Quantitative ^{13}C NMR Analysis of Kraft Lignins*

Göran Gellerstedt[a] and Danielle Robert[b]

[a]Swedish Pulp and Paper Research Institute, STFI, Box 5604, S-114 86 Stockholm, Sweden and [b]Laboratoires de Chimie/DRF, Centre d'Etudes Nucléaires de Grenoble, 85X, F-38041 Grenoble Cedex, France

Gellerstedt, G. and Robert, D., 1987. Quantitative ^{13}C NMR Analysis of Kraft Lignins. – Acta Chem. Scand., Ser. B 41: 541–546.

Three different kraft lignin samples and one native lignin extracted from milled wood have been analysed by ^{13}C NMR. Both the DEPT pulse sequence for CH, CH_2 and CH_3 sub-spectral editing, and an inverse gated decoupling sequence for quantification of the various carbon atoms were used. By employing small correction factors for the integral intensities, the DEPT subspectra were also quantified and this made possible a comprehensive description of structural features in these lignins. The results obtained are discussed with reference to known features of the structure of lignin and the reactions taking place during kraft cooking.

In a previous paper, we described the analysis of two series of kraft lignins by quantitative ^{13}C NMR spectroscopy after acetylation.[2] The results obtained were used to calculate the numbers of different hydroxy groups, viz. primary, secondary and phenolic, and the number of methoxy groups, and to make rough estimates of the amounts of oxygen-linked side-chain carbons and olefinic carbons. The aromatic ring in lignin was used as the base unit.

In order to further explore the possibility of using high-resolution ^{13}C NMR as an analytical tool for the detailed structural elucidation of various lignin preparations, three kraft lignin samples have now been analysed. The same inverse gated decoupling sequence as before was used to obtain quantitative ^{13}C NMR spectra. In addition, the samples were analysed by the DEPT (Distortionless Enhancement by Polarization Transfer) technique.[3] By suitable combination of the various spectra obtained for a sample, a fourth sub-spectrum containing only the quaternary carbon atoms was also constructed. The information provided by the NMR spectra was then used to make quantitative calculations on the chemical structure of the lignin. The results were compared with those obtained from a similar experiment with a native lignin (MWL) preparation.

Results and discussion

^{13}C NMR spectroscopy is a technique well suited to the analysis of lignins, and a large number of papers have appeared on the subject during the last 10 years. The lignin polymer has a heterogeneous structure, and no other analytical method is able to provide the same amount of structural information in one experiment. The possibility of spectral editing by, e.g., use of the DEPT pulse sequence has further substantially increased the amount of structural information which can now be obtained.[4] Attempts to obtain quantitative information about the structural features of a lignin, however, in most instances still involve tedious chemical analyses, leading at best to semi-quantitative data. Although time-consuming, quantitative ^{13}C NMR therefore seems to be the best available method for obtaining reliable information about the structure of a lignin sample.

In the present work, three kraft lignins have been prepared from laboratory kraft cooks of pine wood. One of the samples was obtained from a normal batch cook carried out to a degree of delignification of approximately 70 %, based on wood.[5] The other two samples were taken from a flow-through cook, in which the aqueous

*Part 7 in the series "Structural Changes in Lignin during Kraft Cooking". For Part 6, see Ref. 1.

phase is continuously replaced by fresh cooking liquor so that the dissolved lignin can be withdrawn from the autoclave shortly after it is formed.[2] In this way two different lignin samples were prepared; one was representative of the lignin going into solution during the middle part of a kraft cook and the other was taken out at the end of the cook. The latter sample should therefore be representative of the lignin which is most difficult to remove from the cellulose fibres.

After precipitation of the lignin fractions from the corresponding cooking liquors, the lignins were further purified by removal of low molecular mass materials and polysaccharides according to the procedure described in Ref. 5. The lignins obtained from the flow-through cook (GSK 3 and GSK 6) were acetylated before analysis, whereas the lignin from the batch cook (SK 5) was used without any derivatization.

Acetylated lignins can conveniently be analysed by ^{13}C NMR employing acetone as solvent, whereas underivatized lignins, for reasons of solubility, are usually analysed in dimethyl sulfoxide. In both solvents, virtually all signals in spectra of lignins have been identified.[6-8] In the present work, however, acetonitrile was employed to analyse sample SK 5 since this solvent does not contribute any interfering signals in the 10–50 ppm range.

The three kraft lignin samples and one sample of a native milled wood lignin (MWL) obtained from spruce were analysed by ^{13}C NMR employing, in each case, an inverse gated decoupling sequence[9] as well as sub-spectral editing by means of the DEPT sequence.[3] The former analysis was carried out employing a delay time of 11 sec ($\sim 5 \times T_1$) between pulses in order to ensure complete relaxation of the various carbon atoms.[10] The DEPT spectra were run with a chosen coupling constant of 143 Hz [$(2J)^{-1}$ = 0.0035 s]. This value was taken to be representative of the coupling constants for sp^3-hybridized carbon atoms linked to oxygen, as in ethers and alcohols. In lignin spectra, such carbons give signals in the shift range of approximately 55 to 90 ppm.[6-8] For methoxy carbon signals and all other types of carbon signals present in the DEPT spectra, correction factors were, however, calculated

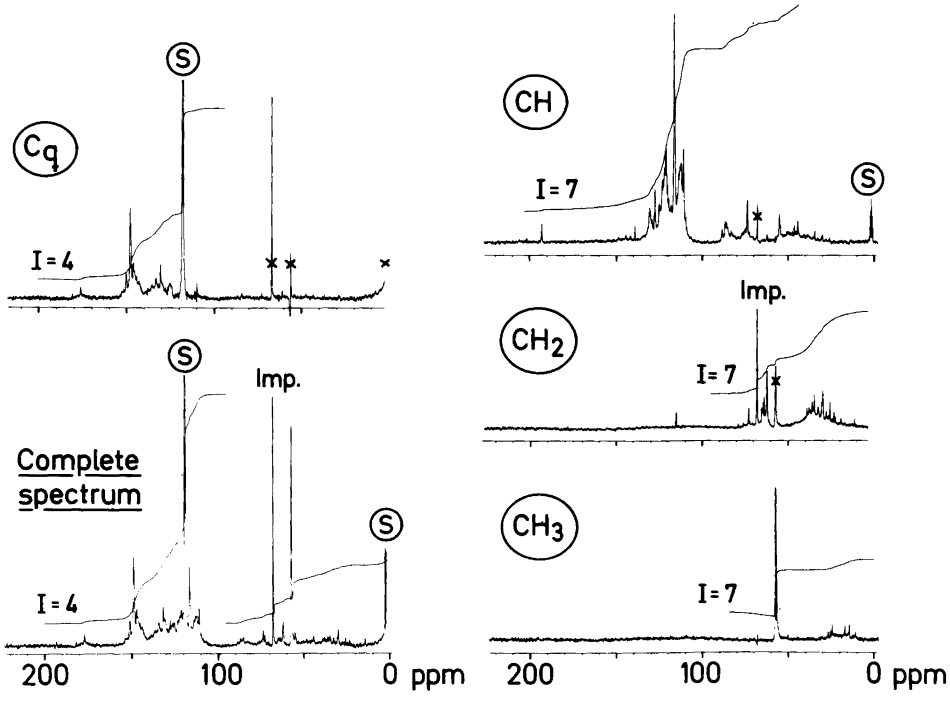

Fig. 1. Complete ^{13}C spectral editing for the kraft lignin sample SK 5 in acetonitrile-d_3. *I* = integral intensity, S = solvent, Imp = impurity of dioxane, X = overlapping peaks.

in order to convert all the various signals to a common intensity level.[11] The integrals obtained from the DEPT sub-spectra of a sample could thus be compared directly with each other. In addition, the integral from the corresponding complete spectrum could be compared with the integrals from the DEPT spectra since they now differed only by a constant. This constant was calculated by comparing the integrals for the methoxy group obtained from the two sets of spectra. In each case, the computer of the NMR instrument was used to add together the three DEPT sub-spectra. The resulting artificial spectrum was then subtracted from the complete spectrum, leaving only the spectrum for the quaternary carbon atoms. This was quantified by integration. An example of complete spectral editing for kraft lignin is given in Fig. 1.

The NMR data obtained were used to quantify the various functional groups present in kraft lignins. For comparison, a lignin preparation from milled wood (MWL) was also analysed and used as a reference. The quantitative calculations were made using the aromatic ring in lignin as a base unit, since virtually all monomeric units constituting the lignin polymer contain this moiety. However, the shift regions for aromatic and olefinic carbon atoms coincide in ^{13}C NMR spectra and, since native lignins and kraft lignins are known to contain small amounts of olefinic carbons, the integral for the aromatic carbon atoms had to be calculated indirectly. For the two lignins (GSK 3 and GSK 6) obtained from a flow-through cook, and analysed as acetates, the calculation was therefore based upon the amount of phenolic hydroxy groups present per aromatic ring. These groups have been quantified previously by chemical means.[2] In the third kraft lignin sample (SK 5), the number of methoxy groups per aromatic ring was used. Previous data based upon chemical analysis are also available in this case.[2]

In the spectra obtained from milled wood lignin, a sharp signal from the carbonyl group in coniferaldehyde structures was visible. Further, it was assumed, in accordance with literature data,[12] that this lignin contained equal amounts of coniferaldehyde and coniferyl alcohol structures, these being the only olefinic groups present. Using these data, the integral for the aromatic carbon atoms could be calculated and thus the integral value for one carbon atom.

With knowledge of the size of the integral corresponding to one aromatic carbon atom in each of the lignin spectra, the amounts of the various functional groups present could be calculated. The results of these calculations (with estimated limits of error of the order of ±5 %), together with some earlier analytical data, are given in Table 1, in which it can be seen, as expected, that the values for all the kraft lignins differ considerably from those for the native lignin. The differences in chemical structure between the kraft lignins are, however, comparatively small. A certain increase in the number of substituted aromatic carbon atoms was found in the lignin withdrawn at the very end of the cook (GSK 6). This result is in accordance with that previously obtained by chemical analysis of the same lignin.[13] It indicates that this particular lignin has a more branched and/or cross-linked structure than the others.

All the kraft lignins exhibited larger amounts of olefinic double bonds than the sample of milled wood lignin. These groups are known to occur in structures such as styrene, stilbene and enol ether derivatives.[14,15] Cinnamaldehyde structures, on the other hand, are not stable under kraft cooking conditions and cannot, therefore, be found. In the CH sub-spectrum of the kraft lignins some small signals centered around 144 ppm were found. These were assumed to be attributable to the β-carbon atom in enol ether structures.[8] By integration of the spectrum for the lignin sample SK 5, a value of approximately 0.05 such structures per aromatic ring was obtained. The remaining olefinic carbon atom signals are found at higher fields and they cannot, therefore, be distinguished with certainty from signals for the CH carbons belonging to the aromatic ring. Recent studies[16,17] on substituent chemical shifts for a variety of lignin-like model compounds suggest, however, that the CH carbon signals found at approximately 127–135 ppm may to a large extent arise from olefinic carbon atoms. In the CH_2 sub-spectrum of sample SK 5, one isolated small signal was also found at 114.4 ppm. This shift value is in excellent agreement with the value obtained for the β-carbon atom in vinylguaiacol (4-hydroxy-3-methoxyphenylethene).[18]

For samples SK 5, GSK 3 and MWL, the CH sub-spectra also contained a sharp signal around 192 ppm which was assumed to be due to the aldehyde group in vanillin structures.[8] Quantification of this signal in the spectra of SK 5 and

MWL gave values of 0.03 and 0.04 aldehyde carbons per aromatic ring, respectively. In the sub-spectrum of SK 5 containing only signals for the quaternary carbon atoms (as well as in the complete spectrum), a broad signal centered at 176.9 ppm was also found. For the other two kraft lignin samples this signal is partially obscured by the strong signals from the carbonyl functions in the acetate groups. The signal can be assigned to carboxy groups, and integration gave 0.13 such groups per aromatic ring. Carboxy groups are known to be present in kraft lignins, and in earlier work their amount has been estimated by conductometric titration to be 0.13 per phenylpropane unit.[19]

The large amount of saturated carbon atoms, resonating in the 0–55 ppm range, is surprising, although several modes of formation of such carbons under kraft cooking conditions have been suggested.[20] The results obtained indicate, however, that, e.g., addition of water across double bonds in coniferyl alcohol and other ring-conjugated structures formed during the cook may play a more dominant rôle than expected. In studies with lignin model compounds, such products, formally arising by addition of water or hydrogen sulfide (polysulfide) across a double bond, have been assumed to be formed as unstable intermediates.[21] The results obtained here tend to indicate, however, that structures resulting from the addition of water or hydrogen sulfide are quantitatively important as end products. The presence of large amounts of CH_2 carbons revealed by the spectra of the kraft lignins supports this view.

In the CH_2 sub-spectrum of the kraft lignin sample SK 5, a sharp signal found at 29.7 ppm can be attributed to the methylene group in diarylmethane structures.[8] Under kraft cooking conditions these can be formed in condensation reactions between phenolic structures and formaldehyde, the latter originating from terminal hydroxymethyl groups in the lignin.[20] For sample SK 5, this type of methylene carbon was calculated to amount to 0.04 per aromatic ring.

Among the various other signals in the CH_2 sub-spectra, some further carbon atom signals were identified on the basis of published shift data obtained for lignin model compounds. A sharp signal found at 72.3 ppm can thus be attributed to the γ-carbon atom in structures of the pinoresinol type.[22] The integrated value for this carbon in sample SK 5 gave 0.02–0.03 units per aromatic ring, In the most prominent structures present in lignins, i.e. the phenylpropane β-aryl ethers, the γ-carbon atom in the hydroxymethyl group gives a rather sharp signal around 60–62 ppm.[23] In sample SK 5, this signal was found at 61.9 ppm, integration giving a value of 0.09–0.12 per aromatic ring for this type of structure.

The aliphatic carbon atom signals in the CH sub-spectra were found in three regions, each containing a large number of individual signals as seen in Fig. 1. Among these, the signal at 54.9 ppm can be assigned to the β-carbon atom in phenylcoumaran structures.[23] In SK 5, the size of this signal corresponds to 0.04 such structures per aromatic ring, whereas in the milled wood lignin sample (MWL) the integral gave a value of 0.13 per aromatic ring. In the CH sub-spectrum of SK 5, the α-carbon atom in phenylpropane β-aryl ether structures was also clearly visible, being situated at 73.6 ppm. The quantitative calculation based on this signal afforded a value of 0.07 per aromatic ring, i.e. a somewhat lower value than that given above. This discrepancy may be due to the presence of a certain amount of dihydroconiferyl alcohol structures in the lignins. Such structures have been found before in both wood and kraft pulps, although no quantitative data are available. The signal for the γ-carbon atom in this type of structure appears at a chemical shift almost identical to that observed for the corresponding carbon atom in phenylpropane β-aryl ether structures.[23]

From the data in Table 1 it can be seen that the amounts of primary hydroxy groups in the various lignins are in fairly good agreement with the amounts of oxygen-linked methylene groups. This indicates that none of these lignins contains any appreciable amount of ether linkages at the γ-position in the lignin side chain. A similar comparison of the amounts of secondary hydroxy groups and oxygen-linked methine groups reveals, however, that all the lignins contain appreciable amounts of ether linkages. Although this type of linkage is frequent in native lignins, its presence in the kraft lignins should, on the whole, be restricted to the residual amounts of phenylpropane β-aryl ether, phenylcoumaran and pinoresinol structures. The quantitative data for these structures given above do not, however, account for all the ether linkages present. It therefore appears that the formation of new ether linkages in lignin takes place during a kraft cook.

Table 1. Number per aromatic ring of various functional groups in kraft lignins (SK 5, GSK 3, GSK 6) and in native lignin (MWL).

Functional group	Number per aromatic ring			
	Degree of delignification (designation)			
	0(MWL)	0–69 % (SK 5)	29–66 % (GSK 3)	93–95 % (GSK 6)
Quaternary aromatic C	3.34	3.42	3.43	3.52
Double bonds	0.06[a]	0.18	0.14	0.22
Hydroxy groups, total[b]	1.29	1.25	1.31	1.32
Hydroxy groups, primary[b]	0.78	0.35	0.44	0.43
Hydroxy groups, secondary[b]	0.31	0.25	0.25	0.21
Hydroxy groups, phenolic[b]	0.20	0.62	0.62[a]	0.68[a]
Aliphatic C, 55–90 ppm, CH	1.34	0.49	0.54	0.51
CH_2	0.84	0.35	0.44	0.35
OCH_3	0.97	0.79[a]	0.91	0.88
Aliphatic C, 0–55 ppm, CH	0.25	0.39	0.32	0.36
CH_2	0.18	0.63	0.55	0.59
CH_3		0.18	n.d.[c]	n.d.[c]

[a]By definition, see text. [b]Data from Refs. 2 and 26. [c]Not determined.

One possibility for the formation of such ether groups could be reaction between quinone methides and secondary alcohol structures in adjacent lignin structures.

A further indication of the presence of condensed structures in the kraft lignins was found by inspection of the amounts of methine carbons resonating in the range 0–55 ppm (Table 1). Thus, in comparison to the milled wood lignin sample, all the kraft lignins contained a considerably higher amount of such carbons. The presence of these is difficult to explain unless some (unknown) reductive mechanism is operative or it is assumed that new carbon–carbon linkages are formed in lignin during the kraft cook. The latter type of reaction has been proposed before, and on the basis of studies with lignin model compounds several possible modes of formation have been identified.[20]

Conclusions

Despite the heterogeneity of lignins, whether native or modified by technical processes, ^{13}C NMR spectroscopy can be used to obtain rather detailed structural information. Spectral editing using the DEPT pulse sequence further greatly facilitates the elucidation of the spectra. The possibility of obtaining ^{13}C NMR spectra, including DEPT spectra, under "quantitative conditions", although time consuming, offers new unambiguous ways of obtaining quantitative data concerning the amounts of various structural units and functional groups. These features are clearly illustrated by the results presented in this work.

From a pulping chemistry point of view, it is interesting to note that the structure of lignin is severely altered by kraft cooking. The major part of these changes seems, however, to take place when, or before, a lignin molecule becomes dissolved, as revealed by the similarity in chemical structure of the three kraft lignins. Furthermore, some strong evidence was obtained for the occurrence of various types of condensation reaction during kraft cooking.

Experimental

Lignin samples. The kraft lignins were obtained from kraft cooks of pine (*Pinus silvestris*) after precipitation from the corresponding cooking liquors. Further experimental details and a thorough description of the work-up procedure are given in Refs. 2 and 5. The sample of milled wood lignin was obtained from spruce (*Picea abies*) and was prepared according to the procedure of Björkman.[24]

^{13}C NMR spectra. The NMR spectra were recorded on a Bruker WM 200 spectrometer equipped with an Aspect 2000 computer and pulse-programmer. Each sample (500–600 mg) was dissolved in 1.6 ml of solvent and the spectrum recorded at 50 °C employing TMS as internal standard. The acetylated kraft lignins GSK 3 and GSK 6 were dissolved in acetone-d_6, the kraft lignin SK 5 in acetonitrile-d_3/deuterium oxide (4:1), and the milled wood lignin in DMSO-d_6. All other experimental parameters and the procedure for quantitative calculation of signal intensities were the same as those described in Ref. 25.

References

1. Gellerstedt, G. and Lindfors, E.-L. *Nordic Pulp. Pap. Res. J. 2* (1987): 2, 71.
2. Robert, D. R., Bardet, M., Gellerstedt, G. and Lindfors, E.-L. *J. Wood Chem. Technol. 4* (1984) 239.
3. Pegg, D. T., Bendall, M. R. and Doddrell, D. M. *J. Magn. Reson. 44* (1981) 238.
4. Bardet, M., Foray, M. F. and Robert, D. *Makromol. Chem. 186* (1985) 1495.
5. Gellerstedt, G. and Lindfors, E.-L. *Holzforschung 38* (1984) 151.
6. Nimz, H. H. and Lüdemann, H.-D. *Holzforschung 30* (1976) 33.
7. Nimz, H. H. *Bull. Liaison Groupe Polyphenols 8* (1978) 185.
8. Kringstad, K. P. and Mörck, R. *Holzforschung 37* (1983) 237.
9. Schoolery, J. N. *Prog. NMR Spectrosc. 11* (1977) 79.
10. Nimz, H. H., Nemr, M., Schmidt, P., Margot, C. and Schaub, B. *J. Wood. Chem. Technol. 2* (1982) 371.
11. Bendall, M. R. and Pegg, D. T. *J. Magn. Reson. 53* (1983) 272.
12. Marton, J. and Adler, E. *Acta Chem. Scand. 15* (1961) 370.
13. Gellerstedt, G. and Gustavsson, K. *J. Wood Chem. Technol. 7* (1987) 65.
14. Gierer, J. and Lindeberg, O. *Acta Chem. Scand., Ser. B 34* (1980) 161.
15. Mortimer, R. D. *J. Wood Chem. Technol. 2* (1982) 383.
16. Hassi, H. Y., Chen, C.-L. and Gratzl, J. *TAPPI Research and Development Conference,* Appleton, USA 1984, Proceedings, p. 249.
17. Hassi, H. Y., Aoyama, M., Tai, D., Chen, C.-L. and Gratzl, J. *Holzforschung. In press.*
18. Mörck, R. and Kringstad, K. P. *Holzforschung 39* (1985) 109.
19. Gellerstedt, G. and Lindfors, E.-L. *Tappi J. 70* (1987): 6, 119.
20. Gierer, J., Imsgard, F. and Pettersson, I. *Appl. Polym. Symp. 28* (1976) 1195.
21. Gierer, J. and Lindeberg, O. *Acta Chem. Scand., Ser. B 32* (1978) 577.
22. Nimz, H. H. and Lüdemann, H.-D. *Makromol. Chem. 175* (1974) 2577.
23. Lüdemann, H.-D. and Nimz, H. H. *Makromol. Chem. 175* (1974) 2393.
24. Björkman, A. *Sven. Papperstidn. 59* (1956) 477.
25. Robert, D., Gellerstedt, G. and Bardet, M. *Nordic Pulp Pap. Res. J. 1* (1986): 3, 18.
26. Robert, D. and Brunow, G. *Holzforschung 38* (1984) 85.

Received April 8, 1987.