Carbohydrate Research, 124 (1983) C1–C4
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Preliminary communication

Instrumental methods for assigning ^{13}C-n.m.r. resonances of carbohydrates measured for the solid state

LAURANCE D. HALL* and TANG K. LIM

Department of Chemistry, The University of British Columbia, Vancouver, B.C., V6T 1Y6 (Canada)

(Received October 6th, 1983; accepted for publication, October 20th, 1983)

The growing interest in ^{13}C-n.m.r. spectroscopy as a tool for studying the structure of carbohydrates in the solid state[1–4] provides a compelling reason for exploration of both the technical and chemical aspects of this area. We now report applications of two methods[5,6] that facilitate the potentially complex task of unravelling and assigning these complex spectra.

The pulse sequence[7] now routinely used[8] for the ^{13}C cross-polarization–magic angle spinning (c.p.–m.a.s.) measurement is summarized in Fig. 1. Without going into

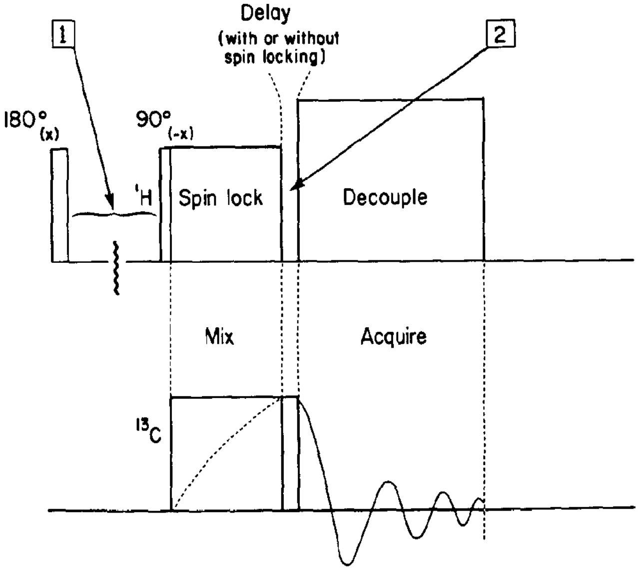

Fig. 1. Pulse sequence used to suppress the Delrin signal, and signals from protonated-carbon atoms in solids.

---

*To whom correspondence should be addressed.

0008-6215/83/$03.00

great detail here, it should be noted that the ^{13}C resonances observed with this procedure are largely induced by transfer of magnetizations from nearby protons of the same domain. Under normal conditions, the timing delays between the individual stages of this sequence are kept as short as possible. Both of the assignment methods which we have followed here depend on introduction into this sequence of selective delays, during which, predictable quantities of nuclear magnetization are permitted to decay, resulting in the effective elimination of certain resonances from the normal, c.p.–m.a.s., ^{13}C-n.m.r. spectrum.

The first method is analogous to the solvent suppression routines that are familiarly used in solution n.m.r. spectroscopy[9-12]; in the present context, it involves suppression of the resonance signal from the sample-spinner. Fig. 2A shows a typical example of the spectrum of a crystalline sample, namely, 1,2:3,4-di-*O*-isopropylidene-6-*O*-tosyl-α-D-galactopyranose, in a Delrin rotor [poly(oxymethylene)]. The method depends on the fact that the magnetization of the protons of Delrin decays far more rapidly than that of the organic substrate. Thus, introduction of a 500–600-ms delay, following the original, 90° pulse at position 1 in Fig. 1, ensures that the subsequent cross-polarization transfers magnetization only to the carbon atoms of the organic substrate. It should be noted that a residual, Delrin signal is usually present; this could be due either to radiofrequency-pulse imperfections or to physical inhomogeneity of the spinner material.

The second method provides, in principle at least, an unequivocal means for discriminating between the resonances of carbon atoms that bear protons from those that bear none. In this case, advantage is taken of the fact that, without spin locking, the magnetization of the protonated carbon atoms decays more rapidly than that of those which are nonprotonated. Thus, insertion of an appropriate delay-time at position 2 in the pulse sequence ensures that, even though all carbon atoms are magnetized to the same extent at the end of the mixing time, only those having, relatively longer, ^{13}C relaxation times retain a measurable magnetization at the start of the carbon acquisition-time. This point is nicely illustrated in Fig. 2C, with the disappearance of the signals for C-1, C-2, C-3, C-4, and C-5, all of which bear a proton (*vide infra*).

Although both of these methods will find widespread use in the rather straightforward roles just depicted, this Communication is prompted by the more subtle possibility of detecting localized motion of individual segments of carbohydrate-containing systems in the solid state. This point may be introduced by noting that, in Fig. 2C, all of the methyl resonances give a substantial signal. This observation appears to contravene the requirements of the foregoing analysis, until it is recognized that the fundamental requirement for observation of a carbon resonance is that it shall have a suitably low relaxation rate, and that it is itself nonprotonated is just one particular circumstance by which this condition can be satisfied. An alternative mechanism whereby protons can relax more slowly is when they have additional degrees of motional freedom, that is, a longer correlation time. In the case of methyl-carbon atoms, this arises from the rapid rotation of a methyl group about its three-fold axids. We consider that this phenomenon will find its most important applications in studying systems in which the carbohydrate is part of a complex "matrix".

Two further features merit comment. From the standpoint of peak assignment, it may be noted in Fig. 2C that specific deuteration[13] can be used in this respect; inter-

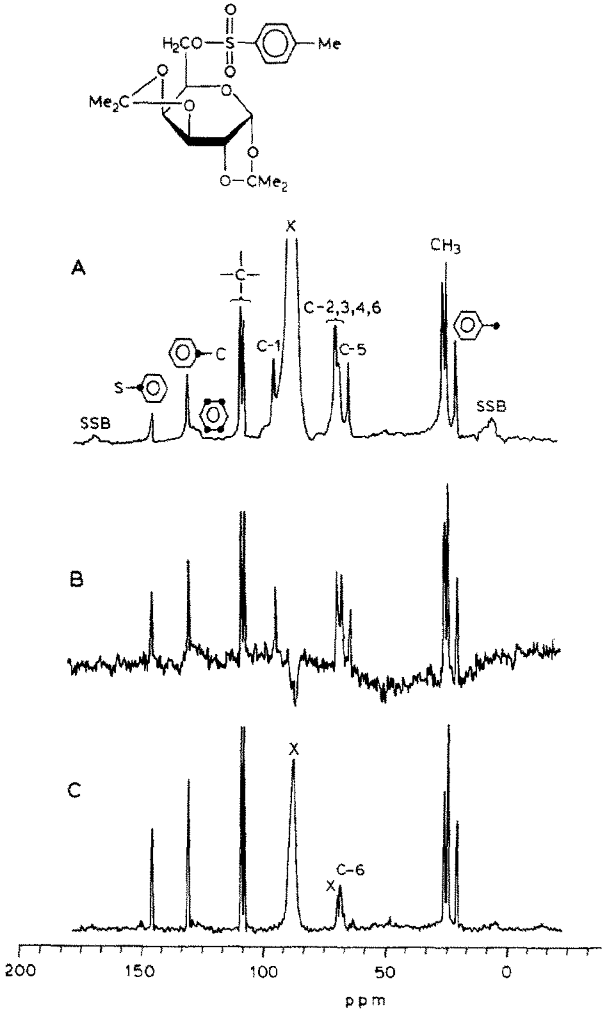

Fig. 2. ^{13}C-N.m.r. spectra of 1,2:3,4-di-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-galactopyranose. [(A) Normal ^{13}C-c.p.–m.a.s. spectrum, obtained with a Bruker CXP-200 F.t. n.m.r. spectrometer operated at 200 MHz for ^{1}H-n.m.r. spectroscopy, with an r.f. field of 1.5 mT, 5-ms contact-time, and 3,350 scans. (B) Delrin-signal suppression, using the pulse sequence of Fig. 1 and a delay time of 500 ms. (C) Nonprotonated-carbon spectrum, obtained by setting a 40-μs period without proton decoupling, prior to ^{13}C data-acquisition. Deuteration at C-6 of the compound is recorded in (B) and (C). Assignments are based on solution spectra, and chemical shifts are relative to external tetramethylsilane. Delrin signals and spinning side-bands are indicated as X and SSB, respectively.]

estingly, in this solid-state experiment, the signal of the deuterated carbon atom remains "visible", whereas, in the solution-state experiment[14,15], it "disappears". In this case, the quadrupole, line-broadening effect[16] on the carbon signal is not large enough to prevent the assignment. As a cautionary note, attention may be drawn to the fact that the signals of the four protonated carbon atoms of the tosyloxy moiety at 129.93 p.p.m. are so broad that they are barely detectable. This is characteristic of other tosylated sugars that we have studied, and several, alternative explanations[17,18] can be cited.

ACKNOWLEDGMENT

It is a pleasure to thank the Natural Science and Engineering Research Council of Canada for their financial support of this work.

REFERENCES

1 W. L. Earl and F. W. Parrish, *Carbohydr. Res.*, 115 (1983) 23–32.
2 C. F. Brewer, J. S. Blanchard, S. Englard, and G. Avigad, *Carbohydr. Res.*, 102 (1982) 294–297.
3 G. A. Jeffrey, R. A. Wood, P. E. Pfeffer, and K. B. Hicks, *J. Am. Chem. Soc.*, 105 (1983) 2128–2133.
4 Y. Inoue, T. Okuda, and R. Chûjô, *Carbohydr. Res.*, 116 (1983) C5–C8.
5 N. Zumbulyadis, *J. Magn. Reson.*, 49 (1982) 329–331.
6 S. J. Opella and M. H. Frey, *J. Am. Chem. Soc.*, 101 (1979) 5854–5856.
7 A. Pines, M. G. Gibby, and J. S. Waugh, *J. Chem. Phys.*, 59 (1973) 569–590.
8 C. S. Yannoni, *Acc. Chem. Res.*, 15 (1982) 201–208.
9 A. G. Redfield and R. K. Gupta, *J. Chem. Phys.*, 54 (1971) 1418–1419.
10 F. W. Benz, J. Feeney, and G. C. K. Roberts, *J. Magn. Reson.*, 8 (1972) 114–121.
11 R. K. Gupta, *J. Magn. Reson.*, 24 (1976) 461–465.
12 A. G. Redfield, S. D. Kunz, and E. K. Ralph, *J. Magn. Reson.*, 19 (1975) 114–117.
13 H. C. Brown, P. W. Weissman, and N. M. Yoon, *J. Am. Chem. Soc.*, 88 (1966) 1458–1463.
14 H. Spiesecke and W. G. Schneider, *J. Chem. Phys.*, 35 (1961) 731–738.
15 P. A. J. Gorin, *Can. J. Chem.*, 51 (1973) 2015–2019.
16 J. G. Hexem, M. H. Frey, and S. J. Opella, *J. Am. Chem. Soc.*, 103 (1981) 224–226.
17 D. L. Vanderhart, W. L. Earl, and A. N. Garroway, *J. Magn. Reson.*, 44 (1981) 361–401.
18 W. P. Rothwell and J. S. Waugh, *J. Chem. Phys.*, 74 (1981) 2721–2732.