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Preliminary communication

Resolution of complex, proton-n.m.r. spectra of carbohydrate derivatives by using "tilted", two-dimensional *J* spectra

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We have previously demonstrated[1–3] some of the diagnostic potential, in organic chemistry, of proton two-dimensional (2D) n.m.r. spectroscopy, using displays derived directly from the frequency-domain data-matrix obtained by double Fourier-transformation of spin-echo, time-domain data. As is clear from the studies of Ernst and coworkers[4–6], the interpretation of 2D *J* spectra is simplified if the 2D spectrum is first tilted through 45° in frequency space, and we have now written a computer routine to effect this "tilt". Its use is illustrated here with results for uridine (**1**) and α,β-D-xylopyranose (**2**), both in D_2O solution.

The fundamental, proton, 2D *J* spectroscopy experiment has been thoroughly described elsewhere[1–9]; it yields a spectrum in two frequency-dimensions f_1 and f_2, signal dispersion in f_2 reflecting the normal, proton spectrum, and, in f_1, multiplet structure only. As a result, multiplets appear on diagonals with $df_2/df_1 = 1$. If this 2D spectrum is "tilted" 45° in frequency space by the transformation $S(f_1,f_2') = S(f_1,f_1+f_2)$, a 2D spectrum is produced in which chemical shifts and multiplet structure are completely separated into orthogonal-frequency dimensions, for weakly coupled spin-systems, so that the multiplet structure for each chemically shifted resonance appears on a separate trace across the f_1 dimension of the 2D spectrum. Strongly coupled spin-systems (such as H-3,4,5*a* and 5*e* of the α anomer of **2**) show complex patterns in 2D *J* spectra, and require numerical analysis[8,9].

Consider first the 2D *J* spectrum of the H-1′ and H-5 resonances of uridine (**1**), shown in Fig. 1A. The projection[6] of this spectrum onto the f_2 axis gives the normal spectrum, showing the overlap of the two doublets. Because of the proximity of these two doublets, a projection of the 2D spectrum onto the f_1 axis yields a partial *J* spectrum in which the two multiplets appear superimposed. Now, consider the projections of the 2D *J* spectrum of Fig. 1B, which was obtained by tilting the original data-matrix by 45° in frequency space, using the transformation already cited. Although the projection onto the f_1 axis is identical to that in Fig. 1A, the projection onto the f_2' axis now gives a singlet for

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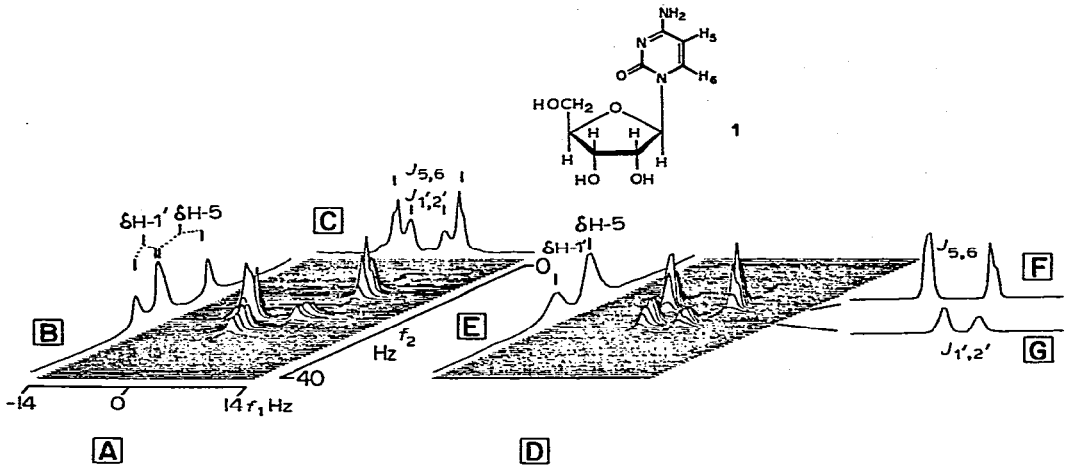

Fig. 1. A, the proton 2D J spectrum of the H-1′ and H-5 resonances of uridine (1), 0.3M in D_2O, obtained by double Fourier-transformation of the time-domain data-matrix; projection onto the f_2 axis (B) gives the equivalent of the normal, one-dimensional spectrum. The partial J spectrum C, obtained by taking the projection onto the f_1 axis, shows the two overlapping multiplets. D shows a 2D spectrum from the same data-matrix as A, after "tilting" the frequency-domain data through 45° in frequency space. Multiplet structure is now restricted to f_1, and f_2' displays chemical shifts only. Thus, a projection onto f_2' (E) now yields two singlets, at the chemical shifts of the two protons, and cross-sections through D at these shifts yield the two, separated, partial J spectra F and G.

each proton at its chemical shift, as the multiplet components now lie parallel to the f_1 axis. Cross-sections[6] through the 2D spectrum at the two chemical shifts now give the two partial J spectra shown, in which the two doublets are completely separated.

The advantages of the 45°-tilt operation become more apparent in complex spectra, such as that of α,β-D-xylopyranose (2). Most of the normal spectrum (see Fig. 2A) is well resolved and can be assigned normally, leaving the region between δ 3.3 and 3.5 which contains the overlapping resonances of five protons. The trace in Fig. 2B shows the "proton-decoupled, proton spectrum" (refs. 5 and 6) obtained by projection of the tilted, 2D spectrum onto the f_2' axis. The cross-section at the chemical shift of H-4β gives the partial J spectrum (inset) for this proton, from which all of the vicinal couplings can be measured.

Spectra were measured at room temperature on a home-built spectrometer operating at 270 MHz for protons, controlled by a Nicolet 1180/293A data system. Typical, data-acquisition times for 0.3M solutions were on the order of 30–60 min, using the Nicolet NTCFT control program. The computer routine for performing the 45° tilt was written in 1180 BASIC; copies are available from the authors on request.

The experimental methods outlined offer a considerable increase in resolving power over conventional, proton n.m.r. techniques, increasing the available information content of proton spectra, and allowing the analysis of the spectra of molecules larger than those for which it is normally possible. The inclusion of a 45° tilt in the processing of 2D J spectra is an important step in their analysis, particularly for complex spectra containing overlapping multiplets, and is in routine use in our studies of di- and oligo-saccharides.

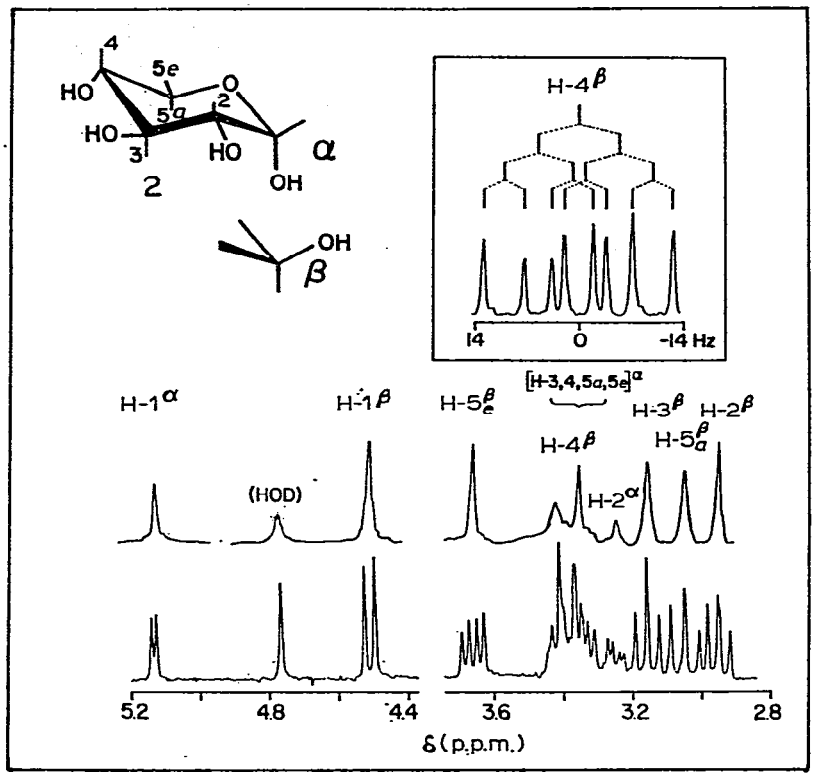

Fig. 2. The lower traces show the normal, 270-MHz, proton spectrum of α,β-D-xylopyranose (2; 0.3M in D_2O). The upper traces show the projection onto the f_2' axis of the tilted, 2D J spectrum of 2, and the inset shows the partial J spectrum for H-4β, obtained by taking a cross-section through the 2D J spectrum at f_2 = 3.35 p.p.m.

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