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Formation and chemical structures of acid-soluble lignin II: reaction of aromatic nuclei model compounds with xylan in the presence of a counterpart for condensation, and behavior of lignin model compounds with guaiacyl and syringyl nuclei in 72% sulfuric acid

Abstract To elucidate the formation and chemical structures of water-soluble material in acid-soluble lignin (ASL), lignin aromatic nuclei model compounds of creosol (I) and 5-methoxycreosol (II) were reacted with xylose or xylan in the presence of apocynol as a counterpart for condensation in 72% sulfuric acid (SA). The reaction of I gave mainly condensation product. However, the condensation reaction of II with apocynol was suppressed because of steric hindrance from the methoxyl group, and II yielded a C-xyloside after refluxing in 3% SA together with condensation products. To obtain information on $CHCl_3$-soluble material in ASL, model compounds of arylglycerol-β-aryl ethers with guaiacyl (VIII) and syringyl (X) nuclei were treated by the Klason procedure. VIII gave only insoluble polymerized product, while X gave insoluble polymerized product and $CHCl_3$-soluble low molecular weight products, which were dissolved in 3% SA. These results prove earlier views that water-soluble material in ASL consists of condensation products formed from syringyl lignin and monosaccharide units in hemicellulose. In addition, the $CHCl_3$-soluble material in ASL appears to be composed of low molecular weight degradation products from SA treatment of Klason lignin with the syringyl nucleus.

Key words Acid-soluble lignin · Klason lignin · Condensation · Lignin carbohydrate complex · Syringyl lignin · Arylglycerol-β-aryl ether

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Introduction

In the quantitative determination of lignin in woody materials, particularly hardwoods, by the Klason method, it is well known that acid soluble lignin (ASL) is formed with a yield of several percent. The formation mechanism and structures of ASL, however, are largely unknown. In addition to the structural complexities of lignin itself, this is due to the great variety of structural changes that lignin undergoes during sulfuric acid (SA) treatment, and to the difficulties encountered in isolating it. The first and sole direct analysis of ASL isolated from beech ASL with an ion exchange resin[1] suggested that it was a resinous material, insoluble in the dilute acid after isolation. It was found to have less carbon content than Klason lignin, indicating a much more highly oxygenated substance. Subsequent research by Swan[2] suggested that ASL is composed of two types of material with different polarities. One is composed of low molecular weight lignin degradation products that are soluble in $CHCl_3$, and the other is a mixture of water-soluble substances with one third the absorption coefficient of pure lignin in their UV spectra. Hardwoods with a higher methoxyl group content have been found to give higher ASL yields,[3] and syringyl lignin model compounds have higher reactivity than the corresponding guaiacyl models[4,5] in dehydrative condensations.

In a previous experiment[6] examining the 72% SA treatment time and ASL content of beech, it was found that wood components yielding ASL are dissolved in 72% SA at an early stage (within 10 min). Furthermore, the soluble lignin produced by 72% SA treatment and subsequent standing in 3% SA yielded insoluble Klason lignin after boiling in 3% SA. These findings suggest that the soluble lignin in 3% SA after standing at room temperature is bonded to hemicellulose with long chains, because of the formation of insoluble Klason lignin after boiling the soluble lignin in 3% SA. In general, a typical water-soluble lignin component in wood is lignin-carbohydrate complex (LCC), in which lignin and hemicellulose are linked by benzylic ether, benzylic ester, and glycosidic bonds. How-

ever, these linkages are most likely unstable under 72% SA treatment conditions. One of the possible formations of the chemical bond between lignin and hemicellulose seems to be a condensation reaction in 72% SA, yielding stable LCC after boiling in 3% SA. On the other hand, the $CHCl_3$ soluble lignin component is believed to be formed by a SA-catalyzed depolymerization of the polymerized lignin (Klason lignin). This article deals with the reactivity of simple lignin model compounds with guaiacyl and syringyl nuclei in the presence of xylose or xylan, the behavior of arylglycerol-β-aryl ethers with guaiacyl and syringyl nuclei in 72% SA, and the characterization of the material dissolved in 3% SA.

Materials and methods

Synthesis of model compounds

The crude products, prepared by reacting 200 mg creosol (I) or 5-methoxycreosol (II) with 100 mg apocynol (III) in 72% SA for 15 min, were purified by column chromatography on silica gel with a mixed solvent of acetone/n-hexane (1:9 v/v) as eluent to isolate 145 mg (84.6%) of model compound IV and 106 mg (63.9%) of model compound V. IV: ^{1}H-NMR (CDCl$_3$) δ: 1.52 (3H, d, $J = 7$ Hz, CH$_3$), 2.17 (3H, s, CH$_3$), 3.80 (3H, s, OCH$_3$), 3.84 (3H, s, OCH$_3$), 4.14 (1H, q, $J = 7$ Hz, CH), 5.49 (1H, s, OH), 5.52 (1H, s, OH), 6.63–6.85 (5H, m, aromatic H). ^{13}C-NMR (CDCl$_3$) δ: 19.2 (CH$_3$), 22.4 (CH$_3$), 40.3 (CH), 55.9 (OCH$_3$), 56.0 (OCH$_3$), 110.3, 113.2, 113.3, 114.2, 120.4, 127.4, 137.3, 138.6, 143.6, 143.8, 144.5, 146.5. Anal. Calcd. for C$_{17}$H$_{20}$O$_4$: C, 67.09; H, 6.62. Found: C, 67.01; H, 6.44. V: ^{1}H-NMR (CDCl$_3$) δ: 1.63 (3H, d, $J = 7$ Hz, CH$_3$), 2.14 (3H, s, CH$_3$), 3.49 (3H, s, OCH$_3$), 3.77 (3H, s, OCH$_3$), 3.81 (3H, s, OCH$_3$), 4.52 (1H, q, $J = 7$ Hz, CH), 5.59 (1H, s, OH), 5.72 (1H, s, OH), 6.44 (1H, s, aromatic H), 6.69–6.83 (3H, m, aromatic H). ^{13}C-NMR (CDCl$_3$) δ: 18.2 (CH$_3$), 20.4 (CH$_3$), 35.7 (CH), 56.0 (OCH$_3$), 56.1 (OCH$_3$), 60.2 (OCH$_3$), 109.2, 110.3, 110.4, 114.0, 119.9, 127.0, 131.4, 137.1, 138.2, 143.4, 145.7, 146.1, 146.5. Anal. Calcd. for C$_{18}$H$_{22}$O$_5$: C, 67.91; H, 6.97. Found: C, 67.77; H, 6.80.

Model compounds (VI and VII)[5] and arylglycerol-β-aryl ethers with guaiacyl (VIII)[7] and syringyl (IX)[8] were synthesized according to the previously published methods.

Reaction of model compounds (I, II, III, and xylose or xylan) in 72% SA

A mixture of 0.25 mmol I, 0.25 mmol II, 0.5 mmol III, and 0.75 mmol xylose or 150 mg xylan (Aldrich) in 0.5 ml 72% SA was stirred with a glass rod at room temperature for 20 min. The suspension was diluted with 19 ml water and heated at 75°C for 1 h. After cooling, the suspension was extracted with benzene. The benzene layer was washed with water, dried over sodium sulfate, and then evaporated to give crude condensation products (IV and V). The aqueous solution was neutralized (pH 6.0) with 2N NaOH and then

evaporated under reduced pressure to yield crude C-xylosides (VI and/or VII). VI: ^{1}H-NMR (acetone-d_6-D$_2$O 9:1 v/v) δ: 2.25 (3H, s), 2.33 (3H, s), 3.54 (1H, m), 3.60 (1H, m), 3.77 (6H, s), 3.80 (1H, m), 4.06 (1H, m), 4.41 (1H, m), 4.53 (1H, d, $J = 10$ Hz), 6.66 (1H, s), 6.68 (1H, s), 6.82 (1H, s), 7.07 (1H, s). ^{13}C-NMR (acetone-d_6-D$_2$O 9:1 v/v) δ: 19.5, 19.7, 44.3, 56.2, 56.3, 63.7, 71.1, 74.9, 75.7, 114.6, 114.9, 116.3, 128.5, 129.0, 133.6, 134.2, 144.6, 146.2, 146.3. VII: ^{1}H-NMR (acetone-d_6-D$_2$O 9:1 v/v) δ: 2.34 (3H, s), 3.33–3.99 (6H, m), 3.80 (3H, s), 3.81 (3H, s), 6.55 (1H, s); ^{13}C-NMR (acetone-d_b-D$_2$O 9:1 v/v) δ: 20.3, 56.2, 61.1, 70.8, 71.1, 72.5, 77.7, 79.8, 110.7, 123.6, 128.9, 138.1, 148.2, 148.5.

Quantitative determination of products (IV–VII)

Each sample of the crude C-xylosides (0.1 mg) in 40 μl pyridine was treated with 20 μl N,O-bis(trimethylsilyl)trifluoro acetoamide (BSTFA) at 50°C for 1 h. The trimethylsilylated derivatives were analyzed by gas liquid chromatography (GLC) using a GL Sciences GC-390B under the following conditions: SE-30 in a 1-m stainless steel column; column temperature 150°–280°C, programmed temperature 3°C/min; carrier gas N$_2$; detector FID.

Compounds (VI, VII) in the water-soluble products were analyzed by high-performance liquid chromatography (HPLC) as follows: about 5 mg of each sample was dissolved in 0.3 ml methanol. Three microliters of the solution was submitted for HPLC analysis on a Shimadzu LC-6A model equipped with an UV (280 nm) detector and an Unisel pack 5C18-300B (i.d. 6 mm × 30 cm, JR Science) with a linear gradient from 0% to 70% solvent B in 40 min at 35°C. Solvent A H$_2$O/MeOH/H$_3$PO$_4$, 940:50:1; solvent B, MeOH/H$_3$PO$_4$, 990:1. Flow rate was 1.5 ml/min.

Treatment of model compounds (VIII and X) in 72% SA

Each sample of compounds VIII and X (35 mg) and 1.0 ml 72% SA were stirred with a glass rod at room temperature for 20 and 60 min, respectively. After dilution with 38 ml water, the suspension (SA concentration 3%) was heated at 75°C for 2 h and then filtered with a filter paper. The insoluble residue was washed with water. The residue was dissolved in ethyl acetate. The ethyl acetate solution was dried over sodium sulfate and evaporated to give insoluble product. A mixture of the aqueous solution and filtration washings was extracted with CHCl$_3$. The CHCl$_3$ solution was washed with water, dried over sodium sulfate and evaporated to give CHCl$_3$-soluble products.

The CHCl$_3$-soluble products from 200 mg X were separated by column chromatography on silica gel with mixed solvents of n-hexane and an increasing proportion of acetone to isolate 2.6 mg 5-methoxycreosol and 4.6 mg compound XII.[4] XII: m.p. 223.5°–224.8°C. ^{13}C-NMR (dioxane-d_6) δ: 18.0 (CH$_3$), 49.7 (α-CH), 56.3 (OCH$_3$), 63.2 (CH$_2$OH), 89.0 (β-CH), 106.4–148.8 (10 aromatic carbons).

Gel permeation chromatography

The molecular weight distribution of reaction products was measured by gel permeation chromatography (GPC) using a Shimadzu LC-6A HPLC equipped with an UV detector (280 nm) and a Tosor pack TSK-GEL α-2500 (i.d. 7.8 mm × 300 mm). The products were eluted with dioxane/water 3:1 (v/v) containing 40 mmol lithium chloride at a flow rate of 0.5 ml/min at 50°C.

Spectrometry

The ^{1}H- and ^{13}C-NMR spectra were recorded with tetramethylsilane (TMS) as an internal standard on a Varian Gemini-2000 (300 MHz) spectrometer.

Results and discussion

Reaction of lignin nucleus models (I and II) and apocynol (III) in the presence of xylose or xylan in 72% SA

The main reaction between lignin aromatic nuclei and hemicellulose in 72% SA is the condensation reaction to form C-glycosidic structures.[5] In the present experiment, creosol (I) and 5-methoxycreosol (II) were selected as lignin nucleus models for guaiacyl and syringyl nuclei, respectively. Another important reaction of the aromatic nuclei in 72% SA is intermolecular condensation[6] with benzylic cations, which were formed by elimination of a secondary hydroxyl group and/or oxygen-containing functional groups such as ether or ester linkages. Apocynol (III) was selected as a counterpart to the condensation reaction. From knowledge of its structure, it is estimated that native lignin is composed of nearly equivalent amounts of aromatic nuclei and benzylic carbons. Therefore the total molar concentration of I and II was adjusted to equal that of III. Furthermore, the ratio of lignin and hemicellulose in hardwoods is about 2:3, so a 1.5-fold molar quantity of a hemicellulose model was used. Selection of xylose as the hemicellulose model is based primarily on the higher reactivity and greater abundance of xylan in hardwood.

Results are summarized in Table 1. Because the model compounds were not dissolved in 72% SA, dioxane was

Table 1. Reaction of mixtures of creosol (I), 5-methoxycreosol (II), apocynol (III) and xylose or xylan in 72% sulfuric acid

| Reaction	Carbohydrate	Product (%)[a]			
		IV	V	VI	VII
1[b]	Xylose	71.4	41.6	0	0
2[c]	Xylose	75.8	37.1	0.6	9.3
3[d]	Xylan	–[e]	–	0.3	3.8

SA, sulfuric acid
[a] % based on I or II
[b] A mixture was suspended in 0.25 ml of dioxane and stirred in 72% SA
[c] 0.5 ml of 72% SA solution was used
[d] 0.75 ml of 72% SA solution was used
[e] Not determined

used for their dissolution. Treatment (reaction 1) of compounds (I, II, and III) in dioxane in the presence of xylose gave only condensation products and no C-xyloside despite vigorous stirring with a magnetic stirrer. This is presumably caused by insufficient contact of two kinds of model compounds with different polarities in the heterogeneous solution. As expected, I and II gave the respective condensation products (IV and V) (Fig. 1). The main reason for low yields of IV and V, i.e., why 200% total yields were not attained, is probably due to shortage of the counterpart by the self-condensation of III. Although the benzene-soluble reaction products contained other products in addition to IV and V, isolation of them was not attempted because they were estimated to be self-condensation products of III. In reaction 2 without any organic solvent, the formation of VI and VII with the C-xylosidic structures was observed in 0.6% and 9.3% yields, respectively. Considerably higher yields of condensation products (IV and V) than those of C-xylosides (VI and VII) are in accordance with the previous reports[5,9] that self-condensation of guaiacylglycerol-β-guaiacyl ether proceeded more rapidly than condensation of I and II with xylose and xylan in 72% SA. In a separate experiment[5] with vanillyl alcohol having a hydroxymethyl group, the syringyl nucleus model (II) showed a higher reactivity for nucleophilic attack on the primary benzyl cation derived from vanillyl alcohol in 72% SA than did the guaiacyl nucleus model (I). The result from reaction 2 (Fig. 1, xylose) can be interpreted as depressed reactivity of the syringyl nucleus (II) toward condensation reaction with the secondary benzylic cation. The C-2,6 positions of the syringyl nucleus are
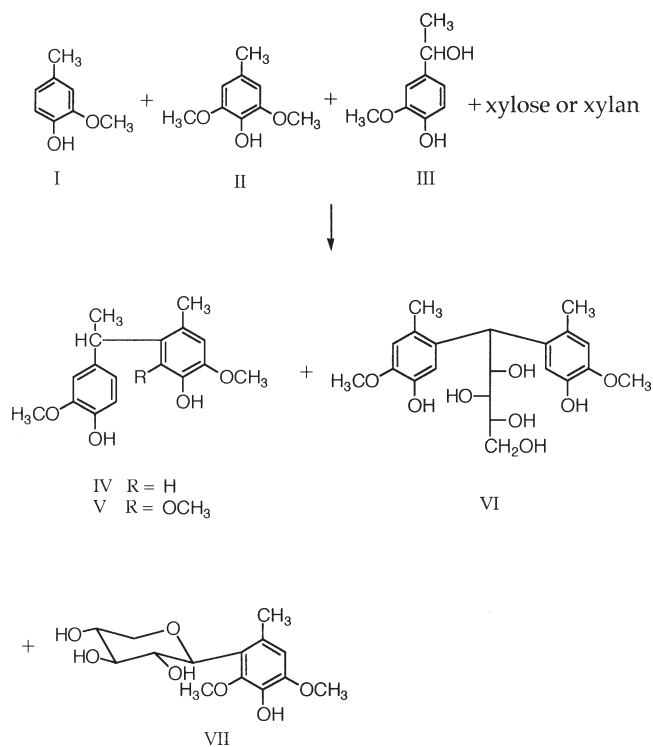
Fig. 1. Reaction of model compounds in the presence of xylose or xylan in 72% SA

sterically crowded because they have two functional groups: a methoxyl group and a side chain at their *ortho* position, and are therefore less reactive toward nucleophilic attack by the secondary benzyl cation originating from III. Thus, the remaining syringyl nucleus (II) has more opportunity to react with xylose to yield VII. Furthermore, because anomeric cations from xylose and xylan have a cyclic structure, they are less subject to steric effects from the functional groups of the aromatic nucleophile. These findings are consistent with the view that hardwood with a higher methoxyl content gives a higher ASL yield.[3] This is because syringyl lignin is believed to yield high amounts of acid-soluble lignin–carbohydrate products with C-glycoside linkages formed by condensation with hemicellulose.

Because the most reactive and abundant carbohydrate component of angiosperm hemicellulose is xylose and its present form is a polymer, xylan was used instead of xylose. C-xyloside (VII) was obtained in a 3.8% yield in reaction 3 (Fig. 1, xylan), about 12 times the yield of the corresponding C-xyloside VI from I. As shown by the reaction mechanism in Fig. 2, the nucleophilic attack of the aromatic nucleus (II) on the terminal anomeric cation of the xylan chain, formed by acid-catalyzed cleavage of the acetal linkage, gives intermediate (i). The initially formed intermediate (i) is temporarily present in 72% SA, but it is then hydrolyzed to yield VII. In fact, refluxing of ASL, separated after 72% SA treatment of beech wood,[6] in 3% SA gave insoluble Klason lignin and stable ASL.

The presently available data are inadequate for a detailed discussion of the formation and structure of ASL or
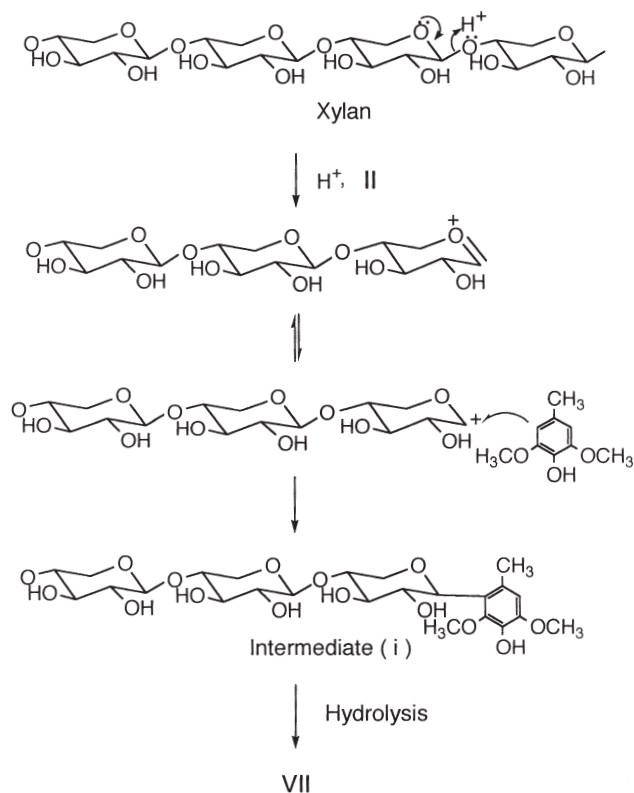

Fig. 2. The formation mechanism of VII

any relationship with native LCC. Further studies are in progress to elucidate the behavior of native LCC in 72% SA.

Behavior of arylglycerol-β-aryl ethers with guaiacyl (VIII) and syringyl (X) nuclei in 72% SA

To reveal CHCl₃-soluble components in ASL, arylglycerol-β-aryl ethers with guaiacyl (VIII) and syringyl (X) nuclei as typical lignin model compounds were treated by the Klason procedure. After the treatment of VIII and X in 72% SA followed by heating in 3% SA, the insolubles were separated by filtration to give material that models insoluble Klason lignin. The filtrates were extracted to give CHCl₃-soluble material that models the CHCl₃-soluble fraction in ASL.

As summarized in Table 2, VIII gave a large proportion of insolubles and a very small amount of CHCl₃-soluble material. The results were very similar to those for red pine using the Klason method.[6] In previous work,[6] the insoluble polymerized products were found to have structure IX (Fig. 3), which is formed by intermolecular dehydrative polymerization among benzylic carbons and C-6 aromatic carbons in ring B. Analysis by GPC and thin layer chromatography (TLC) indicated that the CHCl₃-soluble material contained a very small amount of creosol, a possible degradation product, suggesting the considerable stability of β-aryl ether linkage in polymer IX. X yielded CHCl₃-soluble material in 13%–17% yield together with major insoluble products. The insolubles are believed to have a structure (XI) analogous to IX as determined by ¹³C-NMR analysis (spectrum not shown). Separation of the CHCl₃-soluble material (reaction time 60 min) by column chromatography on silica gel isolated two degradation products (II and XII) in 7.8% and 13.9% yield based on the CHCl₃-soluble product, respectively. The chemical structures of II and XII was confirmed by comparison of ¹³C-NMR spectra with those of authentic samples.[4] The formation of these products can be interpreted as a result of hydrolysis[8] of a methoxyl group to a free phenolic hydroxyl group, followed by the β-syringyl ether bond cleavage of the intermediate with participation of the neighboring hydroxyl group, as shown in Fig. 4. This result suggests a possible hydrolysis of sterically hindered methoxyl groups in syringyl nuclei to free hydroxyl groups. Experimental support for the hydrolysis of a methoxyl

Table 2. Treatment of arylglycerol-β-aryl ethers (VIII and X) by the Klason procedure

| Compound	Reaction time[a] (min)	Yield (%)	
		3% SA-insoluble product	CHCl₃-soluble product
VIII	20	86.6	1.0
	60	84.1	1.5
X	20	74.4	12.7
	60	68.5	16.5

[a] Reaction time in 72% SA

Fig. 3. Behavior of model compounds (VIII and X) in 72% SA
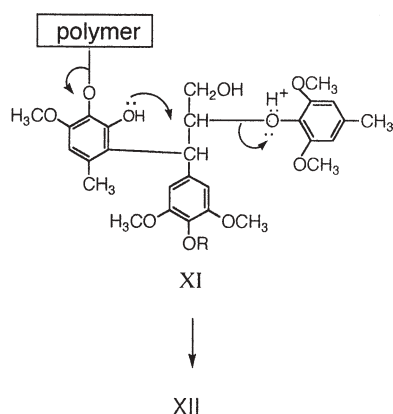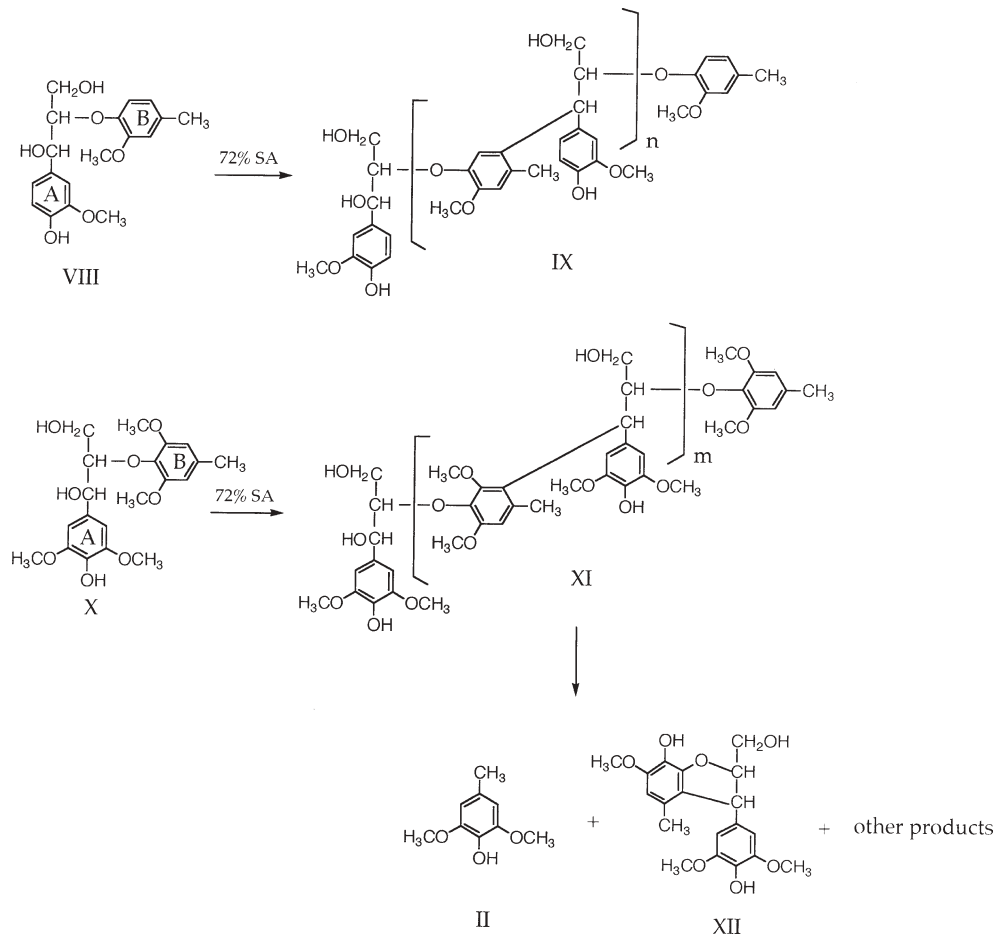
Fig. 4. Degradation of the condensed product (XI) in 72% SA

group has been provided by recording the visible spectra of the 72% SA-treated reaction products of VIII and X. This spectral method is based on the ability of sterically hindered methoxyl groups to undergo hydrolysis and phenolic compounds with a catechol unit to be easily oxidized to give colored material such as *ortho*-quinone. As expected, the latter products showed higher absorption in visible light regions than those of the former.[9] Because phenolic compounds with a catechol unit are known to polymerize in acidic media,[10] resinification[1] of the isolated ASL from
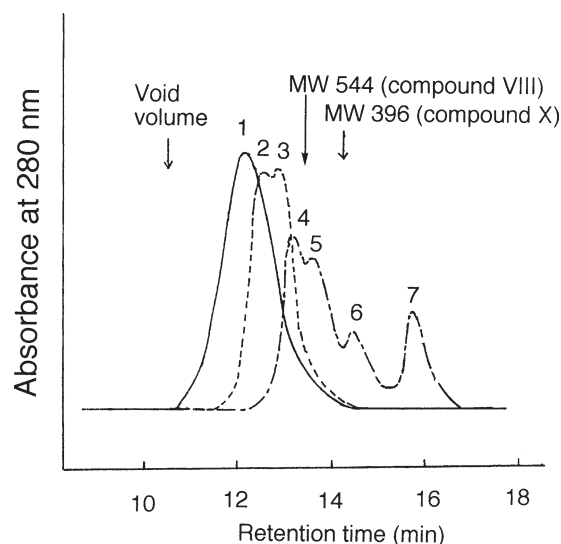
Fig. 5. Gel permeation chromatography of the insoluble products of VIII (IX, *solid line*), the insoluble products of X (XI, *dashed line*), and the CHCl₃-soluble products of X (*hashed line*)

beech may be interpreted as acid-catalyzed polymerization of the catechol units in it.

Gel permeation chromatograms (void volume: MW 5000) of reaction products obtained by the Klason treat-
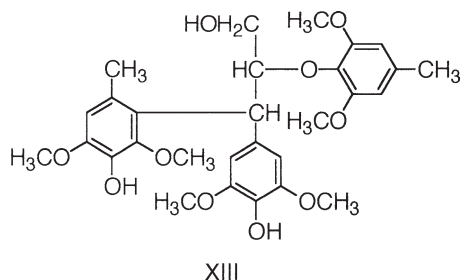
Fig. 6. Structure of XIII

ment of VIII and X are shown in Fig. 5. The molecular weight of the insolubles from VIII (peak 1 with retention time 12.3 min) was very close to those of dehydrogenation polymer[11] (DHP, retention time 12.5 min) prepared from coniferyl alcohol with horseradish peroxidase in the "Zutrop" polymerization. The molecular weight of the insolubles from X (peaks 2 and 3, retention times 12.6, 13.0 min, respectively) was substantially lower than that of the corresponding products from VIII, probably because of the difficulty of forming high molecular weight material and depolymerization of the formed condensation products. The CHCl₃-soluble material showed four peaks. Peaks 6 and 7 with retention times of 14.4 and 15.8 min, respectively, corresponded to those of the isolated products of XII (MW 362) with one phenylpropane unit and 5-methoxycreosol (II) (MW 168). A phenolic compound XIII (MW 544, Fig. 6) that was synthesized by acid-catalyzed dehydrative condensation of X and II at the benzylic position had a retention time of 13.3 min. From molecular weights and retention times of these compounds (II, XII, and the compound with MW 544), peaks 4 and 5 with retention times of 13.2 and 13.6 min appear to correspond to degradation products with molecular weights of about 560 and 480, respectively. These results suggest that the CHCl₃-soluble material was composed of degradation products with a molecular weight below about 600.

Conclusions

The guaiacyl nucleus, even in the presence of xylan in 72% SA, preferentially forms insoluble condensation products through an intermolecular condensation reaction between aromatic nuclei and benzylic carbons. Furthermore, the condensation products are quite stable in 72% SA and yield very few low molecular weight degradation products.

Therefore, guaiacyl lignin seems to yield only small amounts of ASL.

On the other hand, because of the large steric hindrance due to *ortho*-methoxyl groups, the reactive positions at C-2 and C-6 in the syringyl nucleus are considerably suppressed by the nucleophilic condensation reaction with benzylic carbons. Thus, the syringyl nucleus has a longer reaction time with xylan and yields water-soluble C-xyloside together with insoluble condensation products. Moreover, the insoluble condensation products, at least those with β-syringyl ether bonds, undergo degradation during 72% SA treatment to yield CHCl₃-soluble ASL with a low molecular weight.

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