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Preliminary communication

Nulling of the residual-solvent resonance during proton two-dimensional *J* n.m.r. experiments: uridine in water

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Although many important applications of proton, two-dimensional *J* (2-d *J*) spectroscopy[1–4] may require studies to be made in rather dilute solutions, often in aqueous media, we are not aware of any description of the serious problems that are frequently associated with the intense resonance of the residual-solvent protons in a 2-d *J* spectrum. These spectra are conveniently displayed in the absolute mode in order to eliminate complications arising from the "phase-twist" effect[3]. Unfortunately, this introduces a line-broadening effect, and the resulting "wings" associated with any intense resonance can extend over a considerable range of frequency, thereby distorting a substantial region of the 2-d *J* spectrum (see Fig. 1A). To eliminate this problem, we demonstrate here a method that is, for example, well suited for studies of sugars in aqueous media.

The protons of uridine (**1**) in an 0.3M solution in D_2O have a shorter spin–lattice relaxation-time (<2.5 s) than the residual proton (HOD) in the solvent (~ 9.5 s); hence, the HOD signal can be minimized prior to the 2-d *J* pulse-sequence by a conventional, non-selective, inversion–recovery pulse-sequence[5–7]:

$$[180^\circ - \tau_0 - 90^\circ - \tau - 180^\circ - \tau - \text{acquisition}],$$

where τ_0 is set equal to the time required for the HOD resonance to reach its null-point; during this period, the more rapidly relaxing protons of uridine return to thermal equilibrium with the lattice, and can now be studied by the 2-d *J* pulse-sequence. The effectiveness of this approach is clearly discernible in Fig. 1B, where it may be seen that the distortion from the HOD signal has been entirely eliminated.

The impact of this procedure on the partial *J* spectra is demonstrated in Fig. 2; the traces were obtained by projecting sections of a 2-d *J* spectrum onto the f_1-axis. Traces in Fig. 2A show a significant signal at zero frequency due to the residual solvent, even though its resonance is ~270 Hz to low field of H-$5'_B$ (see Fig. 3A); for such weak signals as the H-4′ multiplet, which also shows signals near zero frequency in the f_1-dimension, the wing of the HOD signal completely distorts the partial *J* spectrum (see Fig. 2A). In contrast, the equivalent traces obtained by the solvent-nulling procedure show no zero-frequency component due to the solvent.

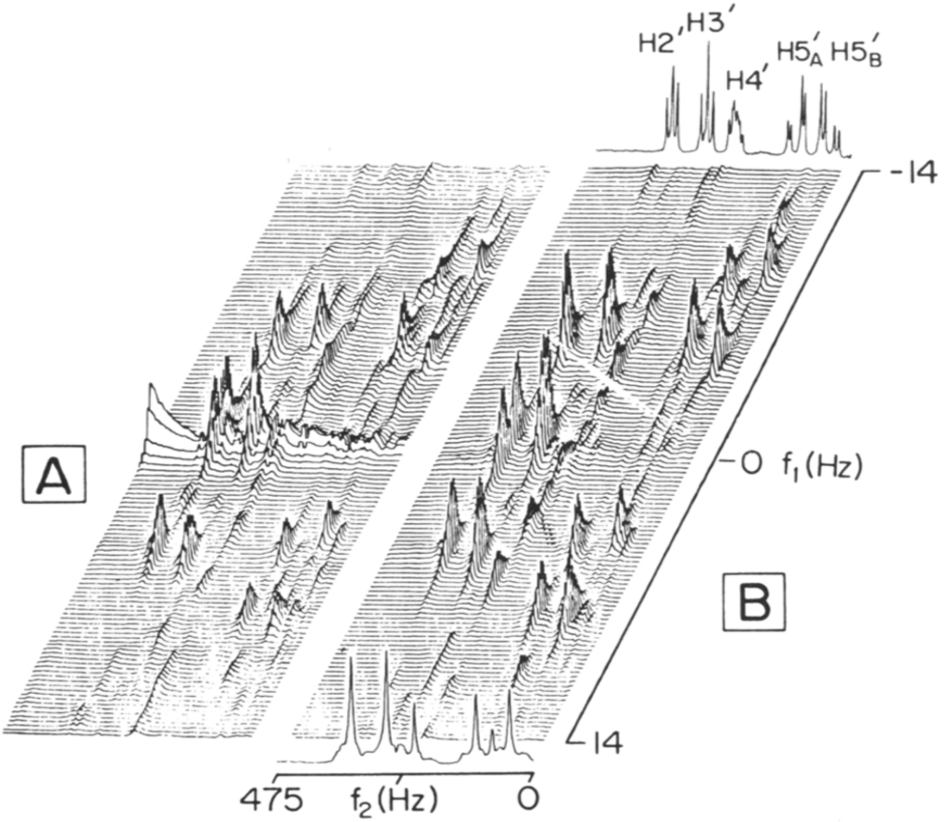

Fig. 1. (A) Proton, 2-d *J* spectrum of the high-field region of uridine (**1**) in D_2O (0.3M), showing the distortion at zero frequency in the f_1-dimension due to the "wing" of the HOD resonance. (B) The same spectral region, from an experiment in which the solvent resonance was eliminated by an inversion–recovery sequence prior to the 2-d *J* pulse-sequence. (The top and bottom traces are the corresponding regions from the conventional spectrum and the 45° skew projections, respectively. Both spectra were plotted by using the power mode, with exponential multiplication of the time-domain signals t_1 and t_2.)

Interestingly, the H-2′ resonance, which, in a normal spectrum (see Fig. 3A), appears as a triplet (4.95 Hz), is clearly resolved as doublets of a doublet ($J_{1,2}$ = 4.51 Hz; $J_{2,3}$ = 5.64 Hz) in a 2-d *J* spectrum; this illustrates the substantial resolution enhancement due to the elimination of magnetic-field inhomogeneity-effects by the technique. Another advantage is demonstrated in the insert in Fig. 3, where chemical shifts and coupling constants of the overlapping H-1′ and H-5 resonances can be measured from the 45°, skew projection[2] and partial *J* spectra, respectively.

One serious limitation of the 2-d *J* technique involves the complex responses given by strongly coupled systems[8], which may show additional lines, some with negative intensities, and others folded over in the f_1-dimension (see the H-4′ and H-5′$_A$, 5′$_B$ signals in Figs. 1B and 2B). However, these spectra can be analyzed by computer stimulation[9,10].

Such dynamic-range problems as the one discussed here may also be minimized to

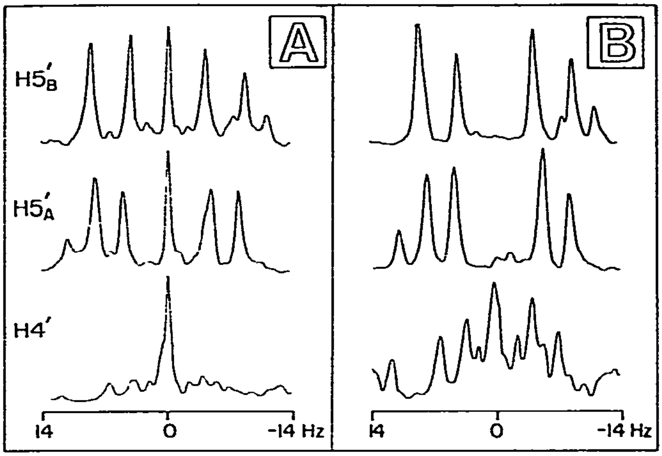

Fig. 2. (A) Partial *J* spectra of H-4′, H-5$'_{\mathrm{A}}$, and H-5$'_{\mathrm{B}}$, obtained by projecting sections of the 2-d *J* spectrum (Fig. 1A) onto the f_1-axis, showing, at zero frequency, the signal from the "wing" of the HOD signal: note the effect of the solvent peak on the H-4′ signals. (B) The corresponding regions obtained from Fig. 1B; each of these partial *J* spectra matches the spectrum simulated by using a computer program[10].

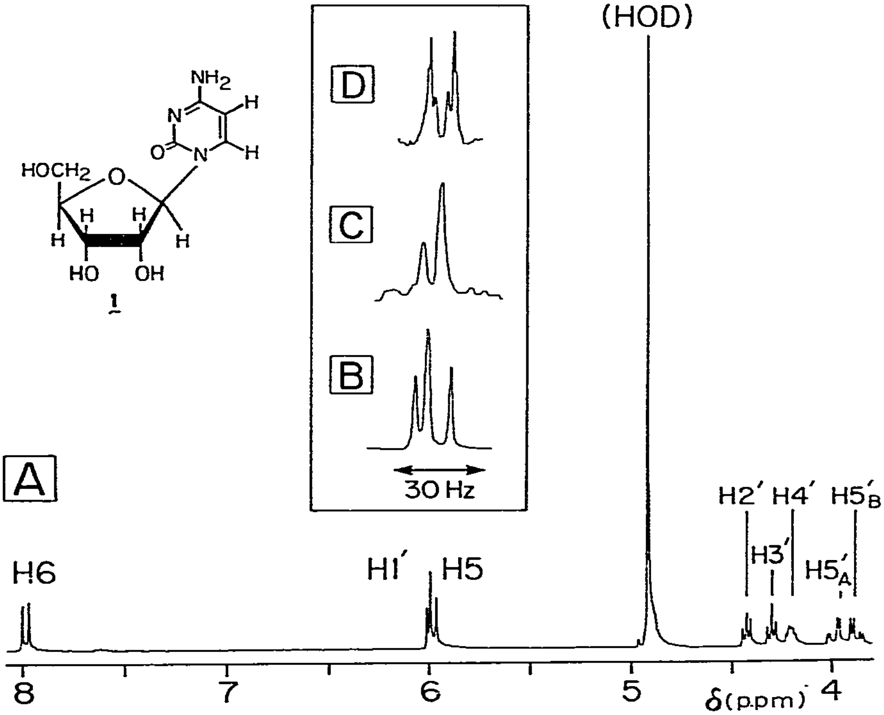

Fig. 3. (A) The conventional (270-MHz), proton spectrum of uridine (1) in D_2O (0.3M), and (B) the overlapping H-1′ and H-5 resonances. The 45° skew-projection (C) gives the chemical shifts of these two protons,and the projection onto the f_1-axis (D) is the partial *J* spectrum of the H-2′, H-5 region with the chemical shifts suppressed, and gives the two coupling constants; signals from both protons are centered about zero frequency.

a certain extent by displaying the power-mode spectrum[11], or by multiplying the time-domain signals, for example, by Gaussian[3] or sine functions[12], to diminish the "wings"; however, these procedures have their limitations[11,12], and the latter routine, for example, shows line-shape and intensity distortions in overlapping multiplets. Other techniques, such as selective irradiation[13,14] prior to a 2-d *J* pulse-sequence, may also be suitable for overcoming these problems; the optimum choice between alternatives depends on the chemistry of the system. It is important to note that some intense resonances of the molecule itself (for example, methyl signals from methoxy or acetoxy derivatives) can produce "wings" of sufficient intensity to prejudice interpretation of a proton, 2-d *J* spectrum unless care is exercised.

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