Two-dimensional *J* Spectroscopy: ^{1}H N.M.R. Spectra of Mono- and Di-saccharides

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Summary ^{1}H N.m.r. spectra (270 MHz) of trideuteriomethyl 2,3,4,6-tetrakis-*O*-trideuterioacetyl-α-D-glucopyranoside (**1**) and 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-glycopyranosyl 3,4-di-*O*-acetyl-1,6-dideoxy-β-D-fructofuranoside (**2**) have been measured using the two-dimensional *J* technique; various integral projections of the complete two-dimensional spectra are presented and discussed.

ONE of the simplest two-dimensional n.m.r. experiments[1,2] is homonuclear two-dimensional *J* (2-D *J*) spectroscopy,[3] which may be used to separate the effects of chemical shifts and scalar coupling in, for example, complex proton spectra. We present here the results of such experiments on two carbohydrate derivatives (**1**) and (**2**),† in order to illustrate some of the advantages and limitations of 2-D *J* spectroscopy applied to organic systems. A 2-D *J* experiment requiring only a few hours of machine time can produce a spectrum in which both resolution and information content are significantly improved over a conventional Fourier transform spectrum. For weakly coupled systems this 2-D *J* spectrum can be processed to provide a one-dimensional spectrum in which all proton–proton scalar couplings have been suppressed.[1c]

Homonuclear 2-D *J* experiments may be performed routinely using the Carr–Purcell method A pulse sequence:[4] [PD–90°–$n\tau$–180°–$n\tau$–Acquisition]$_{\text{NA}}$.‡

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† (**1**) Trideuteriomethyl 2,3,4,6-tetrakis-*O*-trideuterioacetyl-α-D-glucopyranoside; (**2**) 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-glucopyranosyl 3,4,di-*O*-acetyl-1,6-dideoxy-β-D-fructofuranoside.

‡ PD = Pulse delay; NA = Number of acquisitions; $n = 0, 1, 2, \cdots (N - 1)$.

Since the details have been described elsewhere[1,2] it suffices here to comment that the signal $S(t_1t_2)$ measured after the refocusing period is a function of the duration both of the detection period (t_2 ≡ acquisition time) and of the evolution period $t_1 = 2n\tau$. N Free induction decays each with M data points are acquired with a constant increment 2τ in t_1, and the data are stored as an N by M matrix[2c] of time-domain free induction decay signals $S(t_1t_2)$. These signals are then Fourier transformed with respect to t_2 and transposed to give a set of 'interferograms' $S(f_2t_1)$, which are then Fourier transformed with respect to t_1 to give, after a further transposition, the two-dimensional frequency domain spectrum $S(f_1f_2)$ for final presentation.

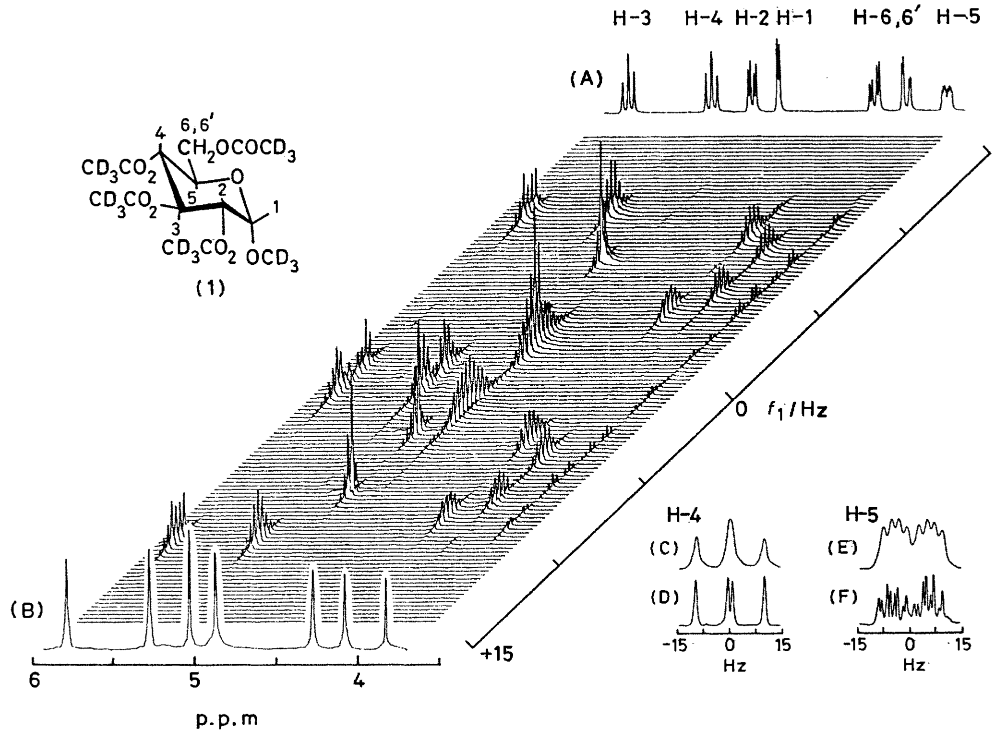

Figure 1. (A) The normal 270 MHz proton spectrum of (**1**) in C_6D_6 solution (0·1 M), which is equivalent to the projection of the 2-D J spectrum on to the f_2-axis. The data were obtained using the following spectrometer settings: sweep width = 1250 Hz; acquisition time = 0·82 s; NA = 4; N = 128; M = 4096; τ = 17·86 ms. (B) A skew projection showing the proton-decoupled proton spectrum. (C) and (E) show respectively the expansions of the H-4 and H-5 resonances of (**1**) obtained from a conventional Fourier transform spectrum. (D) and (F) show the projections on to the f_1-axis of slices taken from the 2-D J spectrum parallel to the f_1-axis; no additional resolution enhancement was used. Note in (D), H-4 shows $J_{3a,4a}$ = 9·38 and $J_{4a,5a}$ = 10·70 Hz, and that in (E) a long-range coupling of 0·91 Hz is resolved. All spectra were run without field-frequency lock.

Although the display of a complete 2-D J spectrum in the form of a stacked plot, such as Figure 1, often reveals interesting new information (in this case that both H-3 and H-4 are doublets of doublets, rather than triplets), considering the time required (*ca.* 2 h in this instance) for plotting the N traces of the 2-D J spectrum, more useful and rapid displays can be obtained by making integral projections§ of the 2-D J spectrum on to various axes. Some of these have limitations; thus the projection on to the f_2-axis gives the equivalent of a normal spectrum (Figure 1,A) which may be somewhat degraded by poor digital resolution and by artifacts[5] generated by instrumental imperfections and the projection of the complete 2-D J spectrum on to the f_1-axis is rather complex because all the resonances are centred about the same frequency-zero and hence are superimposed; this is the J-spectrum.

Novel information concerning the proton chemical shifts can be obtained by making a skew projection of the 2-D J spectrum on to an axis such that $\delta f_2/\delta f_1 = -1$.¶ Viewed from that angle, all the signals of each resonance are aligned directly behind each other and the projection has the appearance of a proton-decoupled proton spectrum (see Figure 1,B). However, care should be exercised in interpreting such spectra because complex projections are obtained from second-order spectra (see the 3F—4F region of Figure 2,B); if any ambiguity should arise it is prudent to simulate the spectrum using an iterative computer program.[6]

Homonuclear spin coupling constants are most accurately obtained by making projections on to the f_1-axis of sections (slices) taken from the complete 2-D J spectrum parallel to

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§ Digital summation of the traces that lie perpendicular to that axis.

¶ If the complete 2-D J spectrum were plotted with the same frequency scale for both f_1- and f_2-axes this axis would be set at 45° to the f_2 axis.

the f_1-axis; typical examples of such partial J-spectra are given in Figure 1,D and F. The enhancement in the resolution of these spectra, compared with that of the normal Fourier transform spectra, is due to the refocusing of the magnetic field inhomogeneity effects at the end of the evolution period. Besides resulting in a more accurate determination of spin coupling constants (compare Figure 1C, and D), partial J-spectra can also reveal smaller couplings not normally visible in conventional spectra (Figure 1,E and F).

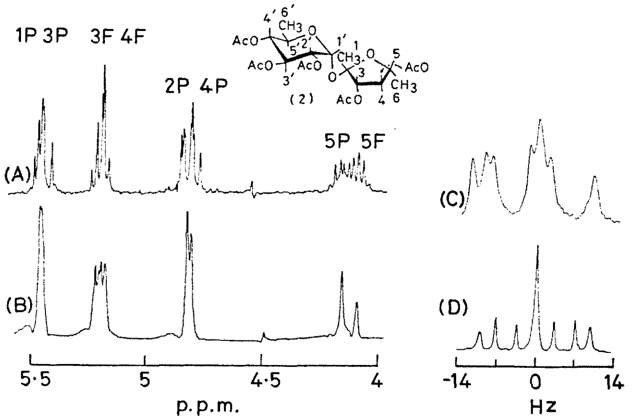

FIGURE 2. Partial proton spectra of (**2**) in $CDCl_3$ solution (0·1 M). (A) shows the normal Fourier transform spectrum and (B) the skew projection of the same region taken from the 2-D J spectrum. The suffix 'P' refers to resonances of the pyranose unit and 'F' to those of the fructofuranose unit. (C) shows an expansion of the 2P—4P region taken from the normal spectrum and (D) shows the equivalent J-spectrum.

If several resonances have similar chemical shifts then their signals will appear in the same partial J-spectrum and, because all the responses are symmetrically disposed about the frequency-zero, it is often possible to assign the signals and hence obtain the coupling constants by inter-comparison with a normal spectrum; in the case of the resonances shown in Figure 2,D, confirmatory evidence was obtained from the differences in the line-widths of the 2P and 4P resonances.

In this study the complications associated with the 'phase twist' effect[2c] were overcome by displaying the spectra in the 'absolute square' (power) mode;[7] this has the advantage over the 'absolute value' mode in that it minimises the 'tail' associated with each line. However, it is important to note that the relative intensities of all signals are the square of their normal values; thus a 1:2:1 triplet now appears as a 1:4:1 triplet (Figure 2,D).

It seems highly probable that the n.m.r. experiments discussed here will find widespread application in structural studies of organic substances. Insofar that the methods are best suited to pseudo first-order spectra it is obviously advantageous to operate at high magnetic fields.

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