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^{13}C - ^{13}C Dipolar Interactions Provide a Mechanism for Obtaining Resonance Assignments in Solid State ^{13}C NMR Spectroscopy

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Cross-polarization magic-angle spinning ^{13}C NMR spectroscopy is becoming an important method for examining the structure and molecular dynamics of crystalline solids (1-4), peptides (5), proteins (6), and polysaccharides (7-10). Although many reports (2, 3, 10, 11) suggest that there is direct correspondence between the isotropic chemical shifts of ^{13}C solution and solid state spectra, recent reports have demonstrated that this is not necessarily always the case (4, 5). Partial assignments are possible, based on differences in C-H dipolar interactions between carbons with and without directly bonded protons using dipolar dephasing (Selpen) experiments (1), and the splitting and broadening of resonances from carbons directly bonded to nitrogen (12). In addition, a new multipulse proton decoupling sequence has been developed to identify shifts through their respective proton coupled multiplicities (13). Unfortunately this powerful technique cannot differentiate resonances that exhibit the same splittings. Such a situation is common in studies of crystalline sugars and polysaccharides since, in most instances, except for ketosaccharides (4) all ring carbons have a single-bonded proton and no bonded nitrogen.

Walker *et al.* (14) have shown in solution studies that ^{13}C enrichment at specific sites can be used to identify adjacent ^{13}C resonances through the observed ^{13}C - ^{13}C scalar coupling interactions. Typically one-bond couplings were found to be approximately 40 Hz while two-bonded interactions were observed as 3-4 Hz doublets in the dilute spin lines (15). To obtain similar information for solid samples we attempted to examine the solid state spectra of molecules specifically enriched with a single ^{13}C atom.

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In the usual high-resolution ^{13}C NMR experiments in solids, the carbon-carbon dipolar interaction is very weak by virtue of the low natural abundance of ^{13}C . The other broadening mechanisms are removed by high-power proton decoupling for the proton-carbon dipolar and scalar interactions and by magic-angle sample spinning for the carbon chemical-shift anisotropy (16, 17). In the exceptional case of materials which have been enriched in ^{13}C , there is a significant probability of having two ^{13}C nuclei bonded to each other. In this case the carbon-carbon dipolar interaction becomes significant and vastly modifies the spectrum obtained. Maricq and Waugh have discussed the effects of magic-angle sample spinning upon such interactions in terms of average Hamiltonian theory (18). In their definitions, the carbon-carbon dipolar interactions are homogeneous and, thus, do not break into narrow spinning sidebands as is seen in inhomogeneous interactions such as chemical-shift anisotropy. In addition, the average Hamiltonian theory predicts interactions due to "high-order" effects, i.e., cross terms between the Hamiltonians of the different interactions. These high-order effects show up as commutators of the Hamiltonians, thus, interactions which commute produce no additional broadening. In the case of carbon-carbon dipolar and carbon chemical-shift anisotropy, the commutator is not generally zero and the result is a term which does not transform as a second rank tensor and, thus, will not spin out at the magic angle. In principle, this interaction produces broadening even under fast spinning conditions. Earlier experiments with doubly ^{13}C -enriched diammonium oxalate have demonstrated the complicated powder pattern obtained in the ^{13}C NMR spectrum under spinning conditions (18).

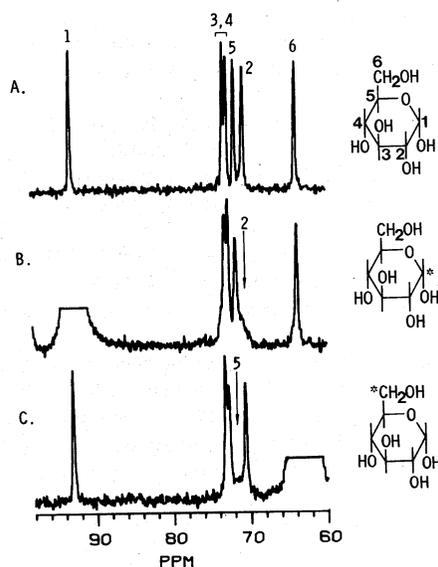


FIG. 1. ^{13}C CPMAS spectrum (37.82 MHz) of (A) unlabeled α -D-glucose after 64 transients, spinning rate ~ 2.5 kHz, 1.0 msec contact time, 25 G decoupling, and 45 sec recycling time; (B) ^{13}C -1-labeled α -D-glucose after 256 transients, 1.0 msec contact time, 25 G decoupling and 30 sec recycling time; (C) ^{13}C -6-labeled α -D-glucose run with the same conditions as B. All shifts are reported relative to *p*-dioxane taken as 67.40 relative to TMS.

If a molecule is enriched with ^{13}C at only one site, the resulting spectral line corresponding to the adjacent natural abundance carbons should be severely affected by the homogeneous interaction and should appear as a broad powder pattern, even under fast spinning conditions. In contrast, the labeled carbon should display a spectrum which contains only from 1–4% of a wide powder pattern, and 99–96% of a narrow line representing those ^{13}C carbons adjacent to ^{12}C . Figure 1 illustrates the effect of the homonuclear dipolar interaction for the example of ^{13}C -6- and ^{13}C -1-labeled α -D-glucose. Figure 1A is the natural abundance ^{13}C NMR spectrum of α -D-glucose. Figures 1B and C are the corresponding spectra of the C-1- and C-6-labeled sugars, respectively. The arrows point to the complete loss of intensity due to broadening of the directly bonded carbon resonances, C-2 and C-5, respectively. The identity of the C-2 shift has been unequivocally verified from the spectrum of authentic ^{13}C -2-labeled α -D-glucose.

The obvious application of this phenomenon for the identification and differentiation of ^{13}C resonances in the solid state is presently being pursued. An in-depth report on the interconversion chemistry of glucose in its different tautomeric and crystalline forms as well as its complete chemical-shift assignments will be published later.

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