

## Oxidative Ammonolysis of the *Eucalyptus Grandis* Kraft Lignin

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A amonólise oxidativa da lignina "kraft" produz um polímero altamente insolúvel de difícil caracterização. O presente trabalho se vale da RMN do  $^{13}\text{C}$  no estado sólido para acompanhar as mudanças ocorridas durante essa reação. Mas espectros de RMN do  $^{13}\text{C}$  em solução da lignina de madeira moída e da lignina "kraft" e de FTIR do polímero formado foram também usados para comparar os resultados. Durante a amonólise oxidativa, a lignina "kraft" se converte num polímero altamente insolúvel que não pode mais ser considerado como lignina. Este produto contém grupos amídicos, alifáticos não oxigenados, amônio e amínicos. A RMN do  $^{13}\text{C}$  no estado sólido com SP/MAS e intervalo entre pulsos próximos de 100s é mais confiável, neste caso, que com CP/MAS que usualmente é empregada para estudos quantitativos de ligninas.

The ammonolysis of kraft lignin produces an insoluble polymer which is very difficult to characterize. This work employed solid state  $^{13}\text{C}$  NMR spectroscopy to follow the structural changes occurring in this lignin during the reaction. The  $^{13}\text{C}$  NMR solution spectra of milled wood lignin and kraft lignin, and the FTIR spectrum of the ammonolytic product were also used. During the ammonolysis, the kraft lignin is converted into a very insoluble product which cannot be regarded as a lignin at all. This polymer contains amide, non-oxygenated aliphatic, ammonium, and amine groups. SP/MAS  $^{13}\text{C}$  NMR spectroscopy with pulse delay close to 100 s is more reliable than the CP/MAS technique for quantitative studies of lignins.

**Keywords:** *kraft lignin, oxidative ammonolysis, eucalyptus lignin, solid state  $^{13}\text{C}$  NMR*

### Introduction

The oxidative ammonolysis of lignins is carried out in a bath reactor where the lignin, in a concentrated ammonium hydroxide solution, is submitted to high pressure oxygen and to temperatures greater than  $100\text{ }^\circ\text{C}^{1-4}$ . This reaction has been intensively investigated in recent years because it offers an alternative way to a more rational use of technical lignins, instead of burning them. The reaction product (called OA-lignin) is being tested as a slow release nitrogen-fertilizer. If approved, the OA-lignins could prevent continual nitrogen application to soils and also leach losses which are frequent with the use of current fertilizers. Trials with potted plants showed that the performance of the OA-lignin, obtained from *Eucalyptus* kraft

lignin, is a little lower than that of urea in maize cultivar<sup>5</sup> and, furthermore, that it is phytotoxic to *Eucalyptus* plants<sup>6</sup>. Another inconvenience of the OA-lignin is its relatively low nitrogen content. So far the fixed nitrogen has not reached 20% for kraft lignin (KL).

Overcoming these hindrances requires a better knowledge of the oxidative ammonolysis of lignins. This reaction is very complex and it is very difficult to characterize the reagent and product. However, the presence of amide, amine and ammonium groups have already been detected in the OA-lignins by chemical and spectroscopic techniques<sup>1-4</sup>.

The present work studies the oxidative ammonolysis of *Eucalyptus* kraft lignin using mainly solid state  $^{13}\text{C}$  NMR spectroscopy<sup>7,8</sup>. The  $^{13}\text{C}$  NMR in a solution of milled wood

lignin (MWL) and KL, and nitrobenzene oxidation data are employed to compare results.

In spite of its relatively low resolution power, the solid state  $^{13}\text{C}$  NMR gives good informations concerning the carbon linkages present in the compounds. These data are of great interest for the structural analysis of insoluble materials such as OA-lignins.

## Materials and Methods

### *Eucalyptus grandis* wood

A 12 year-old tree, grown in the "cerrado" (savannah), near Uberlândia, Minas Gerais state, was cut at the height of 1.5 m. The trunk, with an average diameter of 30 cm, was disk cut and the whole wood was ground to pass through a 1 mm screen. The ground wood was milled in a ball mill for one week and the resulting wood powder was sifted through a 100 mesh screen.

### Milled wood lignin (MWL)

This lignin was isolated from 100 mesh milled wood according to Bland and Menshun's procedure<sup>9</sup> adapted to tropical *Eucalyptus grandis*<sup>10</sup>.

### Kraft lignin (KL)

The lignin was precipitated from kraft liquor with concentrated hydrochloric acid, filtered, washed exhaustively with water until a neutral pH, and then oven dried at 105 °C. The precipitated kraft lignin differs from the lignin solubilized in the kraft liquor. This has a complex structure which is modified with the acid treatment. Hydrogen sulfide release, acidification of carboxylate and sulfide groups and others reactions are involved in this process.

### Nitrobenzene oxidation

Dried lignin (50 mg) were added to a 10 ml stainless steel reactor containing 7 ml of 2M NaOH and 0.4 ml of nitrobenzene. The reactor was sealed and heated at 170 °C for 3.0 h with occasional stirring. After cooling the reactor, the reaction mixture was transferred to a liquid-liquid extractor and extracted continuously with  $\text{CHCl}_3$  for 6 h. The extracted reaction mixture was then acidified to pH 4 and extracted continuously with  $\text{CHCl}_3$  again for 48 h. The oxidation products contained in this chloroform solution were determined by gas chromatography.

### Oxidative ammonolysis

This reaction was carried out according to Meier and coworkers<sup>3</sup>. A sample of lignin (45 g) was dissolved in 50 ml  $\text{NH}_4\text{OH}$  plus 550 ml deionized water and then this solution was poured into the reactor at room temperature. The reactor was then sealed and oxygen-fed up to 16 atm. Then the assembly was heated up to 120 °C for about 60 min, and kept at this temperature for one hour. After

cooling the reactor, the reaction mixture of OA-lignin was dried in a Büchi mini-spray dryer.

### $^{13}\text{C}$ NMR spectra

The  $^{13}\text{C}$  solid state NMR spectra were acquired on a Varian VXR-300 FT-NMR, at a frequency of 75.4 MHz, with a pulse of 7.3  $\mu\text{s}$  (90°) and a spectral width of 50,000 Hz. For the Cross Polarization/Magic Angle Spinning (CP/MAS) experiments, a pulse delay of 5.0 s, a contact time of 500 ms and a number of scans of 1,500 were used.

For the experiments using only MAS (single pulse), pulse delays of 30 s and 100 s were used along with a number of scans of 500. To avoid the nuclear Overhauser effect (NOE), the de-coupler was used in the gate mode.

For both experiments, the samples were spun at 6.600 Hz along the magic angle. For processing, a line broadening of 70 Hz and an apodization function of 0.008 were used. For routine  $^{13}\text{C}$  spectra, a drop of TMS was added to the solution of lignin (200 mg) in  $\text{DMSO-d}_6$ .

## Results and Discussion

A structure for *Eucalyptus grandis* lignin has recently been proposed<sup>11</sup> (Fig. 1). The contents of guaiacyl and syringyl (one and two methoxyl groups per aromatic ring, respectively) are practically equal.

During the delignification of *Eucalyptus* chips submitted to kraft pulping, the lignin macromolecule is degraded and solubilized in the aqueous liquor. The structural changes can be followed by the  $^{13}\text{C}$  NMR solution spectra presented in Fig. 2. Signals 1 and 3 (152.2 and 147.5  $\delta$ ) are respectively assigned to  $\text{C}_3$  and  $\text{C}_5$  aromatic carbons in etherified and non etherified syringyl  $\beta$ -0-4 units; signals 2 and 4 (149.5 and 147.0  $\delta$ ) are respectively assigned to  $\text{C}_3$  aromatic carbon in etherified and non-etherified guaiacyl  $\beta$ -0-4 units. From the intensities of these signals before and after the pulping, it is evident that most  $\beta$ -0-4 ether bonds are split during the pulping process.

The decrease in the intensity of the signals in the 120-110  $\delta$  region (due to  $\text{C}_2$ ,  $\text{C}_5$  and  $\text{C}_6$  of guaiacyl rings), from Figs. 2a to 2b, indicates that important changes occurred on guaiacyl units during the pulping. This fact can also be verified by FTIR spectroscopy (Fig. 3): the characteristic bands of guaiacyl units<sup>12</sup> at 1270 and 1032  $\text{cm}^{-1}$  have lower intensity in fig. 3b.

The nitrobenzene oxidation assay offers another way to confirm the above results (Table 1). The production of vanillin and vanillic acid from the guaiacyl substructures is drastically reduced in KL.

Similar results have been reported for bamboo<sup>13</sup> and aspen<sup>14</sup> kraft pulping. In the former, the occurrence of lignin condensation reactions is suggested as a signal at 128.3  $\delta$  (due to  $\text{C}_2$  of stilbenes) emerges in the  $^{13}\text{C}$  NMR spectrum of bamboo kraft lignin. This signal is not clear in

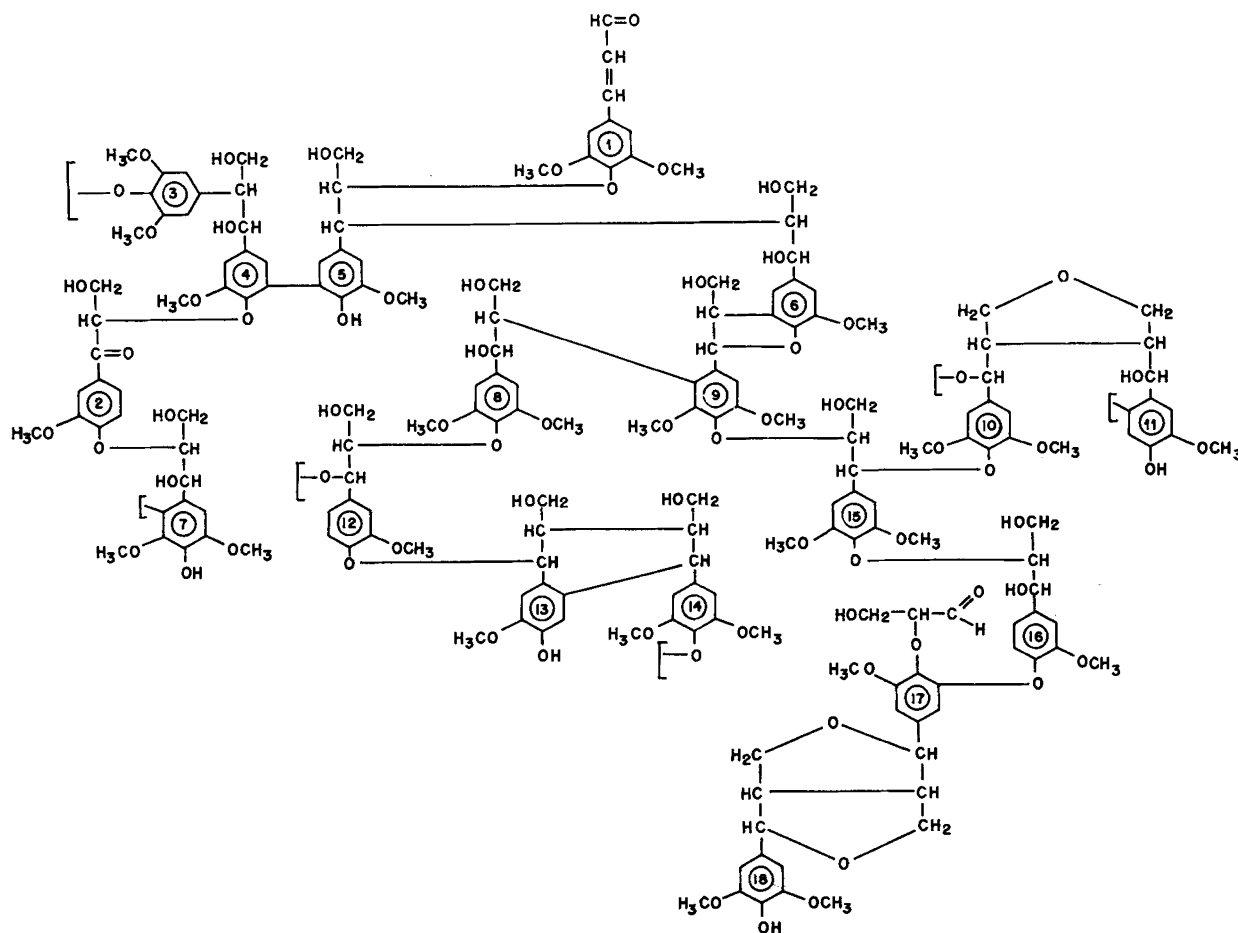


Figure 1. Proposed structure for the *Eucalyptus grandis* milled wood lignin<sup>11</sup>.

the spectrum of aspen kraft lignin<sup>14</sup> nor in Fig. 2b. So far, we are not sure of the occurrence of condensation reactions during the pulping process.

In addition, Fig. 2 clearly shows the changes which occurred in the oxygenated aliphatic region (50-90  $\delta$ ) during the pulping due to the above mentioned  $\beta$ -0-4 ether bond splitting and formation of carboxyl groups (signals close to 170  $\delta$  in Fig. 2b).

These last groups can also be seen in figure 3b (band at 1740.0  $\text{cm}^{-1}$ ). As such, the KL cannot be represented by the

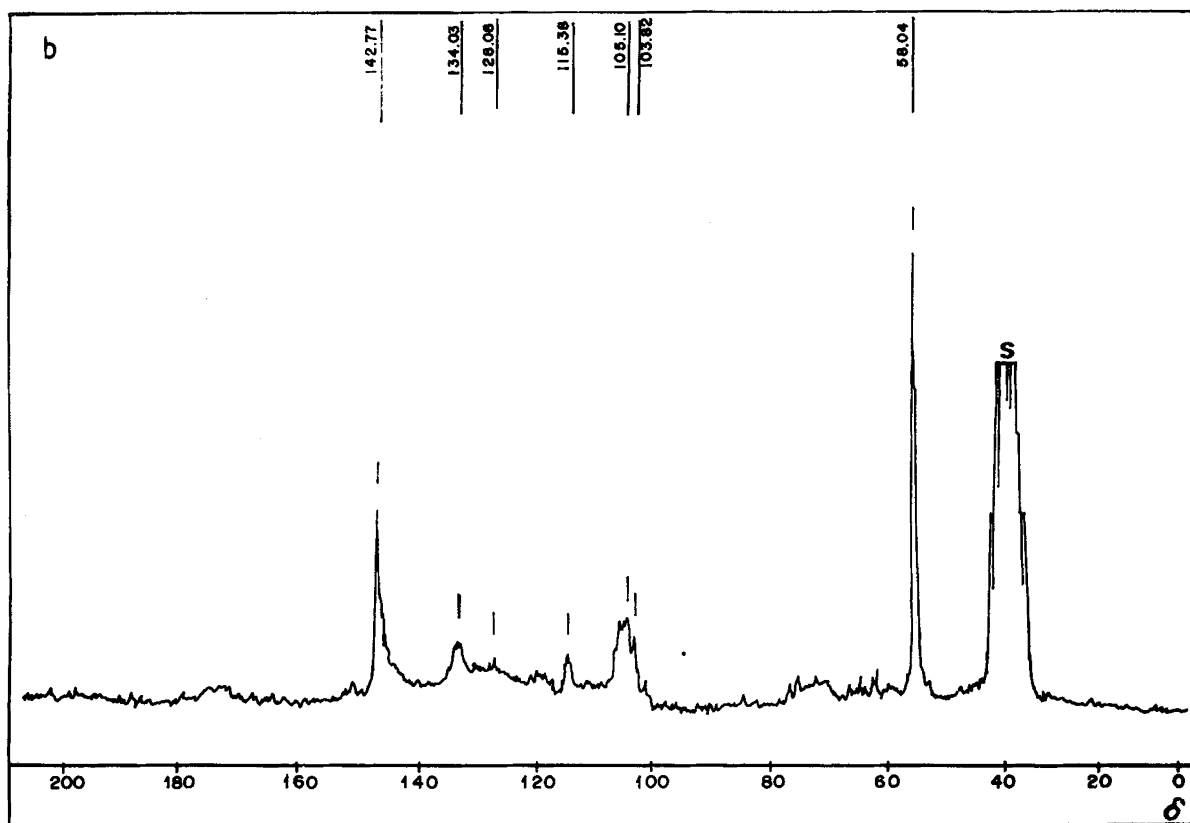
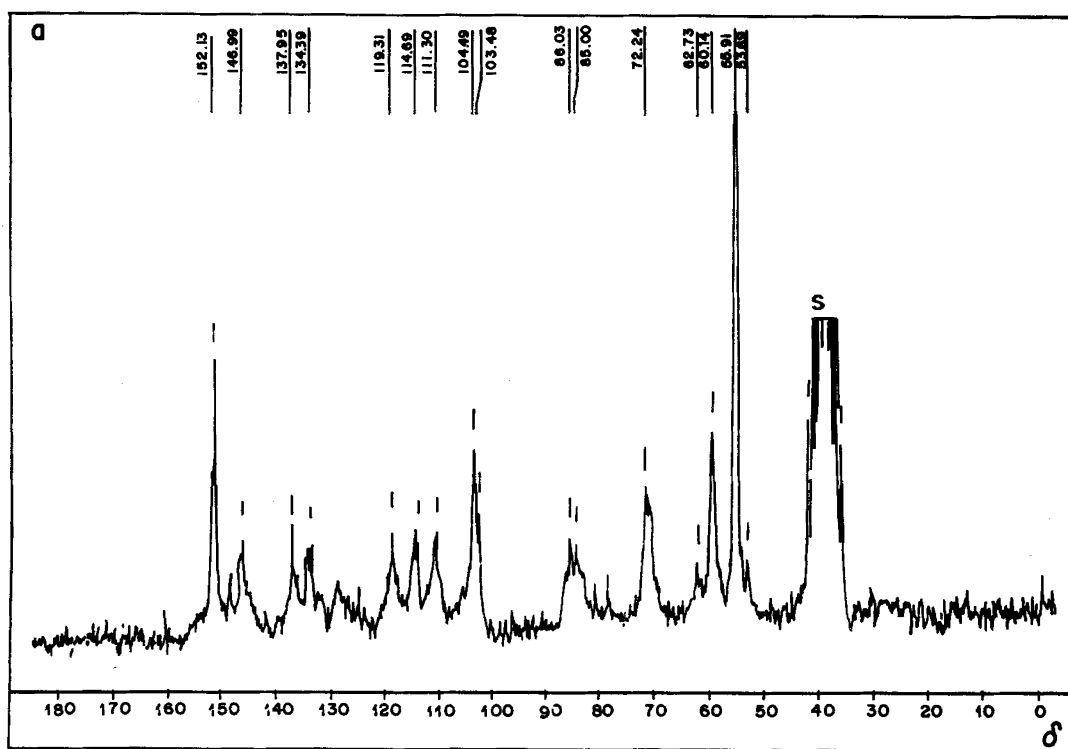
structural formula showed in Fig. 1. To our knowledge, no proposal for *Eucalyptus* KL has been reported so far, and it is precisely this complex material that is the reagent in the reaction under consideration.

The oxidative ammonolysis reaction is very severe, and practically destroys the lignin identity still present in KL. This is confirmed by the elemental analysis of the AO-lignin: C: 42.03%, H: 5.36%, O: 35.79%, and N: 16.28%, which is not characteristic for lignins: C:  $\cong$  60%, H  $\cong$  6%, O  $\cong$  34%.

The changes occurring in KL (C: 59.8%, H: 5.3%, S: 2.3%, N: 0.1% and O: 32.5%) during this reaction can be detected by the solid state C NMR spectroscopy. Figure 4 shows the aromatic and aliphatic signal intensity variation for various contact times for the AO-lignin (CP/MAS sequence). The intensities change with the contact time and, therefore, a reliable quantitative analysis cannot be done by means of a CP sequence. It can be done, however, by pulsing and acquiring the spectrum in the gated mode, in order to avoid the NOE, while the sample is spun at the magic angle. The pulse delay must be long enough to permit the relaxation of all carbon atoms. To attain this, two pulse

Table 1. Nitrobenzene oxidation of MWL and KL.

product	yield (mg / 100 mg of sample)	
	MWL	KL
P-OH-benzaldehyde	0.5	0.8
vanillin	7.1	2.7
syringaldehyde	9.2	8.0
p-OH-benzoic acid		
vanillic acid	6.3	1.2
syringic acid	5.6	3.3



**Figure 2.** Routine  $^{13}\text{C}$  NMR spectra of (a) MWL and (b) kraft lignin in  $\text{DMSO-d}_6$ ; s = solvent peak.

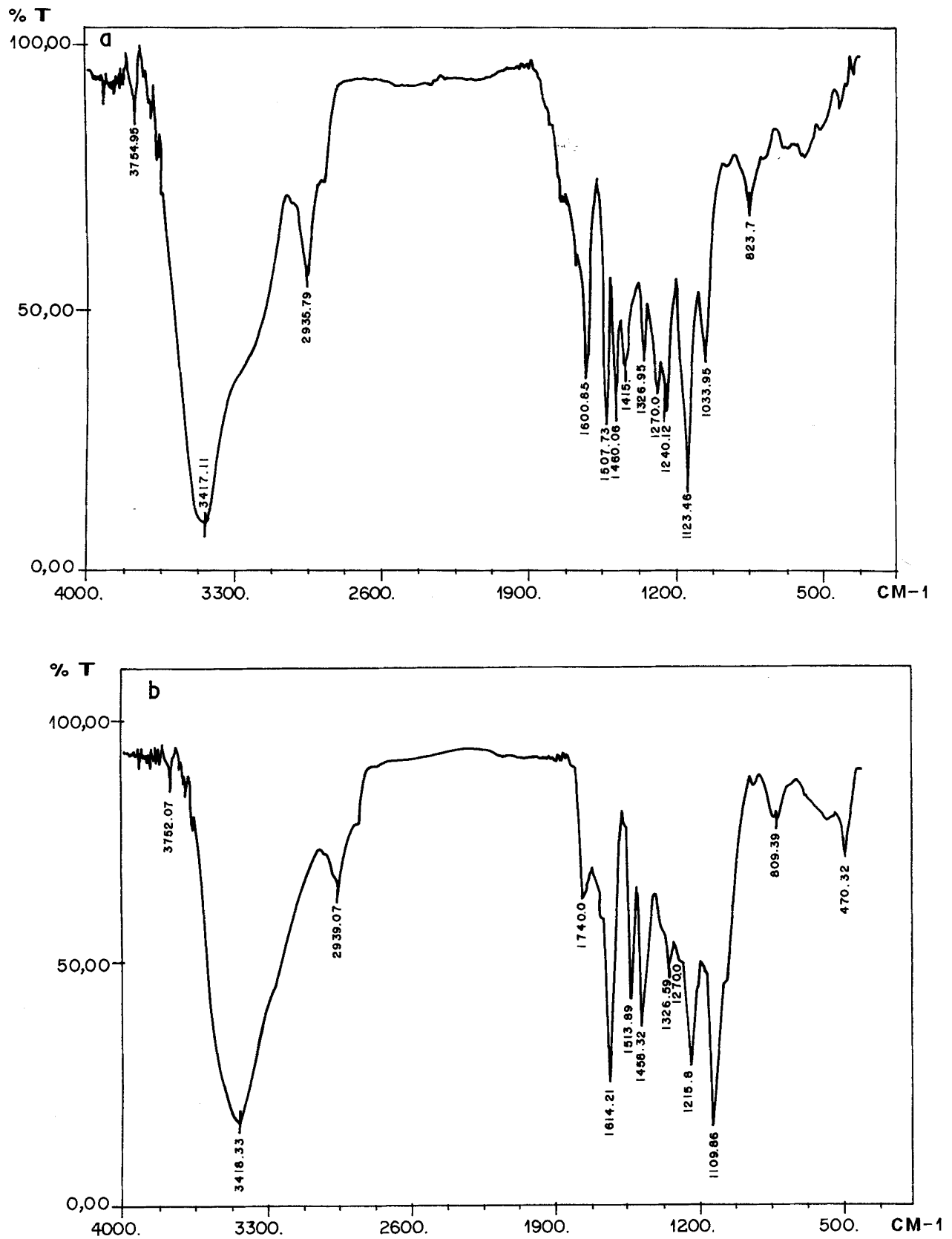


Figure 3. FTIR spectra of (a) MWL and (b) kraft lignin in KBr.

delays were used, 30 and 100 s, and Fig. 5 shows the corresponding single pulse MAS spectra of the ammonolytic product.

Table 2 presents the signal integration for the three main carbon types. In spite of the great time difference between the pulses, the percentages are similar for both cases studied, except for the carbonyl carbons whose intensity has increased significantly; a 200 s pulse delay made no difference over a 100 s delay spectra. We assume these conditions to be quantitatively reliable.

Figure 6 shows the single pulse MAS  $^{13}\text{C}$  NMR spectrum of KL, using a 100 s pulse delay. The comparison of this spectrum with that of the ammonolytic product (fig. 5b) makes evident that many changes occurred in KL during the ammonolysis: in the region of saturated carbons, new signals emerge beside the methoxyl carbon signal at 56  $\delta$  in the AO-lignin spectrum, the aromatic region (160 -

100  $\delta$ ) is more prominent in Fig. 5b, and finally, an intense signal emerges at 164.5  $\delta$  in the AO-lignin spectrum.

The new signals in the 10-90  $\delta$  region (Fig. 5b) can be assigned to aliphatic groups and to C-N bonds. The relative increase in aromatic signal intensities can be attributed to condensation reactions. And the formation of amide carbonyl groups can be confirmed by the intense signal centered at 164.5  $\delta$  (Fig. 5b).

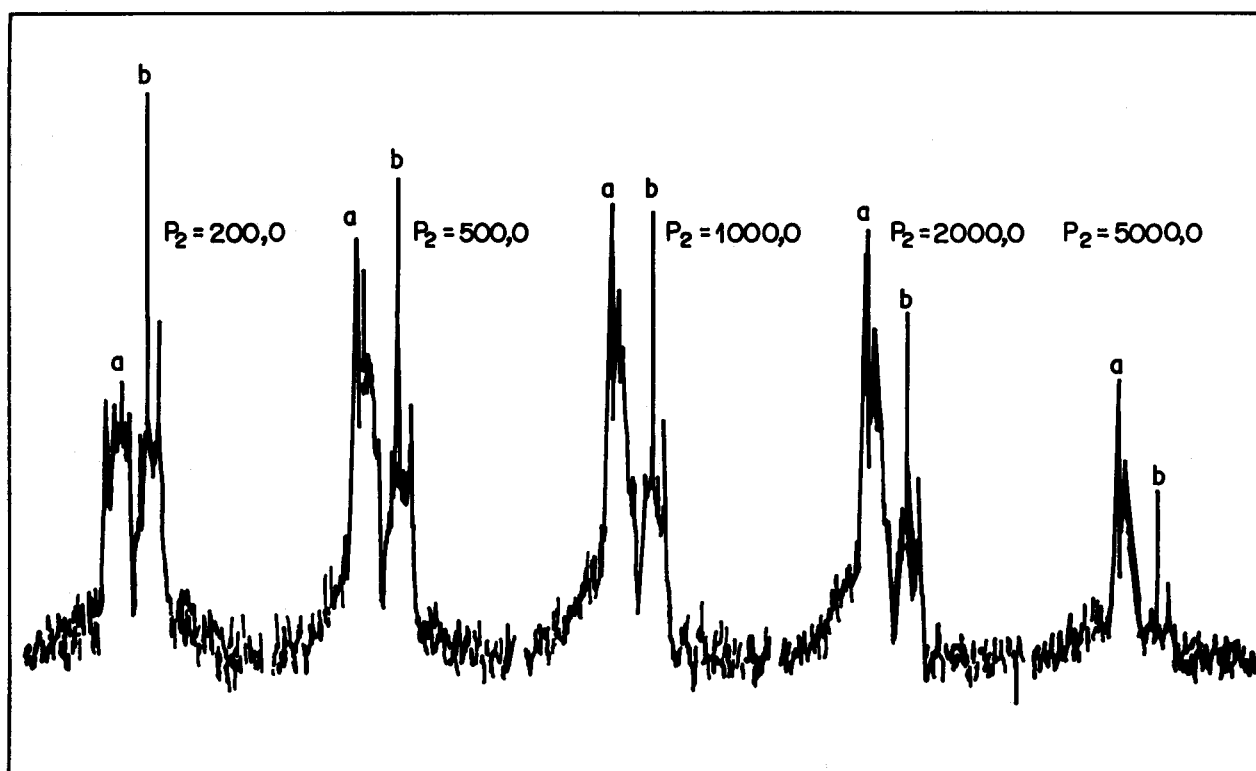
The FTIR spectrum of the ammonolytic product (Fig. 7) presents new bands at 3200, 1659, and 1400  $\text{cm}^{-1}$  which do not appear in the corresponding KL spectrum (Fig. 3b). These new bands also suggest amide and amine formation during ammonolysis. The C-N IR-bands have already been reported elsewhere<sup>3,4</sup>. The reaction product also contains 4% ammonium nitrogen, as determined by the Kjeldahl method.

Figure 8 presents the CP/MAS  $^{13}\text{C}$  NMR spectra of the processes studied from the wood through to the ammonolytic product. The presence of lignin in the wood is moderate, as can be recognized by the signals at 56  $\delta$  (methoxyl carbons) and 110-155  $\delta$  (aromatic carbons) (Fig. 8a). The signal due to syringyl C<sub>2</sub> and C<sub>6</sub> at 104-107  $\delta$  was overlapped by the intense carbohydrate C<sub>1</sub> signal.

Figure 8b shows the MWL spectrum. The contamination by xylans (at 169.6  $\delta$ : -C=O of *o*-acetyls and 20.9  $\delta$ : -CH<sub>3</sub> of *o*-acetyls) and aliphatics is negligible. In spite of the semi-quantitative nature of CP/MAS  $^{13}\text{C}$  NMR spectra,

**Table 2.** SP/MAS spectra of the AO-lignin with pulse delays of 30 and 100 s: percentages of three different carbon atoms.

pulse delay	%C	%C	%C
	saturated	unsaturated	carbonyl
	-95 $\delta$	95-185 $\delta$	185-255 $\delta$
30 s	27.3	67.8	4.9
100 s	28.9	63.7	7.4



**Figure 4.** Aromatic (a) and aliphatic (b) signal intensities for various contact times ( $P_2$ ) in ms; cross polarization sequence.

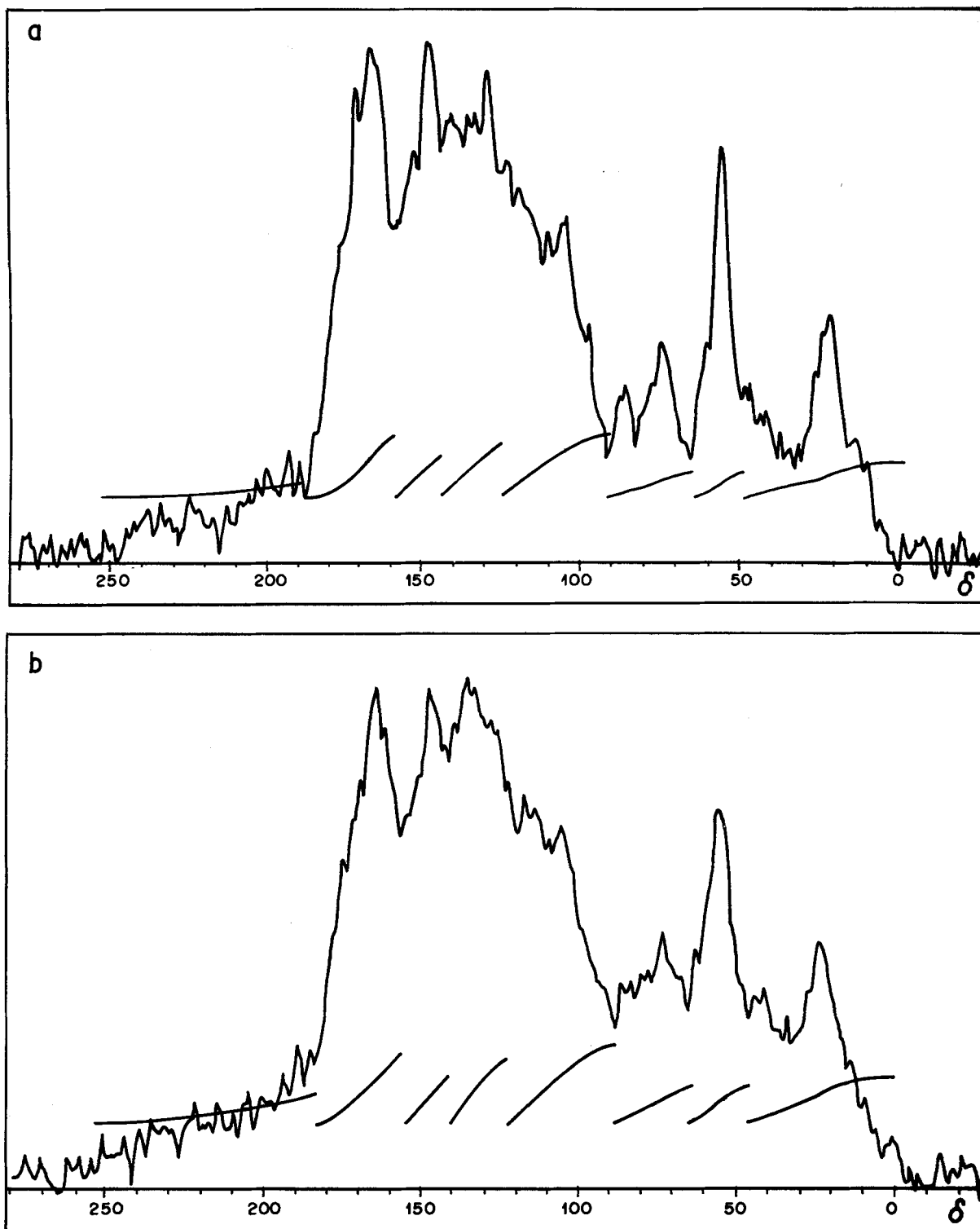


Figure 5. SP/MAS<sup>13</sup>C NMR spectra of the AO-lignin for two pulse delays: (a) 30 s and (b) 100 s.

the integrated methoxyl carbon content reaches 14.0% (52-60  $\delta$  range) and agrees well with the chemical analysis which provides a carbon content of 14.4%. Likewise, the area of the etherified guaiacyl C<sub>3</sub> and C<sub>4</sub> signals at 147.1  $\delta$  is equal to the area of the etherified syringyl C<sub>2</sub> and C<sub>6</sub>

signals at 152.2  $\delta$ , as foreseen by the theoretical C<sub>9</sub> phenylpropan unit: C<sub>9</sub>H<sub>7.90</sub>O<sub>2.73</sub>(OCH<sub>3</sub>)<sub>1.50</sub><sup>11</sup>. This last comparison does not take into account the contribution of non-etherified syringyl and guaiacyl C<sub>3</sub> and C<sub>4</sub> aromatic carbons because the phenolic hydroxyl content is only

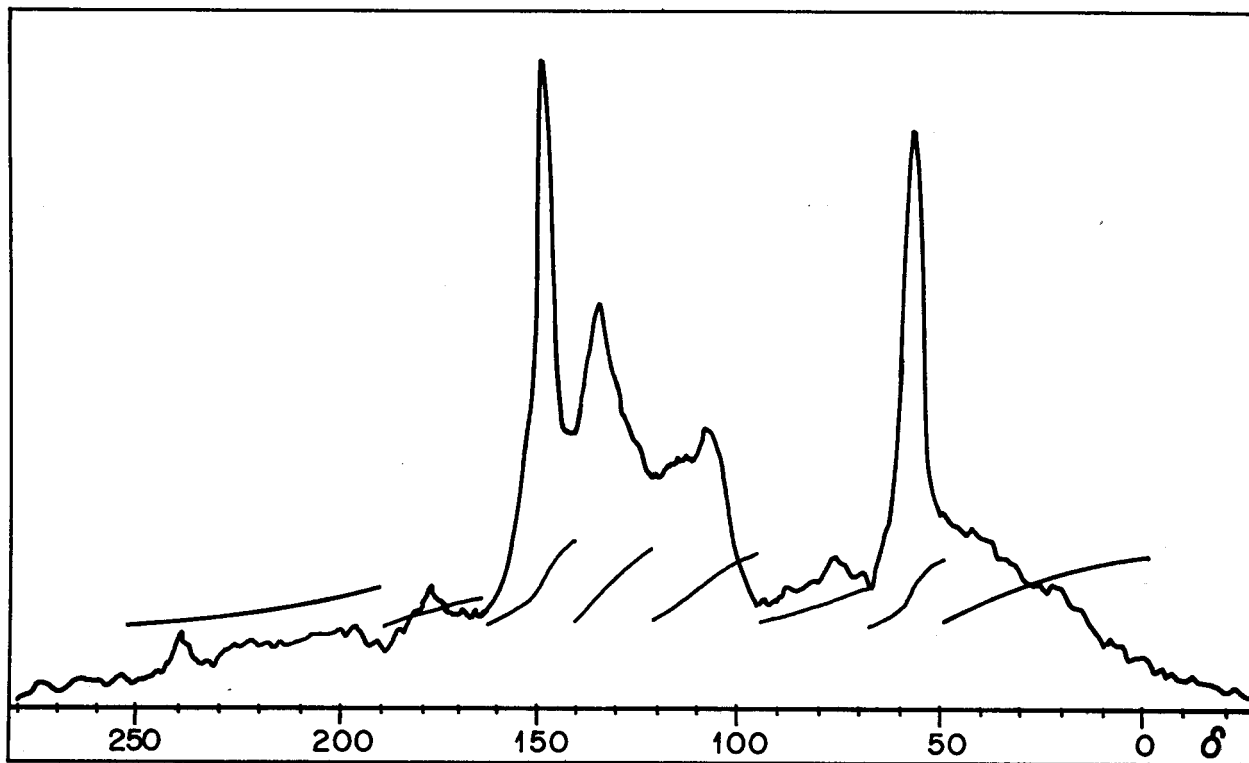


Figure 6. SP/MAS  $^{13}\text{C}$  NMR spectrum of kraft lignin for a 100 s pulse delay.

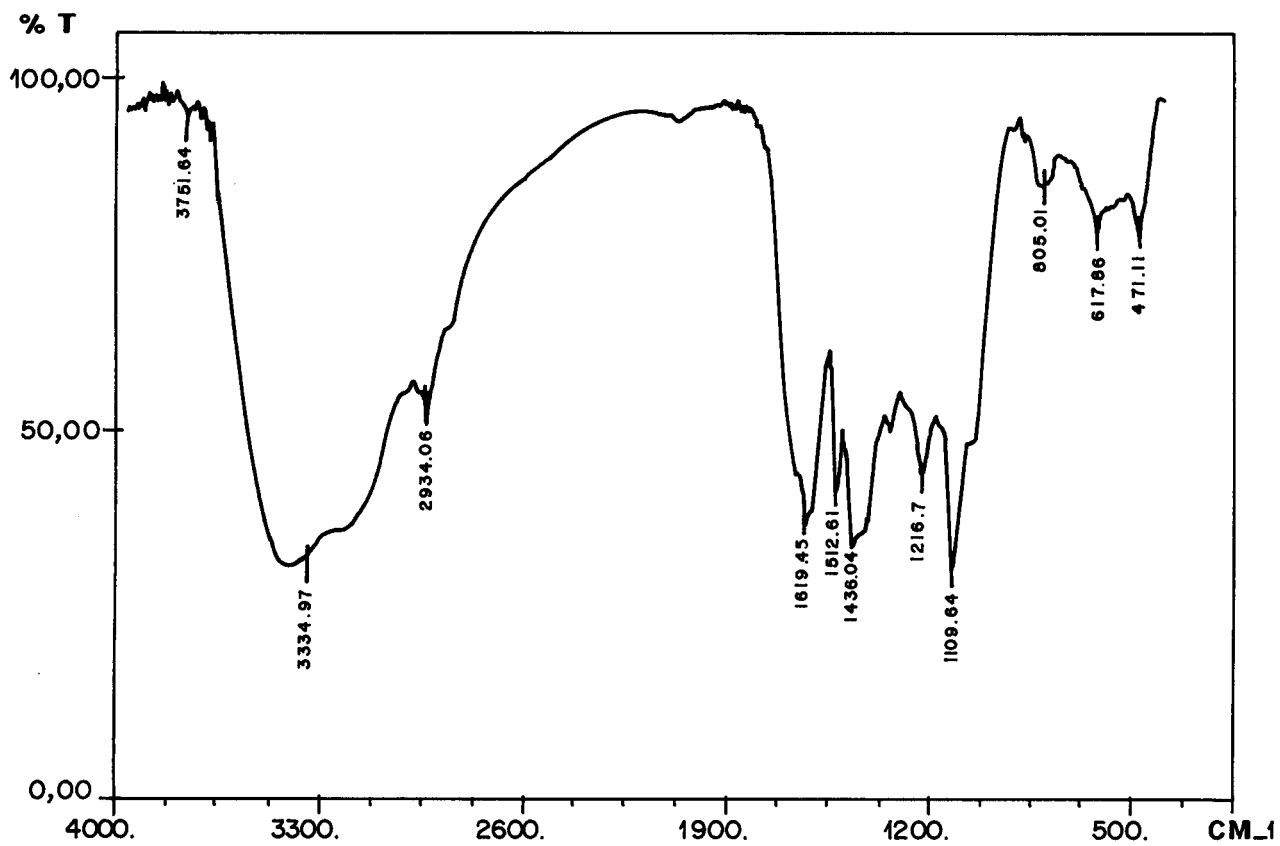


Figure 7. FTIR spectrum of the AO-lignin in KBr.



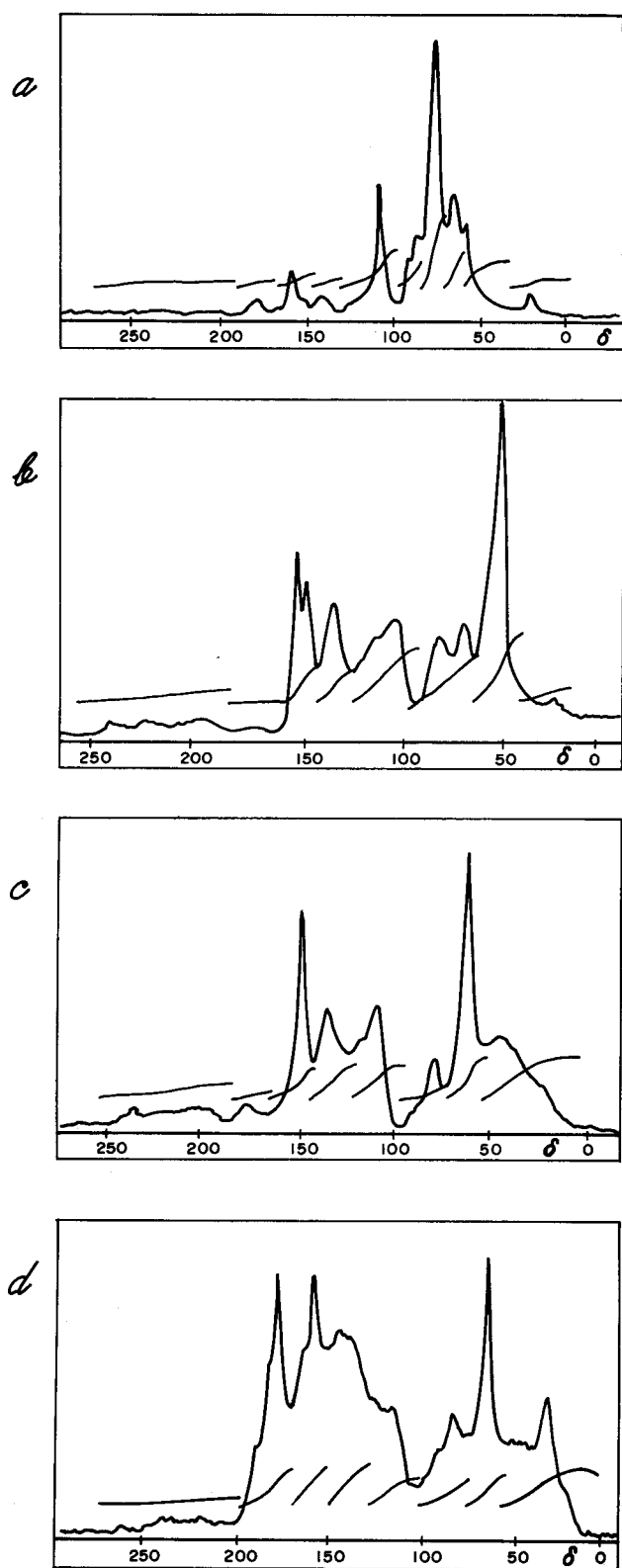


Figure 8. CP/MAS  $^{13}\text{C}$  NMR spectra of: (a) *Eucalyptus grandis* wood, (b) MWL, (c) kraft lignin, and (d) AO-lignin.

2.4%. This relatively good agreement of CP/MAS  $^{13}\text{C}$  NMR results and chemical analysis is already known and therefore the former method is also recommended for quantitative studies<sup>7</sup>.

However, the results of the CP/MAS spectra have to be used carefully. It is absolutely necessary to run a single pulse experiment with a big pulse delay first to see the real quantitative analysis and, after that, to choose the CP/MAS experimental conditions that provide the best results. Even so, the CP/MAS conditions can vary from sample to sample, and the most confident results are achieved by using the single pulse experiment anyway.

CP/MAS, however, can also be used as a quick analysis in the same experimental conditions to compare results from sample to sample.

### Conclusions

The lignin macromolecule is significantly degraded during the pulping: most  $\beta$ -*o*-4 bonds and aliphatic chains are broken and, consequently, the intensities of most characteristic  $^{13}\text{C}$  NMR signals and IR bands of guaiacyl rings decrease.

In the oxidative ammonolysis, the kraft lignin is transformed to a very insoluble polymer which can not be regarded as a lignin at all. This polymer contains amide, non oxygenated aliphatic, ammonium, and amine groups.

SP/MAS  $^{13}\text{C}$  NMR spectroscopy with a pulse delay of close to 100 s is more reliable than the CP/MAS technique for quantitative studies of lignins.

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