Effects of Extraction Time and Different Alkalis on the Composition of Alkali-Soluble Wheat Straw Lignins

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Wheat straw was extracted with 1.5% KOH at 20 °C for 6 h, 1.5% LiOH at 20 °C for 6 h, and 1.5% NaOH at 20 °C for 0.5, 2, 3, 4, 6, 12, 24, 48, 72, 96, and 144 h, respectively. The alkali-soluble lignin fractions were isolated by two-step precipitation, and the chemical compositions in each of the lignin fractions are reported. The physicochemical properties and structural features of these lignin fractions were characterized by GPC, UV, IR, and ^{13}C-NMR spectroscopy and alkaline nitrobenzene oxidation. The most striking characteristics of these lignin fractions are the almost absence of neutral sugars (0.7−0.9%) and their low average molecular weights (1000−1560 Da). The results also showed that these lignin fractions contained roughly equal amounts of guaiacyl and syringyl units with relatively fewer *p*-hydroxyphenyl units and appeared to be very closely associated to hydroxycinnamic acids.

Keywords: *Wheat straw; lignin; phenolic acids and aldehydes; alkaline nitrobenzene oxidation; molecular weight; polysaccharides; infrared spectra; ^{13}C-NMR spectroscopy*

INTRODUCTION

Wood lignins require rather drastic hydrolytic treatments (5% NaOH, 130−170 °C) to become soluble in aqueous alkali solutions, and consequently the alkali lignins obtained by acidification of the hydrolysates are not particularly useful lignin preparations. In contrast, straw and grass lignins can be isolated in substantial yields by mild alkaline treatments, even at room temperature. The mild alkaline treatment is not prone to cause much chemical modification beyond the saponification of ester bonds between *p*-coumaric acid and lignin or ferulic acid and polysaccharides. Surprisingly, the alkali lignins have only rarely been utilized for the characterization of straw and grass lignins (Lai and Sarkanen, 1971). The main reason for this rare application of alkali lignin for structural characterization is that the alkali lignins, isolated by traditional one-step precipitation, contain much higher amounts of nonlignin materials such as polysaccharides, protein, and ash (mainly silica), and the hemicelluloses are the predominant impurities. This high content of polysaccharide, however, can be greatly reduced by the two-step precipitation proposed in this study. In our previous reports (Sun et al., 1995; Lawther et al., 1996), various types and concentrations of alkali, as well as pretreatment time and temperature conditions, have been used for the extraction of wheat straw lignins. The aim of present investigation was carried out on physicochemical characterization of the alkali-soluble lignin fractions which were extracted with 1.5% NaOH at 20 °C for 0.5−144 h and isolated by two-step precipitation.

MATERIALS AND METHODS

Fractionation and Isolation of Alkali-Soluble Lignins. The wheat straw was obtained from Silsoe Research Institute (Silsoe, Bedfordshire). Finely powdered and dried straw (10 g, 2.5 g of straw/100 mL of solution) was extracted under stirring with (a) 1.5% NaOH at 20 °C for 0.5, 2, 3, 4, 6, 12, 24, 48, 72, 96, and 144 h, respectively, and (b) 1.5% KOH and 1.5% LiOH at 20 °C for 6 h.

After filtration, the extracts in each of the fractions were acidified to pH 6.5 with glacial acetic acid, concentrated with a rotary evaporator under reduced pressure at 40 °C, and then mixed with 5 vol of 95% ethanol (24 h, 20 °C) for isolation of crude hemicelluloses or hemicellulose−lignin complexes. The alkali-soluble lignin fractions were then reprecipitated at pH 1.5 with 20% HCl from the supernatant solution (24 h, 20 °C), and the isolated lignin fractions, after filtration, were freeze-dried overnight.

Analysis and Spectroscopy. UV spectra were recorded on a Hewlett-Packard 8452A Diode Array spectrophotometer. Lignin sample (5 mg) was dissolved in 95% (v/v) dioxane−water (10 mL). A 1 mL aliquot was diluted to 10 mL with 50% (v/v) dioxane−water, and the absorbances between 240 and 400 nm were measured.

The average molecular weight of the lignin fractions was determined by gel permeation chromatography on a PLgel 5 μm mixed-D column (300 mm × 7.5 mm; Polymer Laboratories Ltd., England). The samples were dissolved with tetrahydrofuran (THF) with a concentration of 0.2%, and a 200 μL sample in solution was injected. The columns were operated at 40 °C and eluted with tetrahydrofuran at a flow rate of 1 mL min^{-1}. The column was calibrated using polystyrene standards.

IR spectra of the alkali-soluble lignins were obtained on an IR spectrophotometer (Mattson cygnus 100) using a KBr disk containing 1% finely ground samples. The ^{13}C-NMR spectrum of the 1.5% NaOH-soluble (20 °C, 48 h) lignin fraction was obtained on a Brucker 250 AC instrument operating in the FT mode at 62.4 MHz under total proton-decoupled conditions. It was recorded at 25 °C from a 250 mg sample of the above lignin fraction dissolved in 1.0 mL of DMSO-d_6 after 30 000 scans. A 40° pulse flipping angle, a 3.0 μs pulse width, and a 0.85 s acquisition time were used.

The methods of neutral sugar and uronic acid analyses, alkaline nitrobenzene oxidation of lignin, and determination of phenolic acids and aldehydes with HPLC in extracted lignin fractions are described in previous papers (Lawther et al., 1995; Sun et al., 1995, 1996). All nitrobenzene oxidation results represent the mean of at least triplicate determinations, and each oxidation mixture was chromatographed twice. Other experiments were performed in duplicate. The standard errors or deviations were always observed to be lower than

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Table 1. Yield (Percent) of Alkali-Soluble Lignin in the Various Pretreatment Conditions

pretreatment conditions	total	LA[a]	LB[b]	LA/LB
1.5% NaOH, 20 °C, 0.5 h	2.6	1.9	0.7	2.7
1.5% NaOH, 20 °C, 2 h	12.0	9.2	2.8	3.3
1.5% NaOH, 20 °C, 3 h	18.1	14.0	4.1	3.4
1.5% NaOH, 20 °C, 4 h	19.2	15.1	4.1	3.7
1.5% NaOH, 20 °C, 6 h	20.1	16.3	3.8	4.3
1.5% NaOH, 20 °C, 12 h	30.1	24.6	5.5	4.5
1.5% NaOH, 20 °C, 24 h	35.4	29.3	6.1	4.8
1.5% NaOH, 20 °C, 48 h	40.4	33.5	6.9	4.9
1.5% NaOH, 20 °C, 72 h	41.2	34.3	6.9	5.0
1.5% NaOH, 20 °C, 96 h	46.4	38.7	7.7	5.0
1.5% NaOH, 20 °C, 144 h	58.8	49.1	9.7	5.1
1.5% KOH, 20 °C, 6 h	14.1	11.2	2.9	3.9
1.5% LiOH, 20 °C, 6 h	35.5	29.3	6.2	4.7

[a] Obtained by reprecipitation of the supernatant solution with 20% HCl at pH 1.5 after isolation of hemicelluloses. [b] Coprecipitated in the hemicelluloses.

5% except for the variations among triplicate nitrobenzene oxidation (8−16%).

RESULTS AND DISCUSSION

Lignin Yield. Lignin yield was calculated by comparing the amount of lignin reprecipitated (LA) at pH 1.5 with 20% HCl from the supernatant solution after isolation of hemicelluloses and that coprecipitated in the hemicellulosic fractions (LB) with the total amount of acidic chlorite lignin presented in the wheat straw (about 14.1% by weight).

Table 1 shows that extension of pretreatment time tends to favor the alkali-soluble lignin extraction yield as expressed in both lignin rich fraction (LA) and hemicelluloses rich fraction (LB). As expected, the lignin yield of LA was much higher than that of LB as shown by the increase in the LA/LB ratios from 2.7 to 5.1 during the extension time between 0.5 and 144 h. Very low total yield was obtained with 1.5% NaOH at 20 °C for 0.5 h extraction. However, these yields increased from 12.0 to 58.8% with the extension of extraction duration from 2 to 144 h. This result was in good agreement with our previous study (Sun et al., 1995) on lignin yield determined by gravimetric methods from the residues of before and after pretreatments of the wheat straw. We mentioned that the yield of lignin, released during the 1.5% NaOH extractions at 20 °C, increased from 2.9 to 59.2% with the growth of pretreatment time from 0.5 to 144 h. Extractions with 1.5% KOH and LiOH at 20 °C for 6 h produced 14.1 and 35.5% of the alkali-soluble lignins, respectively. The foregoing data indicated that sodium hydroxide and lithium hydroxide are more effective than potassium hydroxide in the release of alkali-soluble lignin from wheat straw, and lithium hydroxide was observed to be the most effective in this respect.

UV Absorption. All the alkali-soluble lignins showed similar UV spectra having two absorption maxima at 316 and 280 nm. The former absorption maximum is attributable to bound hydroxycinnamic acids such as esterified *p*-coumaric and ferulic acids (Kondo et al., 1992), and the latter one originates from the free and etherified hydroxyl group in a hydroxylated benzene nucleus (Ahmad and Khan, 1988). The effect of 1.5% NaOH (20 °C) pretreatment time on the absorbance coefficient of LA is illustrated in Figure 1. As can be seen, the LA fraction, extracted with 1.5% NaOH at 20 °C for 48 h, gave the purest preparation. A slightly lower absorbance coefficient in some samples was probably due to more nonlignin material such as protein, silica, or wax coprecipitating in the isolated LA preparations.

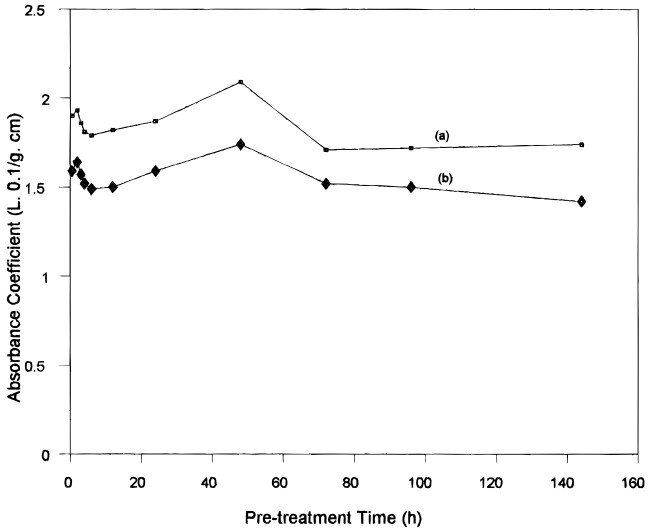

Figure 1. Effect of 1.5% NaOH (20 °C) pretreatment time on the absorbance coefficient of LA preparations: (a) measurement at 280 nm and (b) measurement at 316 nm.

Table 2. Content (Percent Lignin Sample, w/w) of Polysaccharide Sugars and Uronic Acids in LA Fractions

	sugars					uronic	
pretreatment conditions	total	Ara	Xyl	Gal	Glc	acids	total
1.5% NaOH, 20 °C, 0.5 h	1.0	0.2	0.4	0.2	0.2	0.9	1.9
1.5% NaOH, 20 °C, 2 h	0.9	0.2	0.2	0.3	0.2	0.8	1.7
1.5% NaOH, 20 °C, 3 h	0.9	0.1	0.3	0.3	0.2	0.8	1.7
1.5% NaOH, 20 °C, 4 h	0.9	0.1	0.2	0.4	0.2	0.8	1.7
1.5% NaOH, 20 °C, 6 h	0.8	0.1	0.2	0.3	0.2	0.7	1.5
1.5% NaOH, 20 °C, 12 h	0.8	0.1	0.2	0.3	0.2	0.8	1.6
1.5% NaOH, 20 °C, 24 h	0.9	0.1	0.2	0.4	0.2	0.9	1.8
1.5% NaOH, 20 °C, 48 h	0.9	0.1	0.2	0.4	0.2	0.9	1.8
1.5% NaOH, 20 °C, 72 h	0.7	0.1	0.1	0.3	0.2	0.8	1.5
1.5% NaOH, 20 °C, 96 h	0.7	0.1	0.1	0.3	0.2	0.8	1.5
1.5% NaOH, 20 °C, 144 h	0.6	0.1	0.1	0.3	0.1	0.7	1.3
1.5% KOH, 20 °C, 6 h	0.8	0.1	0.2	0.3	0.2	0.8	1.6
1.5% LiOH, 20 °C, 6 h	0.7	0.1	0.2	0.2	0.2	0.8	1.5

Composition of Bound Polysaccharides. All of the LA fractions, obtained by two-step precipitation, contained a rather low level of associated polysaccharides (1.3−1.9%, Table 2). The main reason for this relative absence of polysaccharides in the LA fractions is that alkali treatment would be expected to saponify the hydroxycinnamic esters that have been hypothesized to cross-link lignin to cell wall hemicelluloses (Schwarz et al., 1989). Various isolation and purification procedures have been previously attempted for reducing polysaccharide content in isolated lignin fractions, but none of these methods allowed to obtain the lignin fractions with lower polysaccharide content. Scalbert and co-workers (1986) purified lignin fractions by means of liquid−liquid extraction, phenyl-Sepharose chromatography, and enzymatic hydrolysis with commercial Driselase, and the lignin fractions of milled wheat straw lignin, enzyme lignin, and alkali-soluble lignin still contained 7.1, 16.8, and 18.2% polysaccharides, respectively, which were about 10-fold higher than those of the LA fractions obtained in this study. By comparative study of lignin fractions from alkaline extraction of wheat straw through chemical degradation, analytical pyrolysis, and spectroscopic techniques, Fidalgo et al. (1993) fractionated the alkali-soluble lignins into alkali lignins 1 and 2, which contained 17.3 and 32.9% polysaccharide sugars, respectively. This high content of polysaccharides in alkali-soluble lignin preparations was regarded to be due to specific structural patterns of association between lignin and polysaccharides in wheat straw cell walls (Scalbert et al., 1986). However,

Table 3. Alkaline Nitrobenzene Oxidation Products (Percent Lignin Sample, w/w) of LA Fractions

	1.5% NaOH (20°C) pretreatment time (h)												
phenolic acids and aldehydes	0.5	2	3	4	6	12	24	48	72	96	114	K[a]	Li[b]
gallic acid	0.68	0.71	0.50	0.80	0.91	0.84	1.00	1.16	1.51	1.48	0.58	0.93	1.32
protocatechuic acid	0.070	0.070	0.040	0.072	0.060	0.067	0.069	0.067	0.065	0.080	0.060	0.033	0.081
p-hydroxybenzoic acid	0.47	0.58	0.39	0.24	0.21	0.56	0.47	0.39	0.44	0.43	0.33	0.34	0.29
p-hydroxybenzaldehyde	1.13	1.18	1.02	0.76	0.68	0.85	0.94	1.31	0.80	0.93	1.06	0.82	1.05
vanillic acid	0.89	0.94	0.80	0.57	0.79	0.94	0.87	1.27	1.03	1.13	1.20	0.84	1.11
syringic acid	2.71	2.81	2.41	1.76	1.58	1.75	1.98	2.36	2.29	2.33	2.06	1.54	2.00
vanillin	13.29	14.00	11.90	8.46	8.86	8.10	9.87	12.33	10.83	11.42	10.96	9.40	10.57
syringaldehyde	13.81	14.01	11.94	8.52	8.78	7.94	9.96	12.66	10.35	10.87	10.41	8.64	10.41
p-coumaric acid	0.26	0.40	0.27	0.26	0.23	0.24	0.25	0.24	0.35	0.29	0.20	0.36	0.36
acetovanillone	0.29	0.28	0.24	0.18	0.090	0.075	0.093	0.12	0.14	0.15	0.10	0.18	0.12
ferulic acid	1.27	1.27	1.09	0.78	0.76	0.81	1.05	1.07	0.98	0.50	0.44	0.80	0.80
cinnamic acid	0.073	0.076	0.050	0.041	0.044	0.040	0.060	0.047	0.05	tr	tr	0.04	0.069
total	34.94	36.33	30.65	22.44	22.99	22.21	26.61	33.02	28.84	29.61	27.40	23.92	28.18

[a] 1.5% KOH pretreatment at 20 °C for 6 h. [b] 1.5% LiOH pretreatment at 20 °C for 6 h. tr, traces.

it is easy to obtain the lignin fractions which are relatively free of polysaccharides by using this method of two-step precipitation, proposed in this study, for isolation of alkali-soluble lignins because most of the dissolved lignin in alkaline treatments is free of the linkages with polysaccharides.

Interestingly, as shown in Table 2, there were roughly equal amounts of polysaccharide sugars and uronic acids associated in each of the LA fractions. This relatively high concentration of uronic acids in alkali-soluble lignin fractions obtained in this study was probably due to the ester bonds between lignin and glucuronic acid residue of hemicelluloses in wheat straw cell walls. On the basis of the conjugate acid DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) oxidation of a water-soluble lignin−carbohydrate complex (LCC−WE) from the beech wood, Imamura and co-workers (1994) indicated that the frequency of the ester bond between the lignin and glucuronic acid residue of glucuronoxylan was determined to be 1.6 per molecule of LCC−WE. Occurrence of an ester bond between lignin and glucuronic acid was confirmed by the ^{13}C-NMR spectrum (Figure 4).

Alkaline Nitrobenzene Oxidation Products. The yields and concentrations of phenolic acids and aldehydes obtained by alkaline nitrobenzene oxidation of the LA fractions are given in Table 3. These compounds were identified by comparison with the retention times of standards. The major products were found to be vanillin, syringaldehyde, *p*-hydroxybenzaldehyde, and syringic acid. The predominant degradation products, vanillin and syringaldehyde, resulted from the degradation of noncondensed guaiacyl and syringyl units, respectively. Occurrence of a low amount of *p*-hydroxybenzaldehyde was due to the noncondensed *p*-hydroxyphenyl unit, indicating the incorporation of *p*-hydroxycinnamoyl alcohol in wheat straw lignin.

From Table 3, it was found that wheat straw lignin contained roughly equal amounts of noncondensed guaiacyl (G) and syringyl (S) units with relatively fewer *p*-hydroxyphenyl units. These results coincided with the studies of Nimz et al. (1981) and Scalbert et al. (1986). On the other hand, Billa and Monties (1995) stated that lignin fractions from leaves are relatively richer in guaiacyl units, while lignin from internodes are generally enriched in syringyl units, suggesting a different type of lignification in the wheat plant organs. Moreover, a relatively high S/G ratio value could be obtained by cupric oxide degradation of wheat straw lignin (Fidalgo et al., 1993). Different ratios of G/S in the nitrobenzene oxidation products reported in the literature were probably due to the nature of the sample and

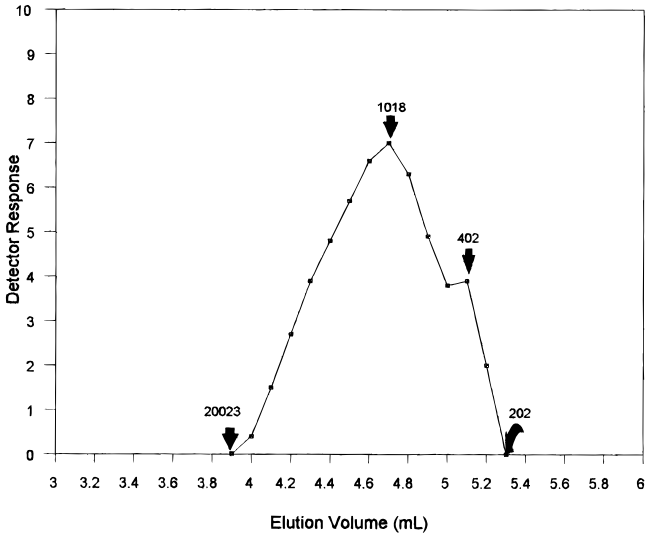

Figure 2. GPC molecular weight distribution of LA fraction extracted with 1.5% NaOH at 20 °C for 48 h.

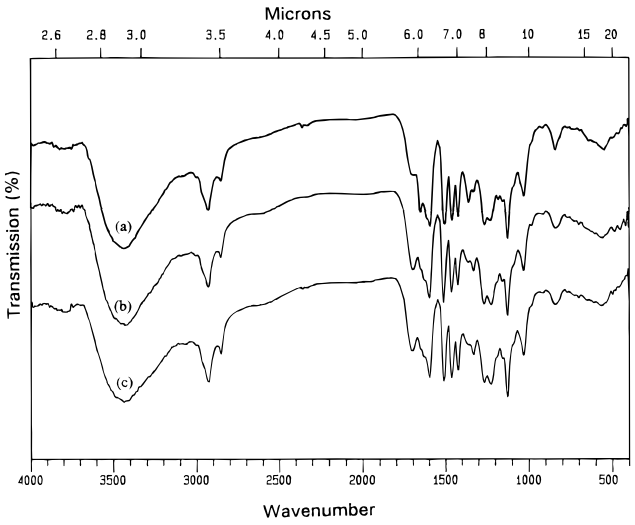

Figure 3. IR spectra of LA fractions extracted with (a) 1.5% NaOH at 20 °C for 0.5 h, (b) 1.5% NaOH at 20 °C for 72 h, and (c) 1.5% NaOH at 20 °C for 144 h.

the various experimental conditions such as reaction mixture, reaction time, and temperature used. In addition, the factors such as soil, climate, botanical variety, etc., can also affect the contents of guaiacyl and syringyl units in wheat straw.

It is noteworthy to compare the low yield of oxidation products found in the case of LA fractions to the corresponding yield of hardwood or softwood. A higher

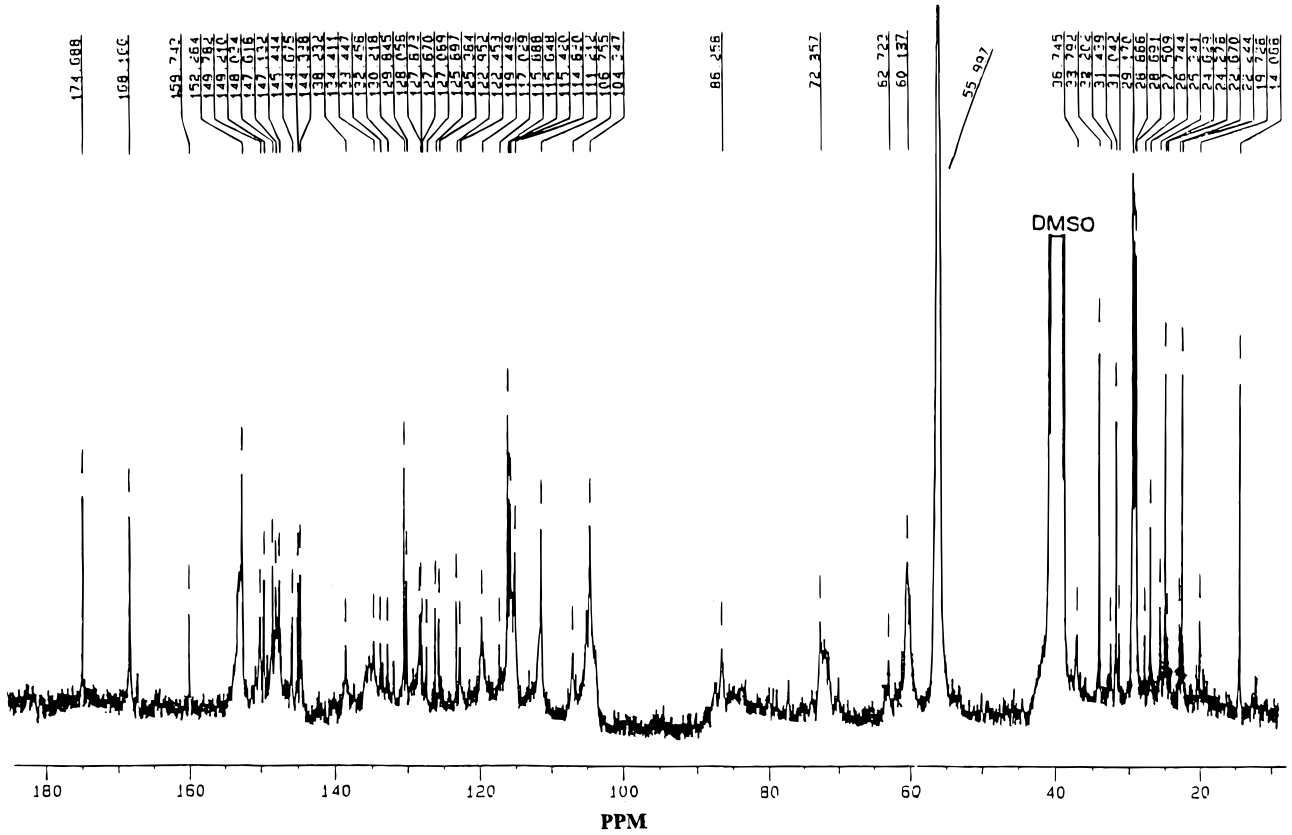

Figure 4. ^{13}C-NMR spectrum of LA fraction extracted with 1.5% NaOH at 20 °C for 48 h (in DMSO-d_6).

condensation degree with fewer β-aryl ether linkages was observed to appear in wheat straw lignins. On the other hand, extractions with 1.5% NaOH at 20 °C for 0.5 and 2 h yielded the relatively high yields of oxidation products and indicated the less condensed fractions. The LA fractions obtained by 1.5% KOH and 1.5% NaOH at 20 °C for 6 h had an equivalent degree of condensation as shown by the roughly same yields of nitrobenzene oxidation, while the LA fraction isolated by 1.5% LiOH at 20 °C for 6 h was confirmed to have the least condensation degree because of the most high nitrobenzene oxidation yield among the three different alkali treatments (20 °C, 6 h).

Molecular Weight Distribution. The weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of each fraction were computed from their chromatograms. It was found that extraction of wheat straw with dilute alkali at room temperature and isolation by two-step precipitation resulted in lignin fractions having low average molecular weights (1000−1560). The results obtained in this study were in good comparison with the corresponding data from the literature except for the studies from Liu et al. (1989) and Ben-Ghedalia and Yosef (1994). Scalbert and Monties (1986) reported that the elution maximum of wheat straw alkali lignin gave a molecular weight at 1450. Kosíková et al. (1990) mentioned that the average molecular weights of one acetone- and two alkali-extractable lignin fractions appeared at 1700, 4700, and 2600, respectively. By comparison of characteristics of soluble lignins from untreated and ammonia-treated wheat straw, Kondo and co-workers (1992) showed that the average molecular weights of untreated and ammonia-treated wheat straw lignins were 2170 and 2200, respectively. However, with successive extraction of wheat straw with 1 M NaOH at 25 °C for 0.5, 2, and 48 h, the obtained three alkali-soluble lignin fractions had average molecular weights at 6624, 13 148, and 19 854, respectively (Liu et al., 1989). Ben-Ghedalia and Yosef (1994) extracted four alkali-soluble lignin fractions from 7, 14, 21, and 28 day ball-milled wheat straw and stated that the average molecular weight ranged between 16 459 and 19 689. These so much higher molecular weights of wheat straw lignins were probably due to the nature of the sample and the various isolation methods used, in which lignin condensation may play a very important factor in affecting the molecular weight.

The GPC molecular weight distribution of LA fraction extracted with 1.5% NaOH at 20 °C for 48 h is shown in Figure 2. As expected, the two-step precipitation is an important factor not only to LA fractions which are relatively free of polysaccharides but also to the molecular weight profile. Elution profile of the lignin showed a wide polymolecularity, ranging from monomer up to polystyrene of molecular weight over 20 000. The elution maximum of the LA corresponded to the polystyrene molecular weight of 1018. The second peak corresponded to very low molecular components, probably dimers.

Infrared Spectra. The IR spectra of the alkali-soluble lignin fractions showed minor changes in the peak intensities (Figure 3) and confirmed that the "core" of the lignin structure does not change dramatically during the various alkaline treatments. The most striking characteristic of the IR spectra of these LA fractions from wheat straw was the presence of peaks at 1705 and 1645 cm^{-1}. The band at 1705 cm^{-1} has been assigned to carbonyl stretching in unconjugated ketone and carboxyl groups. The 1645 cm^{-1} band is also a carbonyl-stretching band due to para-substituted ketones or aryl aldehydes (Jung and Himmelsbach, 1989; Almendros et al., 1992). Aromatic skeleton vibrations in lignin are assigned at 1420, 1503, and 1596 cm^{-1} (Buta et al., 1989). Absorbances for these bands appeared at similar intensities for these different lignin

Table 4. Chemical Shift Value (δ, ppm), Intensity, and Signal Assignment of the LA Fraction Extracted with 1.5% NaOH at 20 °C for 48 h from Wheat Straw

ppm	intensity[a]	assignment[b]	ppm	intensity	assignment
174.6	m	-COOH, aliphatic acids or esters; C-6, GlcA and ester	117.0	vw	C-β, FE ether
			115.9	s	C-3/C-5, PC ester
			115.6	s	same
168.2	m	C-γ, FE ether; C-γ, PC ester	115.4	m	same
			114.8	m	C-5, G
159.7	m	C-4, PC ester	111.2	m	C-2, G
152.3	s	C-3/C-5, S	106.8	vw	C-2/C-6, S with α-CO
149.8	w	C-4, G etherified	104.3	s	C-2/C-6, S
149.2	m	same	86.3	w	C-β, β-aryl ether; C-α in β-β
148.0	w	C-3, G	72.4	w	C-γ in β-β; C-α, β-aryl ether
147.6	m	same	62.7	vw	C-γ in β-O-4; C-5, Xyl internal unit
147.1	m	same	60.1	m	C-γ in β-O-4; β-aryl ether; C-6, Glc; 4-*O*-MeGlcA in xylan
145.4	w	C-4, G in β-5 (nonetherified)	56.0	vs	OCH_3, G S
			36.8	vw	CH_3 group in ketones (conj) or in aliphatic
144.6	m	C-α, PC ester	33.8	m	same
144.3	m	C-α, FE ether	32.2	vw	same
138.2	w	C-4, S etherified	31.4	m	same
134.4	w	C-1, S etherified; C-1, G etherified	31.0	vw	same
			30.1	w	same
133.4	w	C-1, S nonetherified; C-1, G nonetherified	29.2	s	CH_2 in aliphatic side chain
			28.9	m	same
132.5	w	same	28.7	m	same
130.2	s	C-2/C-6, H (PC ester)	27.5	vw	same
129.8	m	same	26.7	w	CH_3 or CH_2 group in saturated side chains
128.1	w	C-2/C-6, H	25.3	vw	same
127.9	vw	same	24.6	m	same
127.1	vw	same	24.3	vw	same
125.9	w	C-1, PC ester	22.7	vw	CH_3 or CH_2 in saturated aliphatic chain
125.4	w	same	22.2	m	same
123.0	w	C-6, FE ester	19.7	w	same
122.5	vw	C-6, FE ether; C-3/C-5, H	14.0	m	γ-CH_3 in *n*-propyl side chain
119.4	m	C-6, G; C-5, G			

[a] Intensity abbreviations: s, strong; m, medium; w, weak; vs, very strong; vw, very weak. [b] Assignment abbreviations: G, guaiacyl unit; S, syringyl unit; H, *p*-hydroxyphenyl unit; PC, *p*-coumaric acid; FE, ferulic acid; Xyl, xylose; Glc, glucose; GlcA, glucuronic acid; 4-O-MeGlcA, 4-*O*-methylglucuronic acid.

fractions, indicating same aromaticity. The 1324, 1270, and 1220 cm^{-1} bands have been assigned to ring breathing with C−O stretch. The 1324 cm^{-1} band has been associated with sinapyl units and the 1270 and 1220 cm^{-1} bands with coniferyl units (Ahmad and Khan, 1988). Two smaller bands at 1360 and 1165 cm^{-1} correspond to aliphatic C−H stretch in CH_3 and C═O ester groups (conjugated), respectively. The bands at 1150, 1120, and 1020 cm^{-1} indicate the aromatic CH in-plane deformation. Aromatic C−H out of plane bending appears at 835 cm^{-1}.

^{13}C-NMR Spectrum. The LA fraction, extracted with 1.5% NaOH at 20 °C for 48 h, was also studied by ^{13}C-NMR spectroscopy (Figure 4). The corresponding δ-values, signal intensities, and signal assignments are listed in Table 4. Most of the assignments could be made according to Himmelsbach and Barton II (1980), Nimz et al. (1981), Scalbert et al. (1985, 1986), McElroy and Lai (1988), Pan et al. (1994), and Kondo et al. (1995) for lignin and phenolic acid signals and to Imamura et al. (1994) for uronic acid signals.

As can be seen in Figure 4, the most striking characteristic of the ^{13}C-NMR spectrum is the disappearance of typical polysaccharide signals between 57 and 103 ppm. Due to a large amount of polysaccharides associated in the extracted wheat straw lignin samples in a number of previous studies (Nimz et al., 1981; Scalbert et al., 1986; Liu et al., 1989; Kosíková et al., 1990), all of the lignin spectra reported earlier showed rather large resonances between 57 and 103 ppm which made the assignments more difficult and overlap. However, because of the very low content of associated polysaccharides (1.3−1.9%) in the LA fraction obtained in this study, the spectrum (Figure 4) did not show any signals between 57 and 103 ppm for polysaccharide sugars. Signals for C-2/C-6 in H and C-3/C-5 (PC ester) in the spectrum obtained by Scalbert et al. (1986) showed only two (130.0 and 127.9 ppm) and one (115.4 ppm) resonance, respectively, while the spectrum of the LA fraction obtained in this study showed five (130.2, 129.8, 128.1, 127.9, and 127.1 ppm) and three (115.9, 115.6, and 115.4 ppm) resonances, respectively. This similar behavior was also found for C-4 in G (etherified) and C-3 in G resonances. Signals for C-α (PC ester), C-1 (PC ester), C-6 (FE ester), and C-2/C-6 in S with α-CO were identified at 144.6, 125.9−125.4, 123.0, and 106.8 ppm, respectively, in the spectrum obtained in this study, whereas none was found in the spectrum of the alkaline lignin obtained by Scalbert et al. (1986).

In the aromatic region (104−160 ppm) of the spectrum, the syringyl, guaiacyl, and *p*-hydroxyphenyl (H) residues were indicated by signals at 152.3, 138.2, 134.4, 133.4, 132.5, 106.8, and 104.3 ppm (S), 152.3, 149.8, 149.2, 148.0, 147.6, 147.1, 145.4, 133.4, 132.5, 119.4, 114.8, and 111.3 ppm (G), and 128.1, 127.9, 127.1, and 122.5 ppm (H), respectively. These signals confirmed that the LA fraction could be justified as GSH-lignin. The signals at 168.2, 159.7, 144.6, 130.2, 129.8, 125.9, 125.4, 115.9, 115.6, and 115.4 ppm showed the esterified *p*-coumaric acid. Etherified ferulic acid was observed with signals at 168.2, 144.3, 122.5, and 117.0 ppm. A very weak signal at 123.0 ppm is expected for esterified ferulic acid. The side chain carbon atoms in *p*-coumarate residues gave signals at 144.6 (C-α) and 168.1 (C-γ) ppm which are, however, overlapped by the signals for etherified ferulic acid. Therefore, it seems very likely that *p*-coumaric acid is linked to lignin by an ester bond,

while the majority of the ferulic acids are linked by their phenolic groups via ether bonds to lignin.

The intensive signals assigned to γ-methyl and α- and β-methylene groups in *n*-propyl side chains appeared in the spectrum between 14.1 and 36.8 ppm. Signals at 14.1 and 22.2−33.8 ppm were also detected for lipid or waxes, indicating a small amount of lipid or waxes in the isolated LA fraction. The very strong signal at 56.0 ppm corresponds to OCH_3 in syringyl and guaiacyl units. The carbonyl resonances from uronic acids and esters, in addition to cinnamic acids and esters, acetyl groups, and other aliphatic esters, may contribute to signals at 174.6 and 60.1 ppm. A signal at 174.6 ppm indicates C-6 in methyl uronates, and the signal at 60.1 ppm originates from the 4-*O*-methoxyl group of the glucuronic acid residue in the xylan, which was overlapped by C-γ in β-O-4 and β-aryl ether singls (Himmelsbach and Barton II, 1980; Imamura et al., 1994).

Based on the above results, it is concluded that the LA fractions, obtained by two-step precipitation, are relatively free of polysaccharide sugars, as indicated by the absence of typical polysaccharide signals in the ^{13}C-NMR spectrum, and contained roughly equal amounts of noncondensed guaiacyl and syringyl units with few *p*-hydroxyphenyl units. They are probably more condensed than typical hardwood or softwood lignins. Meanwhile, the lignins in wheat straw cell walls appear to be very closely associated to hydroxycinnamic acids. It was found that *p*-coumaric acid is ester-linked to lignin at C-α and C-γ, while the majority of the ferulic acids are linked to lignin by ether bonds.

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