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# Residual $^2$H Quadrupolar Couplings in Weakly Aligned Carbohydrates

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**We report a novel two-dimensional NMR pulse scheme for the $^1$H-detected observation of $^2$H in isotopically $^{13}$C, $^2$H-enriched carbohydrates. This scheme is used for the indirect observation of residual quadrupolar couplings in $^{13}$C, $^2$H-enriched methyl-$\beta$-D-glucopyranoside weakly aligned in a dilute lyotropic liquid-crystalline medium comprising 20% (w/v) dihexanoyl-phosphatidylcholine/dimyristoyl-phosphatidylcholine (1 : 3 mol/mol) in $D_2O$. The observed residual quadrupolar couplings are substantially larger than residual dipolar one-bond $^{13}$C–$^1$H couplings under the same experimental conditions. These quadrupolar couplings are thus a useful alternative to dipolar couplings for the structural analysis of small molecules that align very weakly in dilute liquid-crystalline media. Moreover, since the quadrupolar coupling constant is very uniform throughout endocyclic deuterons of the carbohydrate, these data suggest that adoption of a single average value of this parameter in $^2$H relaxation studies on the glycan moieties of glycoproteins and glycopeptides is a valid assumption.** 

*Key Words*: **quadrupolar couplings; dipolar couplings; NMR; carbohydrate; liquid crystal.**

The measurement of residual dipolar couplings in weakly aligned macromolecules is currently of great interest in view of their value as long-range structural restraints. While early studies utilized the very small degree of alignment induced by high magnetic fields (*1–3*), more recently dilute lyotropic liquid-crystalline phases have been used to induce much stronger alignment, giving rise to residual one-bond dipolar couplings tens of hertz in magnitude (*4–6*). By analogy, quadrupolar couplings in $I > \frac{1}{2}$ nuclei are not averaged to 0 in oriented molecules (*1*). Indeed, the residual quadrupolar splitting of the solvent deuterium resonance is a useful tool for confirming magnetic alignment of the liquid-crystalline phase (*6*). A significant barrier to the practical application of residual $^2$H quadrupolar couplings in structural studies concerns the low sensitivity of this nucleus. Here, we illustrate that this difficulty can be overcome in the case of carbohydrates by application of a novel two-dimensional pulse scheme applied to ~97% $^{13}$C, 97% $^2$H-enriched material. Use of highly deuterated material enables a relatively high degree of alignment to be used without significant line broadening from $^1$H–$^1$H dipolar couplings, while at the same time giving rise to substantial residual $^2$H quadrupolar couplings. The pulse scheme for this experiment is illustrated in Fig. 1. Magnetization on a given proton is transferred to the attached carbon by an INEPT sequence, followed by refocusing of the one-bond C–H coupling during the period $2\delta$. Magnetization evolves under the influence of the one-bond $^{13}$C–$^{13}$C scalar coupling $^1J_{CC}$ during the period $2T$, followed by COSY-like transfer to the neighboring carbon(s) in a manner entirely analogous to HCCH-type experiments (*7, 8*). During the period $2\Delta$, carbon magnetization is refocused under the influence of $^1J_{CC}$, and antiphase $^{13}$C–$^2$H magnetization is created during the delay $2\varepsilon$ under the influence of the one-bond $^{13}$C–$^2$H scalar coupling $^1J_{CD}$. This magnetization is then transferred in a COSY-like manner to the attached $^2$H by the simultaneous $^{13}$C and $^2$H 90° pulses. Deuterium magnetization evolves during the $t_1$ period with $^{13}$C decoupling, followed by transfer back to the attached carbon and the neighboring proton by the reverse of the pathway described. The two-dimensional spectrum thus correlates $^2$H resonances in $F_1$ with $^1$H resonances of neighboring proton(s) in $F_2$. In view of the similarity of the magnetization transfer to conventional HCCH spectroscopy (*7, 8*), we suggest the acronym HCCD for this experiment. A typical spectrum obtained for 50 mM 97% $^{13}$C, 97% $^2$H-enriched methyl-$\beta$-D-glucopyranoside in a dilute liquid-crystalline medium comprising 20% (w/v) DHPC : DMPC (1 : 3, w/w) in $D_2O$ is shown in Fig. 2. The high degree of overlap in the $^2$H dimension is overcome in large part by the dispersion afforded in the $^1$H dimension, and resonance assignment of the $^2$H spectrum is straightforward given knowledge of the $^1$H resonance assignments. Despite the small size of the solute, residual quadrupolar couplings of substantial magnitude are observed (Table 1). These are much larger than the residual one-bond $^{13}$C–$^1$H dipolar couplings that can be measured by comparison of $^{13}$C–$^1$H splittings in isotropic vs liquid-crystalline phases in the same sample (Table 1). Accurate measurement of the quadrupolar splittings is very straightforward from the spectrum of Fig. 2 given their magnitude and the narrow $^2$H spectral width. However, unlike $^{13}$C–$^1$H splittings in dilute liquid-crystalline

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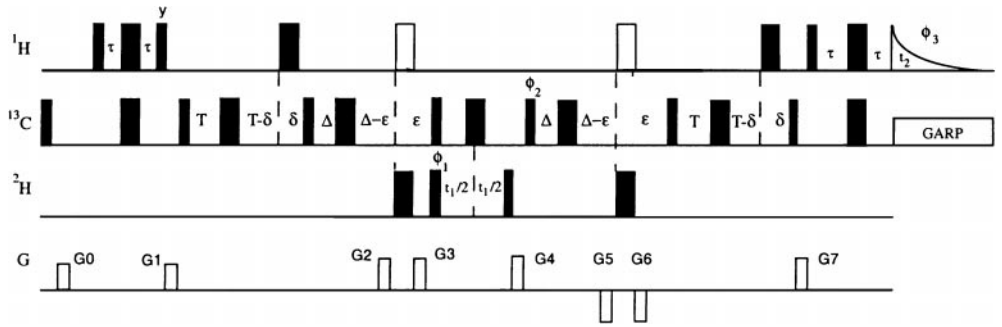


**FIG. 1.** Pulse scheme for the acquisition of proton-detected $^2$H spectra of $^{13}$C (97%), $^2$H (97%)-enriched oligosaccharides. Thin bars represent 90°, thick bars represent 180° RF pulses, and open bars represent gradient pulses along the $Z$ axis. All pulses for which the phase is not indicated are applied along the $+x$ axis. Phase cycling is as follows: $\phi_1 = x, -x, x, -x$; $\phi_2 = x, x, -x, -x$; $\phi_3 = x, -x, -x, x$. Quadrature detection in $\omega_1$ was obtained using the States–TPPI method on $\phi_1$. Decoupling of $^{13}$C during $\omega_2$ was achieved with GARP, using an RF field strength of 3.1 kHz. Delays are as follows: $\tau = 1.65$ ms $[1/(4^1 J_{CH})]$, $T = 3.3$ ms $[1/(8^1 J_{CC})]$, $\delta = 1.6$ ms $[1/(4^1 J_{CH})]$, $\varepsilon = 5.7$ ms $[1/(8^1 J_{CD})]$. The delay $\Delta$ is set to the value $n/(8^1 J_{CC})$ ($n = 3, 5, \ldots$) such that the delay $\Delta - \varepsilon > 0$ where $n = 5$ in this application, giving a delay of 15 ms. Gradient pulses were applied with a duration of 1 ms and with strengths G0 = 5 G/cm, G1 = 5 G/cm, G2 = 4 G/cm, G3 = 4 G/cm, G4 = 6 G/cm, G5 = −4 G/cm, G6 = −4 G/cm, G7 = 5 G/cm. The unshaded 180° $^1$H pulses may be required in samples with $^2$H enrichment lower than that in the present study (e.g., 50%) in order to suppress artifacts from $CH_2$ groupings.

media which are determined by the sum of the one-bond scalar and residual dipolar couplings ($^1J_{C,H} + {}^1D_{C,H}$) (*5*), the splittings observed in the HCCD are pure quadrupolar couplings, derived from the splitting of the two degenerate $m = \pm 1$ transitions by the quadrupolar interaction (*9*). The scalar $^1J_{C,D}$ coupling further splits each transition, giving rise to a doublet of doublets in $F_1$ in the absence of $^{13}$C decoupling. Unlike dipolar coupling measurements, it is thus not possible to determine the sign of the quadrupolar coupling given knowledge of $^1J_{C,D}$. However, the sign of the quadrupolar couplings can be determined in the current example from the signs of the dipolar couplings for each equivalent bond vector—for a given bond vector orientation, quadrupolar and dipolar couplings will be of opposite sign due to the functional dependence of these couplings (*10*), assuming the principal axis of the electric field gradient tensor is parallel to the C–D bond.

The magnitude of the $^2$H quadrupolar coupling constant is determined by the electric field gradient at the deuterium nucleus. The main determinant of this field gradient is the electrons donated by the nucleus to which $^2$H is directly bonded (*11*). In the present example the $^2$H quadrupolar couplings are scaled due to weak alignment of the molecule, in a manner entirely analogous to dipolar couplings (*2, 5*). For an assumed axially symmetric field gradient tensor with a principal axis parallel to the C–D bond, the terms of the Saupe matrix (*12*) that describe the alignment of the molecule are identical for both quadrupolar

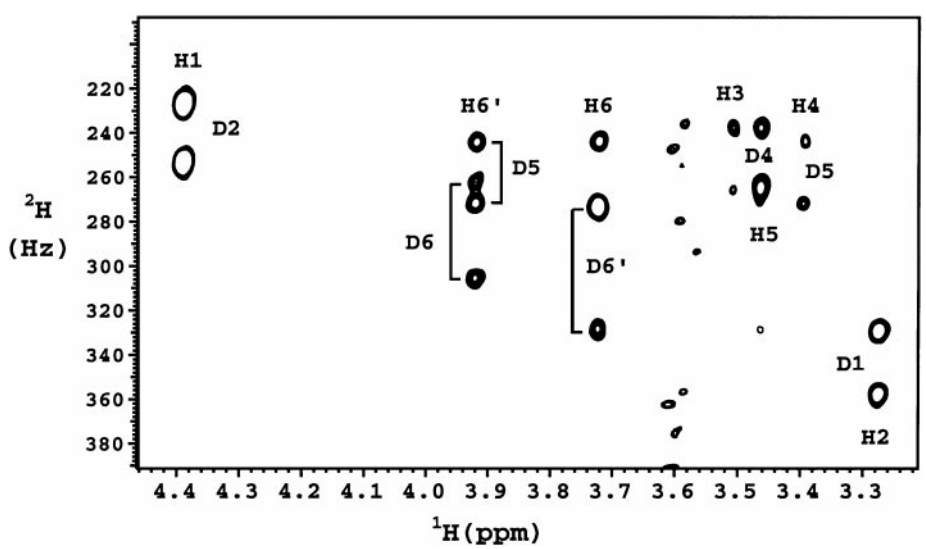


**FIG. 2.** (a) Two-dimensional 500-MHz $^1$H($\omega_1$), $^2$H($\omega_2$) correlation spectrum of uniformly $^{13}$C (97%), $^2$H (97%)-enriched methyl-$\beta$-glucopyranoside (30 mM in 700 $\mu$l 20% (w/v) DHPC : DMPC (1 : 3 (w/w)). Each proton correlates with neighboring deuteron(s), and the relevant assignments are given in the conventional notation.

**TABLE 1**

**Residual Quadrupolar and Dipolar Couplings in Uniformly Enriched Methyl-β-[$^{13}$C (97%), $^{2}$H (97%)] Glucopyranoside in a Dilute Liquid-Crystalline Medium**

| Deuteron | Residual quadrupolar coupling[a] | Residual dipolar coupling[b] |
|---|---|---|
| D1 | −28.6 | 5.3 ± 0.5 |
| D2 | −25.9 | 4.8 ± 0.5 |
| D3 | n/a | 4.0 ± 0.2 |
| D4 | −27.0 | 5.3 ± 0.4 |
| D5 | −27.8 | 5.4 ± 0.2 |
| D6 | 42.3 | −7.6 ± 0.2 |
| D6′ | −55.6 | 9.3 ± 0.4 |

*Note.* n/a: not measurable due to low intensity.

[a] Error determined from three independent measurements to be within digital resolution (±0.15 Hz).

[b] Values reported for the equivalent C–H bond vectors. Error values determined from three independent measurements.

and dipolar splittings, and hence do not appear in the expression for the ratio of these parameters (*10*):

$$\frac{\upsilon_Q}{{}^1D_{CH}} = -0.75\frac{\{e^2qQ/h\}}{\{\gamma_C\gamma_H h/(4\pi^2 r_{CH}^3)\}}.$$

Using this expression with the most accurate residual dipolar coupling in Table 1 (C6–H6′), and with a value for $r_{CH}$ from neutron diffraction studies of 1.1 Å (*13*), gives $\upsilon_Q = 180.6$ kHz, with error bounds of 173.4 and 189.0 kHz. While this value is somewhat larger than the value generally assumed for carbohydrates (164 kHz (*14*)), it is within the range measured very recently by Serianni and co-workers using $^{2}$H spin–lattice relaxation times (*15*). Interestingly, in the present study we find that $\upsilon_Q$ for the anomeric deuteron is significantly larger than that for all other endocyclic deuterons in methyl-β-D-glucopyranoside, whereas in the study of Serianni *et al*. the reverse was found. We rationalize this by noting that in neutron diffraction crystallographic data on methyl-glycosides (*13*), the C1–H1 bond length is significantly shorter than other endocyclic C–H bonds. Overall, however, $\upsilon_Q$ is quite uniform throughout endocyclic deuterons, as would be anticipated for methyl-β-D-glucopyranoside where all endocyclic C–D bond vectors are collinear. Thus the adoption of a single, average value for $\upsilon_Q$ in future relaxation studies on glycoproteins and glycopeptides is valid, as in peptides (*16*). In contrast, the exocyclic couplings differ and cannot be fitted to a single structure due to torsional rotations about the C5–C6 bond.

We should note that the use of 97% $^{2}$H-enriched material in the present study is not mandatory. In situations where a limited quantity of material is available, a much lower (e.g., 50%) level of deuteration improves sensitivity considerably (data not shown). However, in this case it is preferable to include 180° $^{1}$H purging pulses to reduce artifacts as described in the legend to Fig. 1.

In conclusion, we have described a pulse scheme that can be applied in the measurement of residual quadrupolar couplings in weakly aligned $^{13}$C, $^{2}$H-enriched carbohydrates. The magnitudes of these couplings are substantially larger than those of residual $^{13}$C–$^{1}$H dipolar couplings at the same degree of alignment. Quadrupolar couplings can thus be measured to accuracy higher than that of dipolar couplings for small molecules that align very weakly in dilute liquid-crystalline media. They may be of particular value in studies on the bound-state conformations of ligands in the fast-exchange regime, where the fraction of ligand bound is very small due either to very weak binding or to limited concentrations of protein. Although this approach can in principle be applied to other systems amenable to uniform $^{13}$C and fractional $^{2}$H enrichment, application to larger molecules will be limited by $^{2}$H relaxation times.

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## REFERENCES

1. A. A. Bothner-By, *in* "Encyclopedia of Magnetic Resonance" (D. M. Grant and R. K. Harris, Eds.), pp. 2932–2938, Wiley, Chichester (1995).
2. J. R. Tolman, J. M. Flanagan, M. A. Kennedy, and J. H. Prestegard, Nuclear magnetic dipole interactions in field-oriented proteins: Information for structure determination in solution, *Proc. Natl. Acad. Sci*. **92,** 9279–9283 (1995).
3. N. Tjandra, S. Grzesiek, and A. Bax, Magnetic field dependence of nitrogen-proton J splittings in N-15-enriched human ubiquitin resulting from relaxation interference and residual dipolar coupling, *J. Am. Chem. Soc*. **118,** 6264–6272 (1996).
4. N. Tjandra, J. G. Omichinski, A. M. Gronenborn, G. M. Clore, and A. Bax, Use of dipolar 1H-15N and 1H-13C couplings in the structure determination of magnetically oriented macromolecules in solution, *Nat. Struct. Biol*. **4,** 732–737 (1997).
5. N. Tjandra and A. Bax, Direct measurement of distances and angles in biomolecules by NMR in a dilute liquid crystalline medium, *Science* **278,** 1111–1114 (1997).
6. A. Bax and N. Tjandra, High-resolution heteronuclear NMR of human ubiquitin in an aqueous liquid crystalline medium, *J. Biomol. NMR* **10,** 289–292 (1997).
7. A. Bax, G. M. Clore, P. C. Driscoll, A. M. Gronenborn, M. Ikura, and L. E. Kay, Practical aspects of proton carbon carbon proton 3-dimensional correlation spectroscopy of C-13-labeled proteins, *J. Magn. Reson*. **87,** 620–627 (1990).
8. L. Yu, R. Goldman, P. Sullivan, G. F. Walker, and S. W. Fesik, Heteronuclear NMR studies of 13C-labeled yeast cell wall β-glucan oligosaccharides, *J. Biomol. NMR* **3,** 429–441 (1993).
9. A. Abragam, "Principles of Nuclear Magnetism," Oxford Univ. Press, Oxford (1961).
10. A. Mittermaier and L. E. Kay, Measurement of methyl H-2 quadrupolar couplings in oriented proteins. How uniform is the quadrupolar coupling constant? *J. Am. Chem. Soc*. **121,** 10,608–10,613 (1999).

*11.* R. Bersohm, Field gradients at the deuteron in molecules, *J. Chem. Phys.* **32,** 85–88 (1960).

*12.* A. Saupe, Recent results in the field of liquid crystals, *Angew. Chem. Internat. Ed.* **7,** 97–112 (1968).

*13.* S. Takagi and G. A. Jeffrey, Neutron diffraction refinement of the crystal structures of methyl $\alpha$-D-galactopyranoside monohydrate and methyl $\beta$-D galactopyranoside, *Acta Crystallogr. Sect. B* **35,** 902–906 (1979).

*14.* H. C. Jarrell, J. B. Giziewicz, and I. C. P. Smith, Structure and dynamics of a glycerolipid: A $^2$H NMR study of head group orientation, ordering, and effect on lipid aggregate structure., *Biochemistry* **25,** 3950–3957 (1986).

*15.* B. Bose-Basu, J. Zajicek, G. Bondo, S. Zhao, M. Kubsch, I. Carmichael, and A. S. Serianni, Deuterium nuclear spin-lattice relaxation times and quadrupolar coupling constants in isotopically labeled saccharides, *J. Magn. Reson.* **144,** 207–216 (2000).

*16.* A. Mittermaier, L. E. Kay, and J. D. Forman-Kay, Analysis of deuterium relaxation-derived methyl axis order parameters and correlation with local structure, *J. Biomol. NMR* **13,** 181–185 (1999).