Structural Characterization of the Bark and Core Lignins from Kenaf (*Hibiscus cannabinus*)

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Dioxane and milled wood lignins were isolated from the core and bark of kenaf (*Hibiscus cannabinus*), variety Salvador. These lignins were characterized by ^{13}C and ^{1}H NMR, FTIR, and UV spectroscopies, permanganate oxidation, and alkaline hydrolysis followed by GC and GC-MS analysis of the released products and by functional groups analysis. The permanganate oxidation and alkaline hydrolysis was also applied to "in situ" lignins. Isolated and "in situ" lignins showed significant differences in composition and structure. Strong structural differences were observed between bark and core lignins, suggesting their different biosynthetic routes. The β-O-4 type linkages are the main interunit linkages and are more abundant in bark than in core lignin. The core lignin is more "condensed" and shows higher contents of β-β plus β-5 linkages than those in the bark lignin. Permanganate oxidation showed that both core and bark "in situ" lignins are HGS-type lignins with HGS proportions of 15:66:19 and 12:56:32, respectively (H including coumarate structures in the case of core lignin). Coumarates represent about 50% of the H units of core lignin and are absent in bark lignins. The presence of suberin-like aliphatic chains covalently bound to lignin was suggested for bark lignin.

Keywords: *Lignin; kenaf; Hibiscus cannabinus; ^{13}C NMR; ^{1}H NMR; UV spectroscopy; FTIR spectroscopy; permanganate oxidation; phenolic acids*

INTRODUCTION

In recent years, a great focus has been put into alternatives to wood as new sources of vegetable fibers for pulp and papermaking. Kenaf (*Hibiscus cannabinus*), a dicotyledonous annual herbaceous plant, has proved to have excellent characteristics to achieve this task (Kokta et al., 1993; Kaldor, 1992; Thusu and Murthy, 1991). Kenaf can grow in tropical or temperate climates and has long been cultivated in India and East Africa. Investigations on pulp and paper properties of kenaf cultivated in experimental farms in Portugal indicated the potential of these fibers for wrapping and packaging paper (Figueiredo and Baptista, 1994).

The characteristics of kenaf's fibers differ in a marked way, depending on being bark or core fibers. The bark fibers, representing the more interesting kenaf fraction for pulp production, have lengths of ca. 2900 μm while core fibers have lengths of ca. 600 μm (Figueiredo and Baptista, 1994). Also, the chemical composition of these two fractions may differ significantly (Pascoal Neto et al., 1996a).

The structure of the kenaf's lignin has been previously investigated (Abbott and James, 1984; Abbott et al., 1986a,b, 1987; Ralph, 1996). However, the few reported works have not investigated the bark and core lignins in a separate way. In addition, the obtained information is quite disperse and, sometimes, controversial. Our recent results have shown that the two types of lignins in kenaf (bark and core) present significant structural differences (Pascoal Neto et al., 1996a) and should be investigated separately in more detail. To our knowledge, no comprehensive work dealing with the structural characterization of the lignin of core and bark of kenaf has been published. In this paper, we collate structural information on the bark and core lignins, obtained by ^{13}C and ^{1}H NMR, FTIR, and UV spectroscopic characterization, permanganate oxidation, alkaline hydrolysis, and functional group analysis of extracted and "in situ" lignins.

MATERIALS AND METHODS

Preparation of Plant Material. *Hibiscus cannabinus*, variety Salvador, was harvested in September 1994, in Quinta do Canal, Figueira da Foz, Portugal (latitude, 40°08′ N; longitude, 8°51′ W; altitude, 17 m). The stems were separated from 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. Then, it was manually further separated into bark and core. 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-plus protein-free samples by the Klason method according to Tappi standard T 204 om-88. The lignin contents of bark and core fractions were 10.1 and 14.6% (o.d. material), respectively.

Isolation of Lignins. The lignins were isolated from extractive-free plant powder. The milled wood lignin (MWL) was isolated using a centrifugal ball mill Retsch S1 with sintered corundum I jar and balls and purified according to the Björkman method (Björkman, 1956) with minor modifications (Obst and Kirk, 1988). The yield of the MWL obtained from core was 27.5% of the original Klason lignin. The elemental analysis of MWL core lignin gave 53.0% C, 5.8% H,

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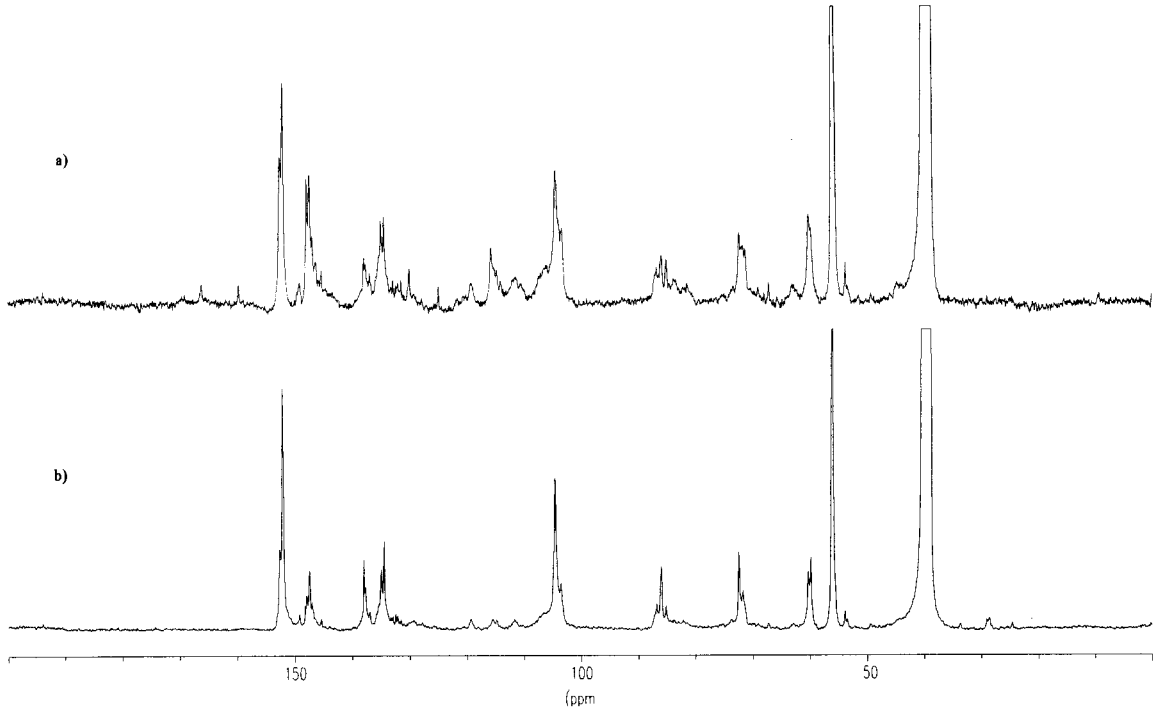

Figure 1. ^{13}C NMR spectra of core (a) and bark (b) dioxane lignins.

and 41.2% O. The isolation of dioxane lignin (DL) was based on methodology described elsewhere (Pepper and Wood, 1962). Extractive-plus protein-free kenaf powder was submitted to three sequential extractions (30 min each) with dioxane−water 9:1 (v/v) solution containing 0.2 N HCl and one extraction with dioxane−water 9:1 (v/v) (without HCl), under reflux and nitrogen atmosphere. The combined extract was concentrated to one-fifth of the initial volume. The lignin was precipitated in water, centrifuged, and washed with water until neutral pH and then with ethyl ether. This lignin from core and bark yielded respectively 63% and 76.2% of the original Klason lignin. The elemental analysis results for the dioxane core lignin and for the dioxane bark lignin were 60.3% C, 6.0% H, 33.8% O and 56.2% C, 6.0% H, 37.8% O, respectively.

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 was recorded at 318 K with TMS as the internal reference. The inverse gated decoupling sequence, which allows quantitative analysis and signal intensity comparisons, was used with the following parameters: 90° pulse angle; 12 s pulse delay; 16K data points; and number of scans 14 000. The DEPT subspectra were taken with a $\theta = 135°$, coupling constant $^1J(^{13}C-^1H) = 150$ Hz, using a 25% solution in 5 mm diameter tubes.

The ^1H NMR spectra of the acetylated lignins in chloroform-d_1 (4−5% 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 μL of pyridine/acetic anhydride (4:4.7 v/v). The mixture was kept at 42 °C for 24 h. Then, methanol (1 mL) and dichloromethane (8 mL) were added to the mixture. After 30 min, the organic phase was washed 3 times with 7% HCl 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 the oven at 42 °C for 24 h.

The assignment of resonances in lignin spectra was based on 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, 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 kenaf material and isolated lignins and the carbohydrate analysis of isolated lignins were performed using previously described methodologies (Pascoal Neto et al., 1996a). The phenolic acids were determined by GC as methyl esters, after alkaline hydrolysis of kenaf material and isolated lignins, according to Chen (1992). Methoxyl group analysis was performed by the modified Zeisel procedure (Girardin and Metche, 1983). The content of phenolic hydroxyl groups 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).

RESULTS AND DISCUSSION

^{13}C NMR Analysis. The ^{13}C NMR spectra and the results of quantitative ^{13}C NMR analysis of dioxane lignin (DL) and milled wood lignin (MWL) of kenaf are given in Figures 1 and 2 and in Table 1.

The spectrum of the core MWL (Figure 2) shows the resonances assigned to hemicellulose contaminations and acetyl groups (169.6, 100−101.5, 92.4, 73−76, and 20.9 ppm) and to residual dioxane (66.4 ppm). The amount of residual carbohydrate, determined by hydrolysis of lignins followed by GC analysis of individual neutral sugars, is nearly 9%, consisting of 88% xylose, 8% glucose, and 4% arabinose. The relatively high amounts of acetyl groups (resonances of corresponding carbonyl carbons at 168−171 ppm and methyl groups at 20.9 ppm) can be partially explained both by the sample contamination with acetylated xylans and by the presence of lignin acetyl groups which may appear during the MWL isolation procedure, which involves a purification step with 90% acetic acid, according to Obst and Kirk modification (Obst and Kirk, 1988). The presence of a signal at 168.9 ppm assigned to the

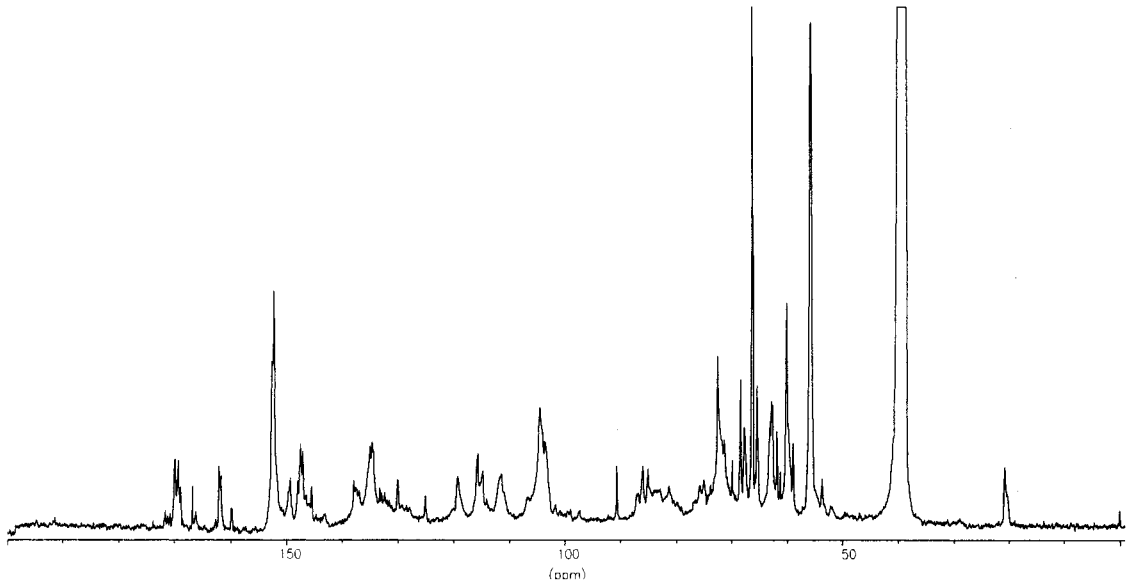

Figure 2. ^{13}C NMR spectrum of core MWL lignin.

Table 1. Quantitative ^{13}C NMR Analysis of Core and Bark Lignins (Number of Structures per Aromatic Group)

		DL	
lignin structures	MWL core	core	bark
S/G	1.37	1.55	3.38
OMe	0.97	0.99	1.43
β-O-4 without C_α=O	0.59	0.39	0.53
β-β + β-5	0.25	0.28	0.12

carbonyl atom of the acetyl group in acetylated phenolic hydroxyls (Robert and Brunow, 1984) supports this last suggestion. The dioxane lignin spectra (Figure 1) do not show typical resonances assigned to hemicelluloses or to acetyl groups, although chemical analyses show that the core and bark dioxane lignins include carbohydrate contents of 2 and 3%, respectively. The presence of very high amounts of acetyl groups in isolated kenaf lignins was previously reported in the literature, and it was claimed that acetates occur naturally in kenaf lignins (Ralph, 1996). As far as the natural acetylation of our kenaf lignins is concerned, our results are not conclusive. Although the core MWL shows a high degree of acetylation, we cannot definitely state that a part, at least, of these acetates occurs naturally in lignin. On the another hand, although the core and bark dioxane lignin spectra do not show typical acetate signals, the hydrolysis and removal of acetates, eventually present in lignin, during the acidolytic isolation of lignin, cannot be excluded. This matter needs further investigation.

In the ^{13}C NMR spectra of both core and bark dioxane lignins, the characteristic tertiary carbon resonances from syringylpropane (S) (signals centered at 104.9 ppm) and guaiacylpropane (G) units (signals centered at 111.5 and 119.3 ppm) may be observed. The approximately calculated S/G ratios for core and bark dioxane lignins are 1.55 and 3.38, respectively, showing that the isolated bark lignin is richer in syringyl content than core lignin (Table 1). The S/G ratios for core dioxane lignin and core MWL are quite similar, suggesting very similar compositions in terms of elementary structural units. The methoxyl contents (as measured by NMR) of both MWL and dioxane lignins are in good agreement with their relative compositions in S and G units (Table 1).

The core lignin also shows the presence of *p*-hydroxyphenylpropane (H) units (characteristic resonances at 130.1, 159.8, and 166.3 ppm) that are absent in the bark lignin. The signals at 159.8 and 166.3 ppm, also found in the ^{13}C NMR spectra of lignins of other annual plants (Pascoal Neto et al., 1997) and grasses in general, are assigned, respectively, to C_4 and ester carbonyl atom resonances in *p*-coumarate type structures (Lapierre, 1993). The CH DEPT spectrum of the core dioxane lignin (Figure 3) shows a positive signal at 144.8 ppm assigned to vinylic Cα in *p*-coumarate type structures (Lapierre, 1993). This resonance was not found in the CH DEPT spectrum of the bark dioxane lignin, confirming that these structures are absent in this lignin or, at least, are present in small amounts.

The lignin structures involving β-O-4 linkages, without a carbonyl group in the α position of the aliphatic side chain, are the most frequent, contributing about 39−59% of the total amount of interunit linkages (Table 1). In addition, the β-O-4 linkages are much more abundant in bark lignin than in core lignin. The weak signal at 63.0 ppm, assigned to C_γ in β-O-4 structures with a C_α=O group in the aliphatic side chain, suggests the existence of these structures in the core and bark dioxane lignins. The inversion of the 63.0 ppm resonance on the CH DEPT (θ = 135°) spectra (Figure 3) confirms the presence of β-O-4 structures with a C_α=O group in the aliphatic side chain in core lignin. However, as follows from the same DEPT spectra, only a negligible amount of these structures is present in bark lignin. The frequency of occurrence of β-O-4 linkages in the core dioxane lignin is lower than that in the corresponding MWL lignin. A tentative explanation could be the cleavage of β-O-4 linkages during the dioxane lignin isolation by acidolysis (Faix et al., 1994). However, the topochemical specificity associated with the MWL isolation procedure (Maurer and Fengel, 1992) cannot be excluded.

The content of β-β plus β-5 linkages, not involved in syringaresinol structures, is also very different in core and bark lignins (Table 1). Particularly, the content of these linkages in core dioxane lignin is twice higher than in bark dioxane lignin. At the same time, in bark lignin, the carbon resonance at 137.9 ppm assigned to

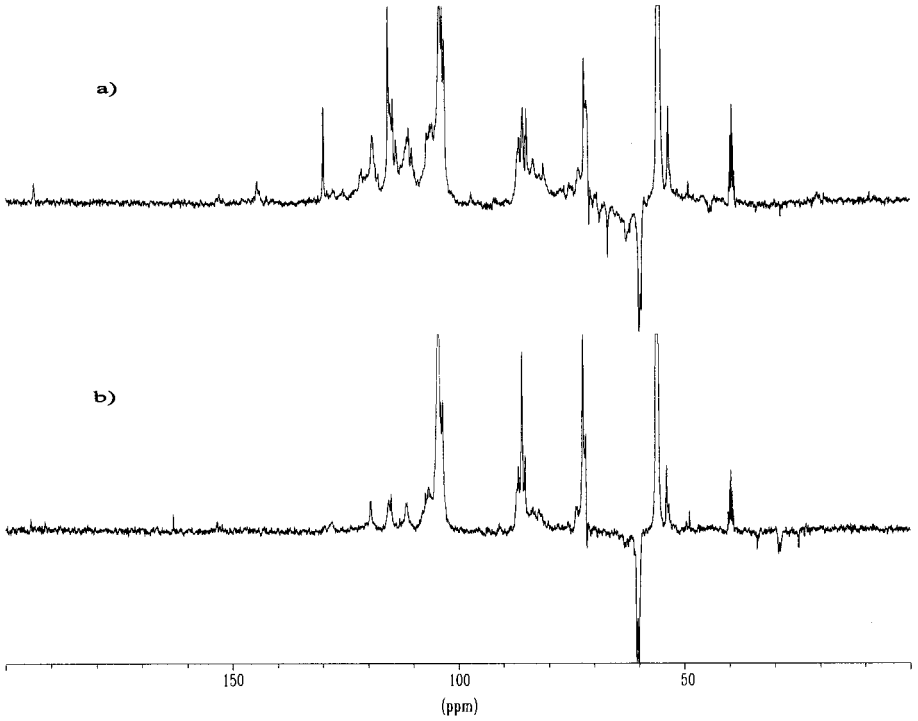

Figure 3. CH DEPT ($\theta = 135°$) spectra of core (a) and bark (b) dioxane lignins.

C_1 of S units involved in syringaresinol structures (Lapierre, 1993) is higher than in core lignin, meaning that bark lignin is richer than core lignin in syringaresinol structures.

The ratio of peak areas at 152.1 and 147.1 ppm provides information about the etherified (S_e) versus the non-etherified (S_{ne}) syringylpropane type structural units, while the same information for guaiacylpropane (G_e and G_{ne}, respectively) units is obtained from the resonances at 149.1 and 145.4 ppm (Robert, 1992). Although these ratios are not rigorously quantitative, they give relevant information on the nature of the end (non-etherified) units of lignins from bark and core. The S_e/S_{ne} values (nearly 3.0 for core and nearly 8.3 for bark dioxane lignins) and G_e/G_{ne} values (nearly 2.7 for core and nearly 2.0 for bark dioxane lignins) suggest that, particularly in bark lignin, the syringylpropane units are mainly internally ether-linked to other lignin units while the guaiacylpropane structures are mainly non-etherified units, located in the end units of the lignin macromolecule.

^{1}H NMR Analysis. Proton NMR spectroscopy of lignin allows additional structural information to be obtained (Lundquist, 1992; Chen and Robert, 1988). ^{1}H NMR spectra of acetylated core and bark dioxane lignins of kenaf are shown in Figure 4.

The ^{1}H NMR gives further evidence for the higher syringylpropane content of bark dioxane lignin when compared with core dioxane and MWL lignin (Figure 4), as follows from the relative intensities of resonances at 6.5−6.8 and 6.8−7.2 ppm, assigned to aromatic protons in syringylpropane and guaiacylpropane structures, respectively.

Although the bark lignin is richer in syringyl units than the core lignin, the quantification of the number of aromatic protons per phenylpropane unit in bark and core dioxane lignins, using the signal of the methoxyl group as an internal reference, gives values of 2.21 and 2.15, respectively. This fact suggests the higher "condensation" degree of the core lignin as compared to the bark lignin.

The presence of signals between 0.75 and 1.60 ppm, assigned to CH_2 and CH_3 in saturated aliphatic chains, deserves some special attention (Figure 4). The existence of suberin-like aliphatic chains, covalently bound to the lignin polymer, was previously reported in the literature for other species (Jung and Himmelsbach, 1989; Pascoal Neto et al., 1996b). To investigate if these aliphatic structures correspond to some suberin-like aliphatic chains ester-bonded to lignin, the dioxane lignin was treated in an alkaline solution (0.3% NaOH, 1 h, reflux). These aliphatic structures are not removed by the alkali treatment, suggesting that they are not ester-bonded to lignin. This finding needs further investigation in order to establish the nature and structural features of these so-called suberin-like aliphatic structures.

The relative abundance of the most important functional groups in bark and core lignins was estimated by using the integral of the signal of methoxyl groups as a "quasi" internal standard (Islam and Sarkanen, 1993). The results of this analysis are presented in Table 2. The content of aromatic and aliphatic hydroxyl groups per C_9 (phenylpropane) unit was calculated based on the integrals of the corresponding aromatic (2.20−2.50 ppm) and aliphatic (1.60−2.20 ppm) acetyl groups. The content of phenolic hydroxyls in the core dioxane lignin is much higher than that found for the corresponding MWL lignin. This result indicates the formation of new OH phenolic groups during the isolation of dioxane lignin by the cleavage of interunit linkages, corroborating the results obtained by ^{13}C NMR. Small differences in the content of aldehyde and carboxyl groups, between dioxane lignin and MWL, and between core and bark lignins were also detected (Table 2). However, it is necessary to be careful in the analysis of these results, because MWL contains notable amounts of sugars that insert an error in the calculations of

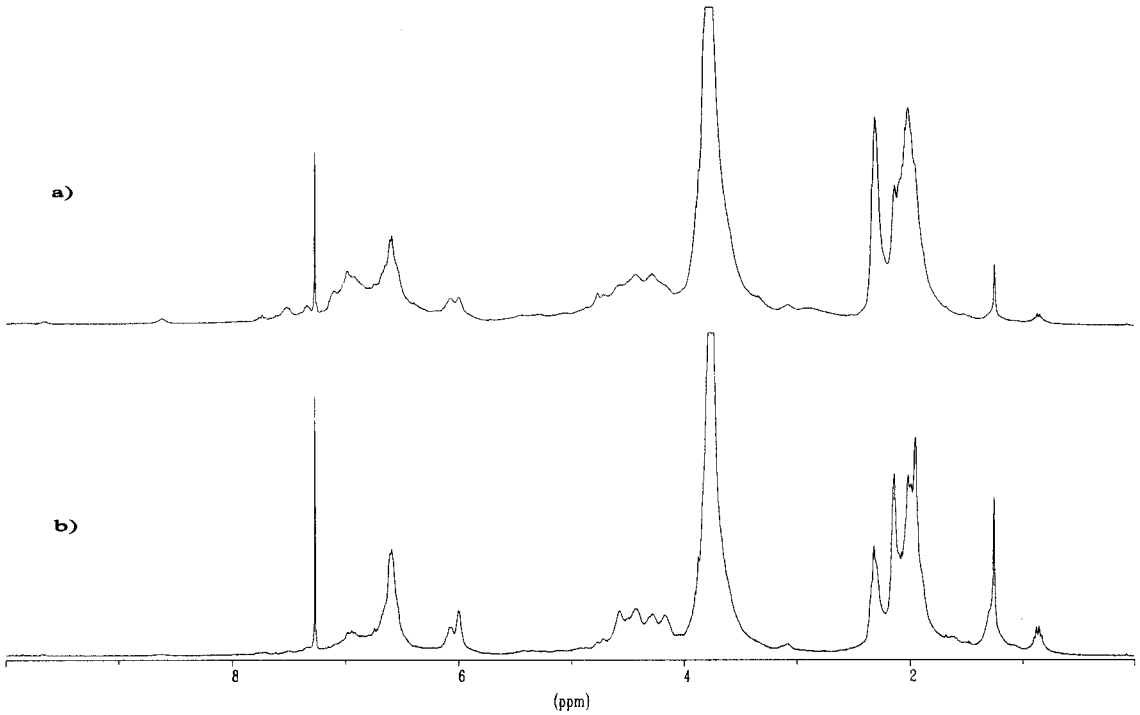

Figure 4. ^{1}H NMR spectra of acetylated core (a) and bark (b) dioxane lignins.

Table 2. Results of ^{1}H NMR Analysis of Lignins (Number of Functional Groups per Phenylpropane Unit)

samples	$OH_{phenolic}$	$OH_{aliphatic}$	CHO	COOH
MWL				
core	0.18	1.03	0.12	0.01
DL				
core	0.43	0.89	0.07	0.03
bark	0.38	1.33	0.08	0.01

Table 3. Functional Group Analysis of Core and Bark Lignins (Number of Functional Groups per Phenylpropane Unit)

samples	OCH_3	OH_{tot}	OH_{ph}	C=O	COOH
MWL					
core	1.11	1.12	0.16	0.24	0.27
DL					
core	1.29	1.40	0.34	0.22	0.08
bark	1.64	1.57	0.24	0.15	0.10

functional groups, based on the quantity of methoxyl groups in lignin as an internal reference. The bark dioxane lignin has slightly less phenolic OH groups than the core dioxane lignin. In contrast, the bark lignin has more free aliphatic hydroxyl groups than the core one. This result can be interpreted by taking into account the presence of suberin-like substances in the bark lignin, as discussed above. The suberin aliphatic chains are constituted mainly by aliphatic hydroxylated chains (Kolattukudy, 1980), thus contributing to the increased content of aliphatic hydroxyl groups in bark lignin.

Functional analysis of lignins was carried out in order to confirm quantitative data from ^{1}H NMR analysis. In general, results obtained (Table 3) agree with those from NMR and provide evidence for the strong structural differences between the core and bark lignins.

UV Spectrophotometric Analysis. The UV spectra of bark and core lignins (Figure 5) were very similar to those obtained from other annual plants (Faix et al., 1989). In the case of bark lignin, the characteristic absorption maximum corresponding to the $\pi \rightarrow \pi^*$ electronic transition in the aromatic ring occurs at 275 nm,

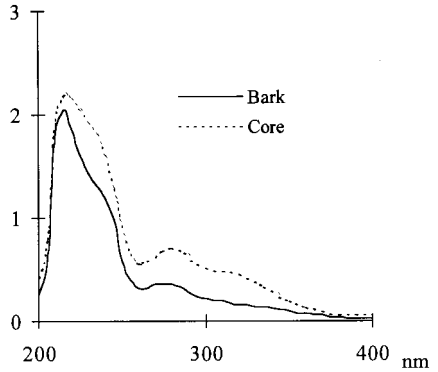

Figure 5. UV spectra of core and bark dioxane lignins.

Table 4. Extinction Coefficients of Core and Bark Dioxane Lignins, at Different Wavelengths

wavelength, nm	core DL	bark DL
215	54.0	63.0
240	37.8	33.3
275/280[a]	16.7	10.3
310	11.9	5.5

[a] 275 nm for bark DL and 280 nm for core DL.

that is, lower than for the core lignin (280 nm). This hypsochromic shift is due to the content of sygingyl units in the bark lignin which is higher than that of the core lignin (Morohoshi, 1991).

The extinction coefficients (ϵ) at 215, 240, 280 (or 275), and 310 nm are indicated in Table 4. The ϵ values at 280 nm for core dioxane lignin are 16.7 L g^{-1} cm^{-1} and for the bark dioxane lignin at 275 nm 10.3 L g^{-1} cm^{-1}. The absorption at 310 nm is normally assigned to the $n \rightarrow \pi^*$ transition in lignin units containing C_α=O groups. The ϵ values at 310 nm for the core lignin (11.9 L g^{-1} cm^{-1}) and for the bark lignin (5.5 L g^{-1} cm^{-1}) suggest that the former has more C_α=O groups, and this is in agreement with ^{13}C NMR results.

FTIR Analysis. FTIR spectra of core and bark dioxane lignins are presented in Figure 6. Based on

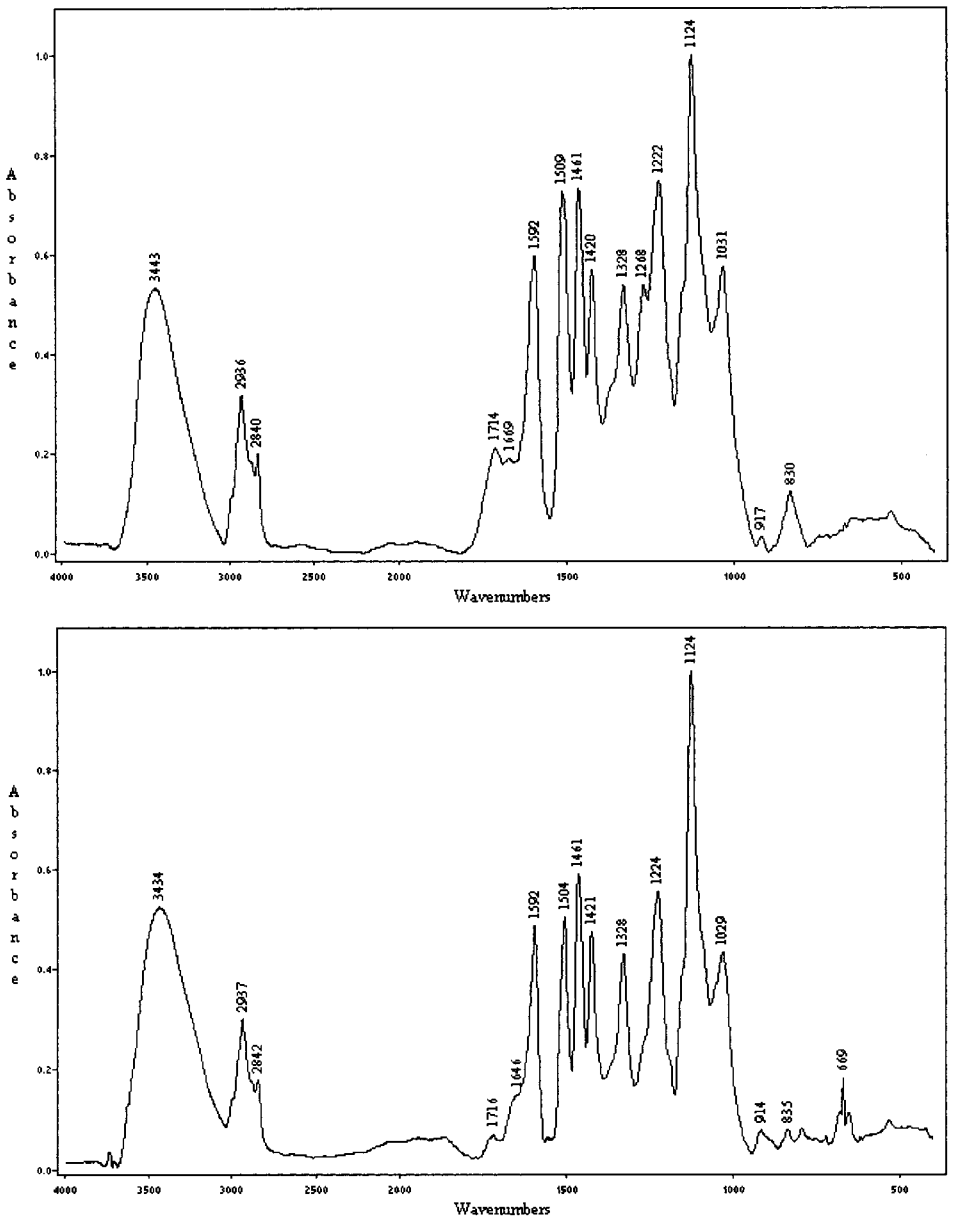

Figure 6. FTIR spectra of core (upper) and bark (lower) dioxane lignins.

previously proposed criteria for the classification of lignins by their FTIR spectra (Faix, 1991), it is possible to roughly classify both core and bark lignins as GS type lignins, although core lignins present some characteristics of the HGS type lignin. The ester carbonyl signal at 1714 cm^{-1} together with the shoulder at 1166 cm^{-1} and the band at 835 cm^{-1} suggests the presence of esterified *p*-coumaric units in core lignin (Faix, 1992). No clear evidence exists for the presence of coumarate structures in the bark lignin, in agreement with previous results.

Other significant differences in the spectra of the core and bark lignins are recognized. The FTIR spectrum of the core lignin shows a small band at 1669 cm^{-1}, not present in the spectrum of the bark lignin, assigned to ketone carbonyls conjugated with aromatic rings, substituted in *para* positions (Vázquez et al., 1997) and with an oxygenated C_β (Abbott et al., 1987). The band at 1268 cm^{-1} in core lignin, not present in the spectrum of bark lignin, is assigned to G units with C=O groups (Faix, 1992). This suggests that such C=O groups are mainly present in G units.

The cross-linking indexes, reflecting the lignin "condensation" degrees, were calculated from the FTIR data using an earlier proposed approach (Faix, 1994). The 0.41 index found for bark lignin vs 0.45 for the core lignin supports the ^1H NMR data concerning the higher "condensation" degree of the core lignin as compared to the bark lignin.

Permanganate Oxidation. The permanganate oxidation of dioxane lignins and "in situ" lignins, followed by GC-MS analysis of methylated oxidation products, allowed the identification and quantification of nine methyl carboxylates, I−IX (Figure 7), and their quantification (Table 5).

The results obtained by permanganate oxidation, in terms of the relative abundance of structural units, show that both core and bark "in situ" lignins are HGS type lignins with H:G:S proportions of 15:66:19 and 12:56:32, respectively. The figures of the relative proportion of H units include coumarate structures which yield, after the permanganate oxidation treatment, product I (Figure 7). The difference, in terms of H:G:S proportions, between the "in situ" lignins and the

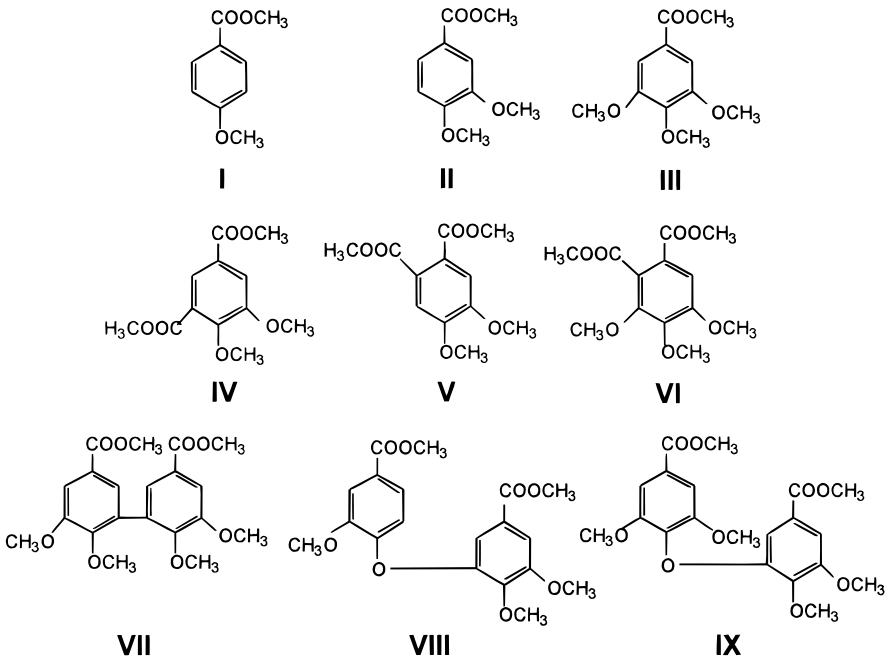

Figure 7. Carboxylic acid methyl esters obtained by the oxidation of lignins with potassium permanganate.

Table 5. Yields and Molar Proportions of the Permanganate Oxidation Products I−IX[a]

samples	I	II	III	IV	V	VI	VII	VIII	IX	η, %
sawdust										
core	15	55	17	3	4	1	2	1	3	18.0
bark	12	52	26	2	0	3	0	0	5	10.8
ext core[b]	7	57	15	8	5	1	2	2	3	8.5
MWL										
core	14	45	22	3	3	2	3	2	6	10.0
DL										
core	8	35	39	2	3	2	2	2	7	18.0
bark	1	29	56	2	2	2	1	tr	7	10.8

[a] See Figure 7 for the structures of products I−IX. [b] Alkali-extracted core; tr, trace.

dioxane lignins can be explained, partially at least, by the acidolysis reactions leading to the formation of new phenolic terminal units, different from those found in "in situ" lignin. This inserts alterations in the permanganate oxidation results, where only the terminal phenolic structural units protected by methylation are accessible for the analysis. A fraction of lignin enriched in S units and with a low content of H units is preferentially extracted by the dioxane method. The core lignin isolated by the Björkman method (MWL) also shows an increased syringyl content, when compared to the "in situ" lignin. The low content of H units in dioxane lignins suggests that a fraction of the *p*-hydroxyphenylpropane units or coumarate structures is linked by acid-labile linkages which are split during the acidolysis treatment. The isolated bark lignins are richer in S units than the core lignins, and the content of H in dioxane bark lignin (1%) is much smaller than in the core dioxane lignin (8%). In conclusion, the differences observed between "in situ" and MWL and dioxane lignins suggest that, in the case of kenaf, the extrapolation of results from isolated lignins to "in situ" lignins should be done with some precautions.

The results obtained by the permanganate oxidation of MWL and dioxane lignins agree, in terms of the relative proportions of structural units, with those obtained by ^{13}C NMR.

The proportion of products IV−IX, arising from oxidation of condensed phenylpropane structures in core and bark lignins, represents 14% and 10%, respectively, in "in situ" lignins and 18% and 14%, respectively, in dioxane lignins (Table 5). From these results, it can be concluded that the core lignin is more "condensed" than bark lignin, which is in agreement with FTIR and ^{1}H NMR results.

The most abundant "condensed" structures in both core and bark lignins are those of diaryl ether type giving, after permanganate oxidation, product IX (Table 5). The second in abundance are the "condensed" structures substituted in positions 5 and 6 of the phenylpropane unit, represented by products IV−VI (Table 5). Concerning the condensation patterns in core and bark lignins, several conclusions may be drawn from the data of permanganate oxidation analysis of "in situ" lignins (Table 5): besides the formation of aryl−O−aryl linkages (4-O-5 type) between guaiacyl and syringyl structural units, the principal way of natural "condensation" of core lignin is the formation of alkyl−aryl linkages envolving guaiacyl units (α-6 and α-5 structures, giving products IV and V), whereas, in bark, the strongest contribution to "condensation" results from the formation of α-6 alkyl−aryl linkages envolving syringyl units (structures giving product VI). Also, in the bark lignin, the formation of aryl−O−aryl linkages between the guaiacyl units (4-O-5 type structures giving product VIII) was not detected.

The different structural features referred to above for core and bark lignins, together with the NMR results, allow us to postulate different biosynthetic routes for these lignins. As has been previously reviewed, the differences in chemical composition and structure between xylem and bark lignins, in angiospermous and gymnospermous trees, are directly related to their different biosynthetic routes (Hemingway, 1981). Although the formation of the cell wall in graminaceous plants proceeds in a quite different manner, the sequence of the main lignification stages is similar to woody plants (Terashima et al., 1993). Detailed investigation on lignin biosynthesis in different morphological

Table 6. Phenolic Acids Released from Alkaline Hydrolysis of Core and Bark Lignins[a]

samples	*Y*, %	%pCA	%FA
sawdust			
core	1.3	1.2	0.1
bark	0.0	nd	nd
MWL			
core	1.1	1.0	0.1
DL			
core	0.4	0.3	0.1
bark	0.0	nd	nd

[a] Y, Yield of phenolic acids (%); pCA, *p*-coumaric acid; FA, ferulic acid; nd, not detected.

regions of graminaceous plants would clarify the observed differences in chemical composition and structure of core and bark lignins of kenaf.

Analysis of Phenolic Acids. The analysis of alkali-labile phenolic acids was carried out by treating the lignins in an aqueous 1 M NaOH solution, for 48 h at room temperature (Chen, 1992). These phenolic acids (referred to in the text as ester-bonded) are attached to the lignin network by ester but also, probably, by alkali-labile ether linkages (for example, by α-O-4 linkage in nonetherified S and G units). The residual alkali-stable phenolic acids are represented by ether-linked structures difficult to split under the referred alkali extraction conditions. The results of GC analysis of the alkali-extracted methylated phenolic acids are shown in Table 6.

The *p*-coumaric acid type structures represent about 90% of the ester-bonded phenolic acids found in "in situ" core lignin, while ferulic acid type structures represent the remaining 10% (Table 6). In the dioxane core lignin, the yield of released *p*-coumaric acid is only about one-fourth that obtained from the "in situ" lignin. This may be assigned to a partial acid hydrolysis of ester-bonded *p*-coumarate during the acid dioxane treatment. No ester-bonded phenolic acids were detected in bark lignins, thus confirming previous suggestions based on NMR results and supporting the proposition that the H units in bark lignins are mainly ether-bonded.

We have seen that at least a part of the H units in core lignin are ester-bonded *p*-coumaric acid. To investigate if other types of H units are present, the "in situ" lignin (sawdust) was submitted to permanganate oxidation analysis, after the alkaline treatment, releasing in this way the *p*-coumarate structures (Table 5). The molar proportion of H units was then reduced to half (Table 5), showing that the H units in core lignin are roughly composed of 50% ester-linked *p*-coumaric acid.

CONCLUSIONS

The results of the present study clearly show strong structural differences between bark and core lignins in kenaf, suggesting a different biosynthetic route for lignins in these two morphological regions. This difference is of theoretical interest from the point view of kenaf physiology, and also of practical interest when dealing with the chemical processing of kenaf biomass. The structural differences are important enough to foresee quite different reactivities for the bark and core lignins. This means that the two kenaf fractions will behave differently when submitted to chemical treatment. For example, the best behavior in soda pulping observed for the bark fraction, as compared to core, previously reported (Pande and Roy, 1996) may now be explained by the higher content of syringylpropane units and lower condensation degree of bark lignin, relative to the core lignin. This difference in structural features of core and bark lignins should be taken into account in the planning of new strategies for the rational utilization of kenaf biomass, particularly for pulp and paper needs.

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