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APPLICABILITY OF THE ^{13}C NMR "INADEQUATE" EXPERIMENT TO LIGNIN, A NATURAL POLYMER.

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Abstract: The ^{13}C INADEQUATE experiment has been applied to lignin, a natural biopolymer.

In the studies of macromolecules , a detailed interpretation of the ^{13}C NMR spectra is often useful, but represents a real challenge; especially in those cases where heterogeneity in the macromolecule is associated with overcrowded spectra. In most cases, assignment can only rely on comparison with model compounds.

Such a situation is encountered in lignins (1) where various monomeric and dimeric models were shown to be very useful (2,3) but do not lead to a total and unambiguous spectral assignment.

In the course of the study of different poplar soluble lignin fractions we have been able to successfully apply some of the various multipulse techniques such as DEPT or spin echo (4) usually restricted to small or medium-sized molecules. Whereas some disappointing preliminary results from polarization transfer experiments on macromolecules were thought to be linked to the unfavorable ^{1}H relaxation times (5), we felt that ^{13}C homonuclear correlation experiments such as INADEQUATE(6) would be successful taking advantage from the fact that the ^{13}C T_1 of polymers are much longer than the ^{1}H ones. We would like to demonstrate here, on the lignins, the perfect applicability of the INADEQUATE experiments to natural or synthetic macromolecules. This approach is, to our knowledge, totally undocumented in this field, although the INADEQUATE technique proved very powerful for structural elucidations (7).

One of its major limitations, its poor sensitivity, has been alleviated in our case by the use of ^{13}C- enriched biological material, obtained here by growing poplars in a controlled $^{13}CO_2$ enriched (20%) atmosphere(4). The overall 11% lignin enrichment proved sufficient for the success of this experiment as exemplified in figure 2 (8), that represents a 100.13 MHz INADEQUATE spectrum.

This spectrum was obtained by a modification (9) of the FREEMAN and MARECI basic pulse sequence (10). Irrespective to the pulse imperfections and offset effects expected from the large spectral width (13 157 Hz), a prerequisite in the study of macromolecules at high field, numerous correlation peaks are visible, without residual peaks along the diagonal.

A powerful attribute of the two dimensional INADEQUATE technique is that it reveals carbon connectivities and often directly leads to signal assignments. Using the one-bond couplings, (average value 55 Hz), we could unambiguously confirm the structure of the major lignin subunits and most of the assignments previously made in ^{13}C enriched lignin spectra (4) from literature data, for both lignin and xylan signals. Almost all one-bond ^{13}C-^{13}C connectivities are revealed in the contour plot, and the corresponding correlations have been drawn for the etherified syringyl ($R_1 \neq H$) (solid lines, upper left), the non-etherified guaïcyl ($R_1 = H$) (dashed lines, upper left) or parahydroxybenzoic ester (solid lines, lower right) subunits as examples.

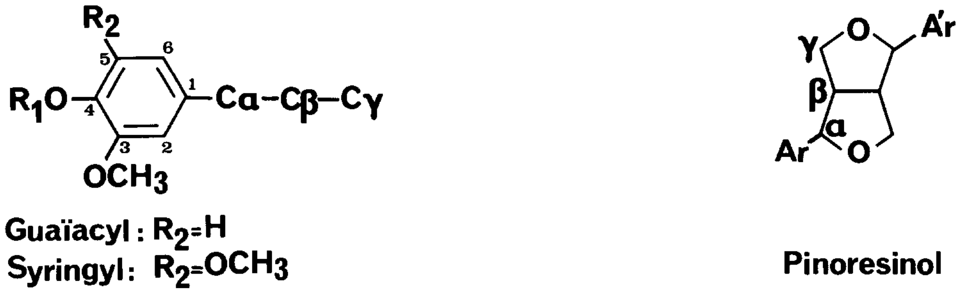

Fig. 1 Usual nomenclature and symbols of lignin subunits.

Many additional correlations can be assigned; various different xylose subunits have been isolated by this method and the corresponding cross-peaks are to be found in the 105-60 ppm range. A detailed interpretation is however beyond the scope of this communication and will be presented elsewhere.

However, a few signals were found to be incorrectly or incompletely assigned,(2-4) as illustrated by the following examples. The nomenclature used here and met in the lignin field is shown in figure 1.

A) Signals A and B, at 152.2 and 147.5 p.p.m. assigned respectively to the C_3-C_5 of etherified ($R_1 \neq H$) or non etherified ($R_1 = H$) syringyl units are coupled to syringyl C_2-C_6 signal C (in agreement with literature results) and, surprisingly, to a single syringyl C_4 signal D at 134.5 p.p.m., contrary to literature data.

B) Signal K is assigned to Cβ in pinoresinol units, by comparison with model compounds (1,4) it is both coupled to signals L and M of the homologous Cγ and Cα. The second coupling observed is not in agreement with literature results. Previously , the pino-resinol Cα was assigned a lower field signal than signal N which is unambiguously assigned to Cβ in β-O-4 structures. This also proves the usefulness of the method in the study of minor structures present in the polymer.

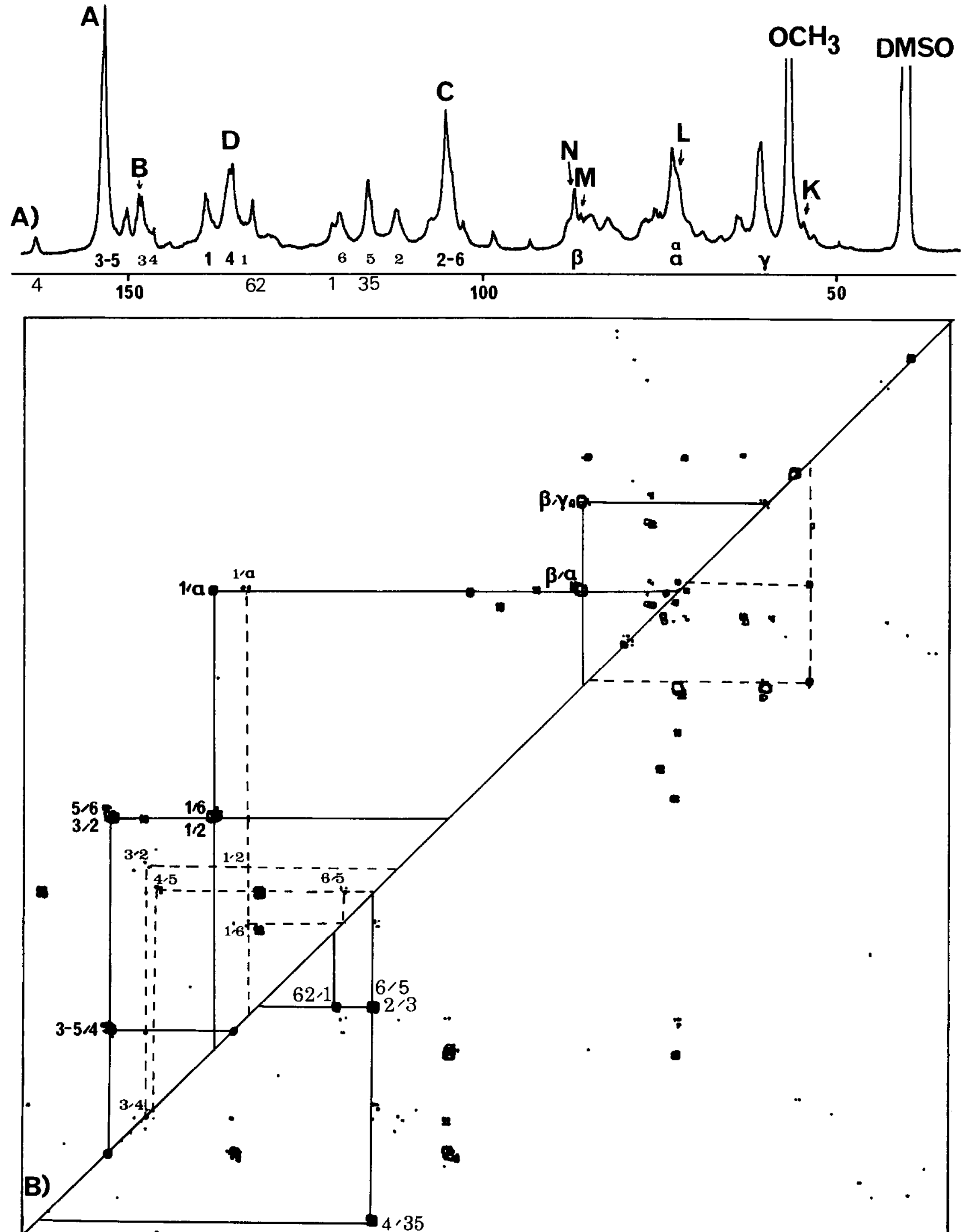

Fig.1 A) 100.37 MHz quantitative ^{13}C NMR spectrum of a poplar lignin solution. B) Contour plot of the corresponding INADEQUATE spectrum.

It should be pointed out that cross peaks intensities are thighly dependent upon ^{13}C relaxation times (11) (and thus upon molecular weight, viscosity, local and global mobility...) but also upon the geminal $^{13}C-^{13}C$ coupling values which range from 30 Hz to to 80 Hz in the lignin case (12). Further information could be gained based on a J-selection.

The results presented here show the applicability of the recently developped INADEQUATE pulse sequence in the extreme conditions (relaxation times, spectral width, signal overlapping) of biological macromolecules NMR. Chemist working in the polymers or the macromolecules fields should be aware of these fascinating possibilities.

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(8) The sample consists in 300 mg ^{13}C enriched poplar lignin dissolved in 1.5 ml DMSO-d_6. The two-dimensional data set is composed of 128 x 1K data point spectra. A 2-s recycle delay was allowed between each scan , the total refocusing time was set to 9.1 ms. 512 scans were acquired for each FID, resulting in an 40 hours total acquisition time. A sine-bell multiplication was applied in both dimensions . The magnitude value is represented Lignin extraction procedure was previously described (4).

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(11)T_1,T_2 but also multiple quanta relaxation times should be included in a general description.

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