A common feature of lignin condensation reactions and phenol/resorcinol formaldehyde curing reactions is the formation of methylene bridges between aryl units. Although this reaction is necessary for resin curing, it may be a detriment to efficient delignification in pulping systems. This condensation reaction of lignin was investigated by treatment of loblolly pine milled-wood lignin with alkali in the presence of formaldehyde or lignin model compounds. By the use of a $^{13}$C-label, it was established that formaldehyde liberated from a lignin model ultimately resulted in methylene bridges between lignin aryl units. Formaldehyde liberation from lignin and model compounds, by reverse-aldol reaction, also resulted in the formation of vinyl ether structures. With modern NMR techniques, it was demonstrated that alkaline treatment of milled-wood lignin at 140 °C resulted in the formation of vinyl ether structures, whereas, after 50 °C treatments, no vinyl ether was detected.

Lignin condensation reactions have been studied for many years, mainly because they possibly interfere with efficient delignification of wood. Numerous model compound studies established the various types of condensation reactions that may occur between lignin fragments (1-5) or between lignin and carbohydrates (1,2,6,7) during alkaline pulping. An important class of condensation reactions involves formaldehyde.

Forty years ago, it was discovered that formaldehyde is released from isolated lignins by either acid or alkaline treatment. It was concluded that the
formaldehyde came from the primary alcohol groups (γ-hydroxymethyl) of the propyl side chain \((\gamma)\). About 20 years later, it was recognized that the formaldehyde that is continuously released during pulping may react with lignin in accordance with the well-known reactions between phenol and formaldehyde \((9)\). Since then, many investigators have published results of studies on model compounds, which are consistent with the elimination of formaldehyde from a quinone methide \((\text{II; Figure 1})\), generated from a phenyl propane unit with a free C-4 phenolic group \((\text{I})\) by a reverse aldol reaction and formation of a vinyl ether \((\text{III})\) \((10)\). Subsequent addition of formaldehyde to free phenolic units (usually at the 5-position) may then occur to give structure \(\text{V}\) [Figure 2]. Ultimately, the new hydroxymethyl groups condense with elimination of water and formaldehyde or condense at a free C-5 position with elimination of water, resulting in formation of a methylene bridge structure \((\text{VI})\) \((10,11)\). One of the most noteworthy studies utilized \(\text{VII}\) as a model compound (Figure 3) with a \(^{13}\)C-label in the γ-hydroxymethyl group. Alkaline treatment of this model compound resulted in a complex mixture. \(^{13}\)C-NMR spectroscopy of the mixture showed that much of the label ended up in methylene bridges between aryl units \((12)\).

Clearly, phenol-formaldehyde type reactions form a common bond between pulping chemistry and adhesives chemistry. In fact, the reactions of added formaldehyde to lignins and the utilization of the phenolic nature of lignin to produce PF resins have been studied extensively \((13-15)\). Although for adhesives PF-condensation reactions are important, they are a nuisance during pulping. The impetus for a more complete understanding of lignin condensation reactions is the hope of increasing the efficiency of delignification by preventing or minimizing condensation reactions during pulping as well as controlling and manipulating them for practical and uniform adhesives. Because of the complexity of lignin, the type and extent of condensation reactions that occur during alkaline pulping are still not well understood. Most of the information regarding these reactions is speculation based on studies with model compounds.

This study was a preliminary effort to detect and characterize structures in the lignin polymer that result from condensation reactions during alkaline treatment. The only method capable of observing this complex polymer with the necessary detail on an atomic scale is \(^{13}\)C–NMR spectroscopy. Enormous progress has been made in the capabilities of NMR especially in the last few years, and very few of the modern techniques have yet been applied to lignin.

In order to facilitate the detection of condensation structures, lignin was treated with alkali in the presence of \(^{13}\)C-labeled formaldehyde or model compounds (Figure 3) enriched with \(^{13}\)C at the γ-position on the side chain. In this manner, the detection of cross condensations between the model and the lignin was facilitated. Conventional and modern NMR experiments were then utilized to examine the treated lignins.
Figure 1. Quinone methide and vinyl ether formation from free-phenolic lignin units.

Figure 2. Condensation of formaldehyde with lignin free-phenolic units.

Figure 3. Lignin model compounds.
Experimental Methodology

**Lignin.** Milled-wood lignin (MWL) from loblolly pine was obtained by 95% dioxane/water extraction of extractive-free vibratory ball-milled wood. The yield was about 20% based on the lignin in the wood.

**Lignin Models.** 1-(4-Hydroxy-9-methoxyphenyl)-2-(2-methoxyphenoxy)propan-1,3-diol (VII). The 4-benzyloxy-α-keto precursor to VII was prepared as previously described (16). Treatment of this compound (70 mg, 0.17 mmole) in THF(2 mL)/H2O(0.1 mL) with 10% Pd/C under H2 (balloon), with stirring, for 3 hours gave a colorless oil (49 mg, 91%) which was shown to be VII by comparison (TLC and NMR) with authentic material. A 70:30 erythro/threo ratio was indicated by 13C-NMR. The 1H and 13C-chemical shifts (CDCl3) have been previously published (17). Compound VII enriched with 13C at Cα was prepared from labeled acetovanilione, which was prepared from guaiacol and acetic acid-1-13C by a procedure analogous with that described previously (16). The γ-13C enriched compound was made by simply utilizing 13C-formaldehyde in the synthesis.

1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)propan-1,3-diol (VIII). Procedures analogous with those described above gave a 94% yield of a 70:30 erythro/threo product as a colorless oil.

**Lignin Treatments.** The MWL (100 mg, 0.5 mmole Cα units) in 0.5M NaOH (3 mL) was placed in a 5 mL stainless steel bomb along with the lignin model compound (10 mg, 0.03 mmole) or formaldehyde (1.7 or 17% soln, 0.03-6 mmoles), and heated in an oil bath at 50 °C or 140 °C. All of the model/lignin runs were at 140 °C.

**Chromatographic Method.** The acetylated reaction product was applied on a 93 cm x 2.4 cm column of Bio-Beads S-X2 (Bio-Rad), which was then eluted with CHCl3.

**NMR Procedures.** All spectra were obtained at 30 °C with a Brucker WM-250 (62.9 MHz 13C-spectrometer controlled by an Aspect 2000A minicomputer. Solutions of lignin product (70-80 mg) in CDCl3 (0.3 mL) containing tetramethylsilane as internal reference were used. For conventional noise-decoupled 13C-spectra, a Bruker power-gated sequence (POWGATE) was utilized with Waltz-16 decoupling (18). Other Bruker standard microprograms were used for the DEPT and QUAT experiments. Generally: 30,000-60,000 free-induction decays (FID’s) of 8K data points were accumulated over a spectral width of 15,000 Hz. For optimum resolution and S/N, the FID’s were zero-filled to 16K points and a 6 Hz line-broadening function was applied prior to Fourier transformation. Resolution-enhanced spectra were obtained by applying a 6 Hz Lorentzian-to-Gaussian function.
Results and Discussion

Direct Formaldehyde Addition. In initial attempts to characterize formaldehyde-promoted condensation reactions, loblolly pine milled-wood lignin was treated with alkali at 50 °C in the presence of either unlabeled or 13C-labeled formaldehyde. The 13C-spectra of the acetylated products are illustrated in Figure 4. The control was a 50 °C alkaline treatment in the absence of formaldehyde. With unlabeled formaldehyde, 10 moles were added per mole lignin (C₉ unit basis). This is actually about a fiftyfold excess, if it is assumed that only about one-fifth of the C-5 positions are activated by a free phenolic group in the milled-wood lignin (13). Even with this enormous excess, it is difficult to detect an appreciable amount of aryl-CH₂-aryl resonances (Figure 4b), possibly due to a relatively large chemical shift spread (30-40 ppm) among the various types. However, new resonances at 62.3 and 63.3 ppm are clearly seen. Also, an expected increase in primary alcohol groups is seen at 170.5 ppm due to the carbonyls of the alcohol acetates. In addition, the missing resonances at 121-123 ppm (Figure 4a expansion) were assigned to tertiary C-5s in guaiacyl units, based on the expected conversion of most of the available tertiary C-5s to quaternary C-5s (replacement of hydrogen with a hydroxymethyl group) and on previous assignments in 9:1 acetone-d₆/D₂O (19). The addition of a large excess of formaldehyde may be appropriate for adhesive research, but certainly is not representative of what occurs during alkaline pulping. However, this extreme case assisted in the assignment of some resonances.

In another experiment, 13C-labeled (98%) formaldehyde was used, but in a much smaller quantity (0.4 mole/C₉ unit) than with the unlabeled (1% natural abundance) 13C-material. The result was an enormous increase in intensity of the formaldehyde, derived resonances (Figure 4c), particularly those centered around 35 ppm that were barely detectable before (Figure 4b). A broad "envelope" of overlapping resonances in this region was also observed with the alkaline treatment of VII in the presence of H₂¹³CO (12). The observation that considerable resonances are still present in the 121-124 ppm region indicates that, with the smaller amount of formaldehyde, there are still many unsubstituted C-5 positions.

All of the new resonances are due to methylene carbons, as shown by a DEPT (Distortionless Enhancement by Polarization Transfer) pulse sequence (20) (Figure 5). This modern NMR experiment distinguishes among carbon types. It is a polarization-transfer experiment in which the 13C-signals are enhanced by transfer of magnetization from attached protons. In this particular version of DEPT, the methyls and methines are positive, methylenes are negative, and quaternaries are not observed. The unusually low field methylenes at 93.3 to 94.1 ppm were assigned to β- or γ-carbons of hemiformal chains, which arise because commercial formalin is a mixture of oxymethylene and hemiformal oligomers (21). The new resonances in the 60-70 ppm region are assigned to the α-carbons of these hemiformal chains, along with the hydroxymethyl carbons.
Figure 4. Alkaline treatments of loblolly pine MWL at 50 °C for 20 hours: a. control; b. unlabeled formaldehyde (10/l); c. labeled formaldehyde (0.4/l).
Figure 5. DEPT spectrum of labeled formaldehyde/lignin product.
Substitution of the acetate group in the hemiformal structures by a second aryl group is also feasible, since the chemical shift of the α-carbon will be little affected (21).

An apparent discrepancy in the intensity of the resonance at 61.3 ppm between Figures 4b (unlabeled) and 4c (labeled) might be explained by differences in the proportions and types of structures due to the large difference in formaldehyde concentration between the two treatments. Further reduction of the formaldehyde level to only 0.05 moles H$_2^{13}$C=O/mole C$_9$ still resulted in appreciable methylene signals (Figure 6). Even after 1 hour at 50 °C, there was a significant methylene bridge content (Figure 6b). The gradual reduction of the 5-hydroxymethyl resonance at 59.9 ppm and the hemiformal resonance at 93.3 ppm with time and temperature is readily apparent from the series of spectra in Figure 6.

Although the NMR spectra were not run under quantitative conditions, a very crude estimate, based on peak areas relative to the methoxyl peak, indicated that the large excess of unlabeled formaldehyde (Figure 4b) resulted in the substitution of about 20 C-5 positions per 100 C$_9$ units with CH$_2$ bridges per hydroxymethyl group in a ratio of 1/3. The smaller amount of labeled formaldehyde (Figure 4c) resulted in considerably less substitution: but there appear to be roughly equal amounts of CH$_2$ bridges and hydroxymethyl groups.

**Lignin Treatment in the Presence of Models.** Loblolly pine milled-wood lignin was treated with alkali at 140 °C for 1 hour in the presence of guaiacyl or veratryl lignin models VII or VIII (Figure 3), representing free and etherified β-O-4-phenylpropane units. In some treatments, models enriched with $^{13}$C at the γ-carbon (98%) were used. After the treatment, the reaction mixtures were acetylated and then fractionated on a polystyrene gel to remove low molecular weight byproducts and unreacted model. Typical chromatograms are illustrated in Figure 7. The high molecular weight (MW) portion (fraction A) accounted for over 85% of the material applied on the column. Fraction B, which was 7-8% of the total, was not investigated. Fraction C is the monomeric portion.

Condensation involving formaldehyde liberated from the γ-hydroxymethyl of the added guaiacyl model is confirmed by the series of partial spectra of the high MW portions (fraction A), illustrated in Figure 8. Although a small difference can be seen between the control (no model compound added) and the unlabeled guaiacyl model/lignin spectra in the diarglmethylene region, the major effect, is that of the label (difference between Figures 8b and 8c). In contrast, with the veratryl model (Figure 8d), the diarylmethylene region (about 35 ppm) is very similar to the control (Figure 8a). This is consistent with the observation that when the phenol is blocked, quinone methide formation is prevented, and liberation of formaldehyde, according to the scheme in Figure 1, does not occur (10,22). The lowest MW fraction (fraction C, Figure 7) in the guaiacyl model/lignin runs was found to be mainly composed of the β-guaiacyl vinyl ether, generated from the free-phenolic model analogous to the scheme illustrated in Figure 1, and accounted for 7 to 9% of the total.

As
Figure 6. Partial spectra of labeled formaldehyde/lignin runs (0.05/1): a. 50 °C/1 hour control; b. 50 °C/1 hour; c. 50 °C/4 hour; d. 50 °C/20 hour; e. 140 °C/1 hour; f. 140 °C/1 hour control.
Figure 7. Typical gel chromatograms from: a. guaiacyl model/ lignin; b. control or veratryl model/ lignin.
Figure 8. Partial spectra of high MW fractions of 140 °C lignin treatments in the presence of a. control; b. unlabeled guaiacyl model VII; c. γ-labeled guaiacyl model VII; d. γ-labeled veratryl model VIII.
expected, there was no evidence of vinyl ether in fraction C of either the control or veratryl model/lignin runs. It is presumed that unreacted veratryl model and its degradation products were present in fraction B, since many sharp resonances present in the spectrum of unfractionated reaction product were absent in both the high MU’ fraction A and the lowest MW fraction C. Vanillyl acetate was detected in fraction C in both the control and veratryl model/lignin runs by mass spectroscopy. Fraction C in these runs accounted for 1-3% of the total material.

Since vinyl ether formation does not result in ether cleavage and a reduction in molecular weight, the lignin vinyl ether’s corresponding to structures III (Figure 1) would be expected to remain in the high MW fraction. The presence of vinyl ether structures in alkali treated lignins is illustrated in Figure 9. Spectrum “a” is a mixture of cis and trans acetylated β-guaiacyl vinyl ether models corresponding to structure III in Figure 1. The resonances of interest are the β-carbons at 142.6 (cis) and 145.2 (trans) ppm. In the 140 °C control spectrum “b”, the corresponding lignin vinyl ether β-carbons can be seen, but are partly obscured by overlapping quaternary carbons. These β-carbons are highly deshielded relative to most of the other protonated carbons of lignin and appear in the quaternary carbon region. This fact becomes an asset when a pulse sequence such as DEPT is utilized. Since this technique does not detect quaternary carbons, the interfering peaks are removed and the deshielded protonated carbons are revealed. For example, the vinyl ether β-carbon resonances can now be cleanly assigned at 142.1 (cis) and 145.0 (trans) ppm in the DEPT spectrum “c”. Spectrum “d” is the product from a guaiacyl model/lignin treatment prior to chromatographic separation. Interestingly, both the lignin vinyl ether and the model vinyl ether resonances (sharp) are observed. The small difference in chemical shift is presumably due to the substitution in the 4’ position in the lignin vinyl ether. Finally, in the 50 °C control spectrum “e”, the absence of vinyl ether resonances can be explained by the fact that quinone methide formation (Figure 1) is too slow at this temperature (1).

Tentative assignments of β-carbon vinyl ether resonances in acetylated kraft lignin preparations have previously been made (23). Values of 144.3-144.4 ppm (trans) and 141.5-142.0 ppm (cis) in CDCl₃ were reported. However, a rather low-field (20 MHz) instrument was used along with the conventional proton noise-decoupled technique, so extreme overlap with quaternary carbons was unavoidable.

Along with the DEPT pulse sequence, a useful complement is the QUAT sequence, which detects only quaternary carbons (24). As illustrated in Figure 10, a QUAT spectrum of a 140 °C control lignin does not detect the protonated β-vinyl ether carbons that are present in both the DEPT and conventional spectra. However, some quaternary resonances do appear in the QUAT spectrum in the same position as does the trans vinyl ether resonance visible in the DEPT spectrum. Thus, it is generally not possible to accurately assign the trans resonance by conventional NMR spectroscopy. A disadvantage of the
Figure 9. Partial spectra illustrating vinyl ether formation in lignin: a. acetylated β-guaiacyl vinyl ether III; b. 140 °C control; c. DEPT spectrum of 140 °C control; d. guaiacyl model/lignin product prior to chromatographic separation; e. DEPT spectrum of 50 °C control.
Figure 10. Spectra of lignin product from 140 °C/1 hour treatment: a. conventional broadband (BB) decoupled; b: DEPT; c. QUAT.
QUAT experiment is that long recovery times are necessary for optimum acquisition of the slowly relaxing quaternary carbons.

Conclusions

Applying modern NMR techniques facilitated the examination of lignin products resulting from alkaline treatment of loblolly pine MWL in the presence of formaldehyde or lignin model compounds. It was determined that $^{13}$C-labeled formaldehyde liberated from a free-phenolic lignin model compound condensed at the C-5 position of the guaiacyl C units. The resulting hydroxymethyl substituted lignin was relatively unstable in base, even at 50 °C, and underwent further condensation leading to labeled methylene bridges between aryl units. As a result of formaldehyde liberation by a reverse-Aldol reaction, relatively stable vinyl ether structures were formed. It was clearly established by the use of the modern NMR pulse sequences, DEPT and QUAT, that vinyl ether structures were present in the lignin macromolecule following 140 °C alkaline treatments and absent in the lignin from corresponding 50 °C treatments.

Literature Cited


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