Fractional and Structural Characterization of Lignins Isolated by Alkali and Alkaline Peroxide from Barley Straw

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A sequential treatment of dewaxed barley straw with sodium hydroxide, different concentrations of hydrogen peroxide, and potassium hydroxide/sodium borate degraded various proportions of the original lignin and solubilized different amounts of the original hemicelluloses. The isolated lignin fractions were subjected to comprehensive structural characterization by UV, FT-IR, and ^{13}C NMR spectroscopy, and their chemical compositions were analyzed by alkaline nitrobenzene oxidation. All of the lignin fractions were typical of grass lignins and had weight-average molecular weights between 1750 and 2190. It was found that the peroxide treatment at low concentrations (≤2.0%) resulted in a slight increase in the amount of carboxyl groups, whereas the treatment at a relatively high concentration of alkaline peroxide, such as at 3.0% H_2O_2, led to a noticeable oxidation of the lignins, as shown by an increase of carboxyl groups. Moreover, the results obtained indicated that the successive treatments with alkali and alkaline peroxide under the conditions used did not significantly affect the *β-O-4* structures of lignins. Substantial amounts of etherified ferulic acids were cleaved by the sequential treatments with alkaline peroxide, as shown in the ^{13}C NMR spectra. The results underscore the structural differences between alkali- and alkaline peroxide-soluble lignins from barley straw.

Keywords: *Lignin; barley straw; alkali; alkaline peroxide; nitrobenzene oxidation, molecular weight; FT-IR; ^{13}C NMR*

INTRODUCTION

In recent years, there has been an increased demand on a world level for paper, and due to exhaustive exploitation of forests, new sources of fibers are of great interest. Annual plant fibers, such as straw materials and grasses, are by far the largest source of nonwood fibers for paper-making. For example, at present China produces more than two-thirds of the nonwood pulp produced worldwide (*1*). In addition, it is possible to operate small pulp units particularly adapted to the needs of developing countries using nonwood species as fibrous resources (*2*). Furthermore, environmental concerns have led to the development of a bleaching process without chlorine or chlorine dioxide, and great interest is presently focused on oxygen-containing bleaching agents such as oxygen, ozone, and hydrogen peroxide (*3*). Unfortunately, oxygen cannot exceed 50% delignification because of its limited selectivity, and ozone exhibits poor bleaching selectivity and low generation efficiency. Because ozone is a very aggressive oxidant and reacts rapidly with pulp, improved equipment will be required to achieve a homogeneous bleaching effect (*4*). Consequently, high-yield pulps produced from cereal straws and bleached with hydrogen peroxide best meet the environmental objectives for pulp manufacture (*2*).

The bleaching effect of hydrogen peroxide has generally been attributed to the action of the perhydroxyl anion, which is a strong nucleophile and reacts with chromophores in the lignin (*5*−*7*). In general, peroxide bleaching is performed in an alkaline solution because the concentration of perhydroxyl anions increases with increasing alkalinity. Under such conditions, however, hydrogen peroxide may decompose to water and oxygen, the latter having little or no bleaching action. This decomposition proceeds predominantly through the formation of radical intermediates, such as hydroxyl radicals ($HO^{\bullet}$) and superoxide anion radicals ($O_2^{\bullet -}$). These radicals oxidize the lignin, leading to depolymerization and the introduction of hydrophilic groups (*3*). The radicals have a somewhat higher reactivity toward the aromatic structure in lignin than polysaccharides, such as cellulose and hemicelluloses, and this promotes the oxidation and degradation of the lignins (*8*). Therefore, it is likely that slight decomposition of hydrogen peroxide may be beneficial because unreactive aromatic structures in the straw lignin can be degraded and oxidized, whereas the hemicelluloses are solubilized or degraded and cellulose is left fairly intact (*3*, *9*).

Lignins are a major obstacle to efficient utilization of plants for paper-making or animal feed (*10*). They are produced by oxidative coupling of three major C_6-C_3 (phenylpropanoid) units, namely, syringyl alcohol, guaiacyl alcohol, and *p*-coumaryl alcohol. Unlike other natural polymers such as proteins, polysaccharides, and

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nucleic acids, which have interunit linkages susceptible to enzymatic and chemical hydrolyses, lignin contains resistant carbon−carbon and biphenyl ether bonds (*11*, *12*). Along with the some 20 different types of bonds present within the lignin itself, lignin seems to be particularly associated with hemicellulosic polysaccharides (*13*). It is well recognized that cleaving ether linkages in lignin, the basis of chemical pulping and various lignin analytical methods, effects a dramatic degree of depolymerization.

Nevertheless, the chemical structure of cereal straws is different from and less studied than that of woods. Although the lignin content of cereal straw cell walls is lower than that in wood cell walls, the chemical structure of its lignin is more complex (*14*). Moreover, the presence of hydroxycinnamic acids, such as *p*-coumaric and ferulic acids, results in a more difficult characterization of the lignins from cereal straw and grass (*2*). In particular, little attention has been paid to the structural changes of the lignins during the new delignifying process with alkaline peroxide. The first study of this dual role of hydrogen peroxide in delignifying and bleaching agricultural residues was reported by Gould (*15*, *16*), who showed that approximately half of the lignin and most of the hemicelluloses present in wheat straw are solubilized when the residue is treated at 25 °C in an alkaline solution of 1% H_2O_2. However, these studies did not investigate the isolation, purity, molar mass distribution, and structural changes of the degraded lignins.

In the present study, we have investigated the chemical changes taking place in barley straw lignin when it is sequentially treated with increasing concentrations of alkaline hydrogen peroxide. The observed differences in lignin structure can be directly related to changes in the alkaline peroxide treatment processes.

MATERIALS AND METHODS

Materials. Barley straw was obtained from the experimental farm of the North-Western Science and Technology University of Agriculture and Forestry (Yangling, People's Republic of China). The straw was milled to pass a 0.8 mm size screen and dried in an oven at 60 °C for 16 h. The straw powder (60 g) was then submitted to extraction with toluene/ethanol (2:1, v/v, 2800 mL) in a Soxhlet for 6 h to remove the wax and other extractives. The composition (w/w) of the straw used was 37.5% cellulose, 37.1% hemicelluloses, 15.8% lignin, 2.6% protein, 2.6% extractives, and 4.2% ash, which contains 68.6% silica.

Sequential Alkali and Alkaline Peroxide Extraction. About 50 g of the dewaxed straw was sequentially treated with 0.1 M NaOH at 45 °C for 3 h; with 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0% H_2O_2 at 45 °C for 3 h at pH 11.5; and with 10% KOH/1% $Na_2B_4O_7{\cdot}10H_2O$ at 28 °C for 15 h under continuous agitation. The extractant-to-straw ratio was fixed at 20 mL/1 g in all of the extracting steps. During the alkaline peroxide treatments, no further adjustments in pH were made. Under these conditions, the reaction pH remained nearly constant for 2 h before slowly rising to a final value of ~12.5. After treatment at the desired concentration for the required time, the reaction was terminated by cooling with cold water and the solutions were filtered through a nylon cloth; the residues were washed with water and ethanol and then dried at 60 °C for 16 h. The solubilized hemicelluloses were isolated from each of the hydrolysates by precipitation of the neutralized hydrolysates (pH 5.5 adjusted with 6 M HCl) with 3 volumes of 95% ethanol. The degraded lignins were obtained by reprecipitation at pH 1.5, adjusted by 6 M HCl, from the corresponding supernatant solutions. The isolated lignin fractions were purified by washing with acidified water (pH 2.0), freeze-dried, and kept at 5 °C until analysis (Figure 1).

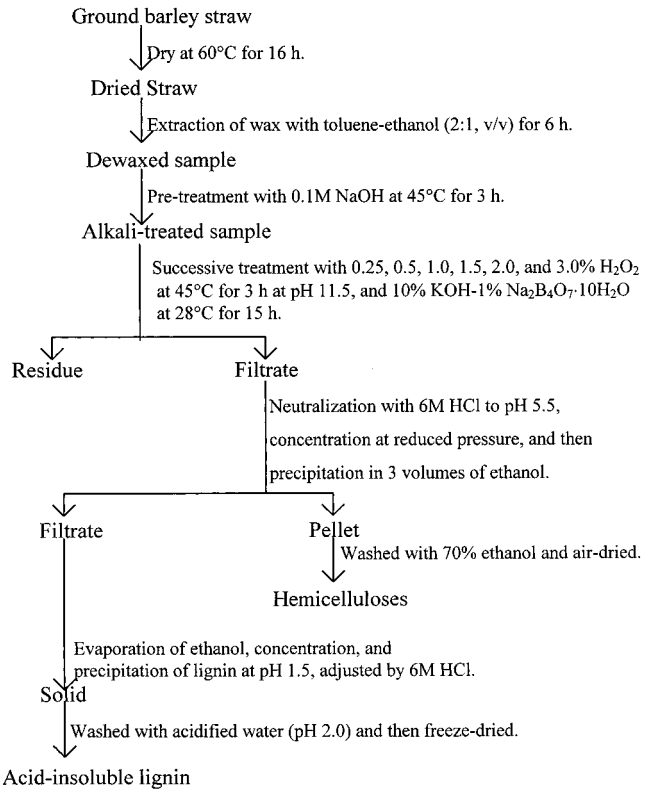

Figure 1. Scheme for isolation of acid-insoluble lignin fraction from the hydrolysates of alkaline peroxide extraction of the 0.1 M NaOH-treated barley straw.

Chemical and Thermal Characterization of the Acid-Insoluble Lignin Fractions. Acid-insoluble lignin fractions were subjected to alkaline nitrobenzene oxidation at 175 °C for 2.5 h. The phenolic acids and aldehydes liberated were separated on a 250 × 4.6 mm i.d. Hichrom H5ODS HPLC column (Phenomenex Co., Beijing, China). Individual compounds were detected at 280 nm and identified by comparison of the retention times and peak areas with the authentic phenolics. The content of contaminating polysaccharides in isolated lignin fractions was determined by their neutral sugar composition with gas chromatography (GC) as their alditol acetates (*17*). Methods for recording UV spectra and determination of molecular-average weights of the acid-insoluble lignin fractions are described in previous papers (*18*, *19*).

FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet 510) using a KBr disk containing 1% finely ground lignin samples. The solution ^{13}C NMR spectra were recorded on a Bruker MSI-300 spectrometer at 74.5 MHz from 180 mg of sample dissolved in 1.0 mL of DMSO-d_6 after 22000 scans.

Thermal analysis of the acid-insoluble lignin preparations was performed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) on a simultaneous thermal analyzer (NETZSCH STA-409). The apparatus was continually flushed with nitrogen. Each sample weighed between 8 and 12 mg and was heated from room temperature to 600 °C at a rate of 10 °C/min.

RESULTS AND DISCUSSION

Yield of Lignin. The yield of lignin fractions was expressed as a percentage of dry starting material. Table 1 shows that successive treatment of dewaxed barley straw with 0.1 M NaOH at 45 °C for 3 h; with 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0% H_2O_2 at 45 °C for 3 h at pH 11.5; and with 10% KOH/1% $Na_2B_4O_7{\cdot}10H_2O$ at 28 °C for 15 h, resulted in a dissolution of 29.1, 15.8,

Table 1. Yields of Lignin (Percent Dry Matter) Solubilized by Successive Treatment of Barley Straw with 0.1 M NaOH, Various Concentrations of Alkaline Hydrogen Peroxide, and 10% KOH/1% $Na_2B_4O_7 \cdot 10H_2O$ at 28 °C for 15 h

		H_2O_2 concn (%)							
lignin	AS1[a]	0.25[b]	0.5[b]	1.0[b]	1.5[b]	2.0[b]	3.0[b]	AS2[c]	total
total solubilized lignin	4.60	2.50	2.30	1.70	0.73	0.51	0.42	0.59	13.35
acid-insoluble lignin[d]	3.51	1.90	1.62	1.10	0.40	0.26	0.22	0.06	9.07
acid-soluble lignin[e]	0.58	0.39	0.40	0.27	0.11	0.07	0.05	0.02	1.89
lignin associated in the solubilized hemicelluloses	0.51	0.21	0.28	0.33	0.22	0.18	0.15	0.51	2.39

[a] Alkali-soluble lignin fraction obtained by extraction with 0.1 M NaOH at 45 °C for 3 h from the dewaxed barley straw. [b] Lignin fractions obtained by successive extractions of the alkali-treated straw with different concentrations of H_2O_2 at 45 °C for 3 h at pH 11.5. [c] Alkali-soluble lignin fraction obtained by extraction with 10% KOH/1% $Na_2B_4O_7 \cdot 10H_2O$ at 28 °C for 15 h from the 3.0% H_2O_2-treated straw. [d] Acid-insoluble lignin fraction obtained by precipitation of the supernatant solution, adjusted by 6 M HCl to pH 1.5 after isolation of the solubilized hemicelluloses. [e] Lignin fraction solubilized in the pH 1.5 supernatant solution after precipitation of the acid-insoluble lignin fraction and calculated by difference (total solubilized lignin − acid-insoluble lignin − lignin associated in the solubilized hemicelluloses).

14.6, 10.8, 4.6, 3.2, 2.7, and 3.7% of the original lignin, respectively. As expected, the sequential treatment also solubilized 21.6, 8.4, 8.9, 8.9, 5.9, 5.4, 5.4, and 26.7% of the original hemicelluloses, respectively. The results revealed that the sequential extractions together resulted in a dissolution of 84.5% of the original lignin and 91.1% of the original hemicelluloses. In addition, the first treatment of the dewaxed barley straw with 0.1 M NaOH at 45 °C for 3 h also removed 81.0% of the original protein and 78.9% of the original ash, with solubilization of 8.0% hemicelluloses and degradation of 4.6% lignin. The acid-insoluble lignin preparation was the major fraction, comprising 51.0−76.0% of the total solubilized lignins except for the acid-insoluble lignin fraction, isolated with 10% KOH/1% $Na_2B_4O_7 \cdot 10H_2O$ at 28 °C for 15 h, which consisted of only 10.2% of the total solubilized lignin. The lignin fraction associated with the solubilized hemicelluloses amounted to 8.4−35.7% of the total released lignins, indicating that the successive treatment with 0.1 M NaOH and various concentrations of H_2O_2 under alkaline conditions significantly cleaved the ether linkages between lignin and hemicelluloses from the cell walls of barley straw. In contrast, in lignin fraction AS2 (Table 1) a substantial amount (86.4%) was found to be chemically linked to the solubilized hemicellulosic fraction, implying a strong linkage between hemicelluloses and lignin in this lignin−hemicellulosic complex fraction. Radical intermediates, such as hydroxyl radicals ($HO^{\bullet}$) and superoxide anion radicals ($O_2^{\bullet -}$) formed in the decomposition reaction, are very reactive and can react with and degraded the unreactive or solubilized lignin structures to more soluble lignin fragments, thus promoting the delignification (*9*). As can be seen from Table 1, about 84, 83, 84, 85, 86, and 88% of the solubilized lignin was recovered as acid-insoluble lignin or associated in the solubilized hemicellulosic fractions after sequential peroxide treatments with 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0% H_2O_2 of the 0.1 M NaOH-treated barley straw, respectively. The remaining 12−17% of the lignin was degraded into water-soluble fragments. These results indicate that treatments with various concentrations of H_2O_2 under alkaline conditions also led to a noticeable depolymerization of the solubilized lignin molecules.

The content of associated hemicelluloses in eight acid-insoluble lignin fractions isolated was determined as their neutral sugar equivalents, and the results are listed in Table 2. The data indicate that all of the lignin fractions contained rather low amounts of bound polysaccharides as shown by a neutral sugar content of 0.20−0.67%, suggesting that the sequential treatments stripped more lignin from most of the neighboring polysaccharide

Table 2. Content of Neutral Sugars (Percent Dry Weight) in Isolated Acid-Insoluble Lignin Fractions Solubilized in Successive Treatments of Barley Straw with 0.1 M NaOH and Various Concentrations of Alkaline Hydrogen Peroxide

		H_2O_2 concn (%)						
sugar	AS1[a]	0.25[b]	0.5[b]	1.0[b]	1.5[b]	2.0[b]	3.0[b]	AS2[c]
arabinose	0.13	0.11	0.12	0.11	Tr[d]	ND[e]	ND	ND
xylose	0.10	0.10	0.13	0.12	0.11	0.10	0.10	0.14
mannose	0.10	Tr	0.10	0.12	0.10	Tr	ND	ND
glucose	0.18	0.19	0.15	0.18	0.15	0.12	0.10	0.18
galactose	0.10	0.11	0.10	0.13	0.10	0.08	Tr	0.12
total	0.61	0.51	0.60	0.67	0.47	0.30	0.20	0.44

[a−c] Corresponding to the acid-insoluble fractions in Table 1. [d] Tr, trace. [e] ND, not detectable.

moieties. In comparison, the two lignin fractions isolated with 2.0 and 3.0% H_2O_2 seemed to be less contaminated than the other samples. Arabinose, xylose, mannose, glucose, and galactose were identified as the major sugar components in all of the fractions. However, the acid hydrolysis probably yielded lower sugar contents than the true values due to the incomplete hydrolysis at 120 °C for 2 h (*20*).

Lignin Composition. The chemical degradation and identification of the fragments obtained are important in a study of lignin composition. Standard procedures for analyzing lignins by chemical degradation techniques, such as alkaline nitrobenzene oxidation degradation, result in information of degradation products, which can be used to derive information about the composition of the original polymer (*21*). The reaction of lignin with alkaline nitrobenzene leads to the formation of aromatic aldehydes and, to a small extent, the corresponding aromatic acids. The yield of degradation products is high, and the method has been extensively used for lignin classification (*22*). Table 3 shows the yields of the monomeric products obtained from the alkaline nitrobenzene oxidation of the seven acid-insoluble lignin fractions. A relatively high yield of the phenolic acids and aldehydes (39.05−52.00%) indicated that all of the lignin preparations were less condensed. The predominant oxidation products were found to be vanillin and syringaldehyde, which resulted from the oxidation of the noncondensed guaiacyl and syringyl units in lignin, respectively, and together comprised 66.8−73.6% of the total phenolics. The presence of less *p*-hydroxybenzaldehyde (1.82−3.53%) and *p*-hydroxybenzoic acid (0.96−1.69%) was considered to be indicative of noncondensed *p*-hydroxyphenyl units, indicating the incorporation of *p*-hydroxycinnamoyl alcohol in barley straw lignin. Interestingly, a general decrease

Table 3. Yield (Percent Lignin Sample) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of the Acid-Insoluble Lignin Fractions

		H_2O_2 concn (%)					
phenolic acids and aldehydes	AS1[a]	0.25[b]	0.5[b]	1.0[b]	1.5[b]	2.0[b]	3.0[b]
p-hydroxybenzoic acid	1.14	0.96	1.08	1.40	1.68	1.69	1.32
p-hydroxybenzaldehyde	2.94	3.26	2.64	3.53	2.58	1.82	1.86
vanillic acid	0.84	1.09	0.83	1.87	1.30	0.94	0.96
syringic acid	2.28	4.16	4.68	5.65	4.58	3.90	5.23
vanillin	22.81	20.33	18.14	16.37	18.45	14.77	17.53
syringaldehyde	15.47	13.24	11.86	15.36	15.17	12.60	15.77
acetovanillone	0.20	0.33	0.31	0.23	0.33	0.12	0.52
p-coumaric acid	3.72	2.38	2.42	1.72	2.80	1.87	3.21
ferulic acid	2.60	1.98	1.64	1.37	1.46	1.34	1.82
total	52.00	47.73	43.60	47.50	48.35	39.05	48.22
molar ratio (G:S:H)[c]	55:34:11	55:34:12	52:36:12	46:43:11	48:39:13	47:41:12	47:43:10

[a,b] Corresponding to the acid-insoluble lignin fractions in Table 1. [c] G represents the relative total moles of vanillin, vanillic acid, and acetovanillone; S represents the relative total moles of syringaldehyde and syringic acid; H represents the relative total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid.

of the G (relative total moles of vanillin, vanillic acid, and acetovanillone)/S (relative total moles of syringaldehyde and syringic acid) molar ratio from 55:34 to 47:43 with an increase in alkaline peroxide concentration from 0.25 to 3.0% during the sequential extractions revealed that the guaiacyl units engaged in *β-O*-4 lignin structures were more easily degraded compared to syringyl units during the successive alkaline peroxide treatments. On the basis of a study of the formation and structure of lignin in monocotyledons, He and Terashima (*23*) stated that the lignification first takes place in the cell walls of protoxylem vessels, progressively proceeds in the compound middle lamellae of fibers and the cell walls of metaxylem vessels, and finally proceeds in the secondary walls of the fibers. Obviously, this high molar ratio of G/S in all of the lignin preparations and decrease as the extraction process proceeded revealed that the guaiacyl-richer lignin fractions, isolated during the initial extraction processes, were released mainly from the primary cell wall, because a larger amount of guaiacyl lignin is formed in the early stage of xylem differentiation than in the later stages of the secondary cell walls, which are rich in syringyl units. Therefore, the composition of lignin in the cell wall changes during cell development, shifting from highly condensed *p*-coumaric acid plus guaiacyl-type monolignols in the middle lamella to predominantly guaiacyl-type lignin through the primary and into the secondary cell wall and then to syringyl-type lignin in a portion of the primary cell wall, but predominantly in the secondary cell wall (*24*). A small amount of *p*-hydroxyphenyl units may also be included in the lignins in every kind of cell wall, as indicated by a constant level of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid in all seven lignin fractions.

In plant tissues, *p*-coumaric and ferulic acids are the most abundant hydroxycinnamic acids, which are bound through an ester linkage to the arabinoxylans of Gramineae or to the pectins of dicotyledons. It is currently hypothesized that the hydroxycinnamic bonding between components of the cell walls shifts from a predominantly ferulic acid ester linkage during the development of the cell wall to a predominantly ether linkage as lignification begins and to a predominantly *p*-coumaric acid ester linkage late in the lignification process (*24*). At this point, the ferulic acid esters are acting as an initiation site for lignin deposition and are critical entities in directing cell wall cross-linking during plant growth and development (*14*). Thus, ferulic acid is laid down in ester linkages to primary cell wall polysaccharides and provides ether linkage initiation sites for lignin, whereas the esters of *p*-coumaric acid cannot serve as initiation sites for lignification because, although some *p*-coumaric acid is esterified to arabinoxylan in the same manner as ferulic acid, most is esterified to the lignin fraction (*24*). A similar observation was found in this study of the seven acid-insoluble lignin fractions. As shown in Table 3, the content of *p*-coumaric acid (1.72−3.72%) was higher than that of ferulic acid (1.34−2.60%) in all seven lignin preparations isolated, although some *p*-coumaric acid and ferulic acid was oxidized to *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid or vanillin and vanillic acid, respectively, during the alkaline nitrobenzene oxidation process. The fact that small amounts of *p*-coumaric and ferulic acids occurred in all of the lignin fractions after nitrobenzene oxidation suggested that the two hydroxycinnamic acid are strongly linked to lignin in the cell wall of barley straw. Our previous study (unpublished results) on barley straw hydroxycinnamic acids showed that the straw contained 0.66% ferulic acid and 0.36% *p*-coumaric acid, in which the bulk of cell wall *p*-coumaric acid (66.7%) was ester-linked to cell wall components, mainly to lignin, and >40% of cell wall ferulic acid was etherified through its phenolic oxygen to the cell wall lignin component in barley straw.

Table 4. Weight-Average ($\bar{M}_w$) and Number-Average ($\bar{M}_n$) Molecular Weights and Polydispersity ($\bar{M}_w/\bar{M}_n$) of the Acid-Insoluble Lignin Fractions Isolated Successively with 0.1 M NaOH at Various Concentrations of H_2O_2 from Barley Straw

		H_2O_2 concn (%)					
	AS1[a]	0.25[b]	0.5[b]	1.0[b]	1.5[b]	2.0[b]	3.0[b]
$\bar{M}_w$	1750	1910	2190	2080	2000	2050	2090
$\bar{M}_n$	1430	1520	1740	1740	1680	1740	1760
$\bar{M}_w/\bar{M}_n$	1.22	1.26	1.26	1.20	1.23	1.18	1.19

[a,b] Corresponding to the acid-insoluble lignin fractions in Table 1.

Molecular Weight. The values of the weight-average ($\bar{M}_w$) and number-average ($\bar{M}_n$) molecular weights and the polydispersity ($\bar{M}_w/\bar{M}_n$) of the seven acid-insoluble lignin preparations are listed in Table 4. These lignin preparations did not show a significant difference in their weight-average molecular weights, which ranged between 1750 and 2190. This relatively low molecular

weight of all the acid-insoluble lignin preparations indicated that the alkali and alkaline peroxide treatment under the conditions used may cleave some β-*O*-4 linkages between the lignin precursors. In addition, a similar low value of the polydispersity of the seven lignin preparations (1.18−1.26) indicated that the lignins have a narrower molecular weight distribution.

Spectroscopic Analysis. A variety of spectroscopic techniques, such as Fourier transform infrared (FT-IR), carbon-13 nuclear magnetic resonance (^{13}C NMR), and ultraviolet (UV) spectroscopies, have been used for structural elucidation and analysis of lignin samples (*25*). In this study we focused on ^{13}C NMR spectroscopy and also to some extent on UV and FT-IR spectroscopies.

The UV absorption spectra of acid-insoluble lignin fractions showed absorption in the 280 nm region assigned to the polylignol, a dehydrogenative copolymer of sinapyl alcohol, coniferyl alcohol, and a small amount of *p*-coumaryl alcohol. Absorption in the 310−320 nm region in the spectra of the first two fractions is mainly due to the esters of hydroxycinnamic acids, such as *p*-coumaric and ferulic acids (*23*). The absence of the latter absorption in the spectra of the last three lignin fractions revealed that the treatment with relatively higher concentrations of alkaline peroxide (1.5−3.0%) or alkali (10% KOH) substantially saponified these ester bonds. It was found that all three structural moieties gave different absorption maxima and extinction coefficients. The extinction coefficient of guaiacyl units at 280 nm is 3.5 times that of syringyl units, and the extinction coefficient of *p*-coumaryl units is lower than that guaiacyl units but higher than that of syringyl units. On the basis of the model compounds, Faix and Schweers (*26*) also observed that the extinction coefficient of *p*-coumaric acid ester is ∼1.5 times that of ferulic acid ester at 320 nm. The absorption coefficient of the lignin fractions decreased with an increase in extracting concentration of the alkali or alkaline peroxide, indicating a decline in guaiacyl units or a rise in syringyl units in the isolated lignin fraction as the extraction process proceeded. This trend corresponded to the results of lignin composition obtained by alkaline nitrobenzene oxidation shown in Table 3.

Figure 2A illustrates the FT-IR spectra of three acid-insoluble lignin samples, extracted successively with 0.1 M NaOH (spectrum a), 0.25 (spectrum b), and 0.5% H_2O_2 (spectrum c) from dewaxed barley straw. The most striking feature of the three lignin fractions is the slight increase in level of carboxylic acid groups at 1710 cm^{-1} as the extraction process proceeds, indicating a small increase in the degree of oxidation of the lignins. In comparison, the two bands at 1710 and 1638 cm^{-1} in the lignin fraction, isolated with 0.1 M NaOH in the absence of peroxide, are rather weak, implying that the lignin fraction obtained during the first alkali treatment may be significantly less oxidized than the lignin fractions obtained from the alkaline peroxide treatments. This was confirmed by a stronger band at 1227 cm^{-1} in the two alkaline peroxide-soluble lignin fractions (spectra b and c) than in the alkali-soluble lignin fraction (spectrum a) because this originated from C−O plus C=O stretching of syringyl and guaiacyl units in lignin. A similar trend of increasing new carboxyl groups at 1718 cm^{-1} by oxidation of lignin with an increment of alkaline peroxide concentration from 1.5 (spectrum a) to 2.0 (spectrum b) and to 3.0% (spectrum c) is clearly observed in Figure 2B. These may result from oxidation of quinones and other conjugated carbonyl structures or direct oxidation of lignin side chains (*27*, *28*). In these reactions, unsaturated and saturated carboxylic acid structures are formed. The introduction of carboxyl groups into the lignin polymer is important because this functional group is hydrophilic and thus facilitates dissolution of the lignin in water (*3*). In contrast, as shown in Figure 2, at the low temperature of 45 °C, an increase in alkaline peroxide concentration from 0.25 to 2.0% resulted in a slow and limited oxidation, but more comprehensive oxidation required a higher concentration of alkaline peroxide such as 3.0% H_2O_2. It should be noted that the observed differences in lignin oxidation by alkaline peroxide concentration under the conditions given are small, and other lignin oxidation reactions may also have a influence on the overall formation of carboxyl groups. The great similarity in the aromatic ring skeleton between the spectra of the alkali-soluble lignin fraction and the alkaline peroxide-soluble lignin preparations revealed that the successive alkaline peroxide treatments under the conditions used did not affect the overall structure of lignin from barley straw except for a small increase of carboxyl and carbonyl groups in lignin side chains.

The structural changes in acid-insoluble lignin fractions during the alkali and alkaline peroxide treatments were also investigated by ^{13}C NMR spectroscopy. Panels A and B of Figure 3 show the alkali-soluble lignin fraction, isolated with 0.1 M NaOH, and the alkaline peroxide soluble lignin fraction, extracted with 0.5% H_2O_2, respectively. Most of the observed signals have been previously assigned in straw and wood lignin spectra (*29*−*34*). The most striking characteristic of the two ^{13}C NMR spectra is the almost complete absence of typical polysaccharide signals between 57 and 103 ppm. In Figure 3A, the spectrum does show three signals at 80.2 (C-4, Glc internal unit), 76.2 (C-3, Xyl non reducing end unit), and 63.0 ppm (C-5, Xyl internal unit) for the associated hemicelluloses. In Figure 3B, the chemically linked polysaccharides are characterized by a small signal at 62.8 ppm (C-5, Xyl internal unit). Nevertheless, all of the peak intensities are rather weak, indicating a trace amount of associated polysaccharides in the lignin fractions, corresponding to the data obtained by sugar analysis.

Another important characteristic of the two ^{13}C NMR spectra is the occurrence of a small band for carboxylic groups at 171.6 ppm (−COOH, aliphatic), assigned to the carboxyl carbons attached to the lignin side chains. It is, therefore, very likely that the treatments favored oxidation of the carbons linked by hydroxyl, aldehyde, and ketone groups in the lignin side chains. As these carbons were oxidized to carboxyl, aldehyde, or ketone groups, the contiguous aryl ethers could be easily cleaved simultaneously and subjected to further degradation. Such an oxidation may be the main reason for the substantial degradation of lignins by alkaline peroxide treatment from barley straw.

In Figure 3A, the signals for the aromatic part of the 0.1 M NaOH-soluble lignin can be observed in the region between 104.3 and 152.2 ppm. The guaiacyl units exhibit signals at 149.2 (C-3 in ether-linked guaiacyl units), 148.0 and 147.1 (C-4 in ether-linked guaiacyl units), 145.4 (C-4 in non-ether-linked guaiacyl units), 135.0 (C-1 in ether-linked guaiacyl units, data not shown), 132.6 (C-1 in non-ether-linked guaiacyl units),

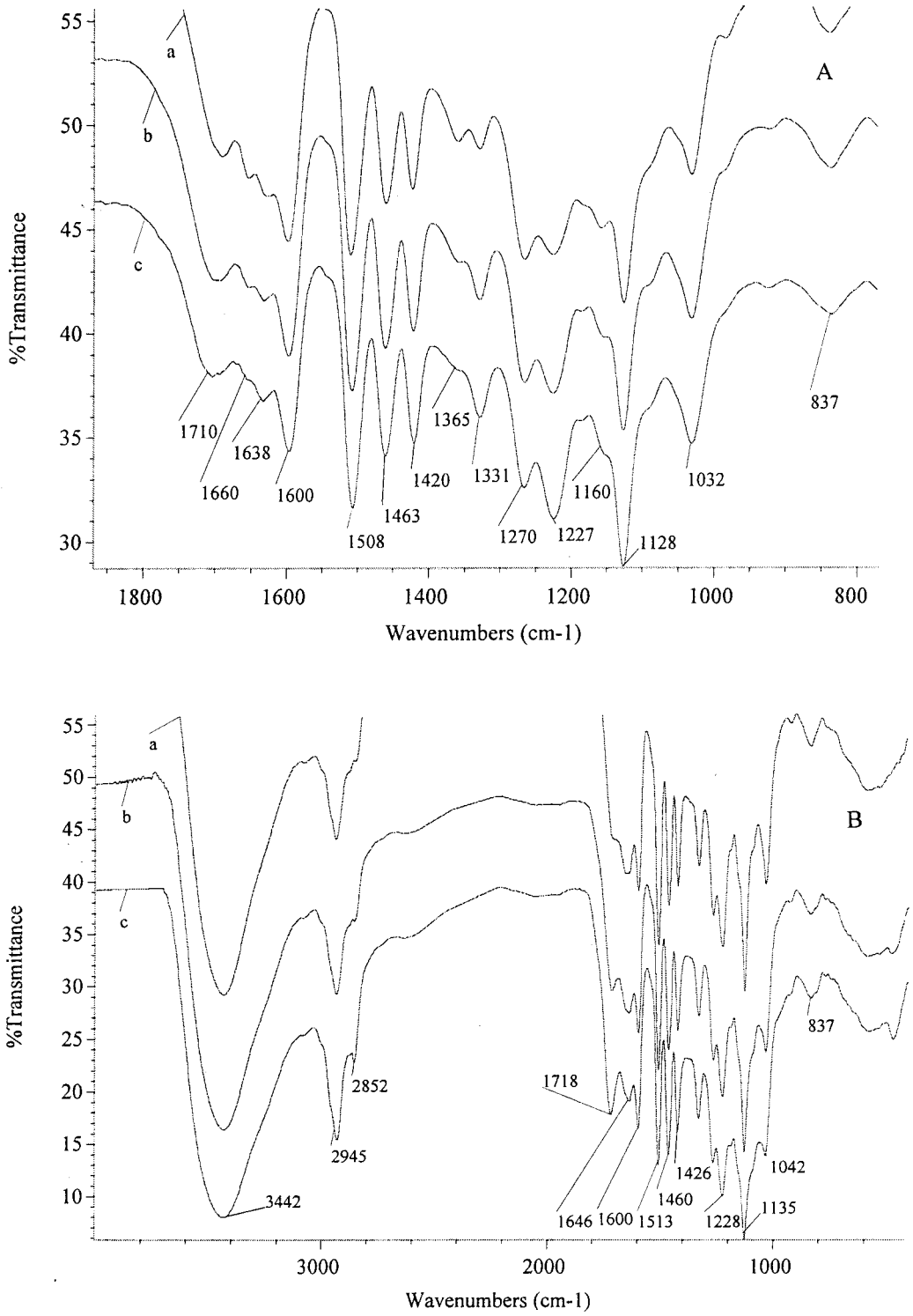

Figure 2. FT-IR spectra of acid-insoluble lignin fractions: (A) lignins extracted successively with 0.1 M NaOH at 45 °C for 3 h (a) and with 0.25 (b) and 0.5% H_2O_2 (c) at pH 11.5 for 3 h at 45 °C from dewaxed barley straw; (B) lignins extracted successively with 1.5 (a), 2.0 (b), and 3.0% H_2O_2 (c) at pH 11.5 for 3 h at 45 °C from the 1.0% H_2O_2-treated barley straw.

119.2 (C-6), 114.8 (C-5), and 111.2 ppm (C-2). The syringyl residues were detected with signals at 152.2 (C-3/C-5), 148.0 and 147.1 (C-3/C-5 in non-ether-linked syringyl units), 138.0 (C-4 in ether-linked syringyl units), 135.0 (C-1 in ether-linked syringyl units), 132.6 (C-1 in non-ether-linked syringyl units), and 104.3 ppm (C-2/C-6). The signals at 159.9 (C-4), 144.6 (C-α), 130.1 (C-2/C-6), 125.8 and 125.3 (C-1), 115.9, 115.7, and 115.4 ppm (C-3/C-5) are attributed to the esterified *p*-coumaric acid. Ether-linked ferulic acid gives signals at 168.1 (C-γ) and 122.7 ppm (C-6), whereas the esterified ferulic acid gives an intense signal at 122.9 ppm (C-6). These signals revealed that *p*-coumaric acid is linked to lignin by ester bonds, whereas ferulic acid is linked to lignin by both ether and ester bonds.

As illustrated in Figure 3A, the β-*O*-aryl ether structures were identified with three resonances at 86.3 (C-β), 72.3 (C-α), and 60.2 ppm (C-γ). The common carbon−carbon linkages, such as $\beta-\beta$ (C-γ, 71.8 ppm), were also present. A very strong signal at 56.0 ppm arises from

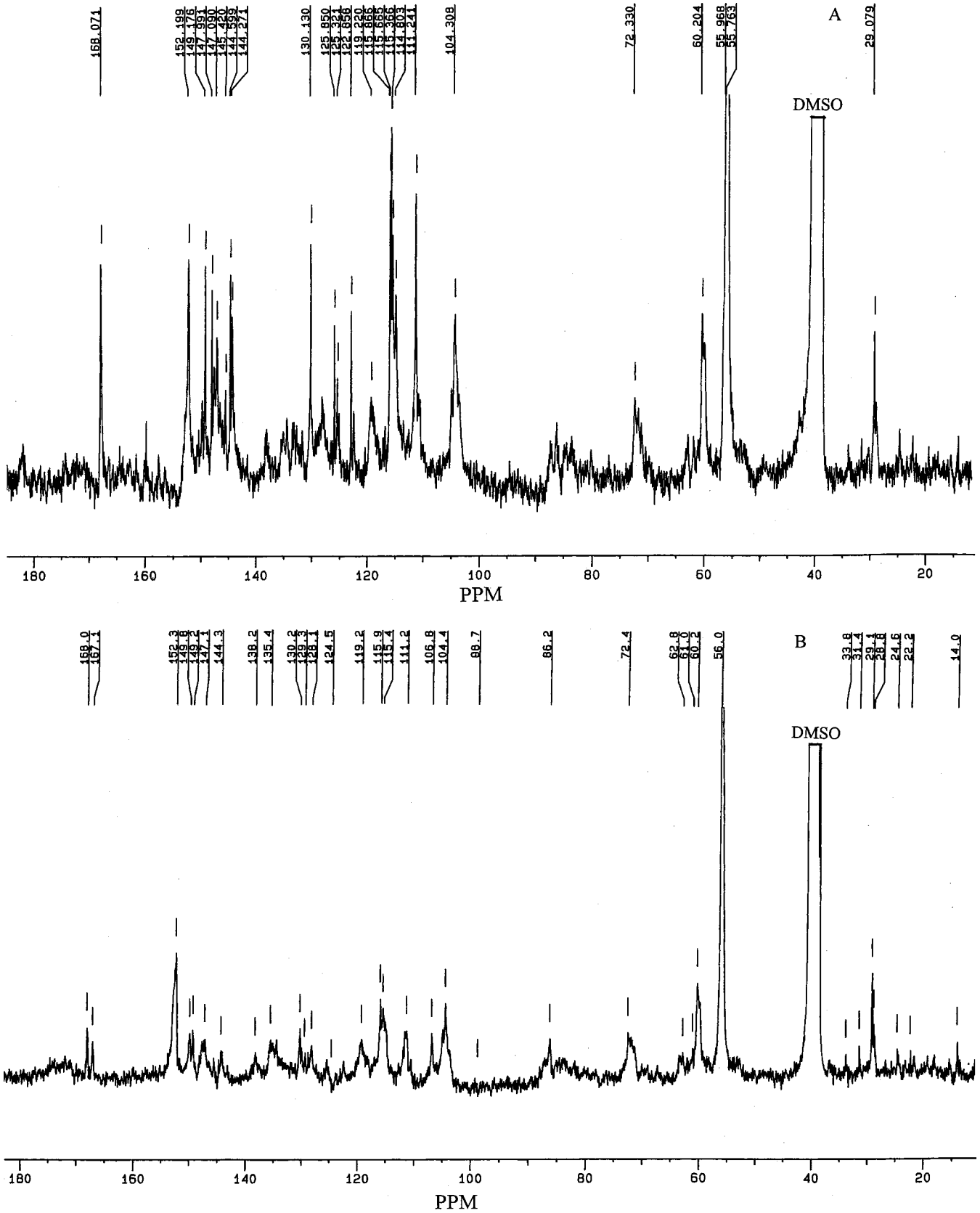

Figure 3. ^{13}C NMR spectrum of the acid-insoluble lignin fractions: (A) lignin extracted with 0.1 M NaOH at 45 °C for 3 h from the dewaxed barley straw; (B) lignin extracted with 0.5% H_2O_2 at pH 11.5 for 3 h at 45 °C from the 0.25% H_2O_2-treated barley straw.

the OCH_3 in syringyl and guaiacyl units. The signals for the γ-methyl and α- and β-methylene groups in *n*-propyl side chains of the lignin fraction appear in the spectrum between 14.2 and 33.8 ppm.

In comparison, the spectrum of the lignin fraction isolated with 0.5% H_2O_2 (Figure 3B) shows a relatively higher intensity of signals for syringyl units (152.3, 147.1, 138.0, 135.4, 106.8, and 104.4 ppm) than for

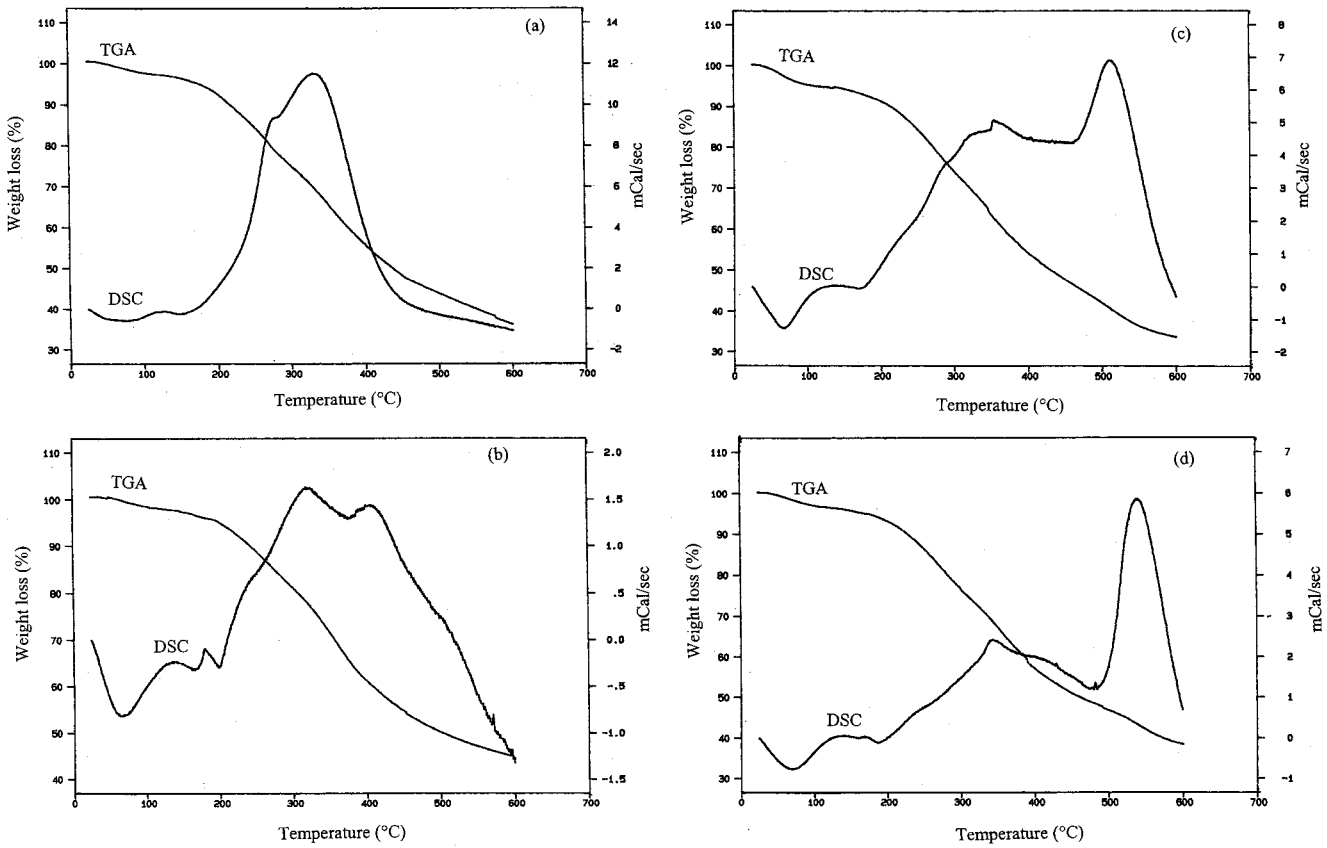

Figure 4. Thermograms of the acid-insoluble lignin fractions extracted successively with 0.1 M NaOH at 45 °C for 3 h (a) and with 0.5 (b), 1.5 (c), and 3.0% H_2O_2 (d) at pH 11.5 for 3 h at 45 °C from dewaxed barley straw.

guaiacyl units (149.8, 149.2, 147.1, 135.4, 119.2, and 111.2 ppm), indicating an increased content of syringyl units in the lignin fraction, which corresponded to the results obtained by alkaline nitrobenzene oxidation (Table 3). In addition, a significant decrease in the ether-linked ferulic acid signal at 168.0 ppm and the absence of two signals for esterified *p*-coumaric acid at 159.9 and 144.6 ppm indicated that 0.5% H_2O_2 treatment under the alkaline condition used substantially cleaved these bonds between lignin and hydroxycinnamic acids, whereas the occurrence of a relatively intense signal at 167.1 ppm in the lignin spectrum (Figure 3B) suggested that the esterified *p*-coumaric acid at the C-γ position of the lignin side chains is strongly linked to the lignin molecules, which is resistant to cleavage by 0.5% H_2O_2 under the alkaline condition used. Furthermore, the presence of a β-*O*-aryl ether structure, identified with signals at 86.2, 72.4, and 60.2 ppm, revealed that treatment with 0.5% H_2O_2 under the alkaline conditions used did not attack the β-aryl ether structure to a significant extent.

Thermal Stability. On the basis of our previous studies on the thermal decomposition of straw hemicelluloses, cellulose, and lignin (*13*), we found that moisture was removed at ~100 °C and that all hemicelluloses were substantially decomposed between 210 and 320 °C. Above 240 °C the decomposition of cellulose occurred showing a maximum peak at ~300 °C, yielding mainly volatile products. The decomposition process of lignin covered a larger temperature range, between 240 and 500 °C, although at temperatures <400 °C only 40% was decomposed giving carbon as the residual product. In this study, the thermal properties of the acid-insoluble lignin fractions were investigated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Figure 4 illustrates the thermograms of lignin fractions extracted successively with 0.1 M NaOH (a) and with 0.5 (b), 1.5 (c), and 3.0% H_2O_2 (d) from dewaxed barley straw. The four lignin fractions showed a similar maximum decomposition temperature ranging between 200 and 500 °C. However, at 10% weight loss the decomposition temperature of the four lignin decomposition temperatures started at 221 °C in Figure 4a, at 241 °C in Figure 4b, at 226 °C in Figure 4c, and at 231 °C in Figure 4d. Similarly, at 50% weight loss the decomposition temperature of the lignins was observed at 443 °C in Figure 4a, at 510 °C in Figure 4b, at 448 °C in Figure 4c, and at 476 °C in Figure 4d. These weight losses corresponded well with the lignin molecular weights given in Table 4, showing a decrease of thermal stability with a decrease in the molecular weight. In addition, the DSC curve of the lignin fraction isolated with 0.1 M NaOH in the absence of hydrogen peroxide (Figure 4a) showed only a large exothermic peak between 200 and 450 °C, whereas the three lignin fractions isolated with alkaline peroxide (Figure 4b−d) gave a small endothermic peak at 72 °C, which corresponded to the dehydration process (*35*). There then appeared two big peaks, due to exothermic reactions of the lignin.

The results here described represent the first comprehensive study on the lignins from barley straw. It is concluded that barley straw lignins are typical grass lignins and comprise substantial amounts of guaiacyl and syringyl units and significant quantities of *p*-hydroxyphenyl units. More significantly, the sequential alkaline peroxide treatment released significant amounts of lignin and hemicelluloses and gave a high-yield pulp

with acceptable brightness, which provides a convenient and environmentally friendly method for pulping of barley straw and other renewable agricultural residues.

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