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Fractional isolation and physico-chemical characterization of alkali-soluble lignins from fast-growing poplar wood

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Abstract

This paper examines the physico-chemical properties and structural features of six alkali-soluble lignin preparations extracted with 5, 7.5, and 10% NaOH at 50°C for 4–12 h from fast-growing poplar wood. The pure lignin (PL) preparations were characterized using UV, FT-IR, ^{13}C-NMR, GPC, and alkaline nitrobenzene oxidation methods. The results showed that all the PL fractions are relatively free of associated polysaccharides and are composed of large amounts of syringyl units together with noticeable quantities of guaiacyl and fewer *p*-hydroxyphenyl units. Their weight-average molecular weights ranged from 4520 to 6900 g mol^{-1}. Noticeable amounts of esterified *p*-hydroxybenzoic acids, minor quantities of esterified *p*-coumaric acid, and traces of both ester and ether linked ferulic acids were identified in the isolated lignin preparations. The lignin fraction, extracted with 5% NaOH at 50°C for 12 h from the dewaxed fast-growing poplar wood, is composed mainly of β-*O*-4 ether bonds together with small amounts of β–β′ and β-5 carbon–carbon linkages between the lignin structural units.

Keywords: Lignin; Fast-growing poplar wood; Alkali

1. Introduction

Lignins are complex phenolic plant polymers essential for mechanical support, defence and water transport in vascular terrestrial plants [1]. They are usually built up by oxidative coupling of three major C_6–C_3 (phenylpropanoid) units, namely, *trans*-*p*-coumaryl alcohol, guaiacyl (coniferyl) alcohol, and syringyl (sinapyl) alcohol in various proportions. For example, hardwood lignins are polymerized from syringyl and guaiacyl units; softwood lignins are essentially composed of guaiacyl units, except compression wood lignins, which are *p*-hydroxyphenyl–guaiacyl copolymers [2]. Unlike other natural polymers such as proteins, polysaccharides, and nucleic acids, which have interunit linkages susceptible to enzymic and chemical hydrolyses, lignin contains carbon–carbon and biphenyl ether bonds. The β-ether interunit linkage predominates in most natural lignin and there is significant evidence for sequences of β-ether linked units of three or more along the polymer chain [3]. Furthermore, the lignin heterogeneity in the same plant is an important problem in relation not only to the lignin chemistry, but also to its biosynthesis, its biodegradation pathways and to the mechanical properties of lignocellulosic products [4].

Due to the very complex constitution of lignins, it is difficult to find a single technique to characterize their structures. It is necessary to combine chemical and physical methods, each providing partial but complementary information [5]. Among the chemical methods, alkaline nitrobenzene oxidation has been used to estimate the extent of uncondensed units in lignins based on the yield of vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde, resulting from three constitutive monomeric lignin units guaiacyl, syringyl, and *p*-hydroxyphenyl, respectively. Indirectly, it may be used to indicate the degree of lignin condensation [6] and to derive information about the composition of the original lignin [7]. On the other hand, several physical non-destructive techniques, used to analyze lignins, such as ultraviolet (UV), Fourier transform infrared (FT-IR), and magnetic resonance spectroscopies (^{13}C-NMR) are complementary to the above degradative procedures since they provide information on the whole structure of the polymer and avoid the possibility of degradation artifacts [8]. Particularly, modern pulsed NMR methods are utilized to assign and authenticate low molecular mass structures and provide databases for classical interpretation of polymer spectra [9]. With regard to lignin, solution-state NMR and solid-state

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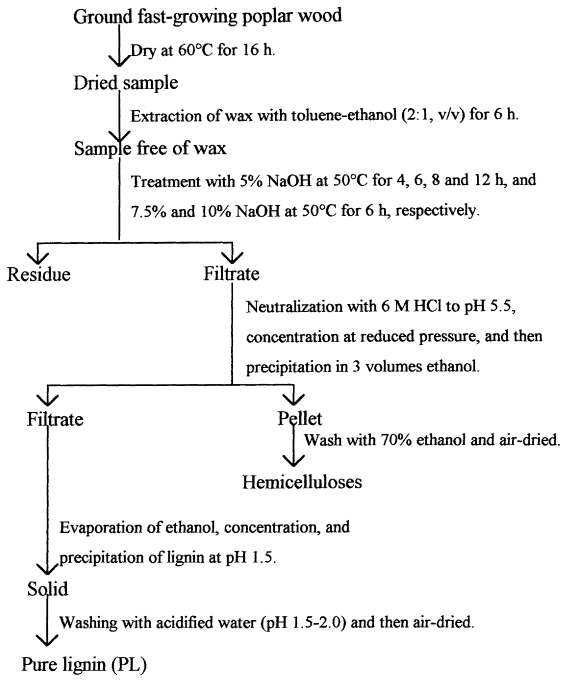


Fig. 1. Scheme for isolation of PL from the alkaline hydrolysates of fast-growing poplar wood.

NMR of specifically labeled substrates have largely been used to confirm information on numerous structural changes in dissolved lignin, including cleavage of β-*O*-aryl ether linkages and formation of unsaturated structures and carboxylic acids.

Fast-growing poplar trees, developed by hybridization of normal poplar trees, which are then bred successively by grafting, have been widely noticed from the viewpoint of an important renewable resource for pulp and paper production and timber [10]. The linkages of wall-bound hydroxybenzoic and hydroxycinnamic acids have been investigated in some detail by Kim and co-workers [10,11], but there is relatively little information on the physico-chemical properties and structural features of lignin in cell walls of fast-growing poplar wood. In this paper, the alkali-soluble lignins from fast-growing poplar wood were extracted with 5, 7.5, and 10% NaOH at 50°C for 4–12 h, respectively. Their physico-chemical properties and structural features were characterized by UV, FT-IR, ^{13}C-NMR spectroscopy, gel permeation chromatography (GPC), and alkaline nitrobenzene oxidation. The effect of alkali treatment time on the physico-chemical properties of the dissolved lignins is also reported.

2. Experimental

2.1. Material

Fast-growing poplar tree, 12 years old, was harvested in the December of 1997, at the University Forest of the North-Western University of Agricultural and Forest Science and Technology (Yangling, China). After the outer and inner barks were peeled off, the log was chipped and dried. The chips were then ground to pass through a 0.6–0.8 mm screen. The ground sample was further dried at 60°C for 16 h. The composition (%, (w/w)) of the dried fast-growing poplar wood is cellulose 43.8%, hemicelluloses 27.1%, lignin 23.3%, ash 1.6%, and wax 2.0%. The material was dewaxed by refluxing with toluene–ethanol (2:1, (v/v)) for 6 h in a Soxhlet apparatus. After being filtered and extensively washed with ethanol and acetone, the residue was dried in a cabinet oven with air circulation at 60°C for 16 h. The dried sample was then kept at 5°C before alkali extraction.

2.2. Isolation of alkali-soluble lignins

The dewaxed sample was treated with 5% NaOH (1 g sample/22 ml extractant) at 50°C for 4, 6, 8, and 12 h, and 7.5 and 10% NaOH (1 g sample/22 ml extractant) at 50°C for 6 h, respectively, under continuous agitation. The hemicelluloses were isolated from the hydrolysates by precipitation of the neutralized hydrolysate in three volumes of ethanol. After filtration, the pellets of the hemicelluloses were washed with 70% ethanol and air-dried. After evaporation of ethanol, the alkali-soluble lignins were obtained by reprecipitation at pH 1.5 from the corresponding supernatants. The isolated lignin preparations were washed with acidified water (pH 1.5–2.0), air-dried, and kept at 5°C until analysis (Fig. 1).

2.3. Characterization of the alkali-soluble lignin preparations PL

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 220 and 350 nm were measured.

The weight-average ($\bar{M}_w$) and number-average ($\bar{M}_n$) molecular weights of PL preparations were determined by GPC on a PLgel 5 μ Mixed-D column. The samples were dissolved in tetrahydrofuran at a concentration of 0.2%, and a 200 μl sample in solution was injected. The column was operated at 40°C and eluted with tetrahydrofuran at a flow rate of 1 ml min^{-1}. The column was calibrated using polystyrene standards.

FT-IR spectra were obtained on a FT-IR spectrophotometer using a KBr disc containing 1% finely ground samples. The solution-state ^{13}C-NMR spectrum was obtained on a Bruker 250 AC spectrometer operating in the FT mode at 62.4 MHz under total proton decoupled conditions. It is recorded at 25°C from 250 mg of sample dissolved in 1.0 ml DMSO-d_6 after 30,000 scans. A 60° pulse flipping angle, a 3.9 μs pulse width and 0.85 s acquisition time were used.

Table 1
Yield of the lignin fractions (% dry matter) isolated with 5, 7.5, and 10% NaOH at 50°C for various periods from dewaxed fast-growing poplar wood

Yield (%)	5% NaOH (50°C) treatment time (h)				7.5% NaOH	10% NaOH
	4[a]	6[a]	8[a]	12[a]	50°C, 6 h[b]	50°C, 6 h[c]
Total solubilized lignin	3.8	4.0	4.4	4.5	4.2	4.8
Isolated PL[d]	2.6	2.7	2.8	2.7	2.7	2.9
Lignin associated in the isolated hemicelluloses	0.8	0.8	0.8	0.9	0.9	0.9
Lignin solubilized in the pH 1.5 solution[e]	0.4	0.5	0.8	0.9	0.6	1.0

[a] The PL fractions extracted with 5% NaOH at 50°C for different periods from dewaxed fast-growing poplar wood.
[b] The PL fraction extracted with 7.5% NaOH at 50°C for 6 h from dewaxed fast-growing poplar wood.
[c] The PL fraction extracted with 10% NaOH at 50°C for 6 h from dewaxed fast-growing poplar wood.
[d] Represents the lignin fraction obtained by precipitation of the supernatant solution with 6 M HCl at pH 1.5 after isolation of the hemicellulose–lignin complexes.
[e] Represents the lignin fraction which is still solubilized in the pH 1.5 solution after precipitation of PLs.

Methods of bound uronic acid analyses and determination of phenolic acids and aldehydes with HPLC in nitrobenzene oxidation mixtures have been described in previous papers [12,13]. Neutral sugar composition in isolated lignin fractions was determined as alditol acetates [14]. All nitrobenzene oxidation results represent the mean of at least triplicate samples and each oxidation mixture was chromatographed twice. Other experiments were performed in duplicate. The standard errors or deviations were observed to be lower than 6.0% except for the variation among the triplicate nitrobenzene oxidation (7–15%). The chemical and structural characterization of the hemicelluloses will be reported elsewhere [15].

3. Results and discussion

3.1. Yield of alkali-soluble lignins

During the alkaline treatment process, some alkali-labile linkages between lignin units, or between lignin and polysaccharides, might be broken by the treatment [16]. In addition, the participation of significant amounts of ester-linked *p*-hydroxybenzoic acid in the cell walls of fast-growing poplar wood is considered to play a very important role in the release of lignin [10]. Generally, the alkali treatment does not seem to have any dramatic consequence on the structure of the solubilized lignins except for the saponification of the ester linkages between lignin or polysaccharides and hydroxycinnamic acids. This technology is, therefore, widespread for the isolation of lignins due to the purity and relatively high yield of the lignin extracted. Previous work has shown that the rate of delignification is governed by diffusion and sorption phenomena as well as by the molecular size, porosity, and structure of the cell wall matrix. It is also known that the rate of diffusion is strongly dependent on the pH of the liquid and extraction temperature. At high pH, lignin reacts with free alkali, especially at elevated temperature. Meanwhile, the swollen fibers under alkaline conditions also have a favorable impact on the transfer of lignin from the fibers [17].

Table 1 gives the yields of total solubilized lignin, isolated pure lignin (PL, acid-insoluble lignin), lignin associated in the isolated hemicellulose–lignin complexes, and the lignin solubilized in the pH 1.5 solution (acid-soluble lignin), obtained from the various alkaline treatment procedures. As can be seen, treatment of the dewaxed poplar wood with 5% NaOH at 50°C for 4, 6, 8, and 12 h, and 7.5 and 10% NaOH at 50°C for 6 h solubilized 3.8, 4.0, 4.4, 4.5, 4.2, and 4.8% lignin (% dry starting material), corresponding to the release of 16.3, 17.2, 18.9, 19.3, 18.0 and 20.6% of the original lignin, respectively. Meanwhile, the treatment also yielded 17.4, 18.2, 18.4, 18.6, 20.3, and 21.9% hemicelluloses, respectively (data not shown). The total yield of dissolved lignin increased from 3.8 to 4.5% with 5% NaOH treatment time increase from 4 to 12 h. Similarly, increase of alkali concentration from 5, to 7.5, and to 10% resulted in an increase of dissolved lignin yields by 5.0 and 20.0%, respectively. These results suggested that extension of alkali treatment duration or increase of alkali concentration favored the lignin release from the cell walls of poplar wood in alkali solution. In addition, as shown in Table 1, the isolated PL was the major lignin fraction, comprising 60.0–68.4% of the total solubilized lignins, while the lignin fraction, associated in the solubilized hemicelluloses, accounted for only 18.2–21.4% of the total released lignins. This observation implied that the alkali treatment under the conditions used significantly cleaved the ether linkages between lignin and hemicelluloses from the cell walls of fast-growing poplar wood.

3.2. UV spectra of PL

The six PL preparations exhibited the basic UV spectrum of typical lignins with a maximum at 236 nm. The second maximum at 272 nm originated from the non-conjugated phenolic groups (aromatic ring) in the lignin, which is known to be characteristic of guaiacyl–syringyl lignin, while the absorbance at 280 nm is characteristic of only guaiacyl lignin [18,19]. The relatively lower absorption in the lignin fraction (spectrum b) extracted with 10% NaOH at 50°C for 6 h is presumed to be due to the slightly higher amounts of co-precipitated non-lignin materials such as ash and salt. The much lower absorption at 310–320 nm in all the PL preparations revealed that the alkali treatments under the conditions given substantially cleaved the ester or ether bonds between hydroxycinnamic acids, such as *p*-coumaric acid and ferulic acid, and lignins.

Table 2
The content of neutral sugars and uronic acids (% dry matter) in PL fractions isolated with 5, 7.5, and 10% NaOH at 50°C for various periods from dewaxed fast-growing poplar wood (Tr = trace)

Sugar/Uronic acids (%)	5% NaOH (50°C) treatment time (h)				7.5% NaOH	10% NaOH
	4[a]	6[a]	8[a]	12[a]	50°C, 6 h[b]	50°C, 6 h[c]
Arabinose	0.045	0.078	0.074	0.064	Tr[d]	Tr
Xylose	0.30	0.32	0.35	0.22	0.58	0.54
Mannose	0.17	0.22	0.24	0.18	0.36	0.34
Glucose	0.41	0.47	0.34	0.33	0.51	0.40
Galactose	0.020	0.018	0.075	0.095	Tr	Tr
Total sugars	0.95	1.11	1.08	0.89	1.45	1.28
Uronic acids	2.06	1.56	1.31	1.25	1.13	1.22

[a] Represents the PL fractions extracted with 5% NaOH at 50°C for different periods from dewaxed fast-growing poplar wood.
[b] Represents the PL fraction extracted with 7.5% NaOH at 50°C for 6 h from dewaxed fast-growing poplar wood.
[c] Represents the PL fraction extracted with 10% NaOH at 50°C for 6 h from dewaxed fast-growing poplar wood.

3.3. Content of chemically linked polysaccharides

To verify the purity of the isolated lignin fractions, the preliminary search for non-ligneous components of the PL preparations was carried out by analyses of the bound neutral sugar composition and the content of associated uronic acids. Table 2 gives the composition of neutral sugars and content of uronic acids. Obviously, all the PL preparations contained only minor amounts of associated polysaccharides as shown by the very low content of neutral sugars, ranging between 0.9 and 1.5% of the dry lignin samples. This finding further confirmed that the alkali treatments under the conditions used removed the lignins from most of their neighboring polysaccharide moieties. In addition, the relatively high amounts of glucose, xylose, and mannose together with traces of arabinose and galactose in PL fractions implied that these bound polysaccharides mainly originated from the hemicelluloses such as xylan and glucomannans in the secondary cell walls of fast-growing poplar wood, not from the pectic polysaccharides in the middle-lamella. It should be noted that the alkali-soluble lignin fraction, prepared by alkali extraction of finely ground fast-growing poplar wood meal followed by purification using Björkman's procedure [20], contained relatively higher amounts of non-lignin materials (14.9%) [10]. It is therefore likely that the convenient method proposed in this present study may be preferred for most studies on lignins.

Interestingly, as can be seen in Table 2, the relatively high content of uronic acids (1.1–2.1%) in all the PL preparations was presumed to be due to the ester bonds between lignin and glucuronic acid or 4-*O*-methyl-glucuronic acid

Table 3
The yield (% lignin sample, (w/w)) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of the isolated PL fractions

Phenolic acids and aldehydes	5% NaOH (50°C) treatment time (h)				7.5% NaOH	10% NaOH
	4[a]	6[a]	8[a]	12[a]	50°C, 6 h[b]	50°C, 6 h[c]
p-Hydroxybenzoic acid	0.34	0.28	0.17	0.18	0.22	0.23
p-Hydroxybenzaldehyde	1.38	1.40	1.32	1.41	1.34	1.30
Vanillic acid	1.62	1.77	1.52	1.73	1.68	1.69
Syringic acid	1.72	1.81	1.61	1.70	1.44	1.43
Vanillin	10.88	11.35	10.80	11.66	10.64	10.78
Syringaldehyde	30.09	32.13	29.80	32.66	29.44	29.79
Acetovanillone	0.26	0.24	0.18	0.20	0.21	0.20
Acetosyringone	1.57	1.64	0.98	1.35	0.98	0.93
p-Coumaric acid	0.020	0.020	0.016	0.018	0.016	0.016
Ferulic acid	0.028	0.029	0.019	0.018	0.021	0.025
Total	47.91	50.67	46.42	50.93	46.00	46.39
Molar ratio (*S*:*V*:*H*)[d]	13:6:1	14:6:1	15:7:1	15:7:1	14:6:1	14:7:1

[a] Represents the PL fractions extracted with 5% NaOH at 50°C for different periods from dewaxed fast-growing poplar wood.
[b] Represents the PL fraction extracted with 7.5% NaOH at 50°C for 6 h from dewaxed fast-growing poplar wood.
[c] Represents the PL fraction extracted with 10% NaOH at 50°C for 6 h from dewaxed fast-growing poplar wood.
[d] *S* represents the relatively total moles of syringaldehyde, syringic acid, and acetosyringone; *V* represents the relatively total moles of vanillin, vanillic acid, and acetovanillone; and *H* represents the relatively total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid.

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 lignin fractions PL isolated with 5, 7.5, and 10% NaOH at 50°C for various periods from dewaxed fast-growing poplar chips

	5% NaOH (50°C) treatment time (h)				7.5% NaOH	10% NaOH
	4[a]	6[a]	8[a]	12[a]	50°C, 6 h[b]	50°C, 6 h[c]
$\bar{M}_w$	4520	5180	5800	5250	5430	6900
$\bar{M}_n$	1600	1850	2020	1950	1940	2330
$\bar{M}_w/\bar{M}_n$	2.8	2.8	2.9	2.7	2.8	3.0

[a] The PL fractions extracted with 5% NaOH at 50°C for different periods from dewaxed fast-growing poplar wood.
[b] The PL fraction extracted with 7.5% NaOH at 50°C for 6 h from dewaxed fast-growing poplar wood.
[c] The PL fraction extracted with 10% NaOH at 50°C for 6 h from dewaxed fast-growing poplar wood.

(MeGlcA) residues of hemicelluloses since it decreased with the increase of alkali treatment duration or increase in the alkali concentration. The occurrence of this ester bond was confirmed by the signal at 174.7 ppm in the ^{13}C-NMR spectrum (Fig. 5).

3.4. Composition of phenolic monomers

Table 3 shows the yield of alkaline nitrobenzene oxidation products from the six PL preparations. From this table, it is interesting to note that there is no significant difference in the total yield of phenolic acids and aldehydes, indicating the same degree of condensation of these lignins. The predominant product was identified to be syringaldehyde, which comprised 62.8–64.2% of the total nitrobenzene oxidation mixtures and resulted from the degradation of non-condensed syringyl units. Vanillin appeared as the second major degradation product and resulted from the degradation of non-condensed guaiacyl units. The presence of small quantities of *p*-hydroxybenzaldehyde was considered most probably to be indicative of non-condensed *p*-hydroxyphenyl units within the lignin core. The relative molar ratios of *S* (the relatively total moles of syringaldehyde, syringic acid, and acetosyringone) to *V* (the relatively total moles of vanillin, vanillic acid, and acetovanillone), and to *H* (the relatively total moles of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid) appeared to be of approximately the same order (13–15:6–7:1), indicating the same original lignin. The high monomeric ratio of *S*/*V* in all the PL preparations reflected that these lignins were released mainly from the secondary wall since a larger amount of guaiacyl lignin is formed in the early stage of xylem differentiation than in the later stages, which are rich in syringyl units in the secondary wall [21]. These results were in complete agreement with the data reported earlier by Kim and co-workers [11] from the comparative study of lignin in fast-growing and normal poplar woods. The authors stated that the lignins from fast-growing poplar wood yielded slightly higher contents of syringaldehyde but lower contents of vanillin than those of normal poplar wood in the alkaline nitrobenzene oxidation products. In addition, small amounts of vanillic and syringic acids, and acetosyringone, together with minor quantities of *p*-hydroxybenzoic acid and acetovanillone were also found in all the PL fractions examined from the nitrobenzene oxidation mixtures. Trace amounts of *p*-coumaric and ferulic acids, detected from the alkaline nitrobenzene oxidation products at 170°C, indicated that considerable amounts of these two acids had been converted to *p*-hydroxybenzaldehyde and vanillin, respectively, or that they appeared in only trace amounts in the cell walls of fast-growing poplar wood.

3.5. Molecular-average weight

The weight-average ($\bar{M}_w$) and number-average ($\bar{M}_n$)

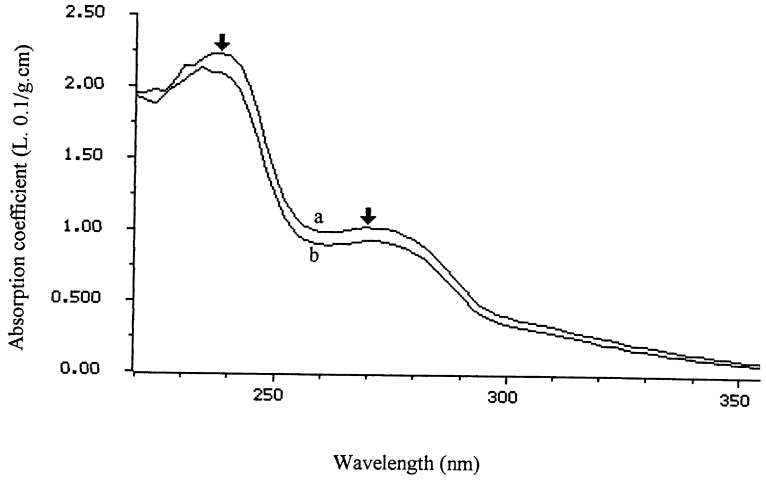


Fig. 2. UV spectra of lignin fractions PL extracted with 7.5% NaOH (spectrum a) and 10% NaOH (spectrum b) at 50°C for 6 h from dewaxed fast-growing poplar wood.

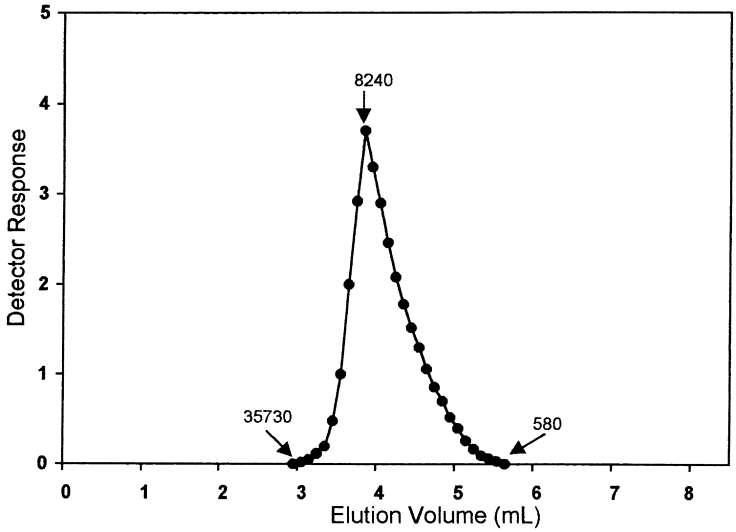


Fig. 3. GPC molecular weight distribution of PL fraction isolated from the hydrolysate of 5% NaOH treatment (50°C, 8 h) of dewaxed fast-growing poplar wood.

molecular weights, and polydispersity ($\bar{M}_w/\bar{M}_n$) of the six PL preparations were computed from their chromatograms and are given in Table 4. The data in Table 4 showed that the lignin preparations had weight average molecular weights ($\bar{M}_w$) between 4520 and 6900 g mol^{-1}, which were higher than those of the lignins isolated from wheat straw in our previous studies [22]. The reason for this relatively higher $\bar{M}_w$ was probably due to the reduced cleavage of the inter-unit linkages in lignin molecules during the 5–10% NaOH treatment processes. Table 4 also showed that $\bar{M}_w$ increased from 4520 g mol^{-1} in 4 h 5% NaOH treatment to 5800 g mol^{-1} in 8 h treatment, from where it decreased to 5250 g mol^{-1} in a maximum treatment time (12 h) used. This observation implied that during the 5% NaOH treatment processes, prolonging the treatment time up to 8 h led to an increasing $\bar{M}_w$ of the released lignin, while it decreased with a further extension of treatment duration from 8 to 12 h, indicating a degradation of the lignins during the 5% NaOH treatment between 8 and 12 h. However, as shown in Table 4, an increase of alkali concentration from 5 to 7.5, and to 10% resulted in an increase of $\bar{M}_w$ from 5180 to 5430, and to 6900 g mol^{-1}, respectively. The reason for this increase in $\bar{M}_w$ was presumed due to the lignin condensation during the treatments at 50°C for 6 h with a relatively high concentration of alkali. The six lignin fractions also gave a fairly similar elution pattern (see Fig. 3 as an example) showing a wide polydispersity ranging from 580 to 35,730 g mol^{-1}. The elution maximum corresponded to a polystyrene molecular weight of 8240 g mol^{-1}.

3.6. FT-IR spectra

To further investigate any differences in the structure of the released lignins, FT-IR spectra were recorded. Fig. 4 illustrates the FT-IR spectra of PL preparations, isolated with 5% NaOH at 50°C for 4 h (spectrum a) and 12 h (spectrum b), 7.5% NaOH (spectrum c) and 10% NaOH at 50°C for 6 h (spectrum d) from the dewaxed fast-growing poplar wood (Fig. 2). The intensity of the bands in the four spectra is rather similar to the typical alkali lignins, indicating that the structure of the lignins does not change substantially under the alkali treatment conditions used. No intense polysaccharide bands were identified, implying a lack of these compounds in the lignin samples. The absence of any detectable peak between 1740 and 1750 cm^{-1} revealed that the ester bonds between *p*-hydroxybenzoic acid or hydroxycinnamic acids and lignins were cleaved by the alkali treatment or the content of these groups is below the detection limit for the spectroscopical methods

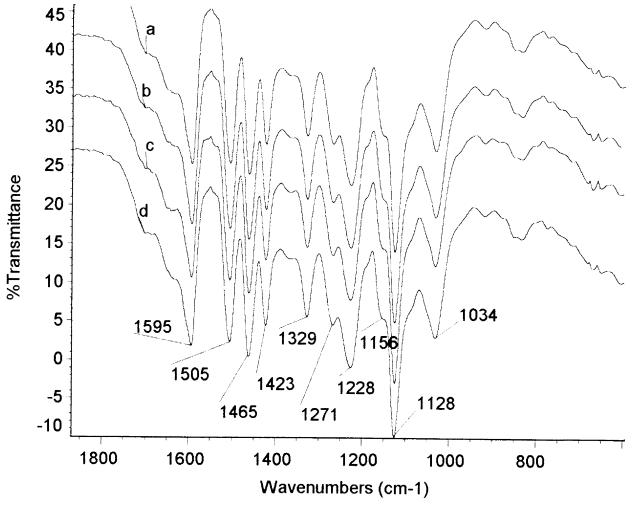


Fig. 4. The FT-IR spectra of PL preparations isolated with 5% NaOH at 50°C for 4 h (spectrum a) and 12 h (spectrum b), 7.5% NaOH (spectrum c) and 10% NaOH at 50°C for 6 h (spectrum d) from dewaxed fast-growing poplar wood.

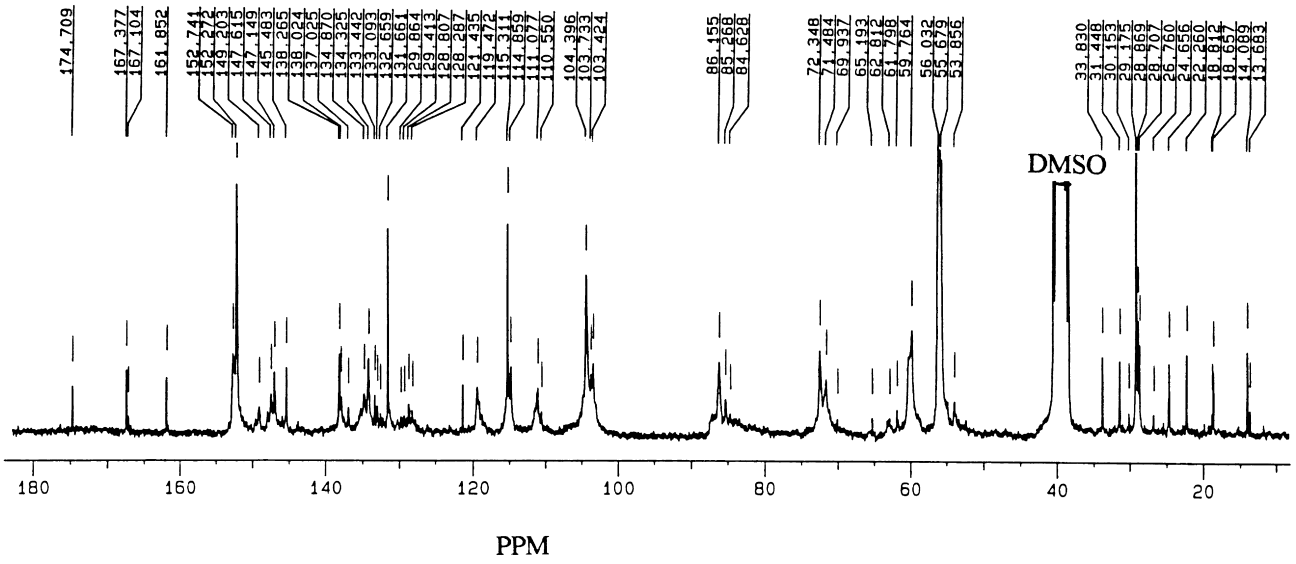


Fig. 5. ^{13}C-NMR spectrum of PL fraction extracted with 5% NaOH at 50°C for 12 h from dewaxed fast-growing poplar wood.

employed. The C=O in unconjugated ketone (β-carbonyl) and carboxylic acid, and C=O stretch in conjugated *p*-substituted aryl ketone (α-carbonyl) were observed in all the spectra at 1703 and 1651 cm^{-1}, respectively [23]. Aromatic skeleton vibrations in the lignin fractions are assigned at 1595, 1505, and 1423 cm^{-1}. Absorption at 1465 cm^{-1} indicates the C–H deformations and aromatic ring vibrations. The strong intensities of the bands at 1329 and 1128 cm^{-1} are associated with syringyl structures in lignin molecules, while the relative weak intensities of the bands at 1228, 1156, and 1034 cm^{-1} are associated with guaiacyl units in lignin molecules. Also the ratio of the intensities at 1271, 1228, and 1128 cm^{-1} has been used to estimate the relative contents of *p*-hydroxyphenyl, guaiacyl, and syringyl units in natural lignin [24]. As can be seen from the figure, the ratio values of $A_{1271}^{-1}/A_{1228}^{-1}$ and $A_{1228}^{-1}/A_{1128}^{-1}$ were rather small in all the spectra, indicating that all the PL preparations contained high syringyl units or low *p*-hydroxyphenyl and guaiacyl units. These results supported those obtained by alkaline nitrobenzene oxidation.

3.7. ^{13}C-NMR spectrum

The characterization of dissolved lignins from the alkaline hydrolysates by ^{13}C-NMR spectroscopy has been shown to be a very informative method [12]. Following previous studies on straw lignins [22], the PL fraction, extracted with 5% NaOH at 50°C for 12 h from the dewaxed fast-growing poplar wood, was also studied by ^{13}C-NMR spectroscopy (Fig. 5). Most of the observed signals have been previously assigned in straw and wood lignin spectra [22,25–27]. As expected, the most striking feature of the lignin is the extremely low level of associated polysaccharides, almost below the detection limit for ^{13}C-NMR between 57 and 103 ppm. The spectrum does show three signals at 65.2 ppm (C-5 in xylose non-reducing end unit), 62.8 ppm (C-5 in xylose internal unit), and 174.7 ppm (C-6 in methyl uronates) for the chemically linked polysaccharides, but the peak intensities are rather weak [19].

In the aromatic carbons area of Fig. 5, from 103.4 to 167.4 ppm, the syringyl, guaiacyl, or *p*-hydroxyphenyl aromatic carbons were detected qualitatively. The syringyl (S) units were identified with signals at 152.7 and 152.3 (C-3/C-5, S), 147.6 and 147.1 (C-3/C-5, S non-etherified), 138.3 and 138.0 (C-4, S etherified), 134.9 and 134.3 (C-1, S etherified), 133.4, 133.1, and 132.7 (C-1, S non-etherified), and 104.4 ppm (C-2/C-6, S). Guaiacyl (G) units gave signals at 149.2 (C-3, G etherified), 147.6 and 147.1 (C-4, G etherified), 145.5 (C-4, G non-etherified), 134. 9 and 134.3 (C-1, G etherified), 133.4,133.1 and 132.7 (C-1, G non-etherified), 119.5 (C-6, G), 114.9 (C-5, G), and 111.1 ppm (C-2, G), respectively. The *p*-hydroxyphenyl (H) units appeared as a very weak signal at 128.3 ppm (C-2/C-6, H). The relative intensities of these syringyl and guaiacyl signals clearly revealed that the ratio of syringyl to guaiacyl units was high in the PL fraction. This qualitative observation was confirmed by similar results obtained quantitatively from comparisons of the non-condensed syringyl to guaiacyl units in the alkaline nitrobenzene oxidation products. However, ^{13}C-NMR data, although not quantitative, give a better image of the whole lignin composition than chemical data dealing only with the non-condensed part of lignin fraction.

The ^{13}C-NMR analysis of lignin also provides a facile means of monitoring the *p*-hydroxybenzoic acid or hydroxycinnamic acids linked to the lignins by integrating the signal from 167.4 to 115.3 ppm. The strong signals at 167.4 and 167.1 (C=O), 161.9 (C-4), 131.7 (C-2/C-6), 121.4 (C-1), and 115.3 (C-3/C-5) ppm represented the esterified *p*-hydroxybenzoic acid. The two signals at 129.9

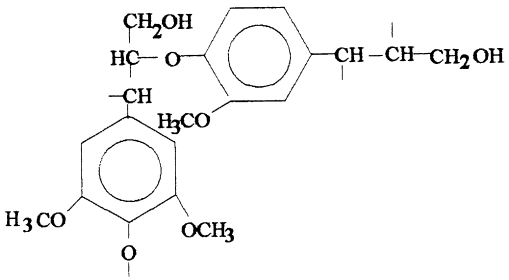
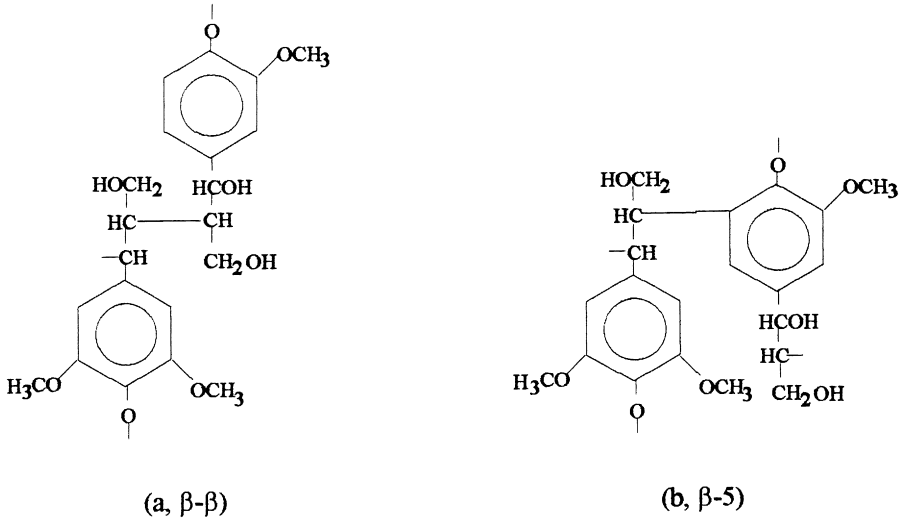


Fig. 6. A simple representative β-*O*-4 ether linkage.

and 129.4 ppm (C-2/C-6, PC ester) indicated the esterified *p*-coumaric acid. Etherified ferulic acid was observed with a small signal at 144.2 ppm (data not shown, C-α, FE ether). The esterified ferulic acid was identified with a very weak signal at 122.5 ppm (data not shown, C-6, FE ester). It is therefore likely that the lignin preparation contained some amount of esterified *p*-hydroxybenzoic acids and minor amounts of esterified *p*-coumaric acid as well as a trace of ferulic acids, which are linked to lignin by both ether and ester bonds. Results here are supported by evidence from the lignins in normal poplar wood, suggesting that *p*-hydroxybenzoic acid is linked to lignin by means of ester linkages in the cell walls [26]. Although chemical analysis such as alkaline nitrobenzene oxidation revealed that the content of this acid was only less than one percent in the lignin preparations, and FT-IR spectroscopy failed to detect any of this acid esterified, its strong signals were found in the ^{13}C-NMR spectrum. Further investigations are still needed to clarify this phenomenon. In addition, as compared to model compounds with a free phenolic function, it could be seen that the phenolic group of the *p*-hydroxybenzoic acid was free [26]. This suggested that in fast-growing poplar woods *p*-hydroxybenzoic acids may not be involved in the ester–ether bridges between lignins and/or between lignin and hemicelluloses. With an extensive study of the pathway of *p*-coumaric acid incorporation into maize lignin as revealed by the application of ^{13}C–^{1}H correlative NMR experiments, Ralph and co-workers [9] unambiguously revealed that *p*-coumaric acid is attached exclusively at the γ-position and none at its α-position. Furthermore, based on the presence of ester–ether bridges through ferulic acids between lignin and hemicelluloses in cell walls of temperate grasses and cereal straws [19,28], it can be presumed that ferulic acids in fast-growing poplar woods are etherified to lignin and also esterified to hemicelluloses, but *p*-coumaric acids are not involved in the ester–ether bridges.

The qualitative ^{13}C-NMR analysis of the lignins also allowed for the quantification of the β-*O*-aryl ether structures (Fig. 6). As shown in Fig. 5, signals at 86.2 (C-β in S β-*O*-4 erythro) and 85.3 (C-β in G β-*O*-4 threo), 72.3 (C-α in β-*O*-4 G and S erythro) and 71.5 (C-α in β-*O*-4 G and S threo), and 59.8 (C-γ in β-*O*-4 S and G threo and erythro) ppm belong to the resonances of C-β, C-α, and C-γ in β-*O*-4, respectively. This observation suggested that β-*O*-4 ether structures were resistant to the alkaline treatment of the fast-growing poplar wood under the conditions given. The common carbon–carbon linkages (Fig. 7) such as β–β (C-α in β–β units, 84.6 ppm; C-β in β–β units, 53.9 ppm) and β-5 (C-β in β-5 units, 52.3 ppm, data not shown in the spectrum) were also present. The signals representing the γ-methyl, α and β-methylene groups in *n*-propyl side chains appeared in the spectrum between 13.8 and 33.8 ppm. Two very strong signals at 56.0 and 55.7 ppm corresponded to the OCH_3 in syringyl and guaiacyl units. These signals revealed that the lignins of fast-growing poplar wood are composed mainly of β-*O*-4 ether bonds together with small amounts of β–β′ and β-5 carbon–carbon linkages. These findings were consistent with previous work reported by Lapierre and co-workers [26] on the lignins obtained from normal poplar wood.



(a, β-β) (b, β-5)

Fig. 7. Simple representative carbon–carbon linkages.

4. Conclusions

The above results showed that all six alkali-soluble lignin fractions are relatively free of associated polysaccharides and are composed of large amounts of syringyl units with noticeable amounts of guaiacyl and fewer *p*-hydroxyphenyl units. Further studies by ^{13}C-NMR revealed that the lignins were chemically linked with noticeable amounts of esterified *p*-hydroxybenzoic acids and minor amounts of esterified *p*-coumaric acid and traces of ferulic acids. It was found that uronic and *p*-hydroxybenzoic acids were esterified to lignin in the fast-growing poplar wood cell walls, while ferulic acids are linked to lignin by both ether and ester bonds. Furthermore, the current results also showed that the alkali-soluble lignin preparation is mainly composed of β-*O*-4 ether bonds, together with small quantities of β–β′ and β-5 carbon–carbon linkages between the lignin structural units.

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