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Article

Piassava Fibers (*Attalea funifera*): NMR Spectroscopy of their Lignin

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Lignina de piaçava (*Attalea funifera*) foi analisada por espectroscopia RMN de hidrogênio e carbono-13. A natureza **HGS** dessa lignina foi confirmada, mas unidades *p*-cumarato tambem estão presentes na estrutura da lignina. Os conteúdos de hidroxilas fenólicas e metoxilas por unidade fenilpropânica, determinados por RMN de ^{1}H, foram 0,57 e 0,68, respectivamente e estão em concordância com os dados obtidos por métodos clássicos. A pequena extensão da condensação da lignina (35%) foi explicada pelo alto conteúdo de estruturas de álcool cinamílico, evidenciadas por RMN de ^{13}C-DEPT. Uma fórmula C_9 estendida foi determinada para a lignina da piaçava:

$$C_{9.00}H^{ar}_{2.25}H^{al}_{3.73}O_{0.89}(OH)^{ph}_{0.69}(OH)^{al}_{0.86}(OCH_3)_{0.57}$$

Lignin of piassava (*Attalea funifera*) was analyzed by ^{1}H- and ^{13}C-NMR spectroscopy. The **HGS**-nature of this lignin was confirmed but *p*-coumarate units are also present in the lignin. Methoxyl and phenolic hydroxyl contents per phenylpropanic unit were determined by ^{1}H-NMR spectroscopy to be 0.57 and 0.68, respectively and are in agreement with wet chemistry methods. A small extent of lignin condensation (35%) was explained by the high content of cinnamyl alcohol structures, evidenced by ^{13}C-NMR-DEPT. An extended C_9-formula for the piassava lignin was established to be

$$C_{9.00}H^{ar}_{2.25}H^{al}_{3.73}O_{0.89}(OH)^{ph}_{0.69}(OH)^{al}_{0.86}(OCH_3)_{0.57}$$

Keywords: piassava fibers, lignin, NMR-spectroscopy

Introduction

In a previous paper lignocellulosic material of piassava fiber (*Attalea funifera*, a palm tree) was characterized by chemical analysis and submitted to alkaline solvolysis in dioxane/water[1]. The results concerning the piassava lignin were remarkable. The Klason lignin content was unusually high (*ca.* 45%) and the distillable oils, obtained by alkaline solvolysis in 40% yield (based on the raw material), consisted mainly of phenol, with some guaiacol, syringol and their alkyl derivatives. Methoxyl group analysis of the lignin showed a content of 0.54 OMe for each phenyl-propane unit, suggesting that the lignin of piassava is rich in hydroxyphenyl units.

In this work piassava lignin was submitted to further in-depth analysis using NMR spectroscopy. ^{1}H- and ^{13}C-NMR spectroscopy has been used as an alternative and non-degradative technique for the characterization of lignocellulosics. As shown in the literature[2], hydrogen signals can be assigned in a ^{1}H-NMR spectrum of acetylated lignin. The assignments of carbon signals in a ^{13}C-NMR spectrum of lignin can also be improved by means of pulse sequence procedures such as DEPT. In the recent literature these techniques were used for monitoring production or reduction of lignin in pulps[3] and for characterizing residual and isolated lignins from pulps[4]. Other modern techniques such as ^{1}H-^{13}C correlation spectra have been used for the characterization of grass lignins[5]. Since piassava lignin has not been well characterized until now, we present here the results of the NMR analysis of this lignin.

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Materials and Methods

Extraction of lignin from piassava fibers

Piassava fibers obtained from palm tree (*Attalea funifera*), used in brooms, were exhautively washed with water and the

extraction was performed with dioxan: HCl 2 mol L^{-1} (9:1) at reflux temperature, for 5 h. After evaporation of the solvent, extracted lignin was obtained and characterized for molecular weight distribution, FTIR and elementar analysis as described elsewhere[1].

Nuclear Magnetic Resonance

The extracted piassava lignin was acetylated by a 1:1 pyridine/acetic anhydride solution at 50ºC for 24 h in a sealed flask[6].

The ^{1}H-NMR spectrum of the acetylated lignin was recorded on a Bruker 300 MHz. The spectrum was measured at 24ºC with 32K data points and 128 scans, using 30 mg of the sample dissolved in 1 mL of $CDCl_3$ with TMS as internal standard. Pulse of 3.5 μs and relaxation delay 1 s. The ^{13}C-NMR spectrum of the non-acetylated lignin was recorded on a Bruker 300 MHz at 24ºC with 32K data points and 19096 scans, using 30 mg of the sample dissolved in 1 mL of DMSO (TMS as internal standard). Total spectrum measured by decoupling ^{1}H at acquisition time (0.88 s), using the POWGATE software (Inverse Gated Decoupling); irradiation pulse of 6.4 ms, relaxation time of 5 s. After the quantitative analysis, the DEPT (Distortionless Enhancement by Polarization Transfer) experiment was performed to assign the regions according to the type of carbon atom. The spectra were measured in the same spectrometer at 24ºC with 32K data points and 11000 scans and acquisition time 0.95 s. Three basic subspectra were recorded with pulses of 45º, 90º and 135º and the CH, CH_2 and CH_3 pure subspectra were calculated and analyzed according to Chen and Robert[2,7].

Results and Discussion

^{1}H-NMR spectrum of acetylated piassava lignin

The analyses of molecular weight distribution and FTIR spectra of the extracted lignin published in the previous work, showed that no contaminants (such as tannins or other extractives) were present in the sample[1].

The results of the semiquantitative analysis of the ^{1}H-NMR spectrum are shown in Table 1, where the hydrogen signals and areas are subdivided into the corresponding regions.

From the elemental analysis of extracted piassava[1] (54.5% C; 5.84% H; 0.52% N), and subtracting the holocellulose content (54.73% measured as $C_6H_{10}O_5$), we obtained the following atomic ratio for the piassava lignin: $C_{2.51}$ $H_{2.42}$ $O_{0.79}$, where the nitrogen content was neglected and the oxygen content calculated by difference. In lignin chemistry the empirical formula of the macromolecule is commonly given as a hypothetical hydroxyphenyl structure unit. This is known as the C_9-formula, with 6 carbon atoms at the benzene ring plus three carbon atoms of the propyl side-chain. Besides these 9 carbon atoms, only the carbon atom of the methoxyl units ($-OCH_3$) is present in the macromolecule. For the calculation of the C_9-formula the first step is to determine the methoxyl content and subtract it from the total carbon. This determination is easily made either by wet chemistry or by integration of the hydrogen signal of the ^{1}H-NMR spectrum, since the methoxyl region is well defined.

The integration of the hydrogen signals (Table 1) results in a total area of 203.2 arbitrary units which corresponds to the 2.42 protons in the above-given ratio. The integration of the methoxyl region (δ 3.55-3.95) results in 0.15 methoxyl units (0.44 hydrogen atoms divided by 3 hydrogen atoms per methoxyl group). Subtracting this value from the total carbon and oxygen atoms as well as subtracting the hydrogen atoms corresponding to the methoxyl units, we can establish a new relation: $C_{2.36}$ $H_{1.98}$ $O_{0.64}$ $(OCH_3)_{0.15}$. Making the carbon atoms to 9.00, the C_9-formula was calculated to: $C_{9.00}$ $H_{7.53}$ $O_{2.44}$ $(OCH_3)_{0.57}$ (Table 1). The calculated value for OCH_3 per C_9 unit is 0.57 which is in agreement with the value of 0.54 obtained in the previous paper[1]. These values are

Table 1. ^{1}H-NMR data of the acetylated piassava lignin (Area and total protons are divided into regions according to Chen and Robert[2]).

region δ (ppm)	attribution	area (arbitrary units)	hydrogens	
			in atom ratio $C_{2.51}$ $H_{2.42}$ $O_{0.79}$	in formula $C_{9.00}$ $H_{7.53}$ $O_{2.44}$ $(OCH_3)_{0.57}$
9.00-12.00	Carboxylic acids and aldehydes	0	-	
6.25-7.90	Aromatic region	49.5	0.590	2.25
5.75-6.25	Noncyclic benzylic region	6.0	0.071	0.27
5.20-5.75	Cyclic benzylic region	5.5	0.065	0.25
3.95-5.20 and 2.50-3.55	Aliphatic region	38.0	0.453	1.73
3.55-3.95	Methoxyl	37.5	0.447	1.70 = 0.57 OMe
2.20-2.50	Aromatic acetoxyl region *	15.2	0.181	0.69
1.60-2.20	Aliphatic acetoxyl region *	19.0	0.226	0.86
<1.60	Nonoxygenated aliphatic region	32.5	0.387	1.48
	sum	203.2	2.42	

* divided by 3

also in agreement with those obtained by Berns *et al.*[8] from analytical pyrolysis of piassava fibers. These authors found a **H/G/S** ratio of 51/40/9, which correponds to an average methoxyl content of 0.58 OCH_3 / C_9 unit.

The condensation degree of the lignin can be calculated from the methoxyl content and the ^{1}H-NMR spectrum. For each C_9 unit a maximum of 4 aromatic hydrogen atoms is expected, since the carbons at positions 1 and 4 are linked to the propyl chain and to the phenolic hydroxyl/ether linkage, respectively. Some of the aromatic positions are occupied by methoxyl groups and for the piassava lignin a maximum of 3.43 aromatic hydrogens (4.00 minus 0.57 OCH_3) is expected. The aromatic region (δ 6.25 to 7.90) corresponds to 2.25 hydrogens per C_9 unit, thus allowing the determination of the condensation degree (34%) by the difference to the maximum aromatic hydrogens. This value is similar to that of lignins obtained by steam explosion, which ranges from 38% to 41%[9]. The conden-sations are mostly due to 5-5 and β-1 linkages between phenylpropanic units.

Determination of phenolic hydroxyl groups by NMR ($0.69/C_9$, Table 1) is also in agreement with the chromatographic method ($0.72/C_9$)[1]. Additionally, the aliphatic OH groups amount to 0.86 per C_9 unit. Thus the C_9 formula can be expanded to

$$C_{9.00}H^{ar}_{2.25}H^{al}_{3.73}O_{0.89}(OH)^{ph}_{0.69}(OH)^{al}_{0.86}(OCH_3)_{0.57}$$

The amount of aldehyde and acidic protons is negligible (signal above δ 9). This corresponds to a small content of terminal γ-carbonyl and carboxyl groups, showing that most carbonyl groups are located in α and β positions of the propyl chains.

^{13}C-NMR spectrum of the piassava lignin

The ^{13}C-NMR spectrum of the extracted piassava lignin is shown in Figure 2. It has characteristic **H** signals found at δ 161.5, 131.3, 116.0 and 121.3[2] and also typical *p*-coumarate signals in the region around δ 150. Furthermore these signals have the highest intensities confirming the high **H** content of this lignin, evidenced by other techniques, or the high incorporation of *p*-coumarate units as attached moieties, as described for other lignins[10,11].

The non-aromatic region (δ 50-103) has a small intensity. Since each C_9-unit contains 6 aromatic and 3 aliphatic carbons, the integration of the non-aromatic region is expected to amount to about half of that of the aromatic region. In order to verify the presence of aliphatic structures such as cinnamic acids in the aromatic region, a DEPT spectrum was recorded to separate CH, CH_2 and CH_3 signals (Figure 2). The aromatic region (δ 103-160) is only contaminated by C-α and C-β of cinnamyl-type structures, and the integration of these signals (δ 122-130.5 and δ 151.5 to 154.5, respectively) must be considered to be part of the non-aromatic region (carbons of propyl side chain). With respect to the C_9-formula the aromatic region must correspond to 6 carbon atoms. The total integration of this region (δ 103 to 155) is 72.0 arbitrary units which corresponds to 12.0 arbitrary units/carbon atom. Table 2 shows the results of the application of this factor to all regions of the ^{13}C-NMR spectrum.

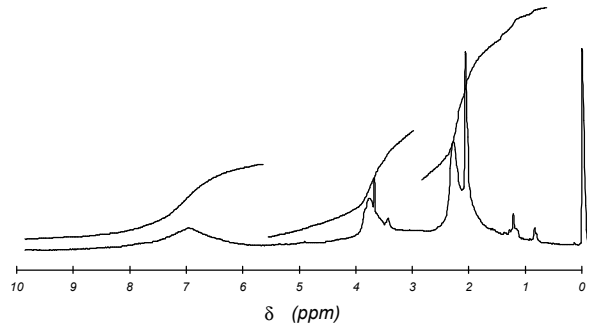

Figure 1. ^{1}H-NMR spectrum of acetylated piassava lignin.

Some information is first obtained by the integration of the region between δ 103-107, which corresponds exclusively to C-2 and C-6 in **s** units (Table 2) and has an integration of 2.5 arbitrary units corresponding to 0.21

Table 2. ^{13}C-NMR data of piassava lignin lignin (Area and total carbons are divided into regions according to Chen and Robert[2]).

chemical shift range (δ in ppm)	region	area (arbitrary units)	carbon atoms per benzene ring
162.0-154.5 and 151.5-130.5	Aromatic quaternary C	39.0	3.25
122.0-103.0	Aromatic tertiary C	33.0	2.75
107.0-103.0	Syringyl C-2/C-6	2.5	0.21
58.0-55.0	Methoxyl	8.0	0.67
154.5-151.5 and 130.5-122.0	Side chain cinnamyl structures	18.0	1.50
99.0-58.0 and 55.0-51.0	Side chain other non-aromatics	13.0	1.08
195.0-190.0	Carbonyls	0.7	0.06

C-2 and C-6 per aromatic ring. This is equivalent to 0.105 **S** units per C_9 unit or 10.5% **S** units in piassava lignin. Analytical pyrolysis furnished a similar value of 9%[8].

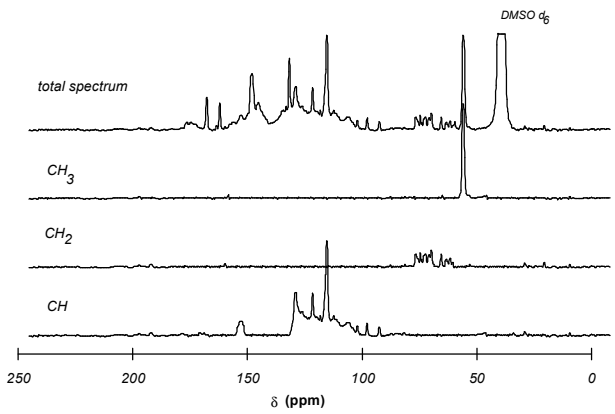

Figure 2. ^{13}C-NMR spectrum and CH_3-, CH_2-, CH-subspectra of piassava lignin.

Methoxyl content is calculated by integration of the δ 55 to 58 region, giving a total area of 8.0 arbitrary units, which correspond to 0.67 OMe/C_9. This value is 24% higher than the 0.54 OCH_3 per C_9 unit found by wet chemistry methods[1].

A close analysis was performed with respect to the aromatic region. Table 2 shows that the integration of CH signals (tertiary carbons) amounts to 2.75 carbons. A corresponding analysis of ^{1}H-NMR spectrum revealed only 2.25 hydrogens for this region. A higher intensity for the tertiary carbons could be attributed to the Nuclear Overhauser Effect, although the difference is high (22%). The values obtained from the ^{1}H-NMR analysis are accepted as being correct.

The aliphatic region containing cinnamyl structures has an unusual integration area, corresponding to 1.5 carbons per C_9 unit. This accounts for the small conden-sation of this lignin. It seems that the original structures were preserved in the piassava lignin biosynthesis, in spite of its high **H** content.

Conclusions

^{1}H- and ^{13}C-NMR spectroscopy was used in this work for piassava lignin characterization and good results were obtained. Information about methoxyl (0.57 OCH_3 per C_9 unit) and phenolic hydroxyl (0.69 OH^{ph} per C_9 unit) contents agree with those obtained by other methods. An extended C_9-formula was obtained $C_{9.00}H^{ar}_{2.25}H^{al}_{3.73}O_{0.89}(OH)^{ph}_{0.69}(OH)^{al}_{0.86}(OCH_3)_{0.57}$. The piassava lignin has a **HGS** nature, with high content of *p*-coumarate, and the presence of cinnamyl alcohol structures was evidenced by ^{13}C-NMR. The degree of piassava lignin condensation is low (34%), which explains its high reactivity under basic hydrolysis conditions.

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