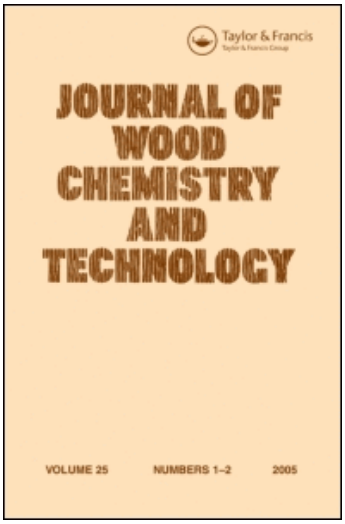

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2D NMR Study of Residual Lignin in Beech Kraft Pulp Combined with Selective Cleavage with Pivaloyl Iodide

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Dedicated to the memory of Kyosti V. Sarkanen

ABSTRACT

Structures of acetylated residual lignin obtained from unbleached beech kraft pulp were investigated by 2D NMR in combination with selective cleavage of non-phenolic α-alkyl ether bonds with pivaloyl iodide generated *in situ* from pivaloyl chloride / sodium iodide. β-O-4 and resinol structures still remained in this polymer lignin. Furthermore, carbohydrates were proved to be linked glycosidically to benzyl carbons of lignin. Possibly this inhibits complete delignification during kraft pulping.

INTRODUCTION

In recent years, it has been claimed that chlorine bleaching produces various organic chlorinated compounds causing environmental problems. Therefore, the development of pollution-free bleaching is one of the most important subjects for the pulping industry. Many studies have been reported on the chemical structures of residual lignin for the better understanding of inhibiting factors of delignification.

Two types of chemical structures have been proposed as inhibiting factors of kraft delignification: lignin carbohydrate (LC) complexes and condensed structures in lignin. Ether bonds between carbohydrates and side-chains of lignin model compounds are stable under kraft conditions except phenolic α-ethers[1]. Yamazaki and his coworkers analyzed the residual lignin obtained by the degradation of cellulose in kraft pulp with cellulase and concluded that the LC bonds were one of the most important inhibiting factors[2]. The existence of LC bonds in native lignin has been claimed by many

researchers. On the other hand, Gierer and his coworkers proposed secondary formation of LC bonds by nucleophilic attack of hydroxyl groups in carbohydrates to epoxide structures formed via the β-aryl ether cleavage3). Iversen and his coworkers analysed carbohydrates in the residual lignin isolated by the same method as Yamazaki, and pointed out the formation of alkali stable lignin-carbohydrates ether bonds during the kraft pulping4). The formation of glycosidic bonds between lignin and carbohydrates may occur during the cellulase treatment, too.

It is well-known that condensed structures are present in native lignin. With respect to the secondary formation of condensed structures, some conflicting experimental results have been reported. Gellerstedt and his coworkers analysed lignin fractions contained in kraft pulps at various cooking stages by ethylation/$KMnO_4$-oxidation and acidolysis, and reported that they did not suffer remarkably from condensation. In addition, they showed a high phenolic hydroxyl group content of 27% and a very low content of β-O-4 structures5),6). However, Chiang and his coworkers reported that their residual lignin was rich in diphenylmethane structures7).

In this report, using various NMR techniques, we analyzed beech residual lignin isolated by the cellulase treatment of kraft pulp and the products obtained by pivaloyl iodide treatment of residual lignin with the aim of getting information on residual lignin and LC bonds.

RESULTS AND DISCUSSION

Structure of Acetylated Residual Lignin

Isolation and fractionation procedures used in this experiment were shown in Figure 1. Residue-2 showed clear bands derived from amide groups (1600-1700 cm^{-1}) in its FT-IR spectrum, suggesting that some amount of enzyme might be still contained not only in Residue-2 but also in other fractions even after the work-up procedure following the cellulase treatment8). However, any more purification process was not applied, mainly because of negligible disturbance of the contaminating enzyme for NMR spectral analyses of acetylated residual lignins.

A gel permeation chromatogram of Fraction-1 detected by the absorption at 280 nm revealed that this fraction contained lignin of low molecular weight. However, a 1D ^1H NMR spectrum showed a lot of sharp signals in the region of 3.5-6.5 ppm, but few signals in the aromatic region (not shown). Clearly Fraction-1 was mainly composed of carbohydrate fragments, which were formed by the cellulase treatment and were still retained irrespective of careful washing of the residues.

A gel permeation chromatogram of Fraction-2 indicated that the fraction had a molecular weight comparable to that of an acetylated birch MWL9). In a 1D ^1H spectrum of Fraction-2 (Figure 2), each peak was much broader than those of the acetylated birch MWL (not shown) and of an acetylated dioxane lignin (not shown), reflecting its large transverse relaxation rate. On the basis of these results the polymer lignin in this fraction was speculated to have a more rigid structure than the acetylated

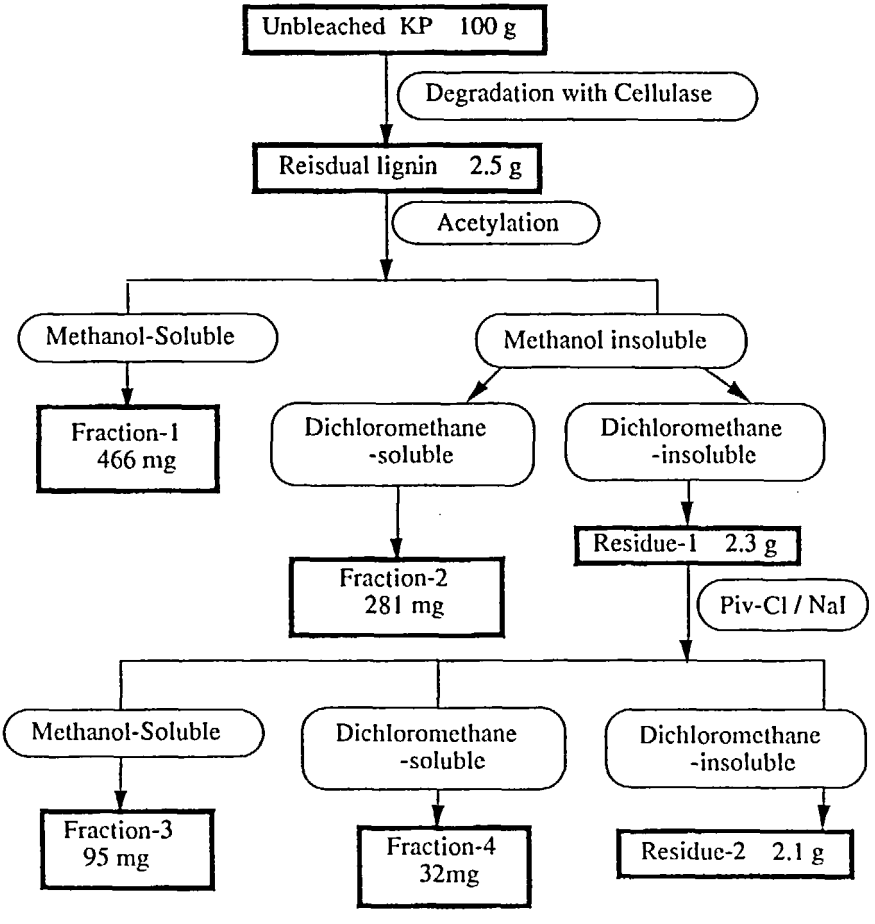


Figure 1. Isolation and fractionation procedure of beech residual lignin

birch MWL and the acetylated birch dioxane lignin. The methyl signal of acetoxyl groups combining with aromatic carbons was clearly observed in Fraction-2 and the ratio of aromatic/aliphatic acetoxyl groups of this fraction was slightly higher than those of the other two lignins.

To obtain detailed information concerning the aliphatic regions, HOHAHA experiment using MLEV-17 mixing[10] (Figure 3) was tried. The coherence transfer from C_1 in xylopyranosyl (nonreducing end) residues to C_5 was not achieved even with mixing time of 60 msec. This insufficient coherence transfer is probably due to the

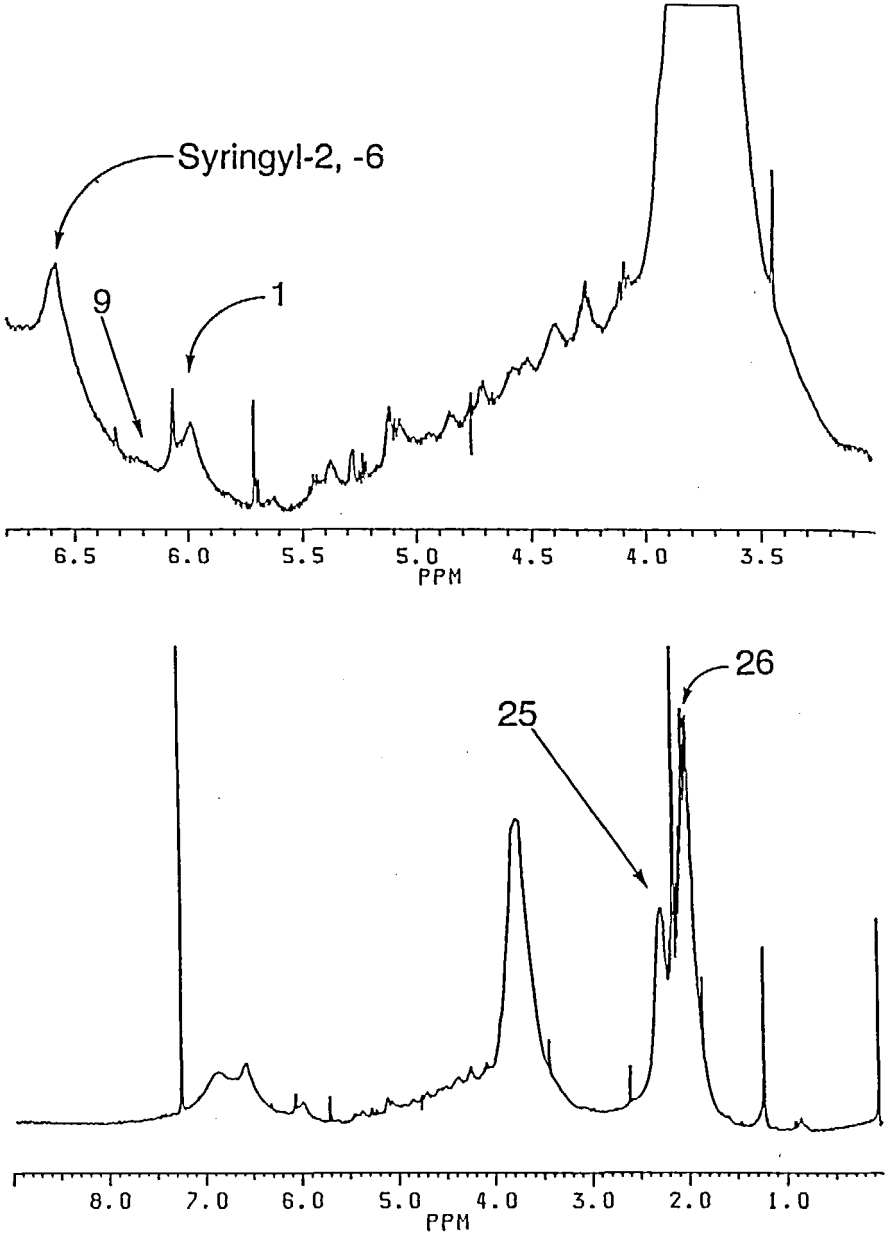


Figure 2. 1D ^{1}H NMR spectrum of Fraction-2. Peak numbers correspond to those in Table 1.

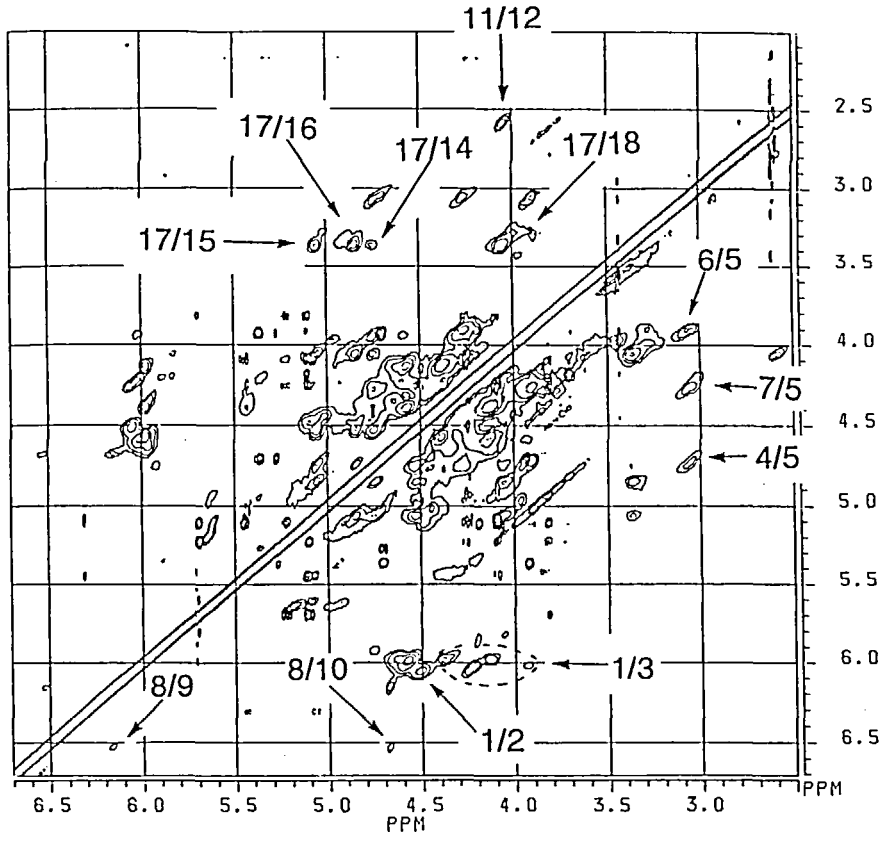


Figure 3. HOHAHA spectrum of Fraction-2 with mixing time of 60 msec. Peak numbers correspond to those in Table 1.

very rapid relaxation of transverse magnetization of this polymer lignin, which is in turn ascribable to its rigid structure. Application of DIPSI-2 and FLOPSY-8, which were reported to be more efficient mixing sequences than MLEV-17, could not improve efficiency of the coherence transfer (not shown)[11,12].

Significant amounts of β-O-4 structures (Table 1, peaks 1-3) still remained in Fraction-2 (Figure 2), although their contents in this fraction were lower than those in the MWL and in the dioxane lignin (estimated on the basis of signal intensities in the 1D spectra). This result agreed with the previous knowledge reported by Gellerstedt and his coworkers[5),6)]. Coniferyl alcohol structures, which can be produced via cleavage of β-

Table 1. Assignment of Signals in the ^1H NMR Spectra of Fraction-2 and -4 (acetylated)

No.	Chemical Shift (ppm)	Assignments
1.	5.9-6.1	β-O-4-α
2.	4.4-4.7	β-O-4-β
3.	3.8-4.4	β-O-4-γ
4.	4.7	Resinol-α
5.	3.1	Resinol-β
6.	3.9	Resinol-γ (axial)
7.	4.2-4.3	Resinol-γ (equatorial)
8.	6.6	Coniferyl alcohol-α
9.	6.2	Coniferyl alcohol-β
10.	4.7	Coniferyl alcohol-γ
11.	4.05	3-Aryl-1-propanol-γ
12.	2.6	3-Aryl-1-propanol-α
13.	4.5	Xylopyranosyl C_1
14.	4.7	Xylopyranosyl C_2
15.	5.1	Xylopyranosyl C_3
16.	5.8	Xylopyranosyl C_4
17.	3.4	Xylopyranosyl C_5 (axial)
18.	4.1	Xylopyranosyl C_5 (equatorial)
19.	4.4	Intermediate xylopyranose residue C_1
20.	4.7	Intermediate xylopyranose residue C_2
21.	5.0	Intermediate xylopyranose residue C_3
22.	3.7	Intermediate xylopyranose residue C_4
23.	3.3	Intermediate xylopyranose residue C_5 (axial)
24.	3.9	Intermediate xylopyranose residue C_5 (equatorial)
25.	2.2-2.4	Methyl of acetoxyl on aromatic carbons
26.	1.8-2.2	Methyl of acetoxyl on aliphatic carbons

ether structures during the kraft cooking, showed weak cross-peaks in a HOHAHA spectrum (Figure 3). The signal intensity at 6.2 ppm due to the β position of this structure in the 1D spectrum was so weak that its detection was hindered by the broad signal of hydrogen atoms at the 2 and 6 positions of syringyl nuclei and that at the α-position of β-O-4 structures (Figure 2).

Resinol structures (Table 1, 4-8) were contained in this fraction. Since phenolic types of the resinol structures are subjected to the ring-opening reaction under the kraft cooking conditions, the resinol structures in this fraction are thought to be non-phenolic types.

The cross peaks of 3-arylpropanol structures (11,12) were also observed in the HOHAHA spectrum (Figure 3).

The coupling networks (17/14, 15, 16, 18), which were also observed for the acetylated birch MWL, were ascribable to xylopyranosyl (nonreducing end) residues. The broad patterns of these cross-peaks and inefficient coherence transfer indicated that these residues had large relaxation rates because of possible bonds with polymers. Whereas in the case of the acetylated MWL the intensities of these cross peaks were much lower than those of intermediate xylopyranose residues, the cross peaks of xylopyranosyl residues were the most intense among those due to carbohydrates in the HOHAHA spectrum of Fraction-2 (Figure 3). Therefore, significant parts of xylopyranosyl residues in this fraction were probably linked to polymer lignin directly. To confirm their bonding sites, phase-sensitive NOESY and ROESY experiments were tried[13),14)]. However, no cross peaks were observed with respect to these xylopyranosyl residues. The reason for these unusual results is not clear.

If these xylopyranosyl residues existed as such before the cellulase treatment, they could not be an inhibiting factor for the delignification during kraft pulping because of hydrophilic character of xylopyranosyl residues. However, if these xylopyranosyl residues were parts of xylan connected to cellulose, in other words, key units of LC bonds and formed by the xylanase activities in cellulase[15)], these LC bonds could play an important role in the resistance of this lignin fraction to delignification.

Selective Cleavage of Non-phenolic Benzyl Ether Bonds with Piv-I

In this decade, various HASB(hard acid/soft base) reagents such as trimethylsilyl iodide have been designed and conveniently used for the cleavage of carbon-oxygen bonds under mild conditions[16-19)]. A typical reaction scheme for the cleavage of an ether-bond with these HASB reagents was shown in Figure 4. Generally, these types of reactions have the following characteristics. i) With respect to R_1 and R_2, relative reaction rates for the cleavage of ether-bonds are the following order; benzyl, allyl > t-butyl >> the other alkyls. ii) Relative yields of R_1-SB and R_2-SB depend on the stability of R_1^+ and R_2^+. iii) Ary-alkyl ethers are much more slowly cleaved than alkyl-alkyl ethers.

In the light of these characteristics, we can expect that in the transition state of these reaction, a positive charge is formed on R_1 or R_2, and that the reaction rate and the regioselectivity depend on the stabilizing ability of R_1 or R_2 for this positive charge. Since aromatic rings in non phenolic units of acetylated lignin have two (in the case of guaiacyl nuclei) or three (in the case of syringyl nuclei) electron-donating groups (methoxyl and propoxyl groups), the positive charge on the α position of a side-chain must be much more stabilized than those on the other side-chain positions. Thus, this type of reagents were expected to cleave selectively non-phenolic α-alkyl ether bonds under appropriate conditions. However, most of these reagents also cleave ester bonds almost to the same extent and may not be suitable for acetylated lignin samples. Oku and his coworkers reported that pivaloyl iodide (Piv-I) produced *in situ* from pivaloyl chloride and sodium iodide cleaves an ether bond selectively to form an alkyl iodide and

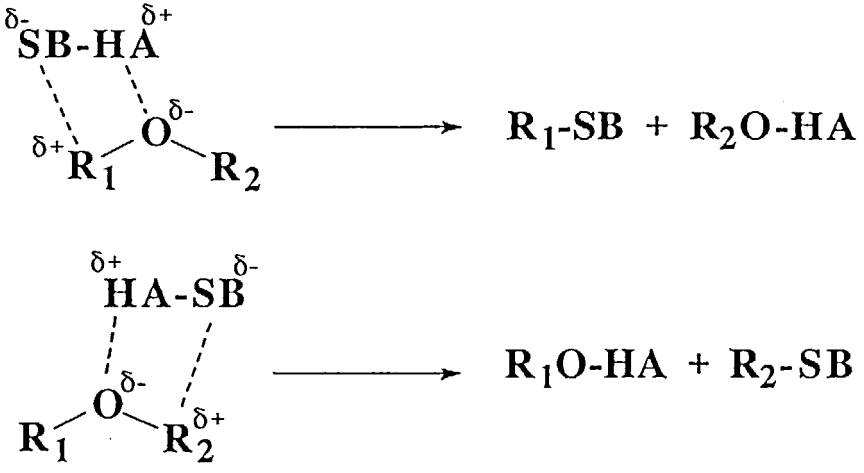
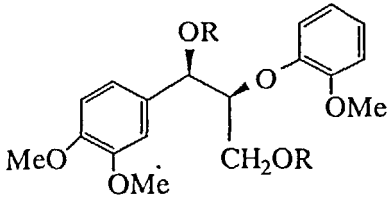


Figure 4 The reaction mechanism for cleavage of ehter bonds with the HASB reagents
HA and SB in figure represents hard acids and soft bases, respectively

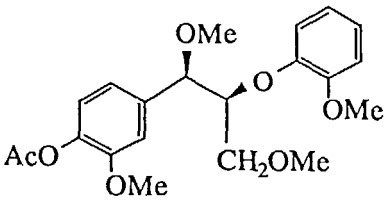
an alkyl trimethylacetate and that ester groups were not involved in the reaction under their conditions[20].

To obtain detailed knowledge about the selective cleavage of non-phenolic α-alkyl ether bonds with Piv-I, this reagent was applied to several lignin model compounds. Recoveries of starting model compounds after reaction (-10°C / 6hr) were shown in Figure 5. The recovery of Compound I was 34%. According to the reaction mechanism above mentioned, a benzyl-iodide (Compound VI in Figure 6) and methyl trimethylacetate should be expected as reaction intermediates. Volatile methyl trimethylacetate was not further pursued in this experiment. Compound VI was so unstable that it condensed to give Compound VII even at the ambient temperature within the order of hours.

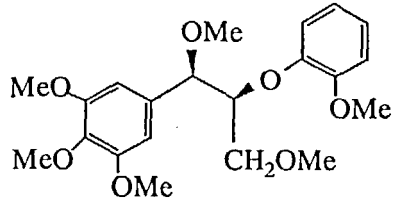
For the application of this reagent to polymer lignin, such a secondary condensation of lignin is not desirable. Therefore, the intermediate iodide needs to be transformed to a stable compound without condensation reactions. During the additional stirring at room temperature for 12hr in a 1/10(v/v) mixture of 1M-Na_2SO_3 and saturated $NaHCO_3$ aqueous solutions, Compound VI was converted to Compound VIII by the nucleophilic attack of hydroxide ion and/or H_2O (Figure 6). After the overall reaction, Compound I gave 55% of Compound VIII and 5% of Compound VII (and 34% of the starting material). A γ-alkyl ether was not affected under these conditions.



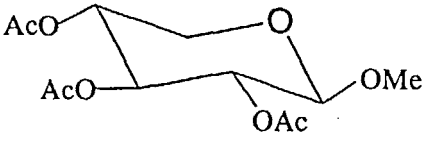
R = Me ; Compound I (34%)
Ac ; Compound II (73%)



Compound III (70%)



Compound IV (94%)



Compound V (93%)

Figure 5 Model compounds for the cleavage of ether bonds with Piv-I. Values in parentheses show the recovery of starting compounds after the treatment with Piv-I(6hr/-10°C)

Compound II was chemically modified only at the benzyl position and gave Compound IX in a yield of 26% (Figure 7). The recovery of the starting material was 73%. Compound IX produced from *erythro*-II was a mixture of *erythro* and *threo*, proving that this compound was not formed via the direct deacetylation by hydrolysis but via the loss of acetoxyl group followed by the same process as that for Compound VI.

Under the same condition, the recovery of compound III, which has an electron-withdrawing acetoxyl group in the aromatic nucleus, turned out to be 70%. The main reaction products, Compound X (Figure 7), was produced via the cleavage of an acetyl group attached to a phenolic hydroxyl group during the stirring in an aqueous solution (the yield was 25%), and α-ether was not affected. However, in the case of Compound IV which was extensively substituted with electron-donating groups, 94% of the starting material remained after the reaction. This unexpected result might be ascribable to the steric hindrance around the α-position caused by methoxyl groups substituted at both the 3 and 5 positions of the aromatic nucleus.

Since the recovery of methyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (compound V in Figure 5) was 93%, glycosidic bonds linked to other positions than benzylic one (the

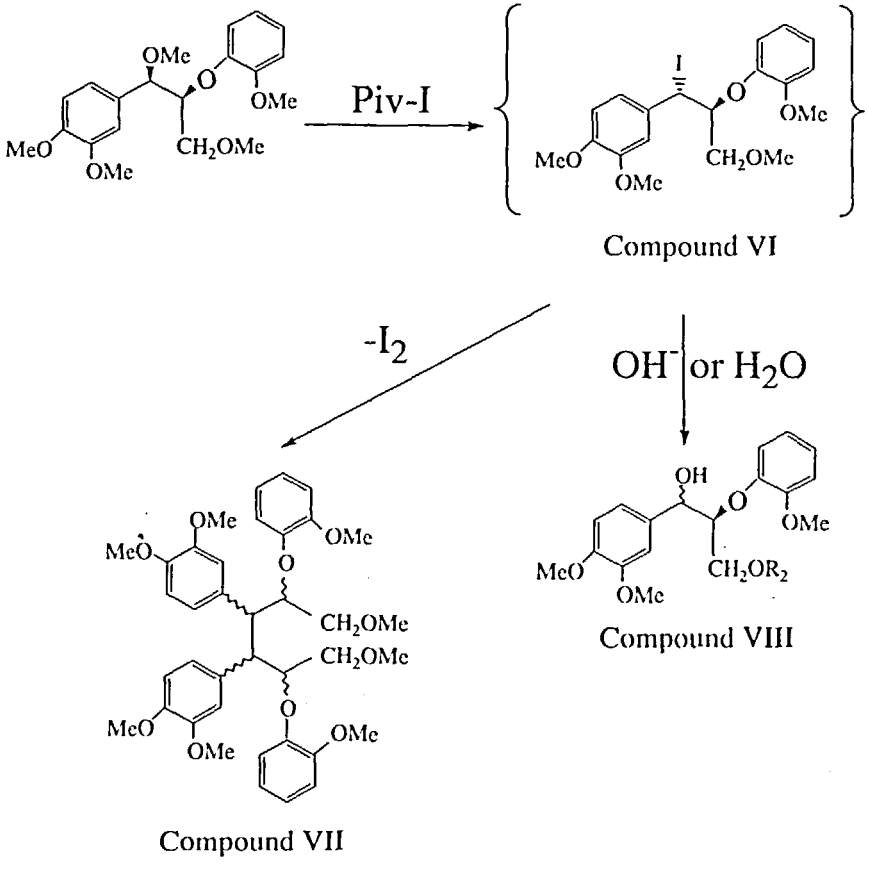


Figure 6 The reaction mechanism for formation of compounds VII and VIII from compound I

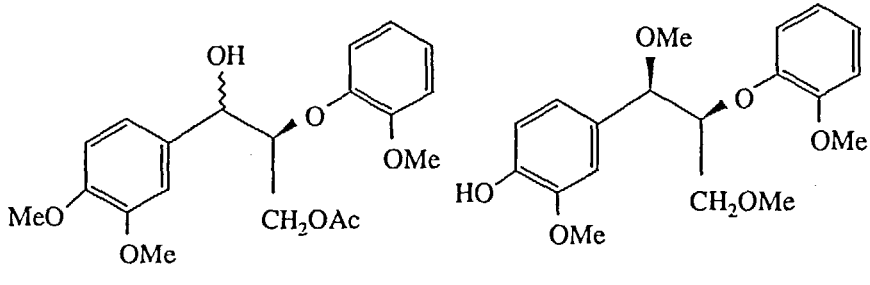


Figure 7 The reaction products from compunds II and III

α-position) are thought to be almost intact under the condition used. On the other hand, as observed for Compound I, those linked to the α-position of non-phenolic units are most likely cleaved significantly under the same condition.

Structure of Piv-I Treated Residual Lignin

Although a 1D ^{1}H spectrum of Fraction 3 showed broad signals derived from lignin in the aromatic region, their intensities were very low compared to those of carbohydrates (not shown). Therefore, this fraction was believed to be mainly composed of carbohydrates. The intense signals ascribable to C_1 position of esterified reducing end units were observed in a HMQC spectrum [21](Figure 8), but no signals ascribable to C_1 position of intermediate units (which should appear in the regions of 4.4-4.6 ppm and 100-105 ppm for ^{1}H and ^{13}C, respectively) were observed. Moreover, while acetylated methylol methylene protons of C_6 position in hexopyranose residues showed intense signals in the HMQC spectrum (^{1}H; 4.0-4.5 ppm, ^{13}C;60-63 ppm, Figure 8), no signals ascribable to methylene protons of C_5 position in pentopyranose residues (which should appear in the region of H; 3.0-4.0 ppm and ^{13}C; 60-65ppm) were observed. Therefore, the carbohydrates still remaining in this fraction were mainly composed of esterified reducing hexopyranoses.

In a HMBC spectrum[22] of Fraction-3 (Figure 9), C_1 protons of β-anomers of esterified reducing end units (5.6-5.7 ppm) showed an intense signal in the pivaloyl carbonyl region (176-177 ppm). C_1-^{1}H of α-anomer (6.3ppm) also showed a signal, although very weak. In the model experiment with methyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside, a 1D ^{1}H spectrum of the reaction mixture showed no signals ascribable to C_1 protons of esterified reducing end units. Therefore, the pivaloyl groups in Fraction-3 were concluded to have been introduced via cleavage of the glycosidic bonds at the benzylic position of lignin with Piv-I (Figure 10). Although clear assignments of each skeleton were not feasible due to the severe overlapping of cross peaks in the HOHAHA spectrum (Figure 11), these results indicate the existence of LC bonds between C_1 of hexopyranose residues and the α-position of guaiacyl components of lignin. These LC bonds might cause the resistance to the delignification, if these hexopyranose residues are connected to cellulose via glycosidic bonds in native lignin.

By the way, the artificial formation of LC bonds during the cellulase treatment was not confirmed by a separate separate experiment of guaiacylglycerol-β-guaiacyl ether with cellulase in the presence of xylan and Avicel (a commercially available cellulose powder) under the same conditions.

A gel permeation chromatogram showed that Fraction-4 had slightly higher molecular weight than Fraction-2. 1D ^{1}H and HOHAHA spectra were shown in Figures 12 and 13. Both β-O-4 structures and xyropyranosyl residues showed signals in these spectra. In addition, several cross peaks which were not confirmed in Fraction-2 were observed, some of which were ascribable to the intermediate xylopyranose residues. The same cross peaks were also observed in the case of the acetylated birch

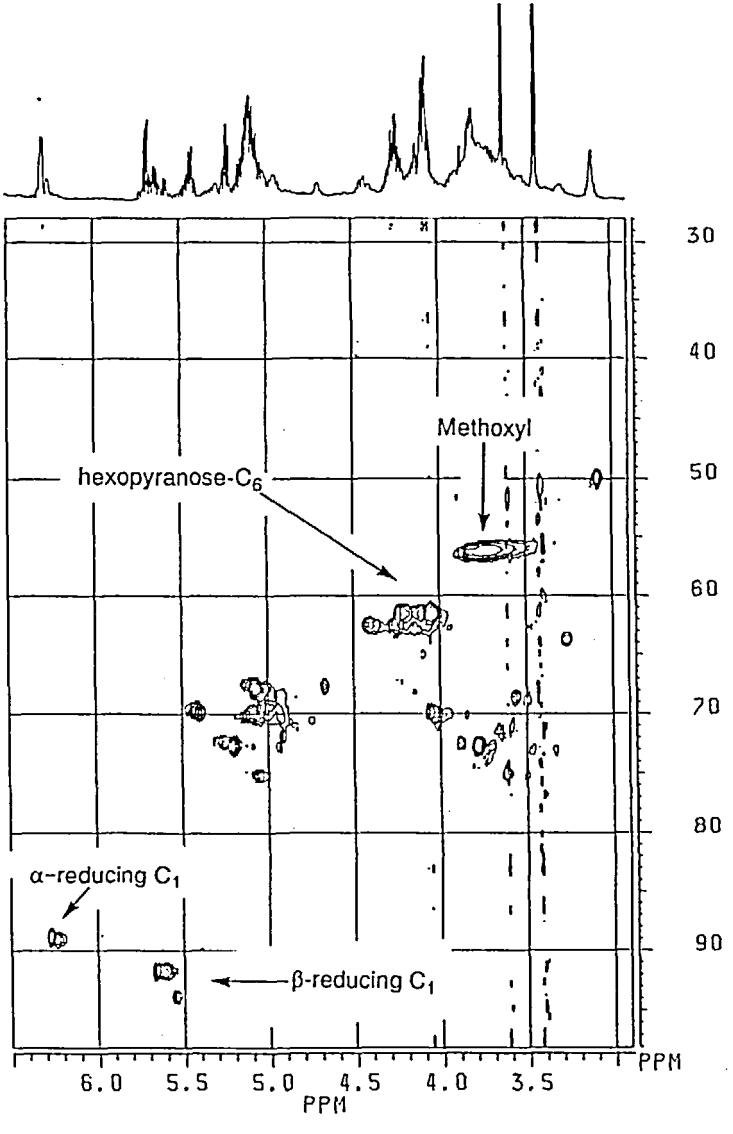


Figure 8. HMQC spectrum of Fraction-3.

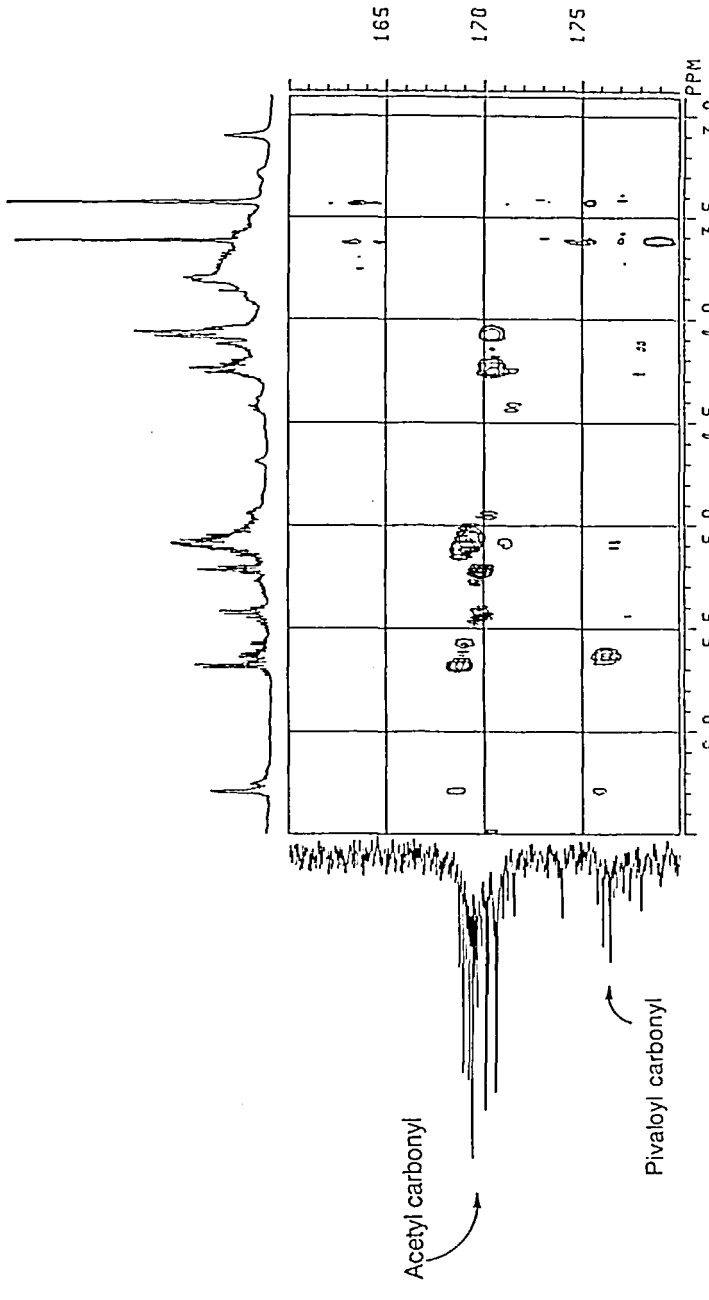


Figure 9. HMBC spectrum of Fraction-3

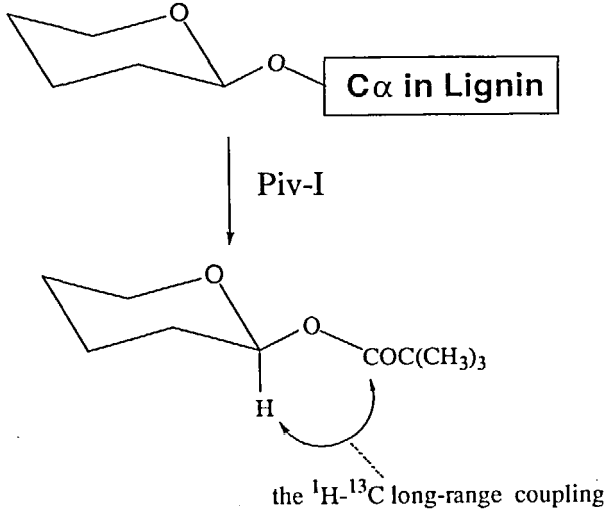


Figure 10. The correlation via the ^{1}H-^{13}C long-range coupling between C_1-^{1}H and Pivaloyl carbonyl ^{13}C introduced via cleavage of a benzylic glycoside bond.

MWL and they were much more intense than those of the xylopyranosyl residues[9]. These results supports the hypothesis that xylan is chemically linked to the benzylic position of guaiacyl components of lignin. Since xylan itself is soluble in an alkaline solution, if the presence of xylan hinder the delignification during kraft pulping, polymer chains of lignin and cellulose seem to be cross-linked through xylan.

EXPERIMENTAL

Isolation and Fractionation of Beech Residual Lignin

To 100 g (oven dried weight) of unbleached beech kraft pulp (Kapper number = 19.4) suspended in 3 l of an acetate buffer (pH = 5.0), 20 g of Meicelase P1(purchased from Meiji Seika Co. Ltd.) was added and stirred at 40 °C. After 48 hr the residue was separated and washed centrifugally with ion-exchanged water. The residue was dried and acetylated with Ac_2O/ pyridine. The reaction mixture was poured into ice-cooled ion-exchanged water and extracted with a 2/1 mixture (v/v) of dichloromethane/acetone. The organic layer was washed with 2N-HCl and brine, and dried over Na_2SO_4. Then the solvent was removed and the residue was dried at 45 °C. The residue was extracted ultrasonically with 50ml of methanol. The methanol solution was evaporated to give 466

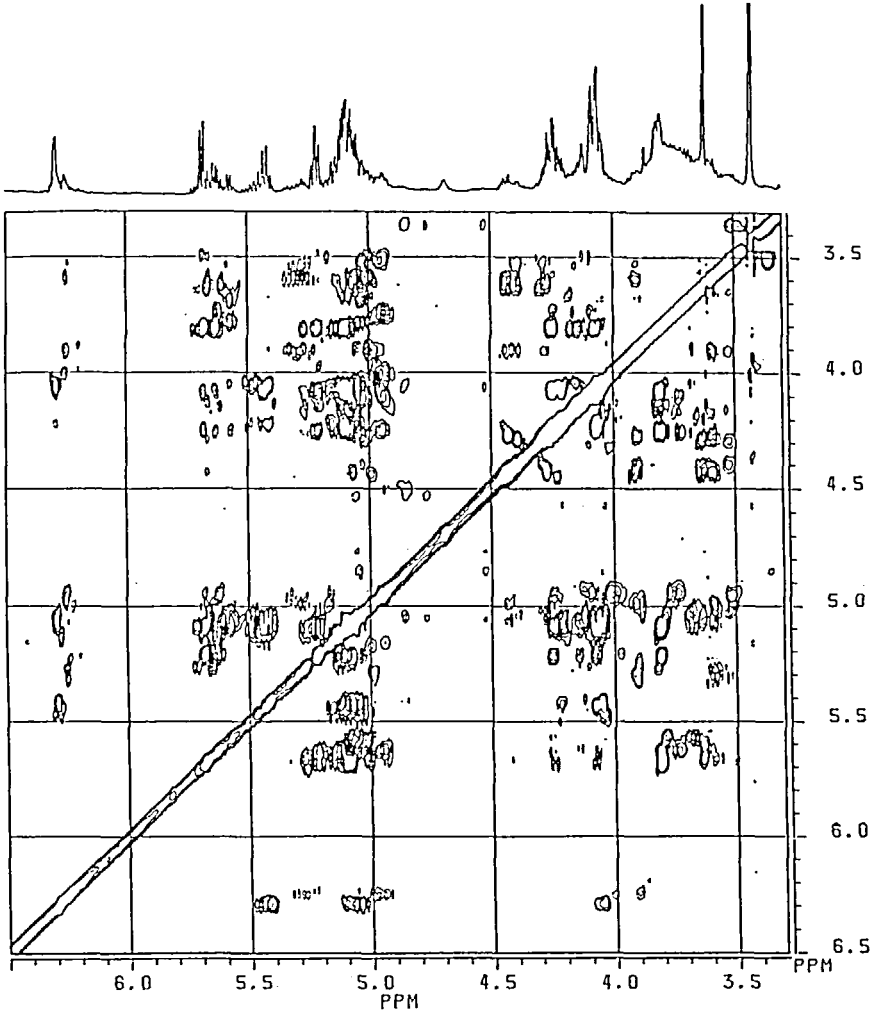


Figure 11. HOHAHA spectrum of Fraction-3

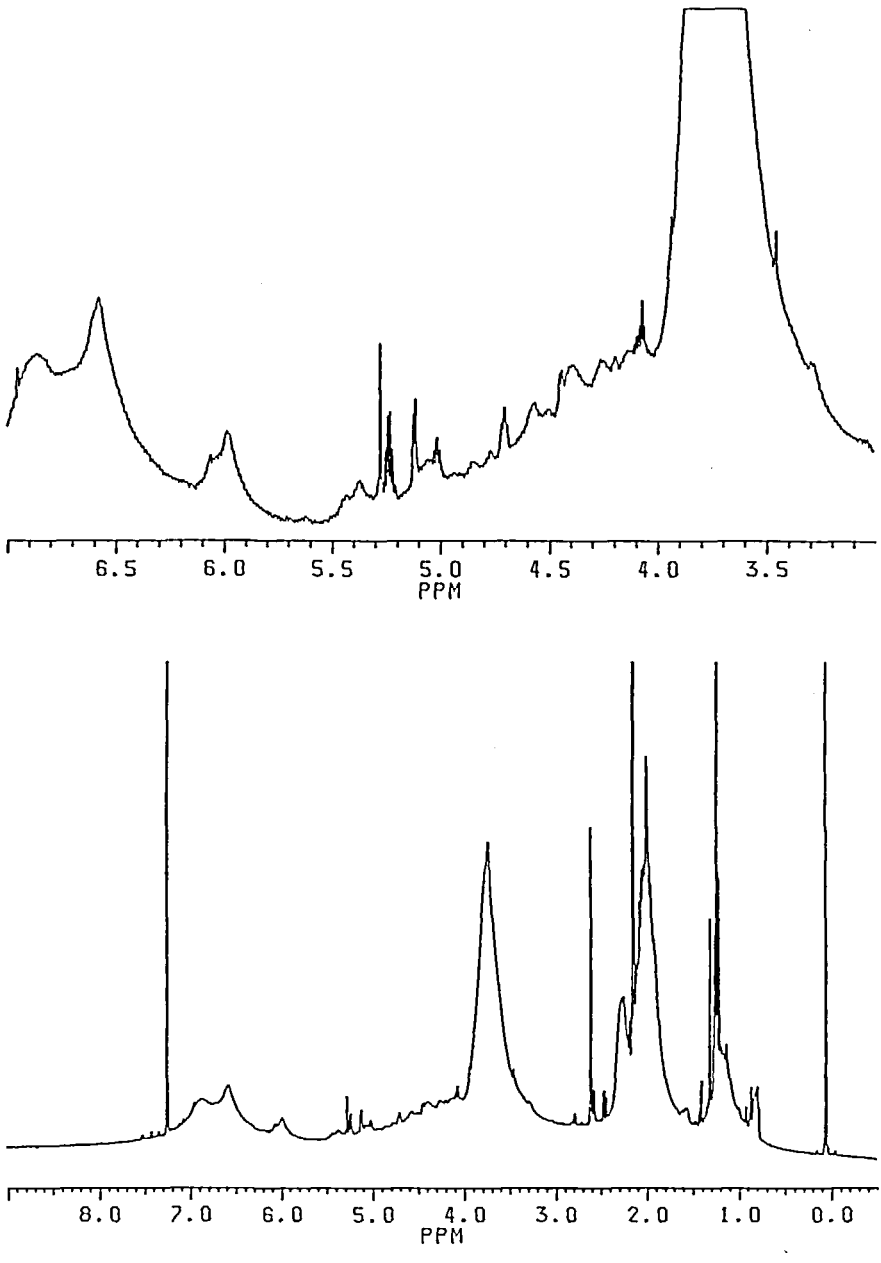


Figure 12. 1D ^{1}H spectrum of Fraction-4.

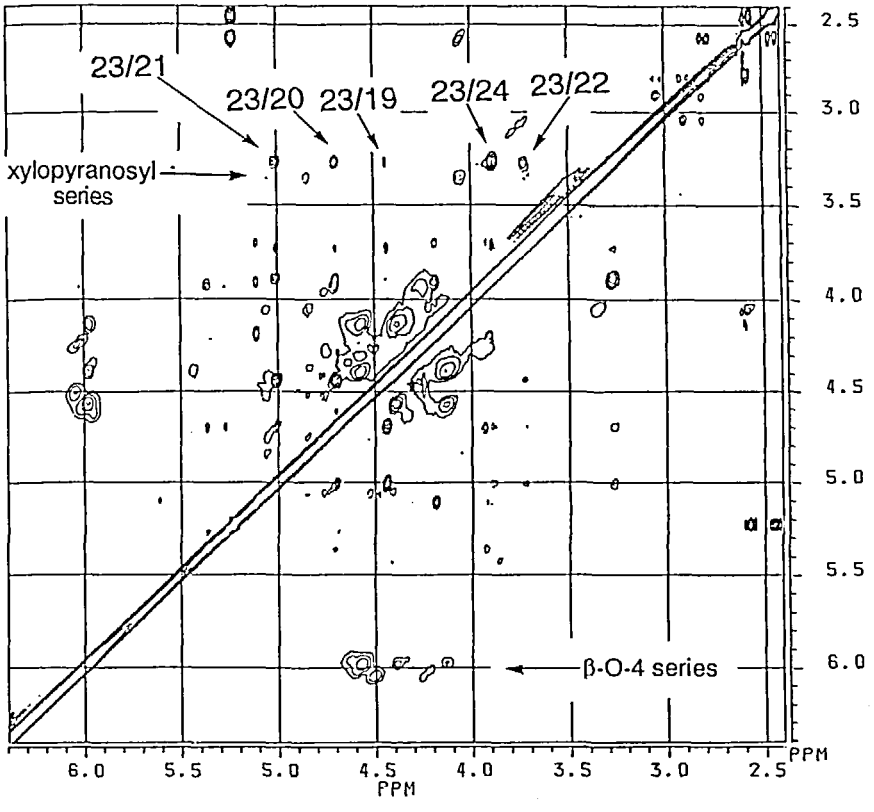


Figure 13. HOHAHA spectrum of Fraction-4. Peak numbers correspond to those in Table 1.

mg of Fraction-1. The methanol insoluble fraction was further extracted with 50 ml of dichloromethane. The dichloromethane solution was evaporated to give 281 mg of Fraction-2. The dichloromethane-insoluble residue (Residue-1) was 2.3 g.

NMR Experiments of Fraction-1 and -2

All NMR spectra of Fraction-1 and -2 were recorded at 30 °C in $CDCl_3$ on a Bruker AM 600. 1D ^1H spectra were acquired on a sample of 40 mg/700 µl accumulating 32 scans.

The phase-sensitive HOHAHA experiment of Fraction-2 was performed using a Bruker pulse program; MLEVDW.AU. The spectra were recorded using mixing time of

60 msec, 27 μsec 90° pulse width, acquiring 400 × 2048 data matrix zerofilling to 1024 × 2048 data size. The largest t_1 value was 35.2 msec, and 32 scans were accumulated per t_1 value. π/4 phase-shifted sinbell-squared windows were applied in both dimensions.

The three phase-sensitive NOESY experiments of Fraction-2 were performed with different mixing times of 50, 150 and 900 msec, using a Bruker pulseprogram; NOESYPH.AU. The spectra were recorded using 8.6 μsec 90° pulse width, acquiring 400 × 2048 data matrix zerofilling to 1024 × 2048 data size. The largest t_1 value was 35.2 msec and 48 scans were accumulated per t_1 value. π/2 phase-shifted sinbell-squared windows were applied in both dimensions.

The phase-sensitive ROESY experiments of Fraction-2 was performed with mixing of 100 msec, using a Bruker pulseprogram;ROESYPH.AU. The same acquisition parameters as NOESY experiment were applied.

Preparation of Model compounds

Compounds I, II, III and IV were synthesized by the method of Nakatsubo and his coworkers followed by methylation (MeI/NaH) and/or acetylation (Ac_2O/ Pyridine).

Compound V (methyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside) was prepared by acetylation of commercially available methyl-β-D-xylopyranoside.

Piv-I Treatment of Model Compounds

To 8 mmoles of NaI and pivaloyl chloride in 10 ml of acetonitrile (distilled over P_2O_5), 1 mmole of a model compound in 10 ml acetonitrile was added dropwise over 15 min at -10 °C and stirred at the same temperature for 6 hr. After 8 ml of 1M-Na_2SO_4 and 50 ml of saturated $NaHCO_3$ were added dropwise at the same temperature, the reaction mixture was stirred for 12hr at room temperature. The reaction mixture was extracted with dichloromethane and the organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed and the residue was subjected to HPLC or GC analyses.

HPLC Analysis of Reaction Mixtures

The reaction mixtures of compounds I, II, III, and IV with Piv-I were analysed by HPLC under the following conditions.

Column: Shimazu Shimpack-CLC-ODS-C18 (10 mm × 150mm).
Eluent: CH_3CN/H_2O=7/3.
Flow rate: 1 ml / min. Detector: UV (280 nm).

GC Analysis of Reaction Mixtures

The reaction mixture of compound V with Piv-I was analysed by GC under the following conditions.

Column: Gasukuro kogyo, OV-101.

Column temperature: kept at 150°C for 1 min and raised to 250°C at the rate of 4°C/min and then kept for 10 min.

Separation and Identification of Reaction Products

The reaction products of Piv-I treatment were separated by preparative TLC (Merck kiesel gel) and HPLC (Shimazu Shimpack-CLC-ODS-C18, 20 mm x 250 mm). ^1H and ^{13}C NMR spectra (in $CDCl_3$) were recorded on a Bruker AC300. EI and FAB (using nitrobenzyl alcohol as a matrix) mass spectra were recorded on a JOEL DX303. The spectral data of each reaction product were as follows.

Compound VII

^1H and ^{13}C spectra of this compound showed very complicated patterns probably because this compound was a mixture of six diastereomers. Thus, clear assignments in NMR spectra were not feasible. However, three peaks of 717($M+K^+$), 701($M+Na^+$) and 678 (M^+) were identified in a FAB-MS spectrum.

Compound VIII (obtained as a mixture of two diastereomers, *erythro* and *threo*)

^1H NMR (δ): 3.30 and 3.31(3H, s, γ-OCH_3), 3.35-3.64 (2H, m, Hγ), 3.82-3.85 (9H, s, aromatic-OCH_3 × 3), 4.08 and 4.32 (1H, m, Hβ), 4.85 (1H, m, Hα), 6.77-7.14 (7H, m, aromatic-H).

^{13}C NMR (δ): 55.7 (aromatic-OCH_3), 59.1(γ-OCH_3), 71.3 and 71.5 (Cγ), 72.9 and 73.8 (Cα), 84.9 and 87.4 (Cβ), 109.6, 109.9, 110.8, 111.9, 112.1, 118.6, 119.5, 120.3, 121.3, 123.5 (tertiary aromatic C), 132.5, 147.2, 148.2, 148.7, 150.7, 151.3 (quarternary aromatic C).

EI-MS (m/z) : 348(M^+, 40), 330(10), 299(13), 224(12), 208(10), 195(25), 179(14), 167(91), 150(100), 139(100), 124(48), 121(55), 109(45), 95(33), 77(35).

Compound IX (obtained as a mixture of two diastereomers, *erythro* and *threo*)

^1H NMR (δ): 1.95 (3H, t, acetyl-CH_3), 3.82-3.86 (9H, s, aromatic-OCH_3 x3), 3.96-4.48 (3H, m, Hβ and Hγ), 4.85 (1H, m, Hα), 6.77-7.14 (7H, m, aromatic-H).

^{13}C NMR (δ): 20.6 (acetyl-CH_3), 55.7 (aromatic-OCH_3), 62.7 and 63.2 (Cγ), 71.9 and 74.1 (Cα), 84.1 and 85.8 (Cβ), 109.3, 109.8, 110.9, 112.1, 118.4, 119.6, 120.2, 120.4, 121.3, 123.4 (tertiary aromatic C), 131.6, 146.9, 147.9, 148.4, 148.9, 150.7, 151.4 (quarternary aromatic C).

EI-MS (m/z) : 376(M^+, 32), 299(13), 210(15), 208(10), 192(18), 181(14), 167(100), 150(100), 139(62), 124(48), 121(42), 109(30), 95(14), 77(17)

Compound X

^1H NMR (δ): 3.26 and 3.49 (6H, s, γ-OCH_3), 3.56-3.73 (2H, m, Hγ), 3.73 and 3.81 (6H, s, aromatic-OCH_3 × 3), 4.38-4.44 (2H, m, Hα and Hβ), 6.77-6.92 (7H, m, aromatic-H).

^{13}C NMR (δ): 55.8 (aromatic-OCH_3), 57.1(α-OCH_3), 59.2(γ-OCH_3), 71.4 (Cγ), 82.5 (Cα and Cβ), 110.2, 112.31, 113.7, 118.0, 120.8, 121.0, 122.2 (tertiary aromatic C), 130.5, 145.2, 146.4, 147.9 (quarternary aromatic C).

EI-MS (m/z) : 348(M^+, 40), 225(113), 193(22), 180(10), 168(100), 152(51), 139(54), 123(16), 109(23), 95(32), 77(33).

Piv-I Treatment and Fractionation of Residue-1

Residue-1(2.0 g) was treated with 16 mmoles of Piv-Cl /NaI under the same conditions as those for model experiments. The reaction mixture was extracted with a 1/2 (v/v) mixture of acetone/dichloromethane. After the same work-up and the fractionation procedure as those for the acetylation of the residual lignin, 95mg of methanol-soluble fraction (Fraction-3), 32 mg of dichloromethane soluble (methanol-insoluble) fraction (Fraction-4) and 2.1g of the methanol- and dichloromethane-insoluble residue (Residue-2) were obtained.

FT-IR Experiment of Residue-2

FT-IR spectrum of Residue-2 was recorded on a Shimazu DR-8000.

NMR Experiments of Fraction-3 and -4

1D ^1H and phase-sensitive HOHAHA spectra of the Fraction-3 and -4 were recorded under the same conditions as those for Fraction-2.

The phase-sensitive HMQC experiment of Fraction-3 was performed using a Bruker pulse program; IND1DP9.AU. The 320 × 2048 data matrix was zerofilling to the 1024 × 2048 data size. The largest t_1 value was 36.5 msec and 32 scans were recorded per t_1 value. $\pi/4$ phase-shifted sinbell-squared window was applied in t_1 dimension, and $\pi/2$ phase-shifted sinbell-squared window was applied in t_2 dimension. ^{13}C decoupling by GARP sequence was applied during t_2 acquisition.

An absorption-mode HMBC spectrum of Fraction-3 was acquired using a Bruker pulseprogram;INVDR2LP.AU. The data point and data size were the same as those for the HMQC spectrum. The delay time for low pass filter was 60 msec. The largest t_1 value was 49.3 msec and 64 scans were accumulated per t_1 value. Sinbell window was applied in both dimensions.

CONCLUSIONS

1. The residual lignin obtained from beech unbleached kraft pulp still contained β-O-4 and resinol type side chains.
2. Glycosidic bonds between lignin and carbohydrates are present in the residual lignin and may play an important role in the resistant nature of this lignin fraction to dissolution into kraft liquor.

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