Pergamon

Tetrahedron Letters, Vol. 35, No. 49, pp. 9267-9270, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)02014-0

Application of Three-Dimensional HMQC-HOHAHA NMR Spectroscopy to Wood Lignin, a Natural Polymer

Ilkka Kilpeläinen* and Erja Ämmälahti

Institute of Biotechnology, P.O. Box 45, FIN-00014 University of Helsinki, Finland.

Gösta Brunow

Organic Chemistry Division, Department of Chemistry, P.O. Box 6, FIN-00014 University of Helsinki, Finland.

Danielle Robert

Centre D'Études Nucleaires de Grenoble, 85X, 38041 Grenoble Cedex, France.

Abstract: The three-dimensional HMQC-HOHAHA experiment was successfully applied to an acetylated ^{13}C-enriched poplar wood lignin preparation. The resolution of this technique proved to be sufficient to give unambiguous assignments for most of the side-chain structures, which overlap heavily in one- and two-dimensional NMR spectra. By this technique it was possible to show, for the first time, that α,β-diaryl ether structures exist in lignin, although in low abundance. These structures have been considered to be of importance in lignin structure, since they may act as reactive cross-linking groups providing the lignin polymer with a branched network structure.

Lignin is the second most abundant terrestrial biopolymer, and it plays a central role in the structure and defence system of vascular plants. Lignification in plants is an enzyme initiated radical reaction, often referred to as dehydrogenative polymerisation.[1,2] Lignin is comprised of phenylpropane units which are linked together to form a polymer network lacking regularity, optical activity or crystallinity. Lignin is unique among natural polymers in that there is little enzymatic control over its biosynthesis and the factors guiding this process are as yet not fully understood.[1,3] The isolation of intermediate products from DHP (dehydrogenation polymer, a synthetic lignin)[1] syntheses, and of oligomeric fragments from lignin, has led to the proposal of a number of side-chain bonding types for lignin (Fig. 1).[1,4,5]

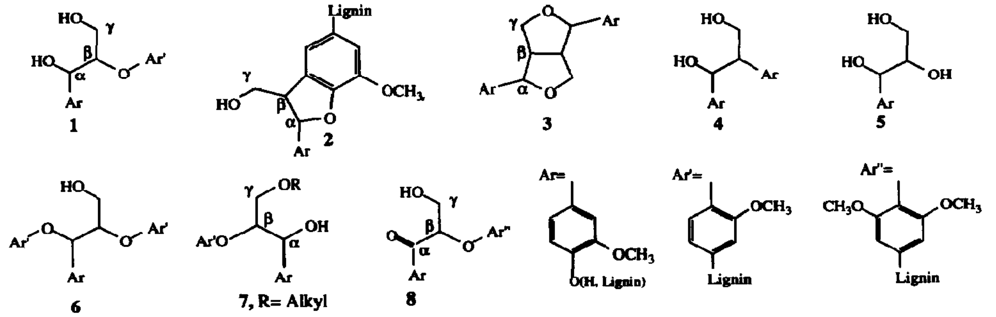

Figure 1. Proposed bonding types in lignin.

By the application of one- and two-dimensional NMR spectroscopy, most of these structures have been reliably identified.[6-15] The one-dimensional NMR techniques suffer from strong signal overlap, and only tentative assignments can be made using reference data from model compounds.[13] Moreover, the ^{1}H linewidths are broader than the scalar coupling constants, therefore preventing the use of coupling information.[13] By two-dimensional techniques,[9-15] especially using inverse detected ^{1}H-^{13}C correlation (HMQC, Fig. 2) and homonuclear Hartman-Hahn (HOHAHA, Fig. 3) in concert,[12-15] it has been possible to trace out most of the side-chain spin-systems present in lignin, and assign them unambiguously. However, even in two-dimensional NMR spectra some of the correlations overlap heavily, and it is not always possible to trace out the whole spin-system.[13,14] In this paper we make a preliminary report of the successful application of the three-dimensional HMQC-HOHAHA experiment to an acetylated ^{13}C-enriched milled wood lignin (MWL) isolated from a hardwood species. It is shown here that α,β-diaryl ether, **6**, structures exist in lignin, but probably at very low abundance. These structures may be of importance in lignin structure (even though their abundance seems quite small), since they can act as reactive cross-linking groups in lignin.[1]

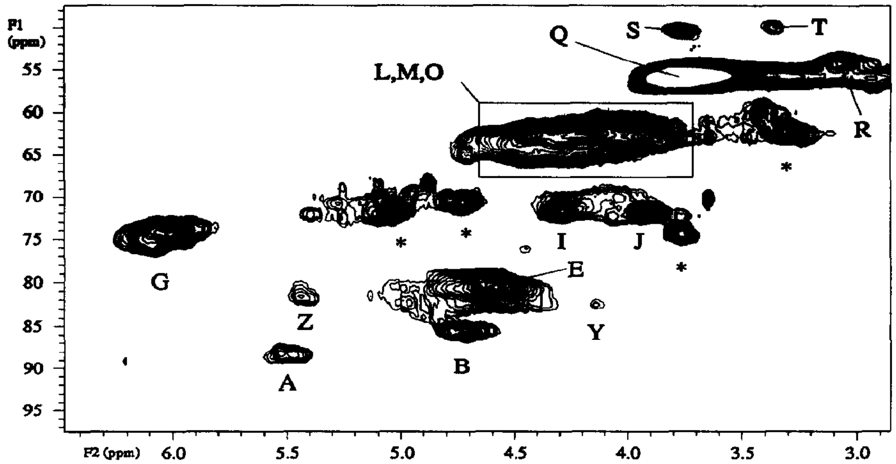

Figure 2. Expansion of the 2D HMQC spectrum of acetylated ^{13}C-enriched poplar wood MWL. The correlations are labelled analogously to our previous publications.[12-14] The asterisks denote correlations that originate from hemicellulose (xylan).[14,15]

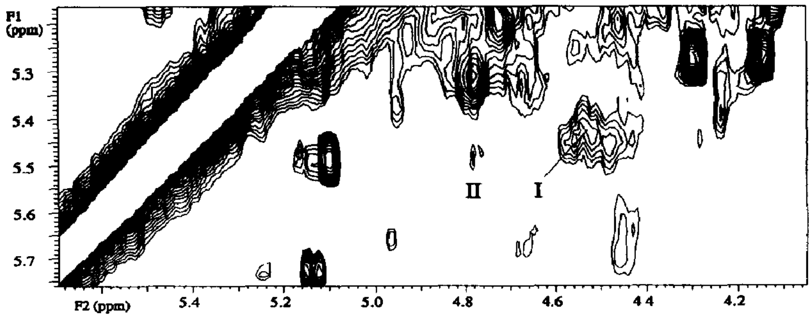

Figure 3. Expansion of the 2D HOHAHA spectrum of acetylated ^{13}C-enriched poplar wood MWL.

As an illustration, the assignments of the side-chain spin-system of β-1 structures, **4**, provide a good example of the resolution power of the 3D HMQC-HOHAHA experiment. In a previous study,[13] it was not possible to assign the chemical shifts of Cα and Cγ as the expected 2D HMQC Hα/Cα and Hγ/Cγ correlations of structures **4** were overlapped by the strong correlations from structures **1** and **2**.[13] In the 2D HMQC spectrum there is a correlation at 3.35/50.5, (T in Fig. 2) which we have previously assigned to the Hβ/Cβ correlation of β-1 structures, **4**.[13,14] The F2F3 plane of the 3D experiment selected at δ of Cβ in structure **4** (50.5 ppm in F1, Fig. 4a) reveals the whole ^{1}H spin-system. The Hβ proton at 3.35 ppm has HOHAHA correlations to 6.07 ppm (III in Fig. 4a) and to 4.12-4.45 ppm (IV in Fig. 4a), which are the correlations of Hβ/Cβ to Hα (III) and to two diasterotropic Hγs (IV), respectively. The chemical shifts of the carbon, to which the protons at 6.05 ppm and 4.12-4.45 are attached to, can now be obtained from the F1F3 planes of the 3D HMQC-HOHAHA spectrum, and are 74.7 (Cα) and 63.7 (Cγ) ppm, respectively (data not shown) in good agreement with model compound data. Thus, the 3D HMQC-HOHAHA experiment provides unambiguous assignments of all protons and carbons belonging to this spin-system.

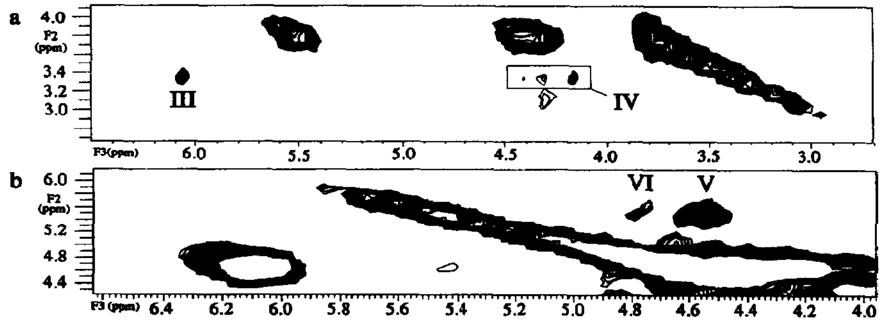

Figure 4. Expansions F2F3 planes from the 3D HMQC-HOHAHA spectrum (mixing time 30 ms) of ^{13}C enriched poplar wood MWL. a) plane at F1=50.5 ppm. b) plane at F1=81.0 ppm.

Of particular interest to us was to trace out the spin-system(s) of the 2D HMQC correlation at 5.42/81.5 (Z in Fig. 1), which we have previously observed in another hardwood (birch) MWL sample.[13] The protons at 5.42 ppm have a HOHAHA correlation to 4.55 ppm (I in Fig. 3) (partially overlapping with the Hα/Hγ correlations of structure **2**)[13], and the HMQC correlation (Z in Fig. 2) was assigned to Hβ/Cβ correlations from structures **8**.[13] However, in addition to the HOHAHA correlation at 5.42/4.55, there is a very small HOHAHA correlation at 5.45-5.53/4.75-4.80 (II in Fig. 3) in this ^{13}C-enriched poplar wood lignin preparation, which could in principle be assigned to structures of type **6**.[16] However, in the 2D HMQC (Fig. 2) spectrum, no resolved correlations from structures **6** were observed. Thus, the assignment of correlation II in the HOHAHA spectrum (Fig. 2) remained uncertain. The F2F3 plane of the 3D HMQC-HOHAHA spectrum at 81.0 ppm in F1 (Fig. 4b) reveals that in addition to structures of type **8**, there is an other spin-system which is giving arise to the HMQC correlation at 5.42/81.5 (Z in Fig. 2) but whose correlations are much less intense than the correlations of Hβ/Cβ to Hγ correlations of structures **8** (V in Fig. 4b). The correlation of 5.47-5.60/80.5-81.0 to 4.73-4.82 ppm in the 3D HMQC-HOHAHA spectrum matches well with expected correlations of Hα/Cα to Hβ in a α,β-diaryl ether, **6**, structures (Hα/Cα = 5.41–5.48/80.2–80.7, Hβ/Cβ = 4.69–4.78/80.7–81.9 and Hγ/Cγ = 4.13-4-7/ 63.4-65.9 for a model compound)[17] and therefore confirm that these structures are present in this lignin preparation, but clearly in very small amounts. Attempts to observe the Hα/Cα correlations to Hγ by using longer mixing times were unsuccessful, probably because the expected correlations may overlap with the correlation of Hβ/Cβ to Hγ (V in Fig 4b) of structures **8**.

In conclusion, the 3D HMQC-HOHAHA NMR experiment offers a very powerful tool for the structural investigation of ^{13}C-enriched soluble lignin samples.[18] In this paper we have shown that α,β-diaryl ether structures exist in lignin (at low abundance) and that by increasing the number of dimensions of NMR spectra it is possible to produce unambiguous assignments for the spin-systems of lignin. Thus, the 3D experiments provide structural information not obtainable by any other available technique.

References and Notes

1. Adler, E. *Wood Sci. Technol. 11* (1977) 169.
2. Freudenberg, K. and Neish, A. Eds. *Constitution and Biosynthesis of Lignin*, Springler Verlag, Berlin 1968.
3. Eberhardt, T.L., Bernards, M.A., He, L., Davin, L.B., Wooten, J.B. and Lewis, N.G. *J. Biol. Chem.* 268 (1993) 21088.
4. Sakikabara, A. *Wood Sci. Technol. 14* (1980) 89.
5. Leary, G. *Wood Sci. Technol. 14* (1980) 21.
6. Lundquist, K. *Acta Chem. Scand. B34* (1980) 21.
7. Robert, D. In: Lin, S.Y. and Dence, C.W. Eds., *Methods in Lignin Chemistry* Springer-Verlag Berlin; Heidelberg 1992, p. 250.
8. Guittet, E., Lallemand, J.Y., Lapierre, C. and Monties, B. *Tetrahedron Lett. 26* (1985) 2671.
9. Bardet, M., Gagnaire, D., Nardin, R., Robert, D. and Vincendon, M. *Holzforschung 41* (1987) 199.
10. Lundquist, K. and Stern, K. *Nordic Pulp Paper Res. J. 4* (1989) 210.
11. Ede, R.M., Brunow, G., Simola, L.K. and Lemmetyinen, J. *Holzforschung 44* (1990) 95.
12. Ede, R.M. and Brunow, G. *J. Org. Chem. 57* (1992) 1477.
13. Kilpeläinen, I., Sipilä, J., Brunow, G., Lundquist, K. and Ede, R.M. *J. Agr. Food Chem.*, 1994, in press.
14. Kilpeläinen, I. *Annales Acadèmiae Scientiarum Fennicae*, Series A, II. Chemica (1994) 255.
15. Fukagawa, N., Meshitsuka, G. and Ishizu, A. *J. Wood Chem. Technol. 11* (1991) 373.
16. Ede, R.M. and Kilpeläinen, I. *Res. Chem. Int.*, 1994, submitted for publication.
17. Sipilä, J. *Annales Academiae Scientiarum Fennicae*, Series A, II. Chemica (1990) 226.
18. ^{13}C-enriched poplar wood MWL sample was obtained from Bardet *et al.*[9], acetylated and dissolved in $CDCl_3$. All NMR spectra were obtained with Varian Unity 500 spectrometer (11.7 T) and referenced to internal TMS. The inverse detected ^1H-^{13}C correlation spectra, HMQC, and the homonuclear Hartman-Hahn spectra, HOHAHA, were measured as described earlier.[13] The three-dimensional inverse detected ^1H-^{13}C correlation-homonuclear Hartman-Hahn spectra, HMQC-HOHAHA, was acquired as described by Wijmenga *et al.*.[19] The spectral width in F2 and F3 was set to 6 kHz, and 25 kHz in F1. 32 scans, 70 time incremets in F1, and 70 time increments in F2 were collected using the hypercomplex method. The delay for polarization transfer between ^1H and ^{13}C was set for an assumed 140 Hz and a relaxation delay of 0.5 seconds and mixing times of 30 to 100 ms were used to obtain the spectra. The time domain data of F1 and F2 was appended to 124 points using forward linear prediction, and the spectra were processed using Π/2 shifted squared sinebell functions in all dimensions prior to Fourier transformation.
19. Wijmenga, S.S., Hallenga, K. and Hilbers, C.W. *J. Magn. Reson. 84* (1989) 634.

(*Received in UK* 12 *September* 1994; *revised* 6 *October* 1994; *accepted* 7 *October* 1994)