# Analysis of lignins by $^1$H NMR spectroscopy

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SUMMARY: The applicability of recently developed NMR techniques in the $^1$H NMR spectroscopic analysis of lignins has been examined. Spectra of acetylated spruce lignin recorded at 500 MHz differ only slightly from those recorded at 270 MHz; peaks due to Hα in β-O-4 structures ($\delta \approx 6.0$) and Hβ in β-5 structures ($\delta \approx 5.5$) are more resolved in the 500 MHz spectra and the shapes of the peaks resemble those obtained in decoupling experiments at 270 MHz. The occurrence of phenolic (*2a*) and etherified (*2b*) β-5 structures in spruce lignin was elucidated.

2D COSY $^1$H NMR experiments (COSY = homonuclear COrrelated SpectroscopY) provided evidence for the occurrence of 3-aryl-1-propanol units in spruce lignin. Cross-peaks which could be attributed to protons in a series of well established lignin structures (β-O-4 structures, β-5 structures and coniferaldehyde units) appeared in the 2D spectrum of acetylated spruce lignin.

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$^1$H NMR spectrometry is a useful tool for the analysis of lignins and lignin products in pulping liquors. A comprehensive $^1$H NMR study of spruce lignin and lignin model compounds was published in 1964 by Ludwig et al. (1). This work together with contributions by other groups [cf. Ludwig (2)] provided a basis for studies of lignins by $^1$H NMR spectroscopy. In most of the early studies the spectra were recorded at 60 MHz. $^1$H NMR spectra of lignins recorded at higher frequency (e.g. 270 MHz) are more resolved than the 60 MHz spectra (3, 4). The use of Fourier transform instruments also constitutes an improvement, since it makes it possible to record spectra with a favorable signal/noise ratio and very accurate spectral data can be obtained. In conclusion, the use of modern instruments strongly increases the possibilities to record $^1$H NMR spectra of lignins which permit an interpretation in structural terms. The present paper includes $^1$H NMR studies of acetylated spruce lignin by the use of 2D COSY technique (400 MHz) as well as by examinations of conventional spectra recorded at 270 MHz and 500 MHz.

## Effects of increased spectrometer frequency

A 500 MHz spectrum of acetylated spruce lignin (*fig. 1*) differs from the corresponding spectrum recorded at 270 MHz (4) primarily in the respect that the peaks at $\delta \approx 5.5$ and $\delta \approx 6.0$ have a more resolved character in the 500 MHz spectrum. A similar resolution of the topical peaks is observed in 270 MHz spectra on decoupling at adequate frequences (ref. 4 and *fig. 2*). Thus the primary changes caused by increased spectrometer frequency can be explained by the fact that the spacing due to spin-spin coupling expressed in δ units is smaller at 500 MHz. This implies that each one of the peaks at $\delta \approx 5.5$ and $\delta \approx 6.0$ is primarily caused by a single type of protons since the effects observed in the above described experiments otherwise hardly would be of detectable magnitude. This is in agreement with previous studies which suggest that the peak at $\delta \approx 6.0$ primarily is due to Hα in β-O-4 structures (*1*) while the peak at δ5.5 primarily is due to Hα in β-5 structures (4).

Fig. 2 shows the effect of decoupling at $\delta \approx 3.76$ (270 MHz spectrum) on the δ5.5 peak. The peak

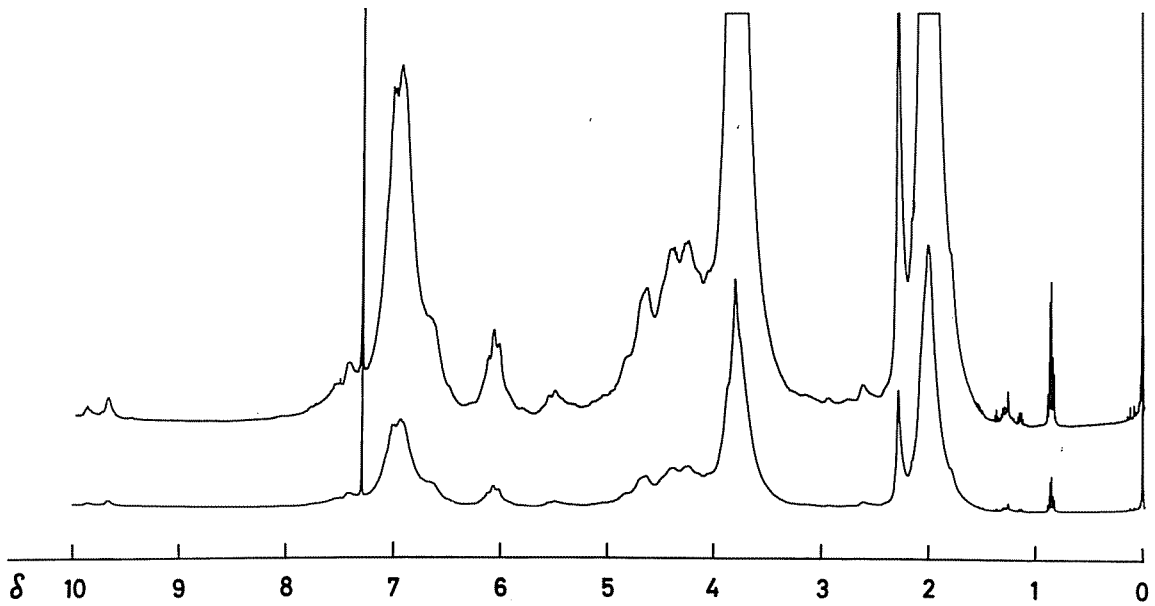


*Fig. 1. $^1$H NMR spectrum (500 MHz) of acetylated spruce lignin.*

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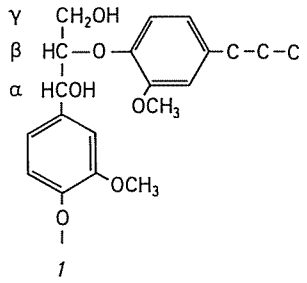


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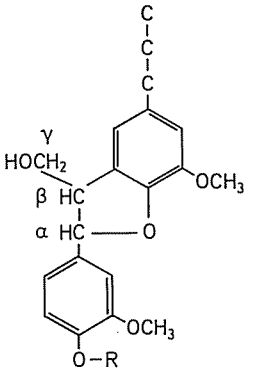



*2a* R=H
*2b* R=C in adjacent lignin unit
*2c* R=$CH_3$

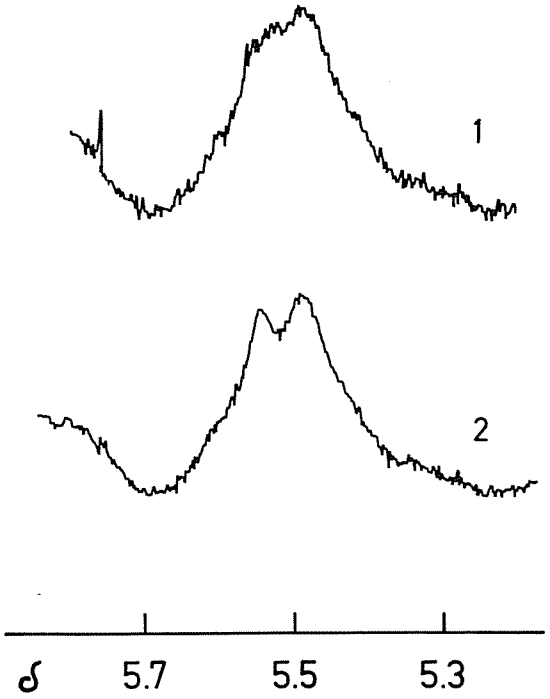



*Fig. 2. Range δ5.3-5.7 of the $^1$H NMR spectrum (270 MHz) of acetylated spruce lignin. Spectrum 2 appeared on irradiation at δ3.76.*

acquires a bimodal character (peaks at δ5.49 and δ5.54); increase of the spectrometer frequency from 270 MHz to 500 MHz causes similar changes (fig. 1, the peaks are located at 5.48 and 5.53). According to model compound studies (5) the two peaks can be attributed to the presence of etherified (*2b*) and phenolic (*2a*) structures of β-5 type. This is supported by studies of a lignin sample with methylated phenolic groups (the methylation procedure is described in ref. 6) which only exhibits one peak in the δ≈5.5 region (*fig. 3*); the position of this peak (δ5.48) agrees with that expected for signals from Hα in etherified structures of the β-5 type (2b and *2c*).

Three peaks (δ6.01, 6.05 and 6.10) can be discerned in the δ≈6.0 region of the 500 MHz spectrum (fig. 1). Peaks which in all likelihood correspond to these peaks (δ6.01, 6.06 and 6.11) are found in the 270 MHz spectrum on irradiation at δ4.65 (the position of signals from Hβ in β-O-4 structures); an interpretation in structural terms is presented in ref. 4.

## $^1$H NMR (400 MHz) 2D COSY experiments

A 2D COSY spectrum of acetylated spruce lignin is shown in *fig. 4*. The cross-peaks are assigned as follows.

*Coniferaldehyde units.* Cross-peaks 6.61/7.42 and 6.61/9.70 can be attributed to the couplings between Hα, Hβ and Hγ in side chains of coniferaldehyde units (for model compound data, see ref. 3). The signals from such units are relatively prominent although there are only ≈4% such units in the lignin (3).

*β-O-4 Structures (1).* Cross-peak 4.64/6.08 can be attributed to the Hβ/Hα coupling in β-O-4 structures (*1*). Such structures constitute the major type of structural element in lignins.

*β-5 Structures (2).* Cross-peak 3.79/5.53 can be attributed to the Hα/Hβ coupling in β-5 structures.

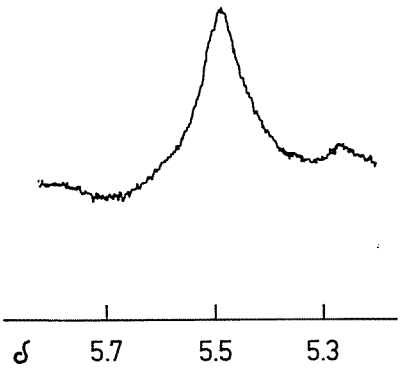
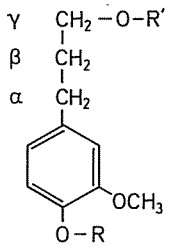
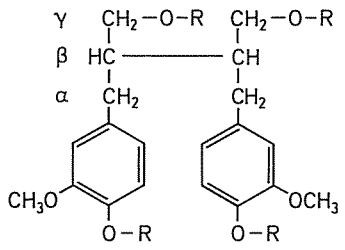



Fig. 3. Range *δ5.3-5.7 of the $^1$H NMR spectrum of methylated and acetylated spruce lignin.*

*Table 1. $^1H$ NMR data for acetate derivatives of lignin model compounds (δ values; J in Hz are given in parentheses). Signals from aromatic protons are found in the range 6.5-7.0.*

| Compound | Hα | Hβ | $H\gamma_1$ | $H\gamma_2$ | $OCH_3$ | $CH_3CO$ |
|---|---|---|---|---|---|---|
| 4 | 2.67 (m) | 1.96 (m) | 4.11 (6.6) | | 3.82 | 2.05, 2.30 |
| 5 | 2.63 (m) | 1.94 (m) | 4.09 (6.6) | | 3.85, 387 | 2.05 |
| 6 | 2.69 (m) | 2.15 (m) | 4.03 (5.8,11.4) | 4.24 (5.6, 11.4) | 3.75 | 2.05, 2.29 |
| 8 | 2.59 (8.4, 13.5)<br>2.69 (5.9, 13.5) | 2.23 (m) | 3.55 (5.9, 8.8) | 3.93 (6.6, 8.8) | 3.79 | 2.30 |



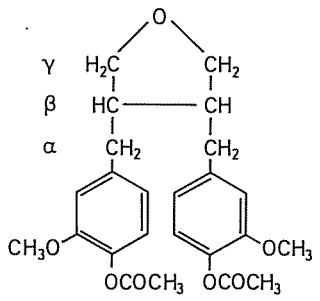

*3* R = C in adjacent unit
R' = H

*4* R = R' = $COCH_3$

*5* R = $CH_3$, R' = $COCH_3$

*6* R = $COCH_3$

*7* R = H

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*3-Aryl-1-propanol units (3).* The positions of the strong cross-peaks 1.92/2.63 and 1.91/4.12 (*fig. 5*) fit well with what could be expected from couplings of protons in propanol side chains in etherified guaiacyl units (3) as judged from the side chain proton data of a model compound; $^1H$ NMR data for some model compounds are summarized in *table 1.* Integrations of signals in a conventional spectrum suggest that the total of 3-aryl-1-propanol units and certain other types of units with "reduced" side chains is small (see Discussion).

## Discussion

It is possible to obtain more resolved $^1H$ NMR spectra of lignins by increasing the spectrometer frequency. Spectra recorded at 270 MHz and 500 MHz differ only moderately. Nevertheless it is possible to draw structural conclusions from comparisons of spectra recorded at 270 MHz and 500 MHz.

$^1H$ NMR studies suggest 1-2% side chains of the type present in secoisolariciresinol structures (*7* attached to the lignin) or 3-aryl-1-propanol units in spruce lignin (4, 7). The 2D $^1H$ NMR studies presented in this study provide qualitative evidence for the occurrence of 3-aryl-1-propanol units in spruce lignin. Support for the presence of such units in lignin has earlier been obtained from studies of lignin hydrolysis products (8, 9). The occurrence of 3-aryl-1-propanol as well as secoisolariciresinol structures and other structural elements of the lignan type has recently been discussed (10). The side chains in 3-aryl-1-propanol units and secoisolariciresinol structures have a lower degree of oxidation than those in *p*-hydroxycinnamyl alcohols. Therefore the occurrence of structural elements of these types in lignins does not fit with the theories for the biosynthesis of lignins which assume an *oxidation* of *p*-hydroxycinnamyl alcohols as the last step.

The 2D NMR studies presented in this work were performed in the course of an investigation of various

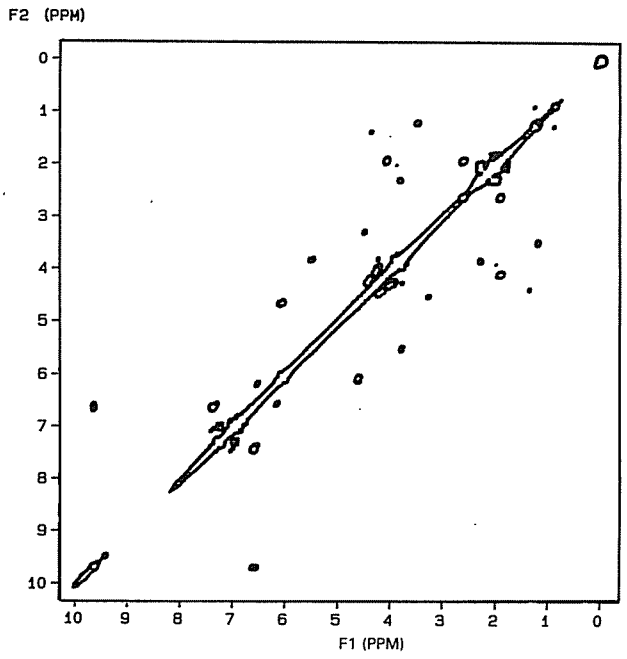


*Fig. 4. 2D COSY $^1H$ NMR spectrum of acetylated spruce lignin.*

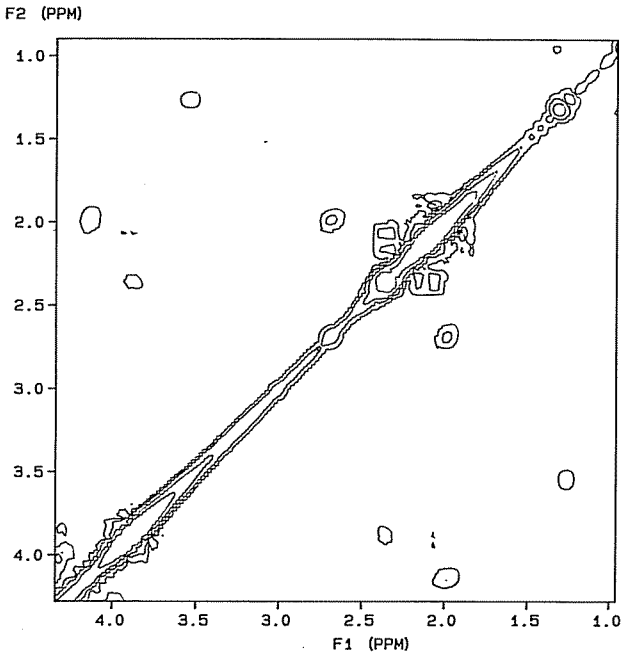


*Fig. 5. Enlargement of a region of the spectrum in fig. 4.*

possibilities of obtaining structural information about lignins by 2D NMR spectroscopic methods (11).

## Experimental

All the $^1$H NMR spectra were recorded with deuteriochloroform as solvent and TMS as internal reference. The temperature was 300 K. For the recording of conventional spectra Bruker instruments (Bruker WH270, Bruker WH500) were used. The 2D COSY spectra were recorded on a Varian 400 XL instrument. The standard COSY-90 pulse sequence was used, since it gave the clearest cross-peaks. The delay time was set to 4 sec; maximum $T_1$ was measured to below 1 sec. A total of 512 increments with 32 scans in each was recorded. The number of data points was set to 2048 with a sweep width of ca. 4200 Hz. It was possible to change the intensity of the cross-peaks by setting a fixed delay time to emphasize smaller couplings.

Experiments with COSY-45 and COSY-60 pulse sequences gave less pronounced cross-peaks. The DQCOSY pulse sequence was also tried, but no improvement compared to the COSY-90 spectrum was observed.

## Acknowledgement

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