 259; ^{1}H NMR ($CDCl_3$) δ: 2.27 (3H, s; CH_3), 3.42–3.88 (2H, m; H_α, H_β), 3.78 (3H, s; OCH_3), 3.79 (3H, s; OCH_3), 4.35 (1H, m; H_γ), 4.55 (1H, m; H_γ), 4.66 (2H, s; CH_2), 6.52–7.23 (9H, m; aromatic H); ^{13}C NMR δ: 21.2 (CH_3), 51.5 (C_α), 55.8 (OCH_3), 56.1 (OCH_3), 61.4 (CH_2), 61.8 (C_γ), 86.6 (C_β), 111.0–154.4.

Hydroxymethylation of compound XVI with 0.6 ml formalin at 60°C and subsequent separation of the products gave di- and trihydroxymethyl products XVIII and XIX, respectively. **XVIII**: MS m/z: 486 (M^+), 289; ^{1}H NMR (acetone-d_6) δ: 2.23 (3H, s; CH_3), 3.41–3.87 (2H, m; H_α, H_β), 3.75 (3H, s; OCH_3), 3.78 (3H, s; OCH_3), 4.35 (1H, m; H_γ), 4.63 (1H, m; H_γ), 4.66 (2H, s; CH_2), 4.70 (2H, s; CH_2), 6.62–7.98 (8H, m; aromatic H); ^{13}C NMR (acetone-d_6) δ: 21.0 (CH_3), 52.6 (C_α), 56.4 (OCH_3), 60.6 (CH_2), 61.9 (CH_2), 62.4 (C_γ), 85.1 (C_β), 111.6–154.6. **XIX**: MS m/z: 516 (M^+), 319; ^{1}H NMR (acetone-d_6) δ: 2.23 (3H, s; CH_3), 3.38–3.87 (2H, m; H_α, H_β), 3.73 (3H, s; OCH_3), 3.75 (3H, s; OCH_3), 4.35 (1H, d, J = 8 Hz; H_γ), 4.62 (1H, m; H_γ), 4.66 (2H, s; CH_2), 4.73 (4H, s; 2 × CH_2), 6.61–7.58 (7H, m; aromatic H); ^{13}C NMR (acetone-d_6) δ: 21.0 (CH_3), 52.6 (C_α), 56.4 (OCH_3), 60.5 (CH_2), 61.8 (C_γ), 62.5 (CH_2), 85.1 (C_β), 111.5–153.1.

The reaction products were submitted to analysis by high-performance liquid chromatography (HPLC) on a Shimadzu LC-10AT model equipped with a Unisil Pack 5C-18 column (6 × 300 mm) and a variable-wavelength ultraviolet (UV) detector. The conditions were as follows: elution solvent methanol-water (1:1 v/v); elution rate 1.0 ml/min; UV detector at 280 nm.

A solution of 30 mg compound XVI in 0.27 ml ^{13}C-labeled formalin and 0.15 ml 1N NaOH was heated for 2 h at various temperatures. The solution was neutralized with 1N HCl and extracted with ethyl acetate. The ethyl acetate solution was dried over Na_2SO_4 and concentrated to give reaction products.

Sulfonation of compounds XVII, XVIII, and XIX

Solutions of 30 mg of compounds XVII, XVIII, and XIX, respectively, in 1 ml dioxane and 9 ml neutral sulfite solution in a stainless steel pressure tube were heated at 130°C for 2 h. After extraction with ethyl acetate, the solutions were acidified with 0.1N H_2SO_4 to pH 2, concentrated under reduced pressure to about 30 ml, and then neutralized with aqueous solution saturated $Ba(OH)_2$ solution to pH 8. Precipitates were removed by centrifugation, and the supernatants were concentrated to dryness. Each methanol-soluble part of the residue was purified by Sephadex G-15 column chromatography using H_2O as an eluent to give sodium sulfonates XX, XXI, and XXII in 74.3%, 85.1%, and 66.3% yields, respectively. **XX**: ^{1}H NMR (D_2O) δ: 2.25 (3H, s; CH_3), 3.63 (3H, s; OCH_3), 3.58–3.82 (2H, m; H_α, H_β), 3.82 (3H, s; OCH_3), 4.02–4.14 (1H, m; H_γ), 4.19–4.26 (3H, m; CH_2, H_γ), 6.72–7.35 (9H, m; aromatic H); ^{13}C NMR (D_2O) δ: 22.2 (CH_3), 53.5 (C_α), 54.0 (CH_2S), 57.8 (OCH_3), 58.3 (OCH_3), 63.8 (C_γ), 85.5 (C_β), 113.6–156.2. **XXI**: ^{1}H NMR (D_2O) δ: 2.25 (3H, s; CH_3), 3.62 (3H, s; OCH_3), 3.55–3.71 (2H, m; H_α, H_β), 3.81 (3H, s; OCH_3), 3.92–4.15 (1H, m; H_γ), 4.15–4.30 (3H, m; CH_2, H_γ), 4.20 (2H, s; CH_2), 6.71–7.41 (8H, m; aromatic H); ^{13}C NMR (D_2O) δ: 20.9 (CH_3), 52.1 (C_α), 52.8 (CH_2S), 53.1 (CH_2S), 56.4 (OCH_3), 57.1 (OCH_3), 62.7 (C_γ), 84.5 (C_β), 112.5–154.2. **XXII**: ^{1}H NMR (D_2O) δ: 2.26 (3H, s; CH_3), 3.62 (3H, s; OCH_3), 3.56–3.72 (2H, m; H_α, H_β), 3.80 (3H, s; OCH_3), 3.93–4.19 (1H, m; H_γ), 4.21–4.33 (3H, m; CH_2, H_γ), 4.25 (4H, s; 2 × CH_2), 6.73–7.38 (7H, m; aromatic H); ^{13}C NMR (D_2O) δ: 20.9 (3H, s), 52.2 (C_α), 52.8 (CH_2S), 53.1 (CH_2S), 56.6 (OCH_3), 57.2 (OCH_3), 63.0 (C_γ), 84.6 (C_β), 112.5–153.0.

A solution of the ^{13}C-labeled hydroxylation products in 1 ml of dioxane and 9 ml of the neutral sulfite solution was heated at 130° and 160°C for 2 h.

Quantitative determination of formaldehyde

Formaldehyde was determined with iodine.[12]

Spectrometry

The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra of compounds were recorded with TMS as an internal standard on a Jeol JNM-EX 270 FT NMR spectrometer. NMR spectra of sodium sulfonates were recorded in D_2O with TSP (sodium 3-trimethylsilylpropionate-2,2,3,3-d_4) as an internal standard on the same spectrometer.

Fig. 2. Reaction of creosol with formaldehyde

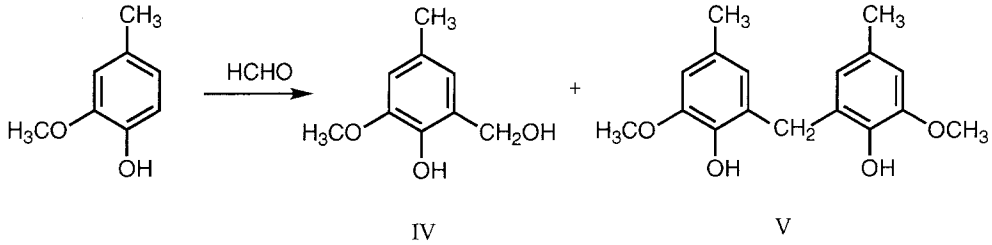
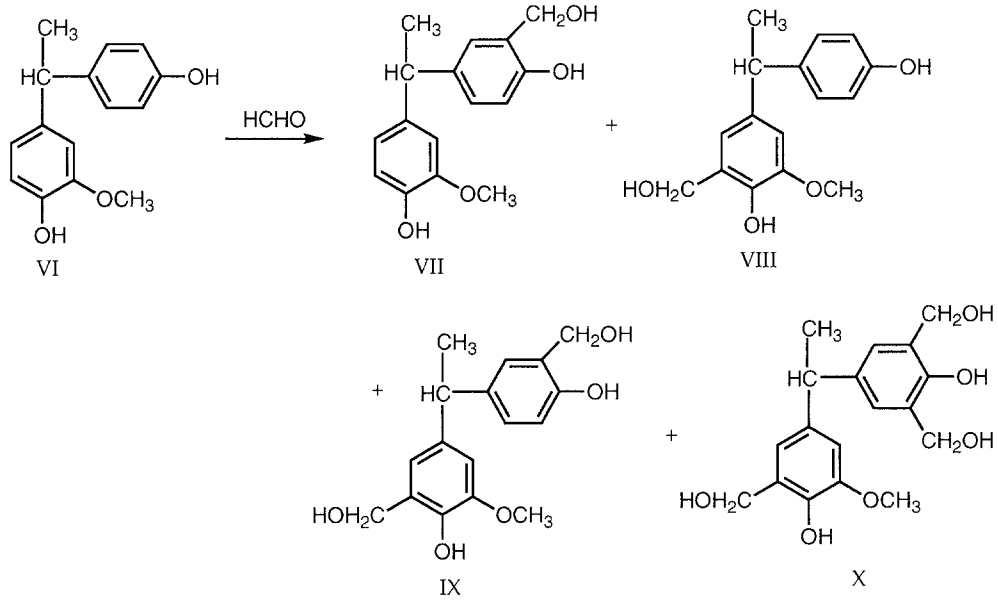


Fig. 3. Hydroxymethylation of condensed-type compound VI with formaldehyde



The MS spectra were recorded on a Jeol JMD D-100 mass spectrometer.

Results and discussion

Formaldehyde is well known to react with phenolic compounds at the *ortho* or *para* positions (or both) to their aromatic hydroxyl groups in alkaline media. The introduced hydroxymethyl group is not necessarily stable under the reaction conditions employed and in part undergoes subsequent condensation with an aromatic nucleus via the corresponding *ortho*-quinonemethide as an intermediate, resulting in formation of a product with diarylmethane structure, as shown in Fig. 2. To convert the hydroxymethyl group into a sulfomethyl group by sulfite treatment, it is necessary to prevent it from transforming to a secondary diarylmethane structure.

Creosol

Table 1 illustrates the results of hydroxymethylation of creosol as a simple guaiacyl nucleus model under various reaction conditions. Undesirable condensation products increased at reaction temperatures over 70°C. As a matter of course, the addition of formalin, a lower reaction temperature, and a shorter reaction time are desirable for yielding the hydroxymethylation product. Therefore the following hydroxymethylation was carried out at 60°C.

1-Guaiacyl-1-*p*-hydroxyphenylethane (compound VI)

Softwood acid lignin possesses an alkyl group at the *ortho* position (C6) of the guaiacyl nucleus to the side chain to a considerable extent. This group, however, can be preferentially substituted for a hydrogen atom in phenolation[10] in the presence of sulfuric acid as a catalyst, and a *p*-hydroxyphenyl moiety is introduced at the α position of the side chain. To elucidate the chemical reactivities of *p*-hydroxyphenyl and guaiacyl nuclei in phenolized sulfuric acid lignin toward hydroxymethylation, 1-guaiacyl-1-*p*-hydroxyphenylethane (compound VI), selected as a simple phenolized sulfuric acid lignin model, was reacted with formaldehyde.

Four products were isolated (Fig. 3). No intense characteristic absorptions near 115 and 129ppm due to

Table 2. Reaction of compound VI with formalin

Treatment	Reaction conditions					Yield (%)				
	VI (mg)	Formalin (ml)	NaOH 1N (ml)	Time (h)	Temp. (°C)	VII	VIII	IX	X	Condensed compound
1	50	0.1 + 0.1[a]	2.5	4	50	0.5	1.4	–	–	–
2	100	0.3 + 0.3[a]	5.0	4	50	4.7	20.3	1.1	–	–
3	50	0.3	2.5	2	60	3.9	15.2	9.7	–	–
4	50	0.3	2.5	4	60	3.9	16.6	3.5	0.3	29.0[c]
5	51	0.6 + 0.6[b]	5.0	2	60	2.5	23.8	40.1	27.9	–
6	50	1.2	5.0	1	60	3.2	22.8	39.8	28.1	–
7	50	0.3 + 0.3[a]	2.5	4	60	–	7.2	5.5	48.6	29.4[c]

[a] Formalin was added at start and after 2 h.
[b] Formalin was added at start and after 1 h.
[c] Absolute weight (mg).

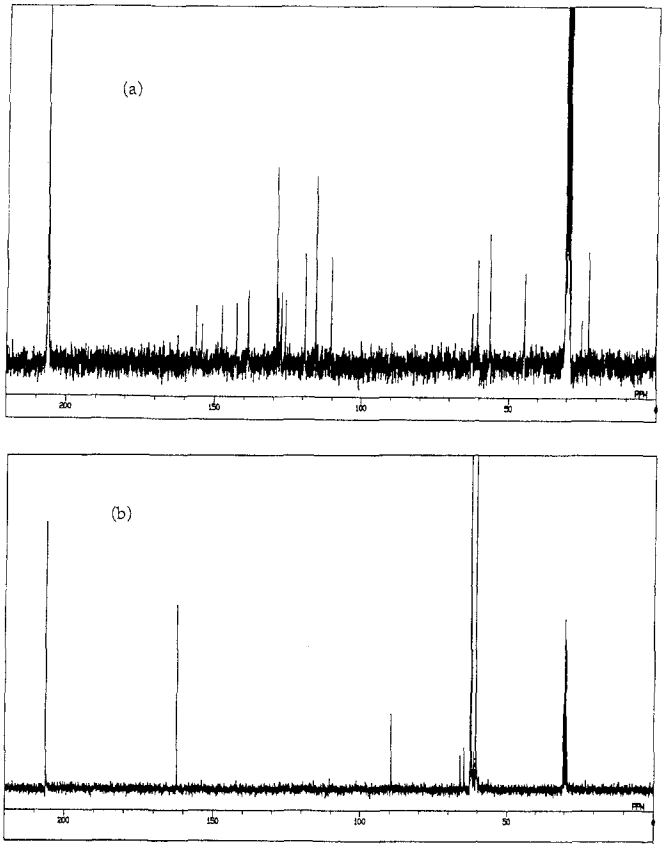
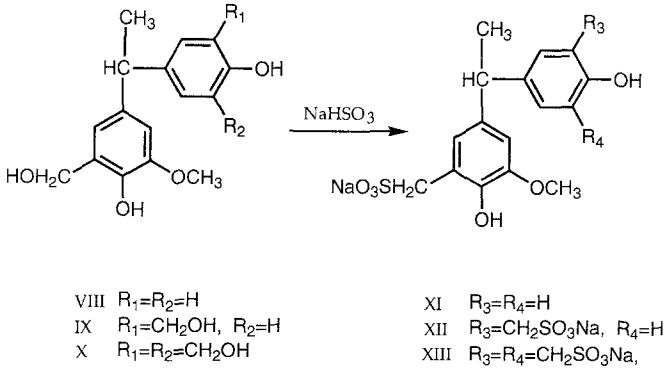


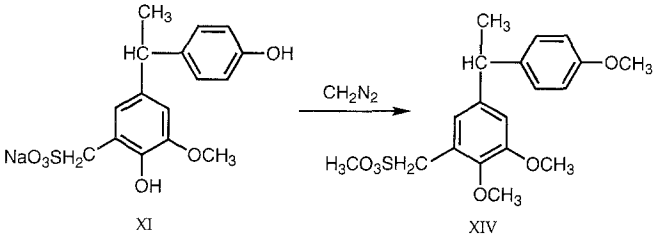
Fig. 4. ^{13}C NMR spectra of total reaction products obtained by treatment of compound VI with nonlabeled (**a**) and ^{13}C-labeled (**b**) formaldehyde



VIII $R_1=R_2=H$
IX $R_1=CH_2OH$, $R_2=H$
X $R_1=R_2=CH_2OH$

XI $R_3=R_4=H$
XII $R_3=CH_2SO_3Na$, $R_4=H$
XIII $R_3=R_4=CH_2SO_3Na$,

Fig. 5. Sulfonation of hydroxymethylation products VIII, IX, and X in neutral sulfite solution



XI XIV

Fig. 6. Methylation of compound XI with diazomethane

p-hydroxyphenyl nucleus, a hydroxymethyl carbon signal at 64.8 ppm, and 12 aromatic carbon signals in the ^{13}C NMR spectrum of compound VII indicate the introduction of one hydroxymethyl group into the *p*-hydroxyphenyl nucleus. Two intense signals at 115 and 129 ppm, an absorption at 62.0 ppm, and 10 aromatic carbon signals suggest that compound VIII has the structure shown in Fig. 3. Two methylene group protons at δ 4.68 and 4.74 in the ^1H NMR spectrum and twelve aromatic carbon signals in the ^{13}C NMR spectrum suggest that compound IX possesses one hydroxymethyl group each in the *p*-hydroxyphenyl and guaiacyl nuclei. The last compound (X) in the separation procedure eluted by means of silica gel column chromatography shows an intense absorption at 126.1 ppm in the ^{13}C NMR spectrum and three methylene group protons at δ 4.64 (2H) and 4.70 (4H) in the ^1H NMR spectrum.

Fig. 7. Synthesis of phenolized β-O-4 lignin compound XVI

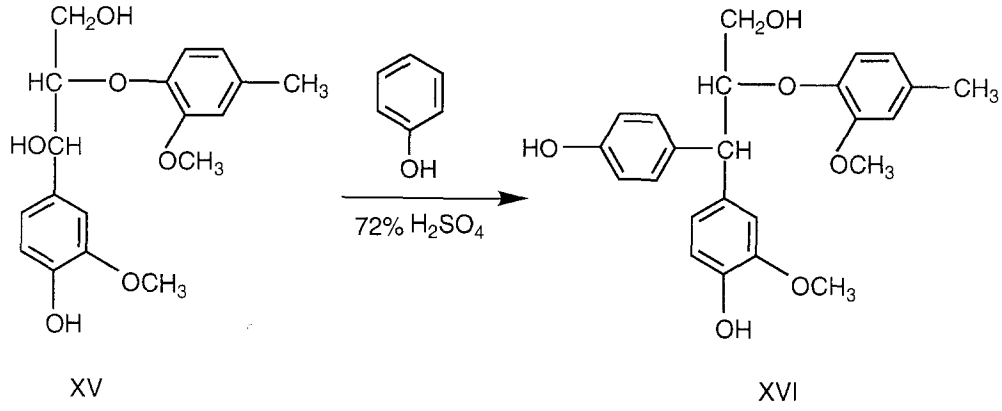
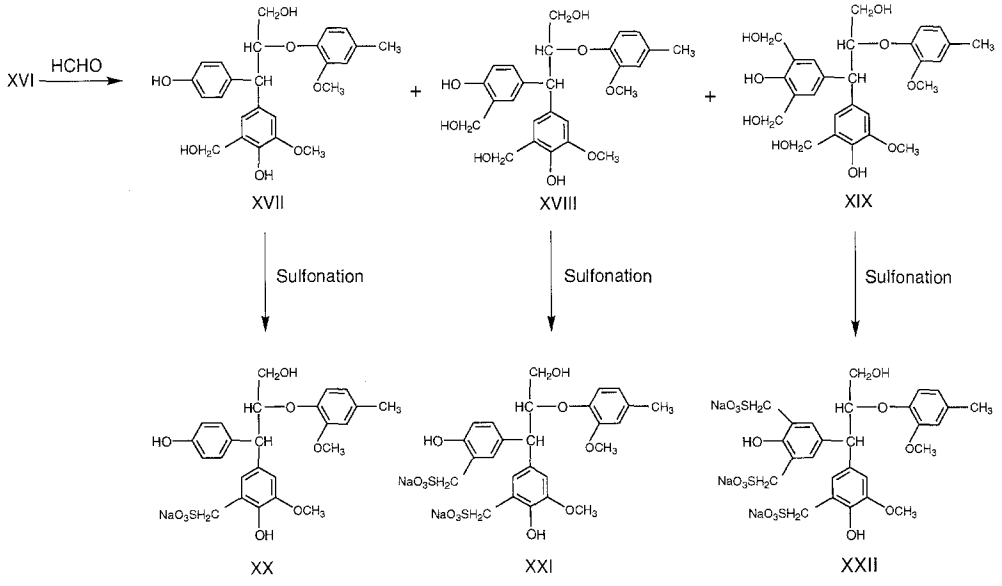


Fig. 8. Hydroxymethylation and sulfonation of phenolized β-O-4 lignin compound XVI



The yields of the reaction products, which were determined by HPLC, are summarized in Table 2. A hydroxymethyl group was observed to be introduced first into the guaiacyl nucleus (treatments 2 and 3) and then into the *p*-hydroxyphenyl nucleus. Contrary to our expectations, the yield of compound VII with a hydroxymethyl group only in the *p*-hydroxyphenyl nucleus was low. A prolonged reaction time increased the amount of fully hydroxymethylated product X. However, no product with a diarylmethane structure was formed in the reaction of creosol with formaldehyde.

To obtain additional information about formation of the diarylmethane structure, compound VI was treated with ^{13}C-labeled formaldehyde (H^{13}CHO) (20% formalin) at 60°C. In the ^{13}C NMR spectrum (Fig. 4b) of the total reaction products, two intense peaks due to the hydroxymethyl carbons were observed at 60.6 and 62.3 ppm, but there was no peak due to diarylmethane carbon at 25–40 ppm. This finding means that the secondary diarylmethane structure was not formed at all, probably because the reactivity of the aromatic nucleus was lower in compound VI than in creosol.

Neutral sulfite treatment of compounds VIII, IX, and X at 110°C gave the corresponding sulfonates XI, XII, and XIII in high yields, as shown in Fig. 5. Methanol extraction of the evaporated residue after removal of hydrophobic material with an organic solvent resulted in isolation of the sulfonates without chromatographic fractionation. In the ^{13}C NMR spectra of the compounds before and after sulfonation recorded in deuteroacetone and D_2O, respectively, the substitution of an alcoholic hydroxyl group for a sulfonate group caused the up-field shift of the methylene carbon by 7–9 ppm.

One method for isolating a sulfonate is chromatographic purification after methylation.[13–15] An experimental attempt suggested low-yield isolation of compound XIV, as shown in Fig. 6. This finding may be interpreted as a loss during the isolation procedure by silica gel column chromatography and as its instability.[12]

Guaiacylglycerol-β-aryl ether (compound XVI)

As shown in Fig. 7, phenolized lignin model compound XVI was synthesized from guaiacylglycerol-β-aryl ether (com-

pound XV) by phenolation in the presence of 72% sulfuric acid. The intense fragment signal at m/z 259 resulted from fisson of a C_α–C_β linkage in the MS spectrum, and the two intense characteristic absorptions at 115.3 and 129.5 ppm due to the *p*-hydroxy-phenyl nucleus indicate that compound XVII has a structure with one hydroxymethyl group on the *p*-hydroxyphenyl nucleus, as shown in Fig. 8. The second product showed an intense signal at m/z 289, corresponding to m/z 259 of compound XVII in the MS spectrum; there were no characteristic signals near 115 and 129 ppm and two hydroxymethyl carbon absorptions at 60.6 and 61.9 ppm in the NMR spectrum, indicating that the product has the structure of compound XVIII. The third product showed an intense signal at m/z 319 in the MS spectrum and two singlets at 4.66 (2H) and 4.73 (4H) due to hydroxymethyl protons in the NMR spectrum, suggesting that the product has the fully hydroxymethylated structure of compound XIX under the mild alkaline conditions used here.

Treatment of compound XVI with formaldehyde yielded three hydroxymethylated products: XVII, XVIII, and XIX (Fig. 8). Although thin-layer chromatography (TLC) of the reaction mixture suggested the possibility of the presence of other products (i.e., compounds with two hydroxymethyl groups on the *p*-hydroxyphenyl nucleus and one hydroxymethyl group on the guaiacyl nucleus), their yields seem low. Furthermore, a secondary product, such as a diarylmethane, which is formed by condensation of the introduced hydroxymethyl group and aromatic nucleus, was not isolated.

Results obtained in the reaction of compound XVI with formaldehyde under various reaction conditions are

Table 3. Reaction of compound XVI (50 mg) with formalin

Treatment	Reaction conditions				Yield (%)			
	Formalin 37% (ml)	NaOH 1N (ml)	Time (h)	Temp. (°C)	XVI	XVII	XVIII	XIX
1	0.23 + 0.23[a]	2.5	4	50	26.7	38.2	26.3	4.6
2	0.23 + 0.23	2.5	4	60	6.1	32.9	35.9	20.6
3	0.23 + 0.23	2.5	4	70	0	3.7	12.2	50.6
4	0.15 + 0.15	2.5	4	60	59.4	20.3	4.0	0.5
5	0.3 + 0.3	2.5	4	60	0	3.9	14.2	58.3
6	0.45	2.5	2	60	32.3	29.2	12.2	2.1
7	0.23 + 0.23	2.5	4	80	0	0.9	4.6	61.8
8	0.45	2.5	2	100	0	0	3.1	19.7

[a] Additional formalin was added after 2 h.

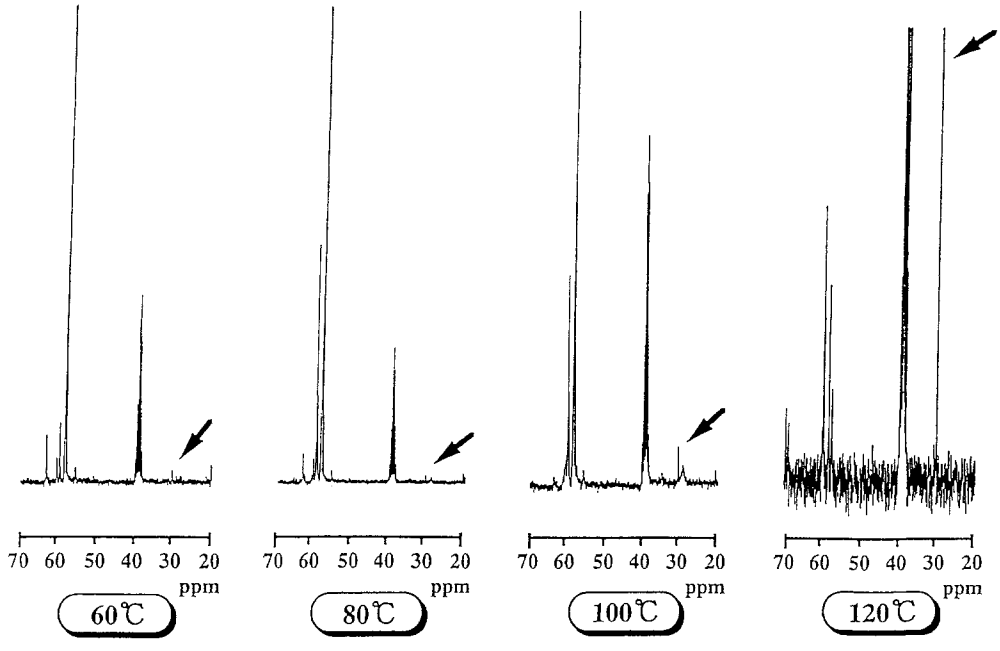


Fig. 9. ^{13}C NMR spectra of total reaction products obtained by hydroxymethylation of compound XVI with ^{13}C-labeled formaldehyde at various temperatures

Table 4. Residual formalin (0.45 ml) in 2.5 ml 1N NaOH solution, over time

Temp. (°C)	Rate of residual formalin (%), at 0.5–4.0 h			
	0.5	1.0	2.0	4.0
50	64.1 (12.6)	45.0 (12.5)	38.2 (12.3)	34.1 (12.0)
60	39.5 (12.5)	28.6 (12.3)	25.5 (12.0)	25.5 (11.5)
70	34.1	31.4	25.5	26.5

Numbers in parentheses are the pH.

summarized in Table 3. A hydroxymethyl group is at first introduced on a guaiacyl nucleus with a free phenolic hydroxy group, as in the case of compound VI. Moreover, the guaiacyl nucleus is more reactive than the *p*-hydroxyphenyl nucleus. As expected, the reaction under stronger conditions gave almost only the three-substituted product XIX (Fig. 8). Hydroxymethylation at 100°C gave compound XIX in low yield, suggesting further conversion to a diarylmethane structure. Lower yields of the products in one-step addition of formalin suggests instability of formaldehyde in alkaline solution, as shown in Table 4, to undergo the Cannizzaro reaction and polymerization.[16] In this experiment, formalin was added every 2 h, but adding it at shorter intervals may result in higher yields.

Figure 9 shows the ^{13}C NMR spectra of the hydroxymethylated reaction products with ^{13}C-labeled formaldehyde ($H^{13}CHO$). Strong absorptions at 60–63 ppm are due to the introduced hydroxymethyl carbons. Absorption at 30 ppm seems to be due to a diarylmethane carbon. In accordance with the reduced yield of compound XIX at 100°C in Table 3, the peak became more intense with increasing reaction temperature, particularly over 100°C.

As expected, neutral sulfonation of products XVII, XVIII, and XIX gave the corresponding sulfonates XX, XXI, and XXII (Fig. 8) in high yields, respectively. To confirm or exclude formation of the diarylmethane structure during sulfonation, neutral sulfonation was carried out at 100° and 160°C. The ^{13}C NMR spectrum (not shown) of the reaction products did not result in any increase in absorption due to the diarylmethane carbon.

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