A DQ/ZQ NMR Experiment for the Determination of the Signs of Small $J(^1\mathrm{H},\ ^{13}\mathrm{C})$ Coupling Constants in Linear Spin Systems

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Unlike most other NMR experiments for the measurement of scalar coupling constants, E.COSY-type NMR experiments offer the additional advantage of containing information on the signs of the coupling constants (*1–8*). The experiments require at least three spins, A, M, and X. The couplings J_{AM} and J_{MX} are measured from the A–X cross peak recorded in an experiment where the third spin M is left undisturbed. The relative signs of the J_{AM} and J_{MX} couplings are encoded in the tilt of the cross-peak multiplet pattern. The absolute signs of the scalar couplings can be determined by relating them, e.g., through a series of E.COSY-type experiments (*9*), to the sign of a large one-bond $^1\mathrm{H}$–$^{13}\mathrm{C}$ coupling which is always positive (*10, 11*). Since heteronuclear three-bond coupling constants can assume positive as well as negative values (*10–12*), the sign information is necessary for accurate dihedral angle determinations based on Karplus curves.

Sign information is also contained in the recently developed double-quantum/zero-quantum (DQ/ZQ) experiment (*13*). The original work showed that the DQ/ZQ scheme offers improved accuracy in the measurement of scalar coupling constants in macromolecules (*13*). The present Communication shows that, unlike E.COSY experiments, DQ/ZQ experiments also yield the relative signs of the couplings J_{AM} and J_{MX} in linear spin systems A–M–X, where $J_{\mathrm{AX}} = 0$. The principle is demonstrated with the determination of the sign and magnitude of the three-bond $^1\mathrm{H}_1$–$^{13}\mathrm{C}_{\mathrm{II}}$ coupling across the glycosidic bond in sucrose at natural isotopic abundance (Fig. 1).

The DQ/ZQ-HC experiment of Fig. 2 is designed for the generation of heteronuclear mixed DQ/ZQ coherence between the spins H_2 and C_{II} during the evolution time t_1, where the DQ and ZQ coherences evolve with the sum and difference of the couplings to the common coupling partner H_1. Using the product-operator notation of Sørensen *et al.* (*14*) and neglecting signs, trigonometric factors, and normalization constants, the coherence between H_2 and C_{II} is generated by the following coherence transfer pathway: H_{1z}—$[90°(^1\mathrm{H})]$—H_{1y}—$[\Delta/2$—$180°(^1\mathrm{H},\ ^{13}\mathrm{C})$—$\Delta/2]$—$\mathrm{H}_{1y}\mathrm{H}_{2z}\mathrm{C}_{\mathrm{II}z}$—$[90°(^1\mathrm{H},\ ^{13}\mathrm{C})]$—$\mathrm{H}_{1z}\mathrm{H}_{2y}\mathrm{C}_{\mathrm{II}y}$. The term $\mathrm{H}_{1z}\mathrm{H}_{2y}\mathrm{C}_{\mathrm{II}y}$ represents the desired mixed DQ/ZQ coherence which evolves during t_1 with passive couplings to H_1. The subsequent $90°(^1\mathrm{H},\ ^{13}\mathrm{C})$ pulses convert it back to $\mathrm{H}_{1y}\mathrm{H}_{2z}\mathrm{C}_{\mathrm{II}z}$. This coherence is detected during t_2 at the frequency of H_1 with a multiplet fine structure which is doubly anti-phase with respect to $J(\mathrm{H}_1,\ \mathrm{H}_2)$ and $J(\mathrm{H}_1,\ \mathrm{C}_{\mathrm{II}})$. If Δ does not perfectly match $1/[2J(\mathrm{H}_1,\ \mathrm{H}_2)]$, the mixed DQ/ZQ coherence term $\mathrm{H}_{1x}\mathrm{C}_{\mathrm{II}y}$ is present during t_1, too. This term cannot be removed by phase cycling, but it would generate cross peaks at F_1 frequencies different from those of the desired cross peaks.
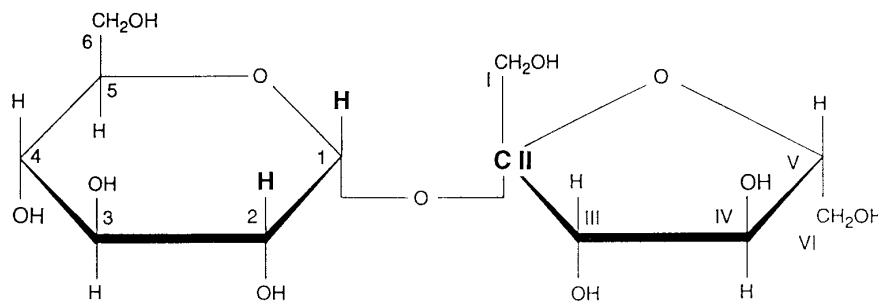

FIG. 1. Atom numbering in sucrose. The atoms involved in the linear spin system H_2–H_1–C_{II} are labeled in bold. Sign and magnitude of the coupling constant $J(\mathrm{H}_1,\ \mathrm{C}_{\mathrm{II}})$ are measured by the experiment of Fig. 2.

To suppress t_1 noise from incompletely suppressed ^{1}H resonances of ^{12}C-bound protons, pulsed field gradients were implemented in the pulse sequence as shown in Fig. 2. The selective $180°(^1H)$ pulse was applied to the H_3 resonance in the middle of t_1 with a rectangular shape. Its duration was adjusted for zero excitation at the frequency of the H_2 resonance ($\delta_{H3} - \delta_{H2} = 0.2$ ppm), and it also does not excite the H_1 resonance ($\delta_{H3} - \delta_{H1} = 1.8$ ppm). Although not required for the experiment, this $180°$ pulse refocuses the large H_2–H_3 coupling, simplifying the multiplet fine structure in the F_1 dimension. Similarly, the selective $180°(^{13}C)$ pulse simplifies the experiment by selecting the correlations with the ^{13}C resonance of C_{II}. If applied as a nonselective pulse, correlations between H_1 and other ^{13}C nuclei would also be observed, requiring a larger spectral width in the F_1 dimension.

Figure 3 shows how the DQ cross peak is split in the F_1 dimension by the sum of the couplings $J(H_1, H_2)$ and $J(H_1, C_{II})$, while the difference between the two couplings does not lead to a resolved multiplet splitting in the ZQ cross peak, mostly because the cross peaks are broadened in the F_1 dimension by unresolved couplings between C_{II} and protons of the fructose ring (Fig. 1). $J(H_1, H_2)$ is readily resolved in a 1D ^{1}H NMR spectrum, so that the coupling $J(H_1, C_{II})$ can be determined accurately from the F_1 multiplet fine structure of the DQ cross peak alone, even if it were significantly smaller than $J(H_1, H_2)$. The
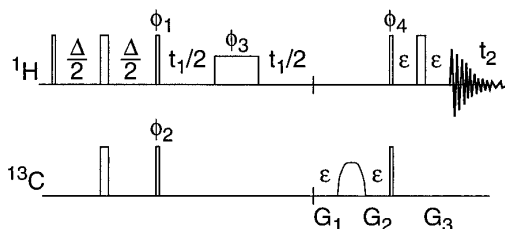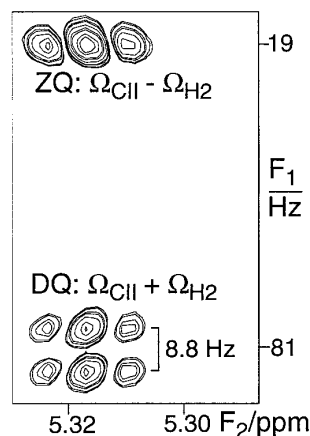

FIG. 3. Spectral region of the DQ/ZQ-HC spectrum of a 200 mM solution of sucrose in D_2O showing DQ and ZQ correlations with the anomeric proton H_1. The multiplet of the DQ peak is split in the F_1 dimension by $J(H_1, H_2) + J(H_1, C_{II})$. The corresponding splitting of the ZQ peak by $J(H_1, H_2) - J(H_1, C_{II})$ is not resolved. $J(H_1, H_2)$ was 3.9 Hz as measured from the 1D ^{1}H NMR spectrum. Consequently, $J(H_1, C_{II})$ is 4.9 Hz and the sign of the coupling constant is the same as that of $J(H_2, H_1)$. Experimental parameters: $\Delta = 124$ ms, $\varepsilon = 1.4$ ms, $|G_1| = 5$ G/cm, ^{1}H carrier 31 Hz upfield from the H_2 signal [i.e., $\Omega_{H2}/(2\pi) = 31$ Hz], ^{13}C carrier 50 Hz upfield from the C_{II} signal [i.e., $\Omega_{CII}/(2\pi) = 50$ Hz], $t_{1max} = 320$ ms, $t_{2max} = 413$ ms, spectral widths in F_1 (F_2) = 200 Hz (2480 Hz), 20°C, total experimental time 1 h, 600 MHz ^{1}H frequency, Bruker DMX 600 NMR spectrometer. The shape of each pulsed field gradient was represented by a histogram of 200 points with a sine-shaped rise over 32 points followed by a plateau of 136 points and a sine-shaped decay of 32 points (*18*). The selective $180°(^{13}C)$ pulse was a 40 ms hyperbolic secant pulse (*19*) applied to the C_{II} signal. The selective $180°(^1H)$ pulse was a 7.2 ms rectangular pulse applied to the H_3 signal, with zero excitation at the H_1 and H_2 resonances. For simplicity, a single spectrum was recorded containing both the DQ and ZQ peaks. Positive and negative levels were plotted without distinction.

FIG. 2. Pulse sequence of the DQ/ZQ-HC experiment for the determination of the long-range $J(^1H, ^{13}C)$ coupling constant in a linear spin system $^1H_2$–$^1H_1$–^{13}C. The experiment determines the absolute sign of the $^1H_1$–^{13}C coupling by relating its sign to the sign of the $^1H_1$–$^1H_2$ coupling. Nonselective 90° and 180° pulses are indicated by narrow and wide bars, respectively. Pulses of lower amplitude denote selective 180° pulses. The selective $180°(^{13}C)$ pulse serves to select the ^{13}C resonance of interest so that a narrow spectral width can be used in the F_1 dimension. The selective $180°(^1H)$ pulse is applied to spins other than H_1 and H_2 to refocus undesired ^1H–^1H couplings. All pulses are applied with the phase x unless indicated otherwise. Phase cycle: $\phi_1 = 4(x, x, -x, -x)$; $\phi_2 = 8(x, -x)$; $\phi_3 = 8(x), 8(-x)$; $\phi_4 = 2[4(x), 4(-x)]$; receiver = $8(x, -x)$. G_1, G_2, and G_3 denote pulsed field gradients of relative intensity 1:−1:0.503 and −1:1:0.503 for N- and P-type signal selection, respectively. Phase-sensitive data are obtained by Fourier transformation in the echo–antiecho mode (*17*). DQ and ZQ coherences can be separated into different subspectra by repeating the experiment with a 90° phase shift for all ^{1}H and ^{13}C pulses before t_1 and taking the sum and the difference between the two data sets, respectively (*13*). $\Delta = 1/[2J(H_1, H_2)]$, $\varepsilon = $ duration of a pulsed field gradient including field recovery delay.

larger splitting of the DQ than of the ZQ peak shows that the couplings $J(H_1, H_2)$ and $J(H_1, C_{II})$ have the same sign. Vicinal ^1H–^1H couplings larger than 3 Hz are invariably positive (*15*); hence $J(H_1, C_{II})$ is also positive. The most frequently used parametrization of the Karplus curve for $^3J_{HC}$ coupling constants in C–O–C–H moieties was based on the assumption of exclusively positive coupling constant values (*16*). The DQ/ZQ HC experiment makes it possible to verify this assumption. Furthermore, the experiment may be helpful in distinguishing $^2J_{HC}$ from $^3J_{HC}$ couplings in organic molecules based on the fact that these couplings are in most cases negative and positive, respectively (*10, 11*).

ACKNOWLEDGMENTS

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