JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Comparative Studies on the Delignification of Pine Kraft−Anthraquinone Pulp with Hydrogen Peroxide by Binucleus Mn(IV) Complex Catalysis

CHEN-LOUNG CHEN,*,† EWELLYN A. CAPANEMA,† AND HANNA S. GRACZ‡

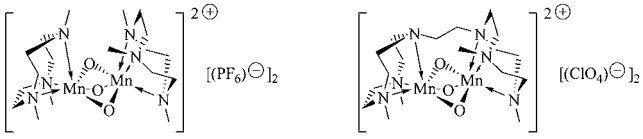
Department of Wood and Paper Science and Department of Biochemistry, North Carolina State University, Raleigh, North Carolina 27695

Pine kraft−anthraquinone (kraft−AQ) pulp was bleached in alkaline solution with hydrogen peroxide catalyzed by either $[L_1Mn(IV)(\mu\text{-}O)_3Mn(IV)L_1](PF_6)_2$ (**C1**) or $[LMn(IV)_2(\mu\text{-}O)_3](ClO_4)_2$ (**C2**) at 60 and 80 °C for 120 min with a catalyst charge of 10 ppm on pulp. The resulting bleached pulp was hydrolyzed with cellulase to obtain insoluble and soluble residual lignins. The alkaline bleaching effluents were acidified to precipitate alkaline-soluble lignins. These lignin preparations were then characterized by 2D heteronuclear multiple-quantum coherence (HMQC) NMR spectroscopic techniques. The results showed that biphenyl (5-5) and stilbene structures of the residual lignin in the pulp are preferentially degraded in both the **C1**- and **C2**-catalyzed bleachings, whereas *β-O*-4, *β*-5, and *β-β* structures undergo degradation to a lesser extent. In both cases, the degradation of the residual lignin increased with the increase in reaction temperature from 60 to 80 °C. Thus, the result of **C1**-catalyzed delignification is not in agreement with the observed decrease in the disappearance rate for substrates in the **C1**-catalyzed oxidation of lignin model compounds with hydrogen peroxide when the reaction temperature is increased from 60 to 80 °C. In addition, the resulting residual lignins in the **C2**-catalyzed bleaching at 80 °C are less degraded than the corresponding lignins in the **C1**-catalyzed bleaching at both 60 and 80 °C. Thus, **C1** is more effective than **C2** as catalyst in the binucleus Mn(IV) complex-catalyzed bleaching of pine kraft−AQ pulp with hydrogen peroxide.

KEYWORDS: Pine kraft−AQ pulp; binucleus Mn(IV) complex; hydrogen peroxide bleaching; catalysis; residual lignins; alkaline-soluble lignin; 2D HMQC NMR; epoxidation; oxidative cleavage

INTRODUCTION

Hydrogen peroxide has been shown to be a more effective delignifying agent at temperatures of <100 °C (*1*−*5*). In addition, it is not cost-effective to carry out the hydrogen peroxide bleaching of pulps at temperatures >120 °C, because of the high decomposition rate of hydrogen peroxide (*3*−*5*). Recently, however, it was demonstrated that upon addition of one of the binucleus Mn(IV) complexes $[L_1Mn(IV)(\mu\text{-}O)_3Mn(IV)L_1](PF_6)_2$ (**C1**), where L_1 is 1,4,7-trimethyl-1,4,7-triazacyclononane [Mn(IV)-TACN], and $[LMn(IV)(\mu\text{-}O)_3Mn(IV)](ClO_4)_2$ (**C2**), where L is 1,2-bis(4,7-dimethyl-1,4,7-triazacyclonon-1-yl)ethane, [Mn(IV)-DTNE] (**Figure 1**), hydrogen peroxide readily oxidizes nonphenolic lignin model compounds such as those with an α-hydroxyl group and double bonds conjugated to aromatic moieties at relatively low temperatures (30−80 °C), which otherwise would not be oxidized by hydrogen peroxide (*6*, *7*). Moreover, kinetic studies on the delignification of pine kraft−anthraquinone (kraft−AQ) pulp with hydrogen peroxide using **C2** as catalyst have shown that the selectivity and reactivity of hydrogen peroxide as an oxidant is improved appreciably (*8*−*10*).



$[L_2Mn(IV)_2(\mu\text{-}O)_3](ClO_4)_2$ (**C-1**)
[Mn(IV)-Me_3TACN]

L = 1,4,7-trimethyl-1,4,7-triazacyclononane

$[LMn(IV)_2(\mu\text{-}O)_3](ClO_4)_2$ (**C-2**)
[Mn(IV)-Me_4DTNE]

L = 1,2-bis-(4,7-dimethyl-1,4,7-triazacyclonon-1-yl)ethane

Figure 1. Structures of binucleus manganese complexes $[L_1Mn(IV)(\mu\text{-}O)_3Mn(IV)L_1](PF_6)_2$ (**C1**) and $[LMn(IV)\ (\mu\text{-}O)_3Mn(IV)](ClO_4)_2$ (**C2**).

It has been postulated that **C1** is less effective than **C2** as a catalyst for the delignification of pulps with hydrogen peroxide in alkaline solution on the basis of a literature review and experimental data (*10*). Very recently, however, it has been demonstrated that in the oxidation of lignin model compounds, **C1** is more effective than **C2** as catalyst in the binucleus Mn(IV) complex-catalyzed oxidation of 1-(3,4-dimethoxyphen-

* Author to whom correspondence should be addressed (e-mail chen-loung_chen@ncsu.edu).
† Department of Wood and Paper Science.
‡ Department of Biochemistry.

10.1021/jf034507f CCC: $25.00
Published on Web 09/12/2003

Table 1. Elemental Analyses and Yields of Lignin Preparations

lignin preparation	elemental analysis C (%)	H (%)	N (%)	yields (%) per residual lignin in pulp[a]	protein contaminants (%) per lignin preparation[b]	yields (%) corrected for protein contaminants[c]
C1-60-ISRL-B	56.71	5.92	<0.02	13.9	negligible	13.9
C1-60-SRL-B	54.98	6.22	4.07	32.0	25.4	23.9
C1-60-ASL-B	53.92	5.49	<0.02	30.0	negligible	30.0
C1-80-ISRL-B	55.98	6.02	<0.02	11.4	negligible	11.4
C1-80-SRL-B	54.64	5.93	4.76	36.8	29.8	25.9
C1-80-ASL-B	50.30	5.13	<0.02	39.8	negligible	39.8
C2-80-ISRL-B	50.01	5.46	<0.02	15.1	negligible	15.1
C2-80-SRL-B	55.96	6.36	3.81	45.6	23.8	34.7
C2-80-ASL-B	57.69	5.84	<0.02	22.3	negligible	22.3

[a] Lignin content in pulp (g) = oven-dried pulp used (g) × [(κ no. × 0.15)/100]. [b] Protein contaminants (%) = 6.25 × N%. [c] Yield corrected for protein contaminants (%) = uncorrected yield (%) × [1 − (6.25 × N%)/100].

yl)ethanol and 1-(3,4-dimethoxyphenyl)-1-propene with hydrogen peroxide but less effective in the oxidation of *E*-diphenylethene (*6*, *7*). In addition, the kinetic studies showed that the disappearance rates of 1-(3,4-dimethoxyphenyl)ethanol and 1-(3,4-dimethoxyphenyl)-1-propene in the first phase of **C1**-catalyzed oxidation with hydrogen peroxide increase up to the temperature range of 50−60 °C and then decrease with increasing reaction temperature. The cause for the decrease in the disappearance rates has not been established so far (*7*).

Recently, the catalyst **C2** has become available, and catalyst charge has been reduced to 10 from 60 ppm on pulp in the bleaching of softwood kraft−AQ pulps with hydrogen peroxide using **C2** as catalyst (*8*−*11*). In addition, reaction mechanisms in the **C2**-catalyzed bleaching of pine kraft−AQ pulp with hydrogen peroxide have been elucidated from the changes of structures occurring in the residual lignin in the pulp in the bleaching, using the two-dimensional (2D) heteronuclear multiple-quantum coherence (HMQC) NMR spectroscopic technique (*9*). In view of these developments, comparative studies were carried out on the structural changes of residual lignin in the **C1**-catalyzed deligninfication of pine kraft−AQ pulp with hydrogen peroxide in alkaline solution to evaluate the effect of reaction temperature on delignification as well as to compare the efficiencies of **C1** and **C2** as catalysts. The 2D HMQC NMR spectroscopic technique was chosen because it is one of the most effective NMR techniques for the characterization of lignin preparations (*12*−*15*).

EXPERIMENTAL PROCEDURES

Pulp, Binucleus Mn(IV) Complexes C1 and C2, and Cellulase. The southern pine kraft−AQ pulp with κ no. 28.7 was provided by the Covington mill of Westvaco Corp. Degussa AG, Hanau, Germany, supplied the catalysts $[L_1Mn(IV)(\mu\text{-}O)_3Mn(IV)L_1](PF_6)_2$ (**C1**) and $[LMn(IV)(\mu\text{-}O)_3Mn(IV)](ClO_4)_2$ (**C2**) used in this study. The cellulase (Fibre Zyme ACL) was purchased from Dyadic International, Inc. (Jupiter, FL). The cellulase preparation had a cellulase activity of 30000 units/mL.

C1- and C2-Catalyzed Bleaching of Pine Kraft—AQ Pulps with Hydrogen Peroxide. Before the H_2O_2 bleaching, the pulp was washed with an H_2SO_4 solution (pH 2). The kraft−AQ pulp (10 g, oven-dried) with a pulp consistency of 3% in deionized water was placed in a 50 mL Erlenmeyer flask, and the pulp slurry was adjusted to pH 2.0 by the addition of concentrated H_2SO_4. The flask was immersed in a constant-temperature water bath at 70 °C for 30 min under vigorous mechanical stirring. The pulp was removed from the bath, filtered, and washed thoroughly with deionized water. The κ no. of the resulting acid-washed pulp was 26.5. The acid-washed pulp (6 g, oven-dried), NaOH (0.12 g, 2% on oven-dried pulp), H_2O_2 (0.24 g, 4% on oven-dried pulp), 0.45 mL of 0.004% **C1** or **C2** solution in deionized water (10 ppm on oven-dried pulp), and appropriate amounts of deionized water to bring the pulp consistency to 10% were placed in a plastic bag. The active concentration of H_2O_2 was determined by addition of the appropriate volumes of 1 N KI and 4 N H_2SO_4 solutions and a few drops of saturated $(NH_4)_2MoO_4$ solution to the sample solution. The I_2 produced was titrated with 0.1 N $Na_2S_2O_3$ solution using starch solution as indicator. The plastic bag was then immersed in a constant-temperature water bath at 60 or 80 °C for 120 min. The starting pH of the reaction mixture was 11.5, and at the end of bleaching, the pH of the reaction mixture was ∼10.8. The variable in all of the experiments was reaction temperature. The concentration of H_2O_2 after the bleaching was determined; it was found that H_2O_2 was almost fully consumed. The κ no., viscosity, and GE brightness were measured according to Tappi standards T236, T230, and T452 (*16*), respectively.

Isolation and Purification of Residual Lignin and Purification of Dissolved Lignins. The residual lignins were isolated from the unbleached and bleached pine kraft−AQ pulps by treatment with cellulase in acetate buffer solution (pH 4.5) according to the procedure of Chang (*17*) to obtain the residual lignin from the pine kraft−AQ pulp (KRL) and the insoluble residual lignin from the pulp bleached at 60 or 80 °C (**C1**-60-ISRL-B, **C1**-80-ISRL-B, or **C2**-80-ISRL-B), respectively. A part of the residual lignins in the bleached pulp was dissolved in the cellulase solution (pH 4.5), which was recovered by acidification with concentrated H_2SO_4 solution to obtain the soluble residual lignin from the pulp bleached at 60 or 80 °C (**C1**-60-SRL-B, **C1**-80-SRL-B, or **C2**-80-SRL-B). The lignin in the alkaline bleaching effluents was precipitated with concentrated H_2SO_4 solution to obtain the alkaline-soluble lignin (**C1**-60-ASL, **C1**-80-ASL, or **C2**-80-ASL). The resulting lignin preparations were purified according to the procedure described by Chang (*17*). The elemental analyses, yields, amounts of protein contaminates in the isolated and purified lignin preparations, and the yields corrected for protein contaminates in the lignin preparations are summarized in **Table 1**. The yield of lignin preparations was calculated as weight percent of lignin preparations isolated per residual lignin content of the corresponding pulps used. The lignin content in a pulp was calculated by the following formula: lignin content in pulp (g) = oven-dried pulp used (g) × [(κ no. × 0.15)/100]. The amount of protein contaminants in a lignin preparation was calculated by the following formula: the amount of protein contaminants = 6.25 × N%. The yield of the lignin preparation was the corrected according to the following formula: yield corrected for protein contaminants (%) = uncorrected yield (%) × [1 − (6.25 × N%)/100]. The yield and elemental analytical data of **C2**-80-ISRL-B, **C2**-80-SRL-B, and **C2**-ASL-B were also described in detail in the previous work (*9*).

^{1}H—^{13}C Correlation 2D NMR Spectroscopy. The NMR spectra were recorded with a Bruker Avance 500 MHz spectrometer with the Oxford narrow-bore magnet after ∼40 mg of each lignin preparation had been dissolved in 0.75 mL of DMSO-d_6 containing 0.01% TMS as internal standard. All measurements were carried out with a 5 mm i.d. ^{1}H/BB (^{109}Ag−^{31}P) triple-axis gradient probe (Nalorac Cryogenic Corp.). The operational frequency for the ^{1}H nucleus was 500.128 MHz, and conditions for analysis included a temperature of 300 K, a 90 ° pulse width of 10 μs, and a 1.5 s pulse delay (d_1).

Table 2. Comparison of Physical Characteristics of Pulps Obtained from the Noncatalyzed and the **C1**- and **C2**-Catalyzed [Mn(IV)-Me_3TACN- and Mn(IV)-Me_4DTNE-Catalyzed] Delignification of Pulps with Hydrogen Peroxide[a]

	delignification			
pulp sample	κ no.	degree of delignification[b]	viscosity (mPa/s)	GE brightness
original pulp[c]	26.5		21.3	25.8
noncatalyzed delignification (60 °C)	19.8	25.3	17.3	30.1
C1 delignification (60 °C)	14.9	43.8	15.4	42.7
C2 delignification (60 °C)	16.4	38.1	15.2	40.3
noncatalyzed delignification (80 °C)	19.6	26.0	16.8	36.2
C1 delignification (80 °C)	13.9	47.5	15.0	42.8
C2 delignification (80 °C)	15.1	43.0	14.9	43.1

[a] Reaction conditions: see Experimental Procedures. [b] Based on κ no. of acid-washed pulp. [c] Acid-washed pulp.

RESULTS AND DISCUSSION

Delignification of Pine Kraft—AQ Pulp with Hydrogen Peroxide Using Binucleus Mn(IV) Complexes C1 and C2 as Catalysts. The results of the delignification (**Table 2**) showed that the noncatalyzed bleaching with hydrogen peroxide removed only ~25% of the residual lignin with a slight viscosity loss and brightness increase. An increase in the reaction temperature from 60 to 80 °C did not practically affect the degree of delignification in the absence of a catalyst but resulted in an additional viscosity loss and brightness improvement.

The catalyst charge used in the present investigation was 10 ppm on oven-dried pulp, 6 times lower than that in the previous study (*8*). In the **C1**- and **C2**-catalyzed delignification of pine kraft−AQ pulp with hydrogen peroxide at 60 °C, the degree of delignification was appreciably increased, from 25.3 to 43.8 and 38.1%, respectively, as compared to the noncatalyzed delignification at the same temperature. In addition, the brightness was also appreciably increased, from 30.1 to 42.7 and 40.3, respectively. However, the viscosity was slightly decreased. Furthermore, the increase of reaction temperature from 60 to 80 °C resulted in a slight increase of the degree of delignification but did not profoundly affect the brightness (**Table 2**). Thus, the catalyst **C1** was more effective in terms of lignin removal and selectivity than **C2** but not in improvement in the brightness. In the absence of a catalyst, hydrogen peroxide has an appreciable capability to act as a nucleophile and attacks the carbonyl groups, resulting in degradation of the chromophoric groups (*1-4*, *18*, *19*). However, the considerable decrease in κ no. and improvement in brightness of the bleached pulps resulting from the addition of either **C1** or **C2** as catalyst in the delignification (**Table 2**) showed that the catalysis improves the efficiency of hydrogen peroxide as an oxidant as well as a nucleophile.

Structural Analysis of Residual Lignin from Unbleached Pine Kraft−AQ Pulp (KRL). Structural characterization of residual lignins from the pine kraft−AQ pulps before and after the delignification gives information on reactions occurring in either the **C1**- or **C2**-catalyzed bleaching of the pulps with hydrogen peroxide. The structure of KRL has been described in detail previously (*9*, *12*−*14*, *20*, *21*). The oxygenated aliphatic region of the HMQC spectrum of KRL (**Figure 3A**) showed clearly the presence of arylglycerol-β-guaiacyl ether structures (β-*O*-4) (**1**), phenylcoumaran (β-5) (**2**), and pinoresinol (β-β) (**3**) structures (**Figure 2**) in the residual lignin. The cross-signals at δ_C/δ_H 71.8/4.76, 87.4/5.48, and 85.2/4.62 correspond to CH-α structures of **1**, **2**, and **3**, respectively. In addition, the cross-signal at δ_C/δ_H 84.6/4.28 corresponds to CH-β of **1** (*22*−*24*). The cross-signals for the CH-β of **2** and **3** (δ_C/δ_H 53.2/3.43 and 53.8/3.03, respectively) were overlapped with the very intensive cross-signal for the CH_3 of the Ar−OCH_3 centered at δ_C/δ_H 55.6/3.73 and were not discernible. The cross-signals at δ_C/δ_H 60.2/3.56 and 71.2/4.13 corresponded to CH-γ of **1** and **3**, respectively. The spectrum did not show the signals in the δ_C/δ_H range of 82−83/5.4−5.5, corresponding to CH-β in the β-*O*-4 moieties with an α-carbonyl group (**10**). The quantity of the structures of type **10** in the KRL was probably very small, below the detection limit (3 C_9 units/100 C_9 units). Moreover, the spectrum showed the presence of lignin−carbohydrate complexes. The cross-signals centered at δ_C/δ_H 81.6/4.56 corresponded to CH-α of the β-*O*-4 structure with a benzyl ether bond with C-6 of a β-hexose unit (**8**) (*25*, *26*), whereas the cross-signals at δ_C/δ_H 63.4/3.19 and 70.0/3.52 corresponded to CH-5 of the xylan backbone (**9**) and CH-6 of the hexose unit in the lignin−carbohydrate complexes, respectively. The cross-signal at δ_C/δ_H 102.6/4.23 corresponded to CH-1 of carbohydrates in sugar units without a reducing end-group. In addition, the cross-signals at δ_C/δ_H 73.6/3.02, 74.2/3.29, 75.1/3.41, and 77.0/3.12 corresponded to oxygenated CH groups of carbohydrates. This indicates that either the kraft−AQ pulping had not degraded the lignin−carbohydrate complexes completely or that the lignin−carbohydrate complexes were formed in the pulping process.

The aromatic region of the HMQC spectrum of KRL (**Figure 3B**) showed that the KRL is a characteristic guaiacyl lignin. The cross-signals corresponding to the CH-2, CH-5, and CH-6 of both etherified and nonetherified guaiacyl groups were observed at δ_C/δ_H 110.4/6.94, 115.4/6.77, and 118.9/6.79, respectively. The lignin also contained a biphenyl (5-5) structure (**4**), a stilbene structure (**5**), and 5-5 structures with an α-carbonyl and/or an α-carboxylic acid group (**7**). The very intensive cross-signal centered at δ_C/δ_H 120.5/6.68 corresponded to CH-6/CH-6′ of **4**, whereas cross-signals at δ_C/δ_H 112.2/7.53 and 126.2/7.59 corresponded CH-2 and CH-6 of etherifed **7** (L_1 = lignin moiety), respectively (*27*−*29*). In addition, the intensive cross-signals at δ_C/δ_H 120.6/7.26 and 128.5/7.2−7.4 corresponded to CH-6 and the conjugated −CH═CH− group of **5**, respectively (*27*). However, no vinyl ether type structures were detected in the KRL. Moreover, no cross-signals for a coniferyl aldehyde structure were detected, which is a potential chromophore. Thus, it is evident that both biphenyl (**4**) and stilbene (**5**) structures are among the abundant structures in the pine kraft−AQ residual lignin (KRL). The observed structures of KRL are in good agreement with these described for KRL in the previous reports (*9*, *12*−*14*, *20*, *21*).

Structural Analysis of Insoluble Residual Lignin from Pine Kraft—AQ Pulp Bleached at 60 °C, Using C1 as Catalyst (C1-60-ISRL-B). The oxygenated aliphatic region of the 2D HMQC NMR spectrum of **C1**-60-ISRL-B (**Figure 4A**) showed that the lignin contains β-*O*-4 (**1**) and β-5 (**2**) structures but not a β-β (**3**) structure. However, the signals for CH-α of **1** and **2** were less intense than the corresponding signals in the spectrum of KRL (**Figure 3A**). The signal for CH-α of **2** was almost not discernible. The spectrum also exhibited cross-signals for the CH-α of structure **8** centered at δ_C/δ_H 82.3/4.49, signals for the CH-6 of a hexose unit in lignin−carbohydrate complexes at δ_C/δ_H 69.5/3.53, and oxygenated CH groups of carbohydrates in the δ_C/δ_H range of 72.0−74.5/3.25−3.55. However, the spectrum did not show the signal for CH-5 of the xylan backbone (**9**) at δ_C/δ_H 62.6/3.21 and the CH-1 of carbohydrates at δ_C/δ_H around 102.6/4.23. This indicates that the lignin−carbohydrate complexes with xylan backbone (**9**) and, to some degree, the benzyl ether bonds in the structural type **8** are degraded in the bleaching. In addition, the spectrum showed new cross-signals in the δ_C/δ_H range of 61.0−67.5/4.00−4.50

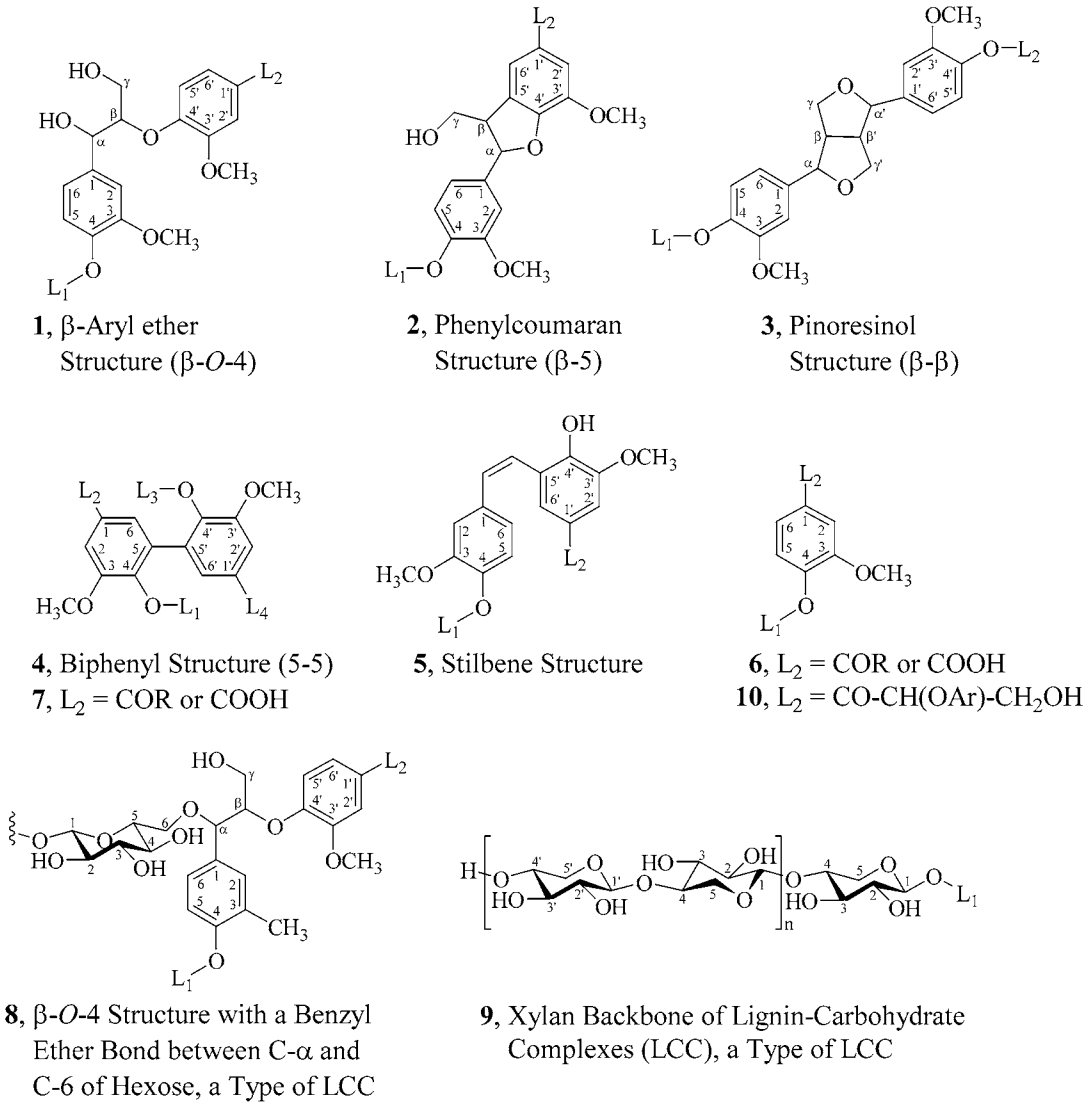


Figure 2. Major substructures in residual lignin preparations isolated from unbleached and bleached pine kraft-AQ pulps and beaching effluent. L_1 = H or lignin moiety; L_2, L_3, and L_4 = lignin moieties.

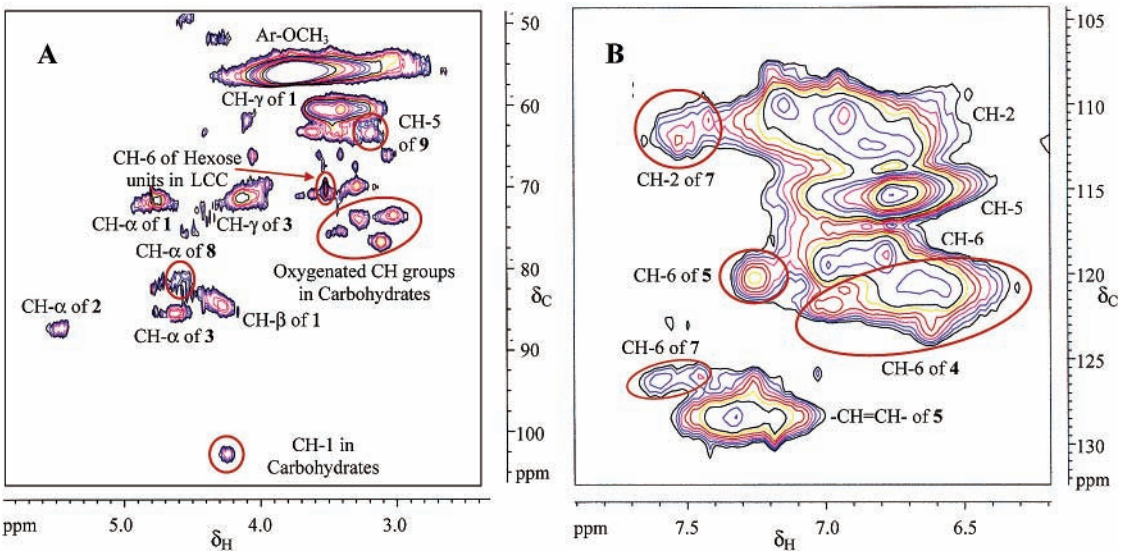


Figure 3. 2D HMQC NMR spectra of residual lignin from pine kraft–AQ pulp (KRL): (A) oxygenated aliphatic region; (B) aromatic region. Solvent: DMSO-d_6.

corresponding to oxygenated CH_2 and CH groups. These signals could be derived mostly by way of oxidative degradation of lignin carbohydrate complexes and/or oxidative cleavage of aromatic rings and side chains.

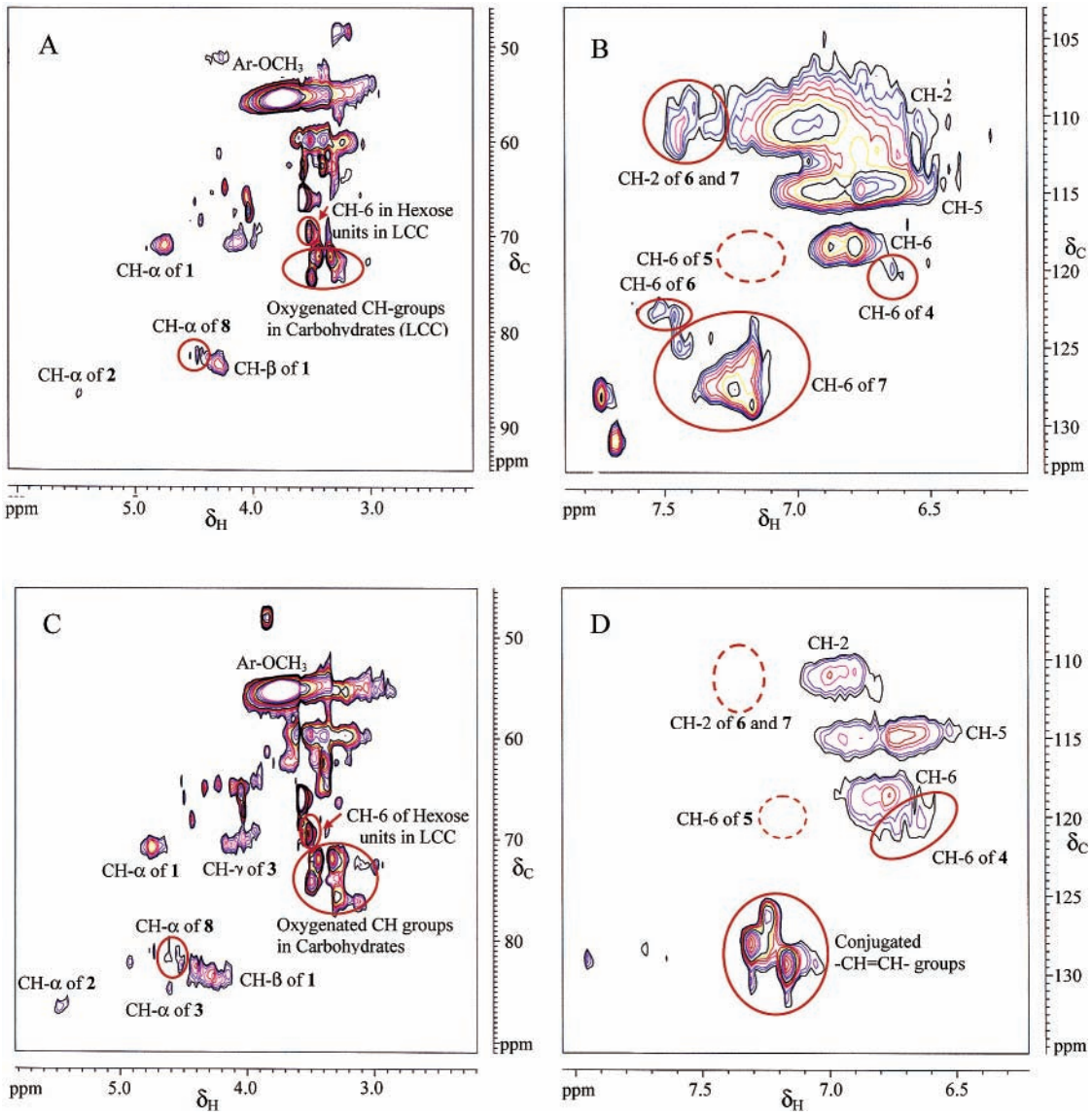


Figure 4. 2D HMQC NMR spectra of insoluble residual lignins **C1**-60-ISRL-B and **C1**-80-ISRL-B from pine kraft–AQ pulp bleached with hydrogen peroxide at 60 and 80 °C for 120 min using **C1** as catalyst: (A) oxygenated aliphatic region of **C1**-60-ISRL-B; (B) aromatic region **C1**-60-ISRL-B; (C) oxygenated aliphatic region of **C1**-80-ISRL-B; (D) aromatic region of **C1**-80-ISRL-B. Solvent: DMSO-d_6.

The aromatic region of the spectrum (**Figure 4B**) exhibited the cross-signals corresponding to CH-2, CH-5, and CH-6 of both etherified and nonetherified guaiacyl groups at δ_C/δ_H 110.7/6.99, 114.8/6.77 and 114.8/6.88, and 118.4/6.79 and 118.4/6.88, respectively. The cross-signal at δ_C/δ_H 120.0/6.65 corresponding to CH-6/CH-6′ of **4** was very weak in intensity as compared to the corresponding signal in the spectrum of KRL. In contrast, the cross-signals centered at δ_C/δ_H 127.8/7.24 corresponding to CH-6 of both etherified and nonetherified **7** (L_I = lignin moiety and H, respectively) and alkenic −CH═CH− group increased considerably in intensity. This cross-signal was shown to correlate to the cross-signal centered at δ_C/δ_H 111.0/7.53 corresponding to the CH-2 of etherified **7** by the corresponding 2D HMBC spectrum (spectrum not shown). However, the signal for CH-6 of **7** was much stronger than the signal for CH-2 of **7**. This indicates that the signal centered at δ_C/δ_H 127.8/7.24 is an overlapped signal consisting of signals for CH-6 of **7** and an alkenic −CH═CH− group of unknown nature. Moreover, the cross-signal at δ_C/δ_H 120.3/7.26 that corresponds to CH-6 of **5** was not discernible, in contrast to the corresponding spectrum of KRL. Thus, the −CH═CH− group of unknown nature is not that of the stilbene type structure **5**. The spectrum also showed rather weak signals at δ_C/δ_H 110.8/7.31 and 122.6−123.0/7.47−7.52 corresponding to CH-2 and CH-6 of an etherified guaiacyl structure with an α-carbonyl and/or α-carboxylic acid group (**6**). The structures **6** could be partly derived from oxidative cleavage of double bonds in the stilbene structure (**5**). This suggests that most of the 5-5 (**4**) and stilbene (**5**) structures undergo intensive oxidative cleavage of both side chains and aromatic rings in the bleaching. The −CH═CH− bond in **5** would undergo epoxidation to give an epoxide intermediate, followed by addition of hydroperoxide anions (HOO^-) under the reaction condition, leading to oxidation cleavage of the −CH═CH− bond with formation of benzaldehyde derivative of the type **6** (R = H) (*6*, *7*, *9*). The weak nature of the signal for CH-6 of structure **6** observed in the spectrum indicates that the amount of **6** in **C1**-60-ISRL-B is rather low and could not account for all of the stilbene structures (**5**) degraded via the oxidative cleavage of conjugated double bond. Consequently, the stilbene structures **5** must also undergo oxidative cleavage of aromatic rings, in addition to the oxidative cleavage of conjugated double bonds. The spectrum also exhibited cross-signals at δ_C/δ_H 128.2/7.74 and 131.1/7.69, the nature of which is not known. However, these signals could

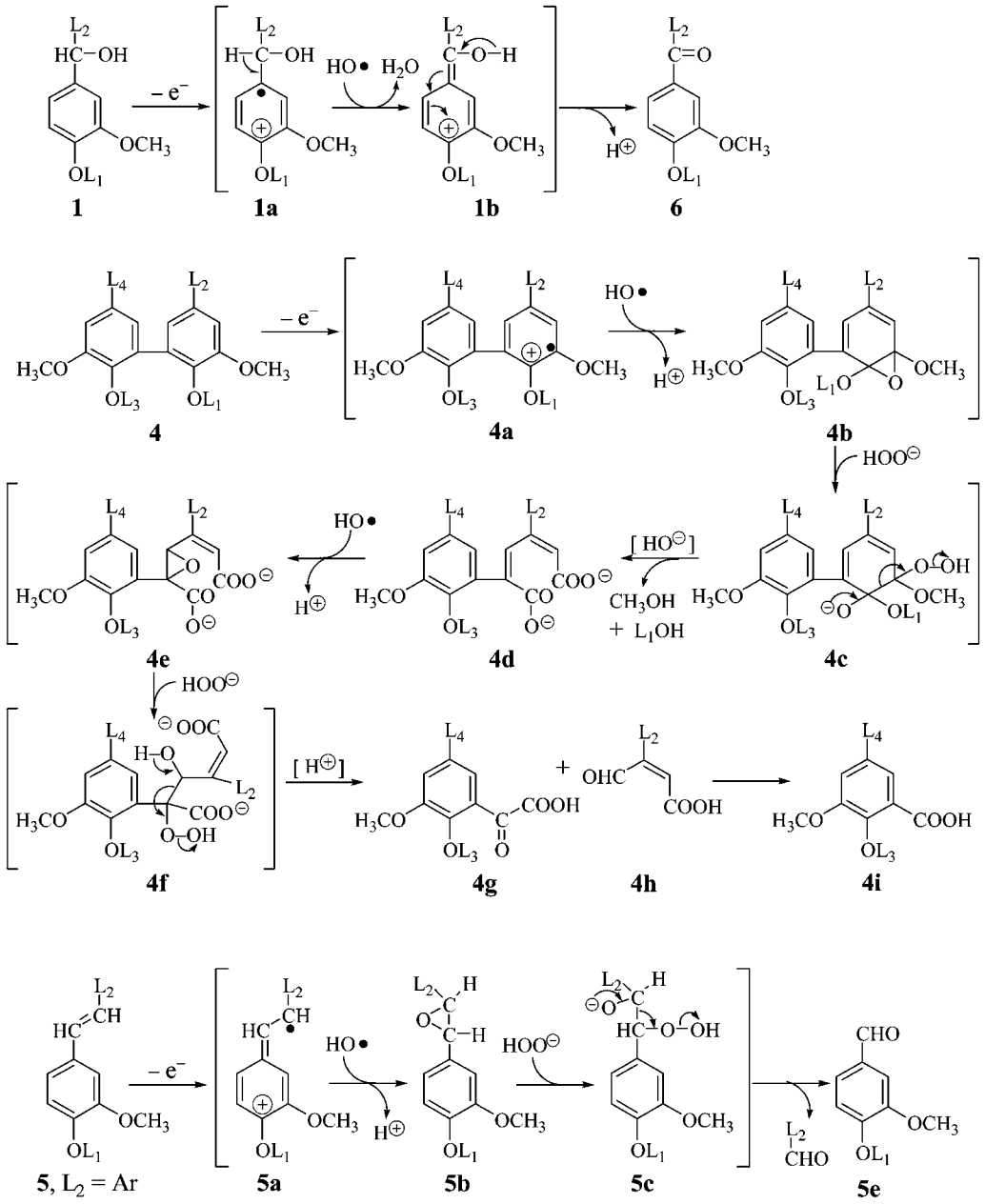


Figure 5. Reaction mechanisms for degradation of α-hydroxyl group in lignin substructures, and biphenyl (5-5) and stilbene substructures in **C1**- and **C2**-catalyzed bleaching of pine kraft–AQ pulp with hydrogen peroxide (*9*).

correspond to −CH═CH− groups conjugated to carboxyl group derived from oxidative cleavage of aromatic rings, in addition to the signals overlapped with the signal for CH-6 of **7**.

Structural Analysis of Insoluble Residual Lignin from Pine Kraft—AQ Pulp Bleached at 80 °C, Using C1 as Catalyst (C1-80-ISRL-B). The oxygenated aliphatic region of the 2D HMQC NMR spectrum of **C1**-80-ISRL-B (**Figure 4C**) showed that **C1**-80-ISRL-B contains *β-O*-4 (**1**), *β*-5 (**2**), and *β-β* (**3**) structures. However, the signals for CH-α of **2** and **3** were hardly discernible. The spectrum also exhibited the cross-signals for CH-α of structure **8** centered at δ_C/δ_H 82.4/4.51 and signals for CH-6 of the hexose unit in lignin−carbohydrate complexes at δ_C/δ_H 69.5/3.52 and oxygenated CH groups of carbohydrates in the δ_C/δ_H range of 71.8−76.0/3.12−3.50. However, the spectrum did not show the signal for the CH-5 of the xylan backbone (**9**) at δ_C/δ_H 62.6/3.21. Thus, the lignin−carbohydrate complexes with xylan backbone (**9**) and, to some degree, the benzyl ether bonds in the structural type **8** also undergo degradation in the bleaching at 80 °C as in the corresponding bleaching at 60 °C.

In the aromatic region of the spectrum (**Figure 4D**), the cross-signals of CH-2, CH-5, and CH-6 of both etherified and nonetherified guaiacyl groups were observed at δ_C/δ_H 110.9/6.99, 115.0/6.73 and 115.1/6.95, and 118.6/6.77−6.84, respectively. As in the corresponding spectrum of **C1**-60-ISRL-B (**Figure 4B**), the cross-signal at δ_C/δ_H 120.2/6.64 corresponding to CH-6/CH-6′ of **4** was also very weak. The spectrum did not contain the cross-signals at δ_C/δ_H 112.2/7.53 and 120.3/7.26 corresponding to the CH-2 of **6** and **7** and the CH-5 of **5**, respectively, indicating that 5-5 (**4**) and stilbene (**5**) structures were almost completely degraded. Thus, the cross-signals at 126.3/7.24, 128.0/7.31, and 129.3/7.17 do not correspond to the −CH═CH− of **5** and CH-6 of both etherified and nonetherified **7** (L_1 = lignin moiety and H, respectively). However, these signals could correspond to conjugated −CH═CH− groups, probably derived from oxidative degradation of the aromatic

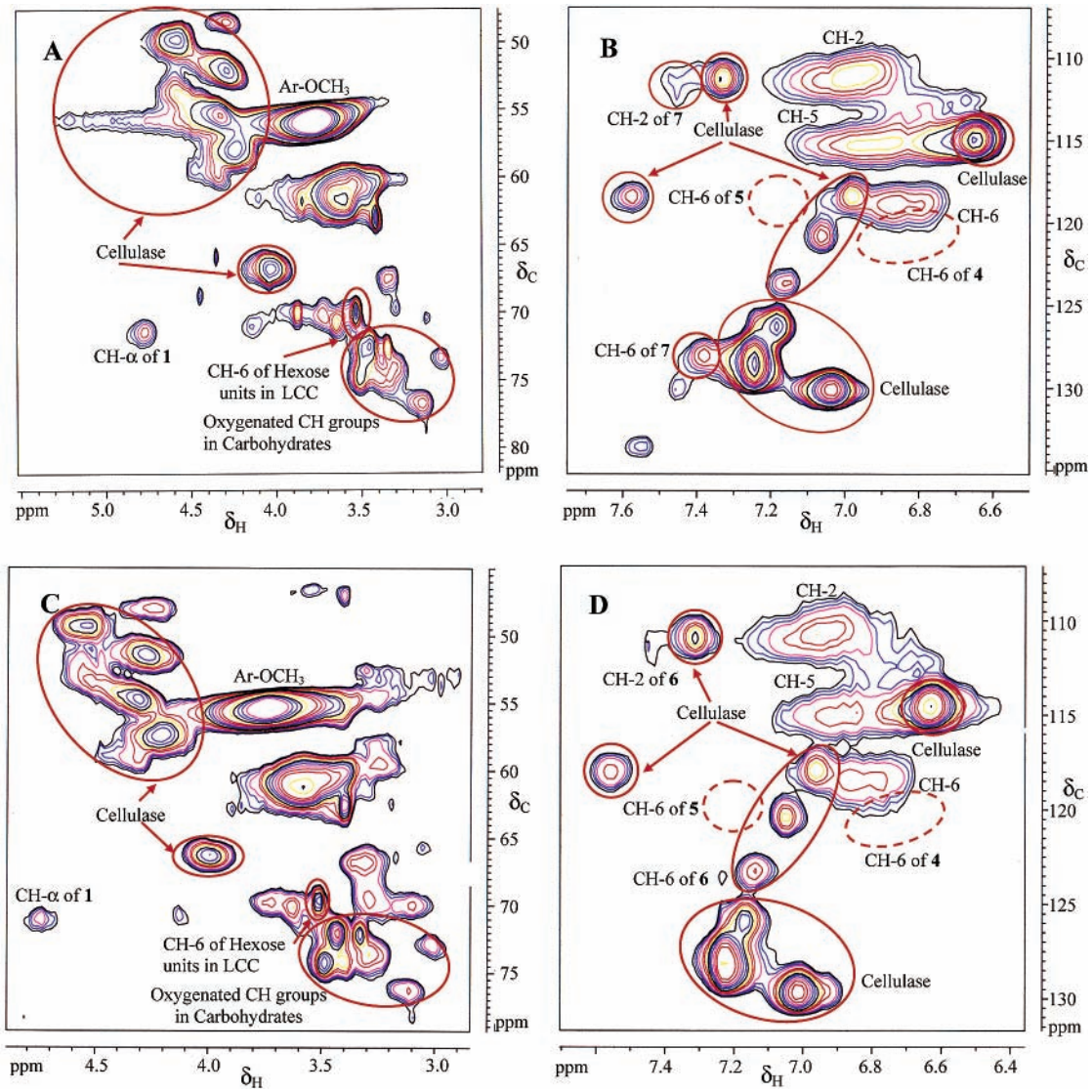


Figure 6. 2D HMQC NMR spectra of soluble residual lignins **C1**-60-SRL-B and **C1**-80-SRL-B from pine kraft–AQ pulp bleached with hydrogen peroxide at 60 and 80 °C for 120 min using **C1** as catalyst: (A) oxygenated aliphatic region of **C1**-60-SRL-B; (B) aromatic region of **C1**-60-SRL-B; (C) oxygenated aliphatic region of **C1**-80-SRL-B; (D) aromatic region of **C1**-80-SRL-B. Solvent: DMSO-d_6.

rings of **4** and **5** (see **Figure 5** for reaction mechanisms). The spectrum did not exhibit the cross-signals at δ_C/δ_H 128.2/7.74 and 131.1/7.69 observed in the corresponding spectrum of **C1**-60-ISRL-B (**Figure 4A**). The structural moieties giving rise to these signals probably undergo further oxidative degradation in the bleaching. Thus, it is evident that **C1**-80-ISRL-B is more intensively degraded than **C1**-60-ISRL-B. This is in good agreement with the fact that the resulting pulp from the **C1**-catalyzed bleaching of pine kraft−AQ pulp at 80 °C has a lower κ no. and viscosity than the corresponding pulp delignified at 60 °C.

Structural Analysis of Soluble Residual Lignin from Pine Kraft—AQ Pulp Bleached at 60 °C, Using C1 as Catalyst (C1-60-SRL-B). The **C1**-60-SRL-B contains ~25% of protein contaminants derived from cellulase, according to elemental analysis. In the oxygenated regions of the spectrum (**Figure 6A**), the cross-signals at δ_C/δ_H 48.6/4.28, 49.8/4.57, 52.2/4.28, 55.6/4.32, 58.0/4.24, and 66.8/4.03 were from the protein contaminants (*20*). Similarly, in the aromatic region of the spectrum (**Figure 6B**), the cross-signals at δ_C/δ_H 111.3/7.33, 114.9/6.65, 118.4/6.98, 118.4/7.57, 120.8/7.07, 123.6/7.16, 126.2/7.18, 128.5/7.24, and 130.1/7.03 were also from the protein contaminates. These were verified by comparing the spectrum of **C1**-60-SRL-B with the corresponding spectrum of cellulase.

Despite the contamination, the spectrum could be analyzed. In the oxygenated aliphatic region of the spectrum (**Figure 6A**), only weak cross-signals corresponding to CH-α and CH-β of the β-*O*-4 structure (**1**) were discernible (spectrum part exhibiting the signal for CH-β of **1** is not shown), but none of the signals for β-5 (**2**) and β-β (3) structures were. In addition, none of the cross-signals for CH-α of β-*O*-4 structure with a benzyl ether bond with C-6 of β-hexose (**8**) and CH-5 of the xylan backbone (**9**) were detected. However, the spectrum exhibited a weak cross-signal at δ_C/δ_H 70.0/3.53 corresponding to CH-6 of the hexose unit in lignin−carbohydrate complexes. In addition, the cross-signals in the δ_C/δ_H range of 72.0−76.0/3.10−3.51 corresponded to oxygenated CH groups of carbohydrates. Thus, these are additional pieces of evidence that the bleaching did affect the degradation of lignin−carbohydrate complexes with xylan backbone (**9**) and benzyl ether bonds of the type **8**, as discussed previously.

In the aromatic region of the spectrum (**Figure 6B**), none of the cross-signals corresponding to CH-6 of 5-5 and stilbene

structures (**4** and **5**) were discernible. However, the cross-signals for CH-2, CH-5, and CH-6 of both etherified and nonetherified guaiacyl groups were observed at δ_C/δ_H 111.2/6.97, 115.2/6.92, and 118.8/6.87, respectively. The absence of a cross-signal for the CH-6 of structure **5** at δ_C/δ_H 120.3/7.26 implied that there is no contribution of −CH═CH− of **5** to the signals in the δ_C/δ_H range of 123−130/7−7.3. Rather weak cross-signals at δ_C/δ_H 111.6/7.46 and 127.9/7.38 corresponded to CH-2 and CH-6 of nonetherifed **7** (L_1 = H), respectively. Consequently, the signals of protein contaminants did not interfere with the analysis of the spectra in this region. It is evident, therefore, that both 5-5 (**4**) and stilbene (**5**) structures undergo very intensive, oxidative degradation in the bleaching in addition to *β*-5 (**2**) and *β*-*β* (**3**) structures. As discussed previously, the stilbene structures (**5**) undergo oxidative cleavage of conjugated double bonds by way of epoxidation as well as oxidative cleavage of aromatic rings. In addition, the absence of 5-5 (**4**) structures and the presence of a rather small amount of structure **7** in the lignin indicate that most of the 5-5 (**4**) structures undergo degradation via oxidative cleavage of aromatic rings (**Figure 5**). This is in good agreement with the high solubility of the **C1**-60-RSL-B in the acetate buffer solution (pH 4.5), suggesting that the residual lignin undergoes rather intensive degradation with formation of hydrophilic groups in the bleaching.

Structural Analysis of Soluble Residual Lignin from Pine Kraft—AQ Pulp Bleached at 80 °C, Using C1 as Catalyst (C1-80-SRL-B). The HMQC spectrum of **C1**-80-SRL-B was similar to that of **C1**-60-SRL-B. However, the oxygenated aliphatic region of the spectrum (**Figure 6C**) exhibited rather strong cross-signals at δ_C/δ_H 66.8/3.30, 69.5/3.29, and 70.0/3.10, which were not present in the corresponding spectrum of **C1**-60-SRL-B (**Figure 6A**). Although the nature of these signals is unknown, they could be oxygenated CH groups derived from the degradation of aromatic rings and side chains.

The aromatic region of the spectrum (**Figure 6D**) did contain the cross-signals for the CH-2, CH-5, and CH-6 of etherified and nonetherified guaiacyl groups, but not the signals for the CH-2 and CH-6 of etherified **7**, in contrast to the corresponding spectrum of **C1**-60-SRL-B (**Figure 6B**). Instead, it contained very weak cross-signals at δ_C/δ_H 111.3/7.44 and 123.6/7.23 corresponding to the CH-2 and CH-6 of structure **6**, respectively. The spectrum also did not exhibit the signals for CH-6 of 5-5 (**4**) and CH-6 of stilbene (**5**) structures. Thus, **C1**-80-SRL-B was more intensively degraded than **C1**-60-SRL-B. Again, the high solubility of the **C1**-80-RSL-B in the acetate buffer solution (pH 4.5) indicated that the residual lignin underwent rather intensive degradation with formation of hydrophilic groups in the bleaching.

Structural Analysis of Alkaline-Soluble Lignin from Pine Kraft—AQ Pulp Bleached at 60 °C, Using C1 as Catalyst (C1-60-ASL-B). The oxygenated aliphatic region of the 2D HMQC NMR spectrum of **C1**-60-ASL-B (**Figure 7A**) showed that the lignin contained moderately intensive cross-signals corresponding to *β*-*O*-4 (**1**), *β*-5 (**2**), and *β*-*β* (**3**) structures. The spectrum also exhibited moderately strong signals for the CH-α of a *β*-*O*-4 structure with a benzyl ether lignin−carbohydrate bond of the type **8** centered at δ_C/δ_H 82.0/4.50, CH-5 of the xylan backbone (**9**) at δ_C/δ_H 62.7/3.18, and CH-6 of hexose units in lignin−carbohydrate complexes at δ_C/δ_H 69.4/3.51. In addition, the cross-signals at δ_C/δ_H 98.9/4.39 corresponded to CH-1 of carbohydrates in sugar units with reducing end-group, whereas the cross-signals at δ_C/δ_H 101/4.27 and 104.8/4.28 corresponded to CH-1 of carbohydrates in internal sugar units. The cross-signals in the δ_C/δ_H range of 71.5−77.5/3.00−3.56 corresponded to oxygenated CH groups of carbohydrates.

In the aromatic region of the spectrum (**Figure 7B**), the cross-signals for the CH-2, CH-5, and CH-6 of both etherified and nonetherified guaiacyl groups were observed at δ_C/δ_H 110.7/6.99, 114.8/6.74 and115.0/6.92, and 118.5/6.79 and 118.5/6.85, respectively. In addition, a very weak cross-signal δ_C/δ_H 119.9/6.64 corresponded to the CH-6 of stilbene structures (**5**), whereas a moderate cross-signal at δ_C/δ_H 119.3/7.20 corresponded to the CH-6/CH-6′ of 5-5 structures (**4**). A very weak signal at δ_C/δ_H 128.2/7.39 corresponded to the −CH═CH− of **5**. However, the spectrum exhibited a moderate cross-signal centered at δ_C/δ_H 111.0/7.45, corresponding to the CH-2 of guaiacyl and 5-5 structures with α-carbonyl groups in structures **6** and **7**. The rather weak cross-signals at δ_C/δ_H 122.6/7.49 and 125.4/7.41 corresponded to CH-6 of **6** and etherified **7** (L_1 = lignin moiety), respectively, whereas moderate a cross-signal at δ_C/δ_H 127.8/7.26 corresponded to the CH-6 of nonetherified **7** (L_1 = H). The structures **6** could be partly derived from oxidative cleavage of double bonds in the stilbene structure (**5**) as discussed previously. Signals corresponding to CH-2, CH-5, and CH-6 of guaiacyl groups were observed. Most 5-5 (**4**) and stilbene (**5**) structures apparently underwent intensive, oxidative degradation including oxidative cleavage of aromatic rings and side chains (**Figure 5**).

Structural Analysis of Alkaline-Soluble Lignin from Pine Kraft—AQ Pulp Bleached at 80 °C, Using C1 as Catalyst (C1-80-ASL-B). The oxygenated aliphatic region of the 2D HMQC spectrum of **C1**-80-ASL-B (**Figure 7C**) showed that the lignin was rather similar to that of **C1**-60-ASL-B (**Figure 7A**). The spectrum exhibited moderate cross-signals of *β*-*O*-4 (**1**), *β*-5 (**2**), and *β*-*β* (**3**) structures. Moreover, the cross-signals at δ_C/δ_H 62.8/3.18 and 69.4/3.51 corresponded to the CH-5 of the xylan backbone (**9**) and CH-6 of hexose units in lignin−carbohydrate complexes, respectively, whereas the very weak cross-signal at δ_C/δ_H 82.8/4.51 corresponded to the CH-α of a *β*-*O*-4 structure of the type **8**. In addition, the cross-signals at δ_C/δ_H 98.7/4.38 corresponded to CH-1 of carbohydrates in sugar units with a reducing end-group, whereas the cross-signals at δ_C/δ_H 101/4.27 and 104.8/4.28 corresponded to CH-1 of carbohydrates in internal sugar units. The cross-signals in the δ_C/δ_H range of 71.5−77.5/3.00−3.56 corresponded to oxygenated CH groups of carbohydrates. Thus, it is evident that the **C1**-80-ASL-B also contained considerable amounts of lignin−carbohydrate complexes by way of oxidative cleavages.

The aromatic region of the spectrum (**Figure 7D**) exhibited the cross-signals for the CH-2, CH-5, and CH-6 of both etherified and nonetherified guaiacyl groups at δ_C/δ_H 110.7/6.99, 114.8/6.77 and 115.0/6.94, and 118.4/6.78 and 118.4/6.86, respectively. A moderate cross-signal at δ_C/δ_H 120.3/7.26 corresponded to CH-6/CH-6′ of 5-5 structures (**4**). However, the spectrum did not show cross-signals at δ_C/δ_H 120.6/7.26 and 128.4/7.32 corresponding to CH-6 and −CH═CH− of stilbene structures (**5**), respectively. The cross-signal centered at δ_C/δ_H 111.2/7.04 corresponded to CH-2 of guaiacyl and 5-5 structures with an α-carbonyl group **6** and **7**. In addition, the cross-signal at δ_C/δ_H 122.6/7.49 corresponded to CH-6 of **6**, whereas those at δ_C/δ_H 125.4/7.42 and 127.0/7.22 corresponded to CH-6 of etherified and nonetherifed **7** (L_1 = lignin moiety and H), respectively. The structures **6** could be partly derived from oxidative cleavage of double bonds in stilbene structure (**5**) as discussed previously (**Figure 5**). Signals corresponding to CH-2, CH-5, and CH-6 of guaiacyl groups were also observed in the spectrum. Thus, it is evident that most of the 5-5 (**4**) and stilbene (**5**) structures undergo intensive, oxidative degradation, including oxidative cleavage of aromatic rings.

Effect of Reaction Temperature in C1-Catalyzed Bleaching. As discussed above, the degradation of residual lignin in pine kraft pulp increased with increasing reaction temperature from 60 to 80 °C. This was also supported by the physical characteristics of the resulting pulps (**Table 2**). Conceivably,

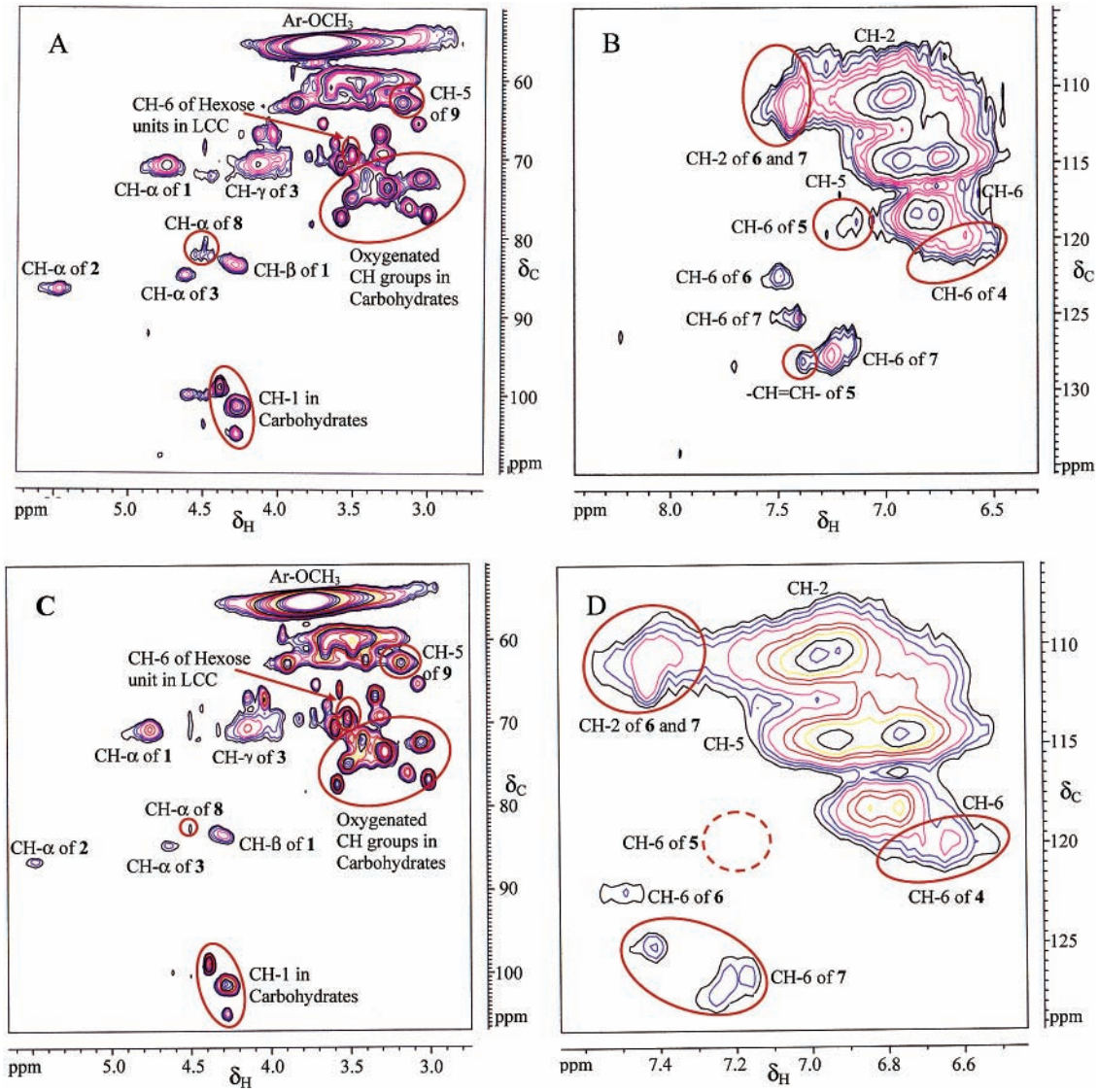


Figure 7. 2D HMQC NMR spectra of alkaline soluble lignins **C1**-60-ASL-B and **C1**-80-ASL-B from the bleaching effluent obtained after bleaching pine kraft–AQ pulp with hydrogen peroxide at 60 and 80 °C for 120 min using **C1** as catalyst: (A) oxygenated aliphatic region of **C1**-60-ASL-B; (B) aromatic region of **C1**-60-ASL-B; (C) oxygenated aliphatic region of **C1**-80-ASL-B; (D) aromatic region of **C1**-80-ASL-B. Solvent: DMSO-d_6.

the increase in the reaction temperature did not change the pathways by which the residual lignin is degraded but increased the rate of degradation reactions. Thus, the results of **C1**-catalyzed bleaching of pine kraft pulp are not in agreement with the observed decrease in the disappearance rate for substrates in the **C1**-catalyzed oxidation of lignin model compounds with hydrogen peroxide when the reaction temperature is increased from 60 to 80 °C in the lignin model compound experiments (*7*). Further investigation is thus required to elucidate the cause for the decrease in the model compound study.

Efficiency of C1 and C2 in Binucleus Mn(IV) Complex-Catalyzed Bleaching of Pine Kraft—AQ Pulp with Hydrogen Peroxide. The structural changes of residual lignin in the **C2**-catalyzed bleaching of pine kraft−AQ pulp with hydrogen peroxide at 80 °C have been previously studied (*9*). The HMQC NMR spectra of the **C2**-80-ISRL-B, **C2**-80-SRL-B, and **C2**-80-ASL-B were compared with the corresponding spectra from the **C1**-catalyzed bleaching of pine kraft−AQ pulp with hydrogen peroxide at 60 and 80 °C. The results clearly showed that the residual lignin in the pulp in the **C2**-catalyzed bleaching at 80 °C was less degraded than in the **C1**-catalyzed bleaching at both 60 and 80 °C. This is good agreement with the results of physical characteristic of pulps obtained including the degree of delignification (**Table 2**). However, the reaction mechanisms for delignication are almost similar in the **C1**- and **C2**-catalyzed bleachings of pine kraft−AQ pulp, and the difference is quantitative rather than qualitative. In general, the *β*-5 (**2**) and *β*-*β* (**3**) structures underwent more intensive degradation than the *β*-*O*-4 (**1**) structure in the **C1**-catalyzed bleaching as compared to that in the **C2**-catalyzed bleaching. The 5-5 (**4**) and stilbene (**5**) structures were also more intensively degraded in the **C1**-catalyzed bleaching than in the **C2**-catalyzed bleaching.

ABBREVIATIONS USED

Me_3TACN, 1,4,7-trimethyl-1,4,7-triazacyclononane; Me_4-DTNE, 1,2-bis(4,7-dimethyl-1,4,7-triazacyclonon-1-yl)ethane; HMQC, heteronuclear multiple-quantum coherence; HMBC, heteronuclear multiple-bond coherence; KRL, residual lignin from unbleached kraft-AQ pulp; AQ, anthraquinone.

ACKNOWLEDGMENT

We are thankful to Degussa AG, Hanau, Germany, for donation of the catalyst Mn(IV)-Me_4DTNE.

LITERATURE CITED

(1) Lachenal, D.; Choundens, C.; Monzie, P. Hydrogen peroxide as delignifying agent. *Tappi* **1980**, *63*, 119−122.

(2) Alphan, C.; Andersson, E.; Andersson, S.; Hook, J. E. High-temperature peroxide bleaching of sulfate pulp. *Svensk Papperstidn.* **1997**, *80*, 480−482.

(3) Lachenal, D. Hydrogen peroxide as a delignifying agent. In *Pulp Bleaching−Principles and Practice*; Dence, C. W., Reeve, D. W. Eds.; TAPPI Press: Atlanta, GA, 1996; Chapter 6, pp 347−361.

(4) Anderson, J. R.; Amini, B. Hydrogen peroxide bleaching. In *Pulp Bleaching−Principles and Practice*; Dence, C. W., Reeve, D. W. Eds.; TAPPI Press: Atlanta, GA, 1996; Chapter 10, pp 347−361.

(5) Gullichsen, J. Fiber line operation. In *Chemical Pulping*; Gullichsen, J., Fogelhom, C.-J., Eds.; Fapet, Oy: Helsinki, Finland, 1999; pp 19−243.

(6) Cui, Y.; Chen, C.-L.; Gratzl, J. S.; Patt, R. A manganese(IV) complex catalyzed oxidation of lignin model compounds with hydrogen peroxide. *J. Mol. Catal. A*: *Chem*. **1999**, *144*, 411−417.

(7) Alves, V. L.; Capanema, E. A.; Chen, C.-L.; Gratzl, J. S. Comparative studies on oxidation of lignin model compounds with hydrogen peroxide using Mn(IV)-Me_3TACN and Mn(IV)-Me_4DTNE as catalyst. *J. Mol. Catal. A*: *Chem*. **2003**, *206* (1−2), 37−51.

(8) Cui, Y.; Puthson, P.; Chen, C.-L.; Gratzl, J. S.; Kirkman, A. G. Kinetic study on delignification of kraft-AQ pine pulp with hydrogen peroxide catalyzed by $Mn(IV)_2$-Me_4DTNE. *Holzforschung* **2000**, *54*, 413−418.

(9) Chen, C.-L.; Capanema, E. A.; Gracz, H. S. Reaction mechanisms in delignification of pine kraft-AQ Pulp with hydrogen peroxide using Mn(IV)-Me_4DTNE as catalyst. *J. Agric Food Chem.* **2003**, *51*, 1932−1941.

(10) Odermatt, J.; Kordsachia, O.; Patt, R.; Kühne, L.; Chen, C.-L.; Gratzl, J. S. A manganese-based catalysts for alkaline peroxide bleaching. In *Oxidative Delignification Chemistry−Fundamentals and Catalysis*; Argyropoulos, D. S., Ed.; ACS Symposium Series 785; American Chemical Society: Washington, DC, 2001; Chapter 14, pp 234−254.

(11) Capanema, E. A.; Balakshin, M. Yu.; Chen, C.-L.; Gratzl, J. S.; Gracz, H. S. Studies on kraft pulping lignins using HMQC NMR techniques. In *Proceedings of 7th Brazilian Symposium on the Chemistry of Lignins and Other Wood Components*, Belo Horizonte, MG, Brazil, Sept 2−5, 2001; pp 61−68.

(12) Tamminen, T. L.; Hortling, B. R. Isolation and characterization of residual lignin. In *Advances in Lignocellulosics Characterization*; Argyropoulos, D. S., Ed.; Tappi Press: Atlanta, GA, 1999; pp 1−42.

(13) Ralph, J.; Marita, J.; Ralph, S. A.; Hatfield, R. D.; Lu, F.; Ede, R. M.; Peng, J.; Quideau, S.; Helm, R. F.; Grabber, J. H.; Kim, H.; Jimenez-Monteon, G.; Zhang, Y.; Jung, H. J. G.; Landucci, L. L.; MacKay, J. J.; Sederoff, R. R.; Chapple, C.; Boudet, A. M. Solution-State NMR of Lignins. In *Advances in Lignocellulosics Characterization*; Argyropoulos, D. S., Ed.; Tappi Press: Atlanta, GA, 1999; pp 55−108.

(14) Ammalahti, G.; Brunow, G.; Bardet, M.; Robert, D.; Kilpelainen, I. Identification of side-chain structures in a poplar lignin using three-dimensional HMQC-HOHAHA NMR spectroscopy. *J. Agric. Food Chem.* **1998**, *46*, 5113−5117.

(15) Zhang, L.; Gellerstedt, G. Detection and Determination of Carbonyls and Quinones by Modern NMR Techniques. *Proceedings of the 10th International Symposium on Wood and Pulping Chemistry*, Yokohama, Japan, June 7−10, 1999; Vol. II, pp 164−170.

(16) Tappi. *TAPPI Test Methods 1996−1997*; TAPPI Press: Atlanta, GA, 1996.

(17) Chang, H.-M. Isolation of lignin from pulp. In *Methods of Lignin Chemistry*; Lin, S. Y., Dence, C. W., Eds.; Springer-Verlag: Heidelberg, Germany, 1992; pp 71−74.

(18) Pan, X.; Lachenal, D.; Lapierre, C.; Monties, B. Structure and reactivity of spruce mechanical pulp lignins. Part III. *J. Wood Chem. Technol*. **1993**, *13*, 145−165.

(19) Pan, X.; Lachenal, D.; Lapierre, C.; Monties, B. Structure and reactivity of spruce mechanical pulp lignins. Part IV. *J. Wood Chem. Technol*. **1994**, *14*, 483−506.

(20) Balakshin, M. Yu.; Capanema, E. A.; Chen, C.-L.; Gratzl, J. S.; Kirkman, A. G.; Gracz, H. Biobleaching of pulp with dioxygen in the laccase-mediator system−Reaction mechanisms for degradation of residual lignin. *J. Mol. Catal. B: Enzymatic* **2001**, *13* (1−3), 1−16.

(21) Capanema, E. A.; Balakshin, M. Yu.; Chen, C.-L.; Gratzl, J. S.; Gracz, H. Structural analysis of residual and technical lignins by $^1H-^{13}C$ correlation 2D NMR spectroscopy. *Holzforschung* **2001**, *55*, 302−308.

(22) Ralph, S. A.; Ralph, J.; Landucci, L. L. NMR database of lignin and cell wall model compounds; 2001; available at URL http://www.dfrc.wisc.edu/software.html, accessed January 2003.

(23) Okusa, K.; Miyakoshi, T.; Chen, C.-L. Comparative studies on dehydrogenative polymerization of coniferyl alcohol by laccases and peroxidases. *Holzforschung* **1996**, *50*, 15−23.

(24) Miyakoshi, T.; Chen, C.-L. ^{13}C NMR spectroscopic studies of phenylcoumaran and 1,2-diarylpropane type lignin model compounds. *Holzforschung* **1991**, *45* (Suppl.), 41−47.

(25) Freudenberg, K.; Grion, G. Beitrag zum Bildungsmechanismus des Lignins und der Lignin-Kohlenhydrat-Bindung. *Chem. Ber*. **1959**, *92*, 1355−1363.

(26) Tokimatsu, T.; Umezawa, T.; Shimada, M. Synthesis of four diastereomeric lignin carbohydrate complexes (LCC) model compounds composed of a β-*O*-4 lignin model linked to methyl β-D-glycoside. *Holzforschung* **1996**, *50*, 156−160.

(27) Kringstad, K. P.; Mörck, R. 13C-NMR spectra of Kraft lignins. *Holzforschung* **1983**, *37*, 237−244.

(28) Drumond, M. G.; Lemos de Morais, S. A.; Piló-Veloso, D.; Santos Cota, S. D.; Do Nascimento, E. A.; Chen, C.-L. Biphenyl type lignin model compounds: Synthesis and ^{13}C NMR substituent chemical shift additivity rule. *Holzforschung* **1992**, *46*, 127−134.

(29) Alves, V. L.; Drumond, M. G.; Stefani, G. M.; Chen, C.-L.; Piló-Veloso, D. Synthesis of new trimeric lignin model compounds containing 5-5′ and β-*O*-4 substructures, and their characterization by 1D and 2D NMR techniques. *J. Braz. Chem. Soc.* **2000**, *11*, 467−473.

Received for review May 15, 2003. Revised manuscript received August 1, 2003. Accepted August 5, 2003. This research project was supported by an USDA National Research Initiative Competitive Grand under Cooperative Agreement 98-35504-6781, for which we are grateful.

JF034507F