Structural Characterization of the Lignin from the Nodes and Internodes of *Arundo donax* Reed

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Milled wood lignin (MWL) and dioxane lignin (DL) from different morphological regions (nodes and internodes) of *Arundo donax* reed were subjected to a comprehensive structural characterization by ^{13}C, ^{1}H NMR, FTIR, and UV spectroscopies and functional analysis. The permanganate and nitrobenzene oxidation methods were also applied to the in situ lignins. Both node and internode lignins are HGS-type lignins, with a significant amount of H units (including *p*-coumaric acid type structures). The S/G ratio (1.13−1.32), the weight-average molecular weight (20400−24500), the methoxyl group content (0.90−0.98), the phenolic hydroxyl group content (0.23−0.27), and the aliphatic hydroxyl group content (1.00−1.09) are not very different in the lignins from nodes and internodes. However, some structural differences between node and internode lignins were observed. The former has much more phenolic acids (*p*-coumaric and ferulic), 8.8% in node versus 1.2% in internode and less β-O-4 (0.32 and 0.49 per aromatic unit in node and internode, respectively). In situ node lignin is more condensed than internode lignin.

Keywords: *Lignin; reed; Arundo donax; ^{13}C NMR; ^{1}H NMR; UV spectroscopy; FTIR spectroscopy; permanganate oxidation; nitrobenzene oxidation; phenolic acids; functional analysis; gel permeation chromatography*

INTRODUCTION

In the past decades, fast growing plants have received particular attention as wood alternative sources of vegetable fibers. *Arundo donax* reed, a monocotyledonous plant with a segmented tubular structure like bamboo, growing naturally in Mediterranean countries with high biomass production rates (20−25 ton/ha/year) (Faix et al., 1989), is among those plants. Its potential applications include the use as a source of fibers for printing paper (De Coudens, 1966; Mela et al., 1994; Paavilainen and Hemming, 1995) and as a source of biomass for chemical feedstocks and for energy production (Faix et al., 1989). To improve the utilization of this plant, it is necessary to broaden the knowledge of structural features of its components. Previous chemical research on *Arundo donax* include chemical composition, general features of macromolecular components (Pascoal Neto et al., 1997), and the structures of isolated hemicelluloses (Driss et al., 1973; Joseleau and Barnoud, 1974, 1975, 1976). A few studies on the lignin of *Arundo donax* showed that it is composed of guaiacyl- and syringylpropane units with minor amounts of *p*-hydroxyphenylpropane units (Faix et al., 1989) and associated with phenolic acids (Tai et al., 1987). It was also shown that the lignin content in *Arundo donax* internodes greatly increases from the younger to the older parts of the plant (Joseleau and Barnoud, 1976; Joseleau et al., 1976). However, to our knowledge, no comprehensive work dealing with the complete structural characterization of the lignin from the different morphological regions of *Arundo donax* has been reported until now.

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This paper reports the detailed chemical characterization of milled wood lignin (MWL), dioxane lignin (DL), and in situ lignin from the nodes and internodes of *Arundo donax* by FTIR, UV, ^{1}H NMR, and ^{13}C NMR, spectroscopies, functional analysis, and chemical degradation methods.

MATERIALS AND METHODS

Preparation of Plant Material. The harvest of *Arundo donax* samples was performed as previously described (Pascoal Neto et al., 1997). The stems were separated from the foliage, air-dried, and cut in three fractions with the same length. The fraction corresponding to the middle part of the stem was used in this study. This part of the stem was manually further separated into nodes and internodes. The plant material was milled in a Retsch cross-beater mill SK1, sieved to 40 mesh, and air-dried. The plant powder was then submitted to successive extractions with petroleum ether, acetone, ethanol, and water (8 h each). Proteins were removed by treating extractive-free samples with 1% pepsin solution in 0.1 N HCl at 40 °C overnight, followed by hot water washing until neutrality. The lignin content was determined in extractive- and protein-free samples by the Klason method according to Tappi standard T 204 om-88 and found to be 17.2% for node and 19.8% for internode fractions (o.d. material).

Isolation of Lignins. The milled wood lignin (MWL) was isolated from extractive-free plant powder. The MWL was isolated using a centrifugal ball mill (Retsch S1) with a sintered corundum I jar and balls and purified according to the Björkman method (Bjorkman, 1956) with minor modifications (Obst and Kirk, 1988). The yield of the MWL obtained was 45.8% (of Klason lignin) for node and 25.2% for internode. The elemental analysis of MWL from node and internode gave 58.4% C, 6.4% H, 35.2% O and 57.0% C, 6.5% H, 36.5% O, respectively. The isolation of dioxane lignin (DL) from the internode region was based on the methodology described elsewhere (Pepper and Wood, 1962). Extractive- plus protein-

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free plant powder was submitted to three sequential extractions (30 min each) with a dioxane−water 9:1 (v/v) solution containing 0.2 N HCl under reflux and a nitrogen atmosphere. At the end, the powder was washed with dioxane−water 9:1 (v/v) (without HCl) and the combined extract was concentrated to 1/5 of the initial volume. The lignin was precipitated in water, centrifuged, and washed with water until neutral pH and then with ethyl ether. Nearly 86% of Klason lignin in internode was extracted. The elemental analysis of dioxane internode lignin gave 58.4% C, 5.7% H, and 35.9% O.

NMR Analysis. The ^{13}C NMR spectra were recorded on a Bruker AMX 300 spectrometer operating at a carbon frequency of 75.2 MHz. Lignin samples were dissolved in DMSO-d_6 and placed into 10 mm diameter tubes and the spectra were recorded at 318 K with TMS as an internal reference. The inverse-gate decoupling sequence, which allows quantitative analysis and comparisons of signal intensities, was used with the following parameters: 90° pulse angle, 12 s pulse delay, 16 K data points and number of scans 14 000. The distortionless enhancement by polarization transfer (DEPT) subspectra were taken with a $\theta = 135°$, coupling constant $J(^{13}C-^{1}H) =$ 150 Hz, using a 25% solution in 5 mm in diameter tubes. S/G ratios were calculated taking into account that H units, in addition to G units, also contribute to the integral at 110−122 ppm.

The ^{1}H NMR spectra of the acetylated lignins in $CDCl_3$ (3−4% concentration) were obtained using the same spectrometer and operating at $\nu_H = 300$ MHz at room temperature. The pulse experiment with a probe angle of 90° and a 2 s delay was run. The preparation of 100 mg of acetylated lignin was carried out using 2 mL of pyridine/acetic anhydride (4:4.7 v/v) solution. The mixture was kept at 42 °C for 24 h. Then 1 mL of methanol was added to the mixture (for anhydride decomposition) followed by 8 mL of dichloromethane. After 30 min, the organic phase was washed 3 times with HCl 7% and 2 times with water and then dried with anhydrous Na_2SO_4. The solvent was evaporated to dryness, and the dry residue was placed in oven at 42 °C for 24 h.

The assignment of resonances in lignin spectra was based on the comparison of their chemical shifts with those of lignin model compounds and other assigned spectra (Chen and Robert, 1988; Lundquist, 1992; Robert, 1992; Lapierre, 1993). The calculations of the amounts of different structural groups per aromatic group were based on signal integration (102−106 ppm, excluding contribution of vinylic carbons on coumarate structures) using previously published methods (Chen and Robert, 1988; Evtuguin et al., 1994).

UV and FTIR Analysis. Ultraviolet (UV) spectra were recorded in 2-methoxyethanol on a Hitachi 2000 UV/Vis spectrophotometer using 1 cm cells. Infrared (FTIR) spectra were obtained on a potassium bromide pellet (1.5/300 mg) using a Mattson 7020 FTIR spectrometer. The spectra resolution was 4 cm^{-1}, and 64 scans were averaged.

Chemical Analysis. The permanganate oxidation of in situ, MWL and dioxane lignin as well as the alkaline nitrobenzene oxidation of in situ lignin were performed as previously described (Gellerstedt, 1992; Chen, 1992). The phenolic acids were determined by GC as methyl esters, after alkaline hydrolysis of plant material (1 N NaOH, 48 h, room temperature), according to Chen (1992). Methoxyl group analysis was performed by the modified Zeisel procedure (Girardin and Metche, 1983). The content of the phenolic hydroxyl group was determined by aminolysis (Mansson, 1983). The determination of the total amount of hydroxyl (acetylation method) and carboxyl groups (chemisorption method) was conducted according to previously published procedures (Zakis, 1994). The analysis of neutral sugars in the lignin samples was performed as previously described (Blakeney et al., 1983; Coimbra et al., 1996). Elemental analysis was carried out with a Leco CHNS 932 analyzer. The oxygen content was calculated by subtraction. Number-average molecular weights of lignins dissolved in dimethylacetamide were determined by GPC using a PL-GPC 110 chromatograph equipped with two Plgel 5 μm Mixed D 300 × 7.5 mm columns, a refractive index detector, and calibration with lignin preparations previously characterized by ESI-MS.

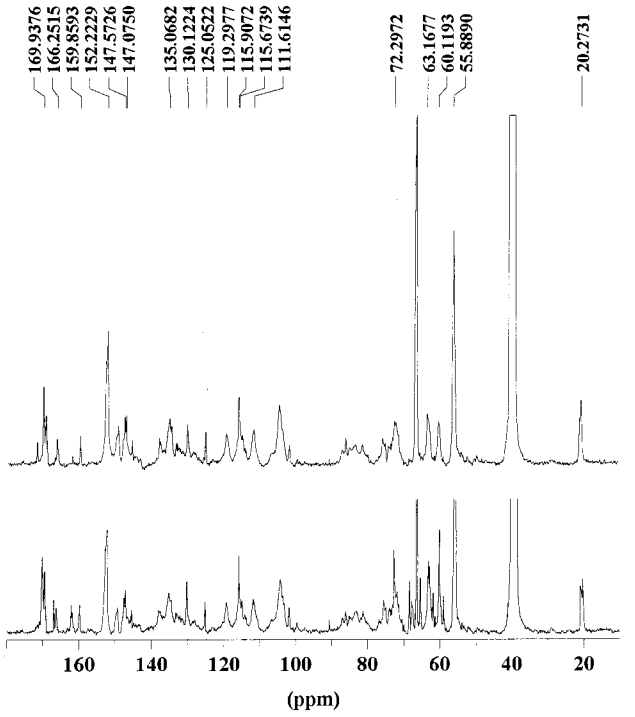

Figure 1. ^{13}C NMR spectra of of node (a) and internode (b) MWL lignins.

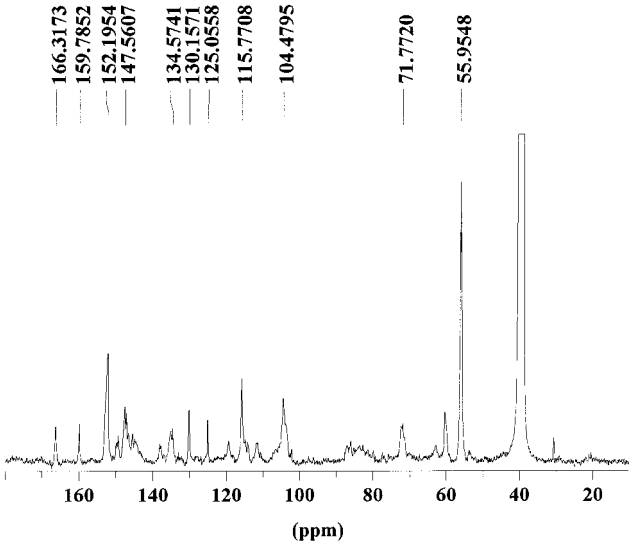

Figure 2. ^{13}C NMR spectra of internode dioxane lignin.

RESULTS AND DISCUSSION

^{13}C NMR Analysis. The ^{13}C NMR spectra of MWL and dioxane lignin of *Arundo donax* are given in Figures 1 and 2, respectively. The spectra of MWL lignins show a high level of the impurities as residual dioxane (66.3 ppm), carbohydrates (101−100 ppm, 75.6−73.0 ppm, 62 ppm), and acetyl groups (methyl carbon at 20.8 ppm and carboxyl carbon at 169.0−169.9 ppm). A relatively high content of acetates was previously reported also for MWL from *Hibiscus cannabinus*, a dicotyledonous annual herbaceous plant. There are two possible explanations for this abundance of acetyl groups in MWL: (i)

Table 1. Neutral Sugars in MWL and Dioxane Lignins (%)

samples	% total sugars	arabinose	xylose	glucose
MWL				
node	11.8	10.4	82.4	7.2
internode	10.5	4.8	88.0	7.2
DL				
internode	3.4	23.1	60.7	16.2

they can proceed from acetylated xylans, (ii) they may be introduced during the isolation procedure of MWL, which involves a purification step with 90% acetic acid (Obst and Kirk, 1988), or (iii) they may exist naturally on native lignin. As far as the first tentative explanation is concerned, the sugar analysis showed a carbohydrate content (xylose, glucose and arabinose) of 11−12% node and internode MWL (Table 1). The abundance of sugars in lignin does not justify a significant acetyl group content. On the other hand, the presence of a signal at 168.9 ppm in the ^{13}C NMR spectra, assigned to carbonyl carbon of the acetyl group in phenolic hydroxyls (Robert and Brunow, 1984), suggests a partial acetylation of −OH groups in aromatic rings during the lignin isolation procedure. As far as the natural acetylation of lignin is concerned, our results are not conclusive. It could be argued that if acetates exist in MWL but not in dioxane lignin, their origin would be essentially in the purification step during the MWL isolation procedure and would not exist naturally in lignin. However, even if dioxane lignin does not bear acetyl groups (Figure 2), we cannot conclude that they do not exist in native lignin because they should be removed, partially at least, during the acidolysis procedure. Further investigation is required on this topic.

The node and internode lignins of *Arundo donax* have high contents of *p*-hydroxyphenylpropane (H) units, as usual in monocotyledonous plants (Scalbert et al., 1985). This is clearly shown in the ^{13}C NMR spectra by strong signals at 115.5 and 129.9 ppm assigned to C3,5 and C2,6 and at 159.8 ppm assigned to C4 in the aromatic nuclei of H units. The presence of *p*-coumarate-type structures is also confirmed by the signal at 166.2 ppm assigned to ester carbonyl carbon conjugated with the vinylic moiety and to the signal at 144.2 ppm in the DEPT spectrum of dioxane internode lignin (Figure 3) assigned to C_α in coumaric acid-type structures. Previous calculations on ^{13}C NMR spectra showed that H units in *Arundo donax* lignin are composed mainly of esterified *p*-coumarate structures (Pascoal Neto et al., 1997).

Table 2. Quantitative ^{13}C NMR Analysis of Node and Internode Lignins (Number of Structures per Aromatic Unit)

	MWL		DL
lignin structures	node	internode	internode
S/G	1.13	1.23	1.32
β-O-4 without C_α=O	0.32	0.49	0.34
β-β + β-5	0.17	0.21	0.20
C_{ar}−H	2.54	2.94	2.71
C_{ar}−C	1.52	1.35	1.46

The results on ^{13}C NMR quantification of different types of structures and linkages in *Arundo donax* lignins are summarized in Table 2. Node and internode lignins have similar S unit content, with S/G ratios of 1.13 and 1.23, respectively.

β-O-4 linkages without a carbonyl group in the α position of the side chain are the most frequent (32−49%) interunit linkages (Table 2). Internode MWL lignin has more β-O-4 linkages than node MWL lignin, while internode dioxane lignin has less β-O-4 linkages than internode MWL lignin. The differences in β-O-4 content between MWL and dioxane lignins was previously reported for other species (Seca et al., 1998) and may be explained by the cleavage of β-O-4 linkages in acidic medium (Faix et al., 1994) and by the topochemical specificity of the MWL isolation procedure (Maurer and Fengel, 1992). β-O-4 linkages with a Cα=O were detected only in internode dioxane lignin by the signal at 62.7 ppm in the ^{13}C NMR spectra, assigned to Cγ in those structures (Figure 2) and by the inversion of the 62.7 ppm resonance on the CH DEPT (θ = 135°) spectra (Figure 3). In MWL lignins, this signal is overlapped by the signals from sugar contamination (Figure 1).

Quantitative ^{13}C NMR spectra (Figure 1) show that node lignin is more condensed than internode lignin:

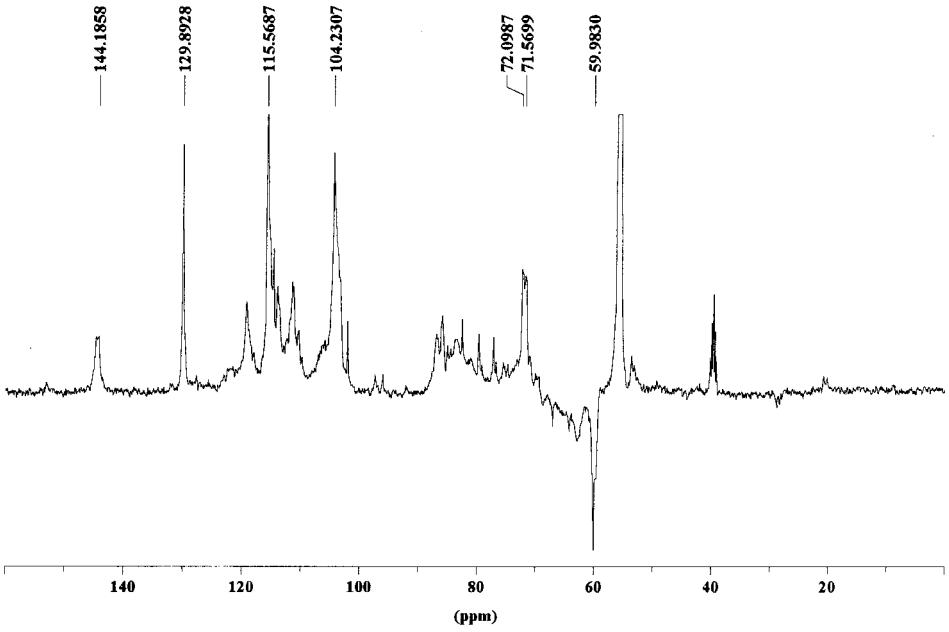

Figure 3. DEPT CH (θ = 135°) spectra of internode dioxane lignin.

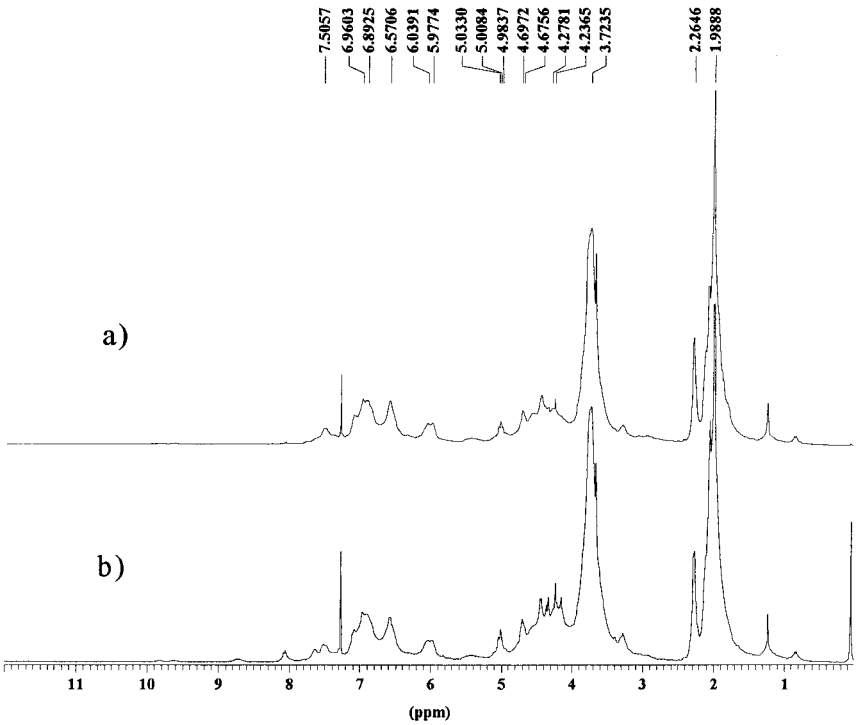

Figure 4. [1]H NMR spectra of acetylated node (a) and internode (b) MWL lignins.

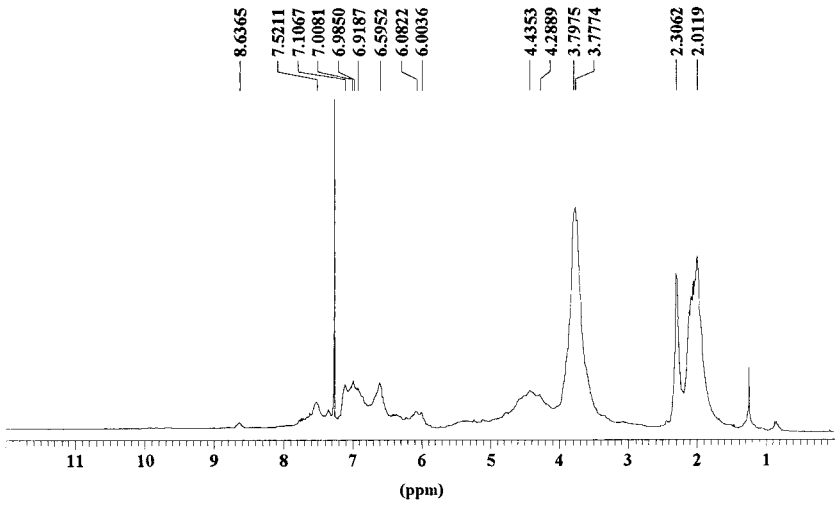

Figure 5. [1]H NMR spectra of acetylated internode dioxane lignin.

the former has fewer tertiary carbons per aromatic ring and more $C_{ar}-C$ linkages than internode lignin (Table 2). Also, it may be concluded that dioxane lignin is more condensed than MWL lignin. No significant difference in the content of $\beta-\beta$ plus β-5 linkages not involved in syringaresinol structures was observed between MWL and dioxane lignin.

The proportion of etherified (S_e) and non-etherified (S_{ne}) syringylpropane-type structural units can be estimated by the ratio of peak areas at 152.2 and 147.1 ppm, while the same information for guaiacylpropane (G_e and G_{ne}, respectively) units is obtained from the resonances at 149.4 and 145.5 ppm (Robert, 1992). The S_e/S_{ne} values (nearly 2.3 for node and 2.2 for internode MWL lignins and 1.9 for internode dioxane lignin) and G_e/G_{ne} values (nearly 1.3 for node and 1.7 for internode MWL lignins and 1.1 for internode dioxane lignins) suggest that the syringylpropane units are more involved in ether linkages with other lignin units than the guaiacylpropane structures.

[1]H NMR Analysis. Proton NMR spectroscopy of lignin enables one to obtain additional structural information to that obtained by [13]C NMR (Lundquist, 1992; Chen and Robert, 1988). The [1]H NMR spectra of acetylated MWL and dioxane lignins are presented in Figures 4 and 5. The signal at 5.0 ppm, assigned to carbohydrate impurities, is evident in node and internode MWL but not in internode dioxane lignin, which is in agreement with [13]C NMR analysis.

In MWL and dioxane lignins [1]H NMR spectra (Figures 4 and 5), the integrals of signals centered around 6.6 and 6.9 ppm assigned to aromatic protons in syringylpropane and guaiacylpropane structures, respectively (Faix et al., 1994), suggest the presence of similar relative contents of S and G in both these types of lignins. The broad signal around 7.3−7.6 ppm can be assigned to the aromatic protons in positions 2 and 6, in structures containing a $C\alpha{=}O$ group (Lundquist, 1992), to aromatic protons in positions 2 and 6 of H units conjugated with a double bond, and to the proton in

Table 3. Results of ^{1}H NMR Analysis of Node and Internode Lignins (Number of Functional Groups per Phenylpropane Units)

samples	$OH_{phenolic}$	$OH_{aliphatic}$	CHO	COOH
MWL				
node	0.20	1.09	0.07	0.01
internode	0.17	1.00	0.09	0.02
DL				
internode	0.45	1.12	0.32	0.03

Table 4. Functional Groups of Node and Internode Lignins (Number of Functional Groups per Phenylpropane Units)

samples	OCH_3	OH_{tot}	OH_{ph}	C=O	COOH
MWL					
node	0.98	1.24	0.23	0.15	0.08
internode	0.90	1.01	0.27	0.20	0.16
DL					
internode	1.29	1.35	0.40	0.23	0.10

Table 5. Yields and Molar Proportions of the Nitrobenzene Oxidation Products before and after Alkaline Hydrolysis of in Situ Node and Internode Lignins

samples	η %[a]	H:V:S[a]	η %[b]	H:V:S[b]
sawdust				
node	42.1	10:43:47	29.1	4:42:54
internode	51.2	11:44:45	30.3	4:44:52

[a] Before alkaline hydrolysis. [b] After alkaline hydrolysis.

$HC_\alpha{=}C_\beta$ structures, confirming the presence of *p*-coumarate-type structures and $C_\alpha{=}O$ groups in both lignins.

The average number of each functional group per C_9 unit can be estimated using the integral of the OMe signal as a reference (Islam and Sarkanen, 1993). Results from this analysis are shown in Table 3. Small differences were found between node and internode MWL lignins. Dioxane lignin of an internode has more phenolic and aliphatic hydroxyl groups than the corresponding MWL lignin, suggesting again the partial hydrolysis of lignin during the acidolytic treatment.

In general, ^{1}H NMR results are in agreement with those obtained by chemical analysis (Table 4) carried out in order to confirm quantitative ^{1}H NMR data.

Nitrobenzene Oxidation and Phenolic Acids. Table 5 summarizes the yield and molar proportion of syringaldehyde (S), vanillin (V), and *p*-hydroxybenzaldehyde (H) from nitrobenzene oxidation of node and internode in situ lignin before and after alkali hydrolysis. The S/V molar ratio before alkaline hydrolysis is in agreement with reported results for whole reed MWL (Tai et al., 1987) and in situ lignins (Higuchi et al., 1967). No significant difference was found between the S/V ratio in node (1.09) and internode (1.02), even after alkali hydrolysis (1.28 to node, 1.18 to internode). However, the yield of nitrobenzene oxidation, which is suggested by some authors to be related to the degree of condensation of lignin (Lam et al., 1990; Chen, 1992), is higher in internode than in node in situ lignin (Table 5), indicating that node lignin is more condensed than internode lignin. This is in agreement with results from ^{13}C and ^{1}H NMR analysis.

The amount of ester-bonded phenolic acids, released during alkaline hydrolysis, may be estimated by GC analysis of the alkaline hydrolysate (Chen, 1992). The amount of phenolic acids in node lignin, 8.8%, is much higher than that in internode lignin, 1.2%. The phenolic acids content, similar to that of nodes lignin, was previously reported for the whole stem of other monocotyledonous plants such as *Miscanthus condensatus* and *Coix lachryma* (Higuchi et al., 1967). Coumaric acid (isomers cis and mainly trans) represents ca. 70% and ca. 80% of the ester-bonded phenolic acids fraction in nodes and internodes lignin, respectively, the remaining being constituted by ferulic acid (isomer trans) (Table 5).

Results from nitrobenzene oxidation of *p*-coumaric and ferulic acids (pure compounds), using the same conditions as for lignins, showed that these acids are only partially converted to *p*-hydroxybenzaldehyde (52%) and vanillin (63%), respectively, during the oxidative treatment. Noting the predominance of *p*-coumaric acid in the phenolic acids fraction, the H units content in the H:V:S proportions (Table 5) is, hence, underestimated, particularly for node lignin.

On the basis of the yields of oxidation products and phenolic acids released from alkaline hydrolysis and taking into account the partial conversion of *p*-coumaric and ferulic acid to the corresponding aldehydes, some interesting conclusions may be drawn from nitrobenzene oxidation and phenolic acids analysis. When comparing the yields of *p*-hydroxybenzaldehyde obtained by nitrobenzene oxidation of node sawdust before and after alkaline hydrolysis, there is a fairly good correlation between the decrease in *p*-hydroxybenzaldehyde yield and the yield of *p*-coumaric acid released from alkaline hydrolysis. Hence, the lignin fraction dissolved during the alkaline treatment corresponds essentially to phenolic acids. However, in the internode sample, the yield of *p*-coumaric and ferulic acids released during alkaline hydrolysis is significantly lower than the decrease in the yield of *p*-hydroxybenzaldehyde and vanillin obtained from nitrobenzene oxidation of internode before and after alkaline hydrolysis. This suggests that, in the case of internode, in addition to the hydrolysis and dissolution of phenolic acids, a significant fraction of alkali labile lignin dissolves during the mild alkaline hydrolysis treatment.

After alkaline hydrolysis, nearly one-half of the initial H units is retained in node and internode lignins, suggesting the presence of alkali-resistant *p*-hydroxyphenyl units involved, for example in ether or carbon−carbon linkages.

Permanganate Oxidation. The permanganate oxidation of in situ node and internode lignin, followed by GC-MS analysis of methylated oxidation products allowed the identification and quantification of 11 carboxylic acid methyl esters, I−XI (Figure 6; Table 7).

The relative proportion of H, G, and S structural units showed that both node and internode in situ lignins are a HGS-type lignin with H:G:S proportions of 31:60:9 and 36:60:4, respectively. These proportions are very different from those obtained by nitrobenzene oxidation or NMR analysis. Such differences are not necessarily surprising. In the permanganate oxidation method, only phenolic phenylpropane units (17−20% of total units in MWL lignin and 45% in dioxane lignin, Table 3), previously protected by methylation toward the oxidation, are actually accessible to this analysis. In addition, as shown by ^{13}C NMR analysis, the syringylpropane units are more esterified than guaiacylpropane units. Such features may partially explain the differences observed between the H:G:S proportions obtained by permanganate oxidation and by ^{13}C NMR and nitrobenzene oxidation.

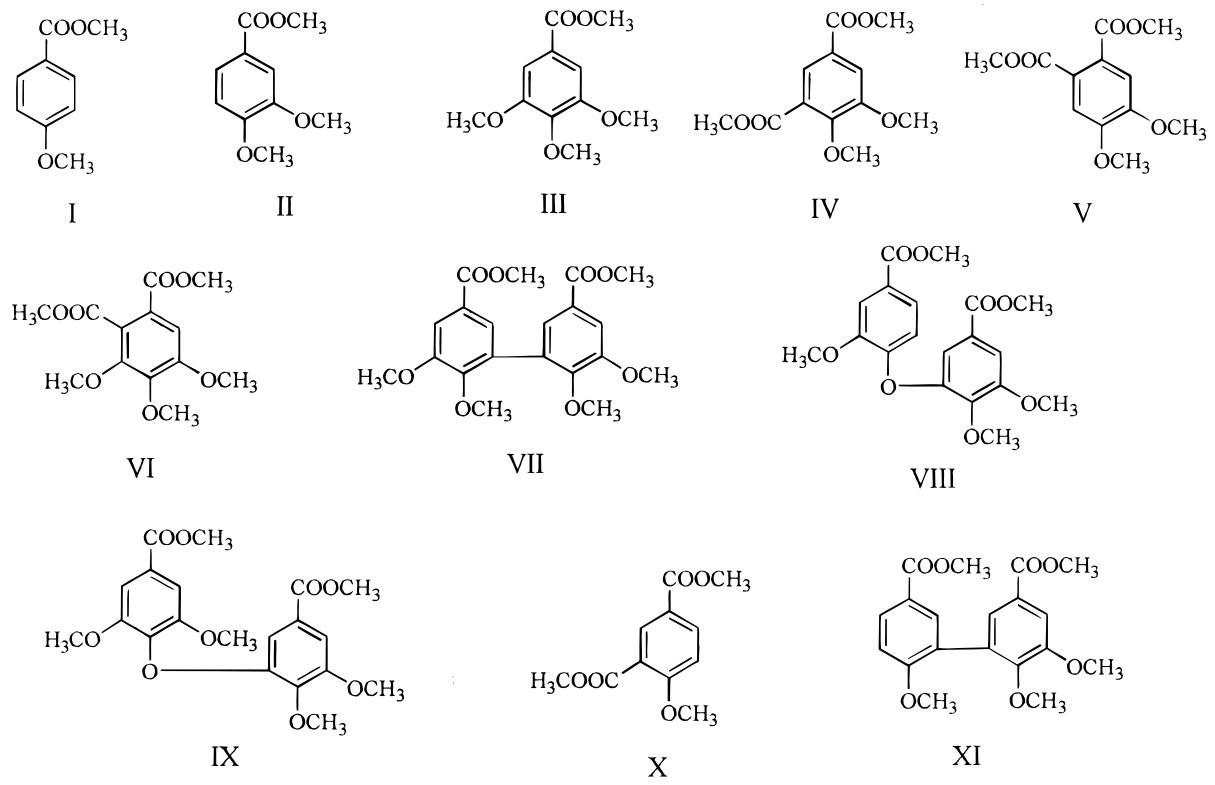

Figure 6. Carboxylic acid methyl esters obtained by the oxidation of *Arundo donax* lignin with permanganate.

Table 6. Phenolic Acids Released from Alkaline Hydrolysis of in Situ Lignins of the Node and Internode Reed[a]

samples	η %	%PC	%FA
sawdust			
node	8.8	6.4	2.4
internode	1.2	1.0	0.1

[a] η: Yield of phenolic acids (%). PC: *p*-coumaric acid. FA: ferulic acid.

Table 7. Yields and Molar Proportions of the Products of the Permanganate Oxidation[a]

samples	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	η %
sawdust												
node	31	51	8	4	1	tr	2	1	2	tr	tr	21.6
internode	36	51	4	4	1	tr	2	1	1	tr	tr	19.0

[a] See Figure 8 for the structures of products I−XI. tr: trace.

The figures of the relative proportion of H units also include coumarate structures which yield, after the permanganate oxidation treatment, product I (Figure 6). In addition, permanganate oxidation gave compounds X and XI (Figure 6), showing that some *p*-hydroxyphenyl units in lignin exist as C5 condensed structures.

The results obtained by permanganate oxidation of in situ node and internode lignins agree, in terms of the relative proportions of structural units, with those obtained for MWL lignin (Pascoal Neto et al., 1997). However, they are in apparent conflict with previously reported results for internode kraft lignin (Joselau et al., 1976), where a lower proportion of H and a higher proportion of S type structures were found. Those differences may be assigned to the specific nature of kraft lignin, which prevents comparisons from being made with MWL and dioxane lignins.

UV and FTIR Spectrophotometric Analysis. The UV spectra of MWL lignins (Figure 7) are very similar

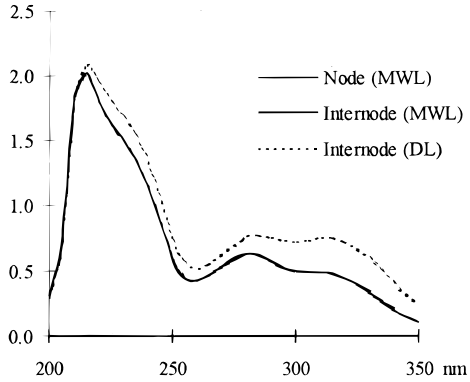

Figure 7. UV spectra of node and internode MWL lignins and internode dioxane lignin.

to those obtained from lignin of whole plant (Faix et al., 1989). The spectra show three maxima, one at 208 nm corresponding to the $\pi \rightarrow \pi^*$ electronic transition in the aromatic ring, one at 282 nm assigned to the free and etherified hydroxyl groups and one at 308 nm assigned to the $n \rightarrow \pi^*$ transition in lignin units containing C_α=O groups and $\pi \rightarrow \pi^*$ transitions in lignin units with C_α=C_β linkages conjugated with aromatic ring. No significant differences are observed between the UV spectra of node and internode lignins. The extinction coefficients (ϵ, L g^{-1} cm^{-1}) for node and internode are, respectively, 15.9 and 15.2 L g^{-1} cm^{-1} at 282 nm and 12.3 and 11.6 L g^{-1} cm^{-1} at 308 nm. The UV spectrum of dioxane lignin is very different from that of MWL lignin. The two maxima for dioxane lignin are at 285 and 315 nm, and the extinction coefficients are 19.5 and 19.0 L g^{-1} cm^{-1}, respectively. The high value of ϵ reveals that this lignin has more phenolic groups and C_α=O and C_α=C_β linkages conjugated with the aromatic ring, in agreement with previous results. The maxima observed at 282−285 nm is similar to that of other monocotyle-

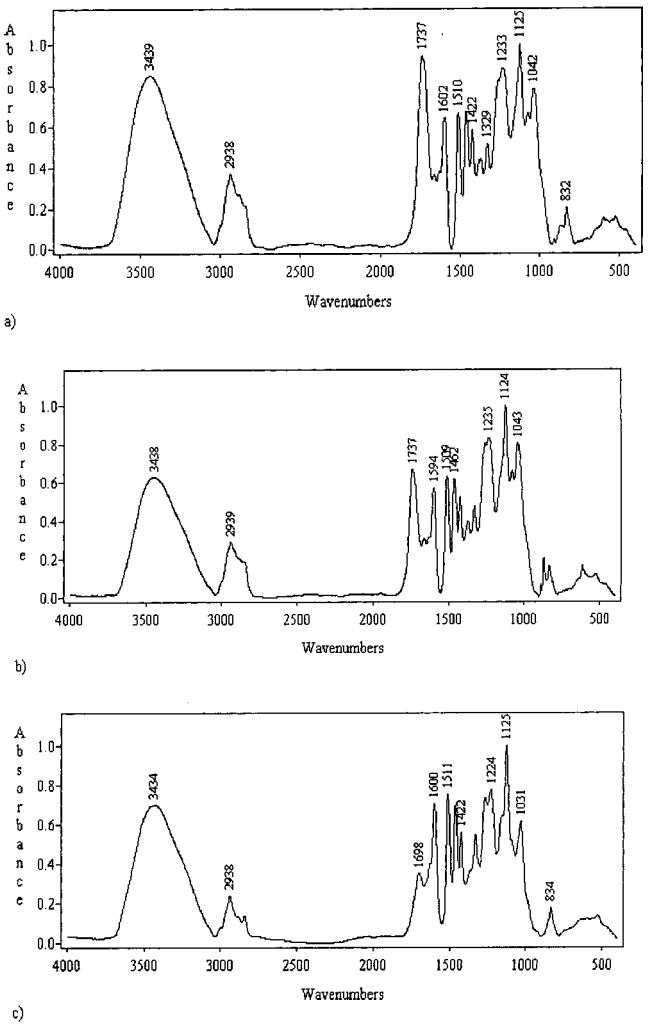

Figure 8. FTIR spectra of node (a) and internode (b) MWL lignins and internode dioxane lignin (c).

Table 8. Number-Average Molecular Weights (M_n) of Node and Internode Lignins

samples	M_n
MWL	
node	2200
internode	2600
DL	
internode	1800

donous (Higuchi et al., 1967) and softwood lignins (Sakakibara, 1991) and is consistent with the relatively high content of guaiacyl units.

FTIR spectra of node and internode MWL lignin and internode dioxane lignin are shown in Figure 8. Both node and internode *Arundo donax* lignin show the spectral features of HGS type lignins, namely, the bands at 1125 and 832 cm^{-1} and the shoulder at 1152 cm^{-1} (Faix, 1991). Spectra of MWL lignins show a strong absorbance centered at 1737 cm^{-1}, assigned to C=O in unconjugated ketones, carbonyls, and ester groups (Faix, 1991). Esterified phenolic acids and acetyls are the contributors to this absorption band. This band does not appear in internode dioxane lignin, suggesting the hydrolysis of these structures.

Number-Average Molecular Weights Analysis. Results of lignin number-average molecular weights (M_n) determined by GPC are shown in Table 8. Because calibration was carried out using polystyrene standards, figures shown on this table can be used only in comparative analysis. The M_n of internode lignin is higher than that of node lignin. Dioxane lignin has a M_n lower than that of MWL. This difference is assigned to the cleavage of β-O-4 linkages by acid hydrolysis during the isolation of dioxane lignin.

The results here described represent the first comprehensive study on the lignins from nodes and internodes of *Arundo donax*. It is concluded that *Arundo donax* lignins are typical grass lignins composed of guaiacyl, syringyl, and a significant amount of *p*-hydroxyphenylpropane units. *p*-Hydroxyphenylpropane units exist as ester-linked *p*-coumaric acid, particularly in node but also incorporated in the core lignin.

In general, the structure and composition of lignins, including the relative proportion of H, G, and S units, is not remarkably different in nodes and internodes. However, some differences may be observed between the two lignins. The degree of condensation in nodes is higher than that in internodes and the β-O-4 linkages content in internodes is higher than in node lignin. The phenolic acids content in nodes lignin is higher than that in internodes (8.8% and 1.2%, respectively), with *p*-coumaric acid dominating over ferulic acid. Such differences are important enough to suggest different lignin biosynthetic routes in these two morphological regions of *Arundo donax* reed, resulting in different responses for nodes and internodes, when submitted to chemical processing.

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