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Preliminary communication

Assignment of proton n.m.r. spectra of carbohydrates, using two-dimensional techniques: COSY and SECSY

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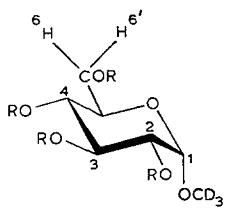
Previous reports have demonstrated[1,2] that proton two-dimensional (2-D) *J*-spectroscopy can provide unprecedented dispersion of the ^{1}H-n.m.r. spectra of carbohydrates; unfortunately, that method leaves unsolved the assignment of the individual resonances. This is a particularly awkward problem for those carbohydrates that have numerous overlapping resonances within a narrow range of chemical shifts, and couplings of similar magnitudes; as a result, matching of spectral splittings is ambiguous, and spin-decoupling restricted[3]. We now confirm the utility of two related variants of the original, Jeener 2D-spin echo experiment[4] for making assignments, namely, "2D correlation spectroscopy" (COSY)[5–7] and "spin-echo correlated spectroscopy" (SECSY)[8]. In order to illustrate the two kinds of experiment, we have chosen trideuteriomethyl 2,3,4,6-tetra-*O*-(trideuterioacetyl)-α-D-glucopyranoside (**1**) as a simple exemplar.



1 R = $OCOCD_3$

Both experiments involve a non-selective, two-pulse sequence (see Fig. 1). After a relaxation delay (RD), the first (*preparatory*) pulse is applied. An evolution period follows. In the COSY experiment[5–7], the *mixing* pulse is applied at the end of the evolution period, and this is immediately followed by detection (during t_2). With the SECSY experiment[8,9], the mixing pulse is applied half-way through the evolution period.

In both experiments, t_1 is systematically incremented in n small steps (Δt_1) over a total time-range ($n\Delta t_1$) which is of the order of the spin–spin relaxation-time. In this way, a single data-matrix $s(t_1,t_2)$ is built up; time-averaging is used to develop an adequate signal-intensity.

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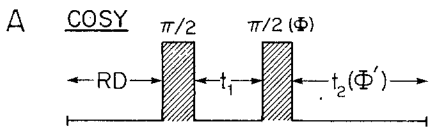
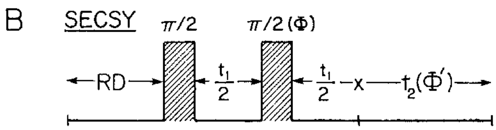


CYCLE	Φ	Φ'
1	0	+
2	90	+
3	180	+
4	270	+



CYCLE	Φ	Φ'
1	0°	+
2	90°	−
3	180°	−
4	270°	+

Fig. 1. The pulse sequence for (A) COSY and (B) SECSY experiments, with the phase-cycling indicated.

In order to detect connectivities involving small coupling constants, large data-matrices are required; typically, 256(n) spectra, each of 1024 size before Fourier transformation. The digital resolution in F_1 is determined by the number of increments used (n), and the effective sweep-width (SW_{F_1}), which is set by the value chosen for Δt_1; that of F_2, by the sweep width (SW) (in the absence of zero-filling) used for each data acquisition. Data manipulation is relatively fast (~1 h for a 256 × 1024 data matrix) with appropriate software, and is straightforward, requiring a Fourier transformation, a data transposition, and a second Fourier transformation which includes conversion into either the absolute value or power-mode. Then, with SECSY, a second transposition is advisable. The final data matrix $S(F_2,F_1)$ or $S(F_1,F_2)$ is best displayed as a contour plot, and, in our hands, we have found that the clearest display is obtained following sine-bell apodization[10] in both dimensions.

COSY. – This experiment is performed by following the modification by Freeman *et al.*[6,7] of the Jeener echo experiment[4,5]. Quadrature detection is employed in both dimensions, to produce a single, symmetrical data-matrix (Figure 2). The mixing pulse is phase-cycled by 90°, with the preparatory pulse and receiver phases held constant. The sweep width in F_1 is typically set equal to that in F_2 by making Δt_1 equal to the F_2 dwell-time*.

Two types of response are produced. Each proton resonance gives a matrix of peaks along the principal diagonal. Each pair of scalar-coupled protons gives a pair of off-diagonal responses that are symmetrically disposed with respect to the chemical shifts of the coupled protons. This connectivity information is readily retrieved by direct inspection, and can be mapped out as illustrated in Fig. 2.

SECSY. – This experiment is based on the procedure of Nagayama and co-workers[8,9], and the four-cycle scheme requires single-phase detection in t_2, and the phase-cycling given in Fig. 1. The F_1-dimension** now represents *differences* between

*$\Delta t_1 = (2 \times \pm SW)^{-1}$.
**$1/2\Delta t_1 = (4 \times \pm SW_{F_1})^{-1}$.

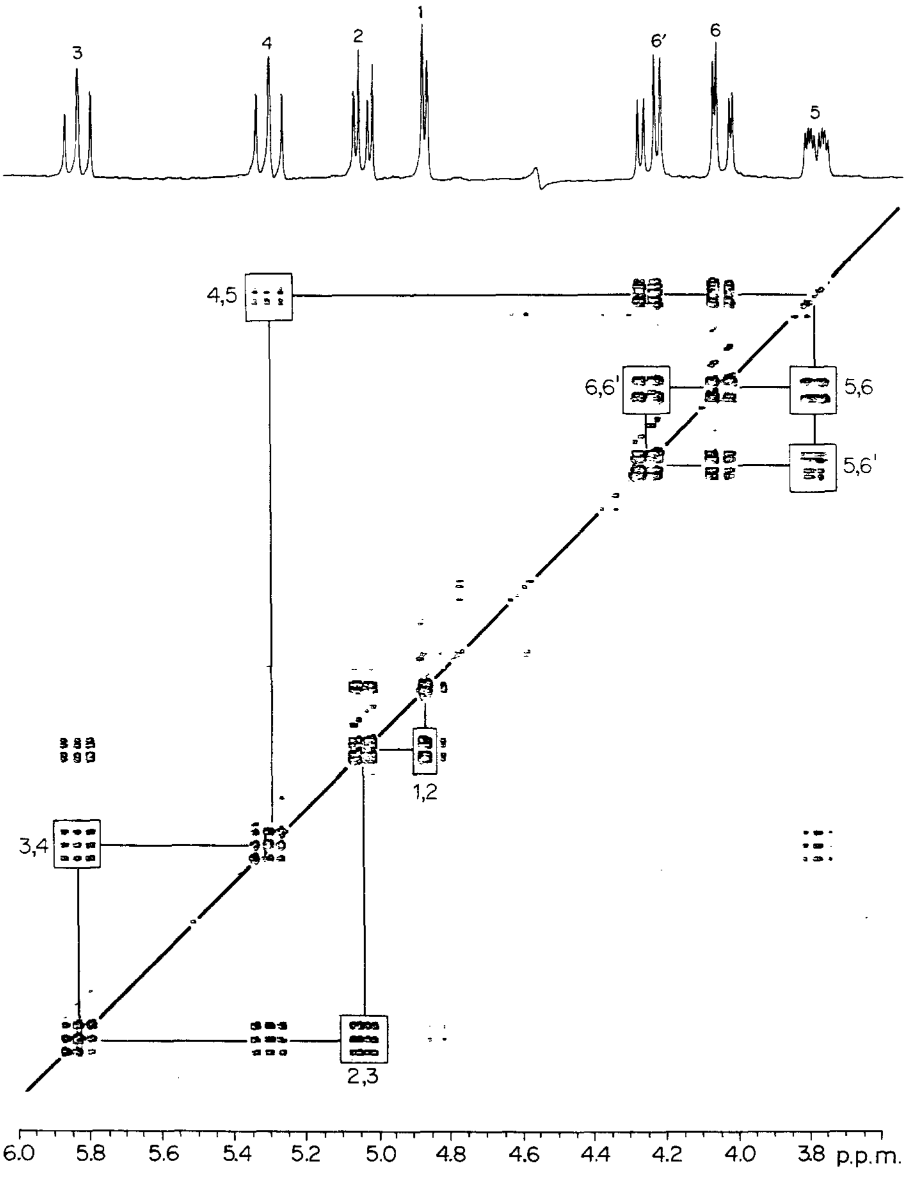


Fig. 2. COSY spectrum of **1** (0.1M, in C_6D_6). $SW_{F_1} = SW_{F_2} = \pm350$ Hz. One hundred and twenty-eight values (n) of t_1, with an incremental delay-time, $\Delta t_1 = 1.42$ ms, were used to build up a 256 × 1024 data matrix. Hence, the "acquisition time" in t_1 was 366 ms. The receiver acquisition-time, t_2, was 732 ms. Four transients were accumulated for each t_1 value; a recycle time of 2.7 s was used. The total accumulation time was ~36 min. The final, digital resolution was 2.7 Hz/pt in F_1 and 1.4 Hz/pt in F_2.

the chemical shifts of the scalar-coupled nuclei. Each proton gives a complex response along the principal axis at $\Delta\delta = 0$, together with one off-axis correlation-response for each resonance with which it shares a scalar coupling. Care should be taken to select SW_{F_1} larger than the largest chemical shift difference between coupled spins.

Information retrieval from this experiment is marginally less convenient than

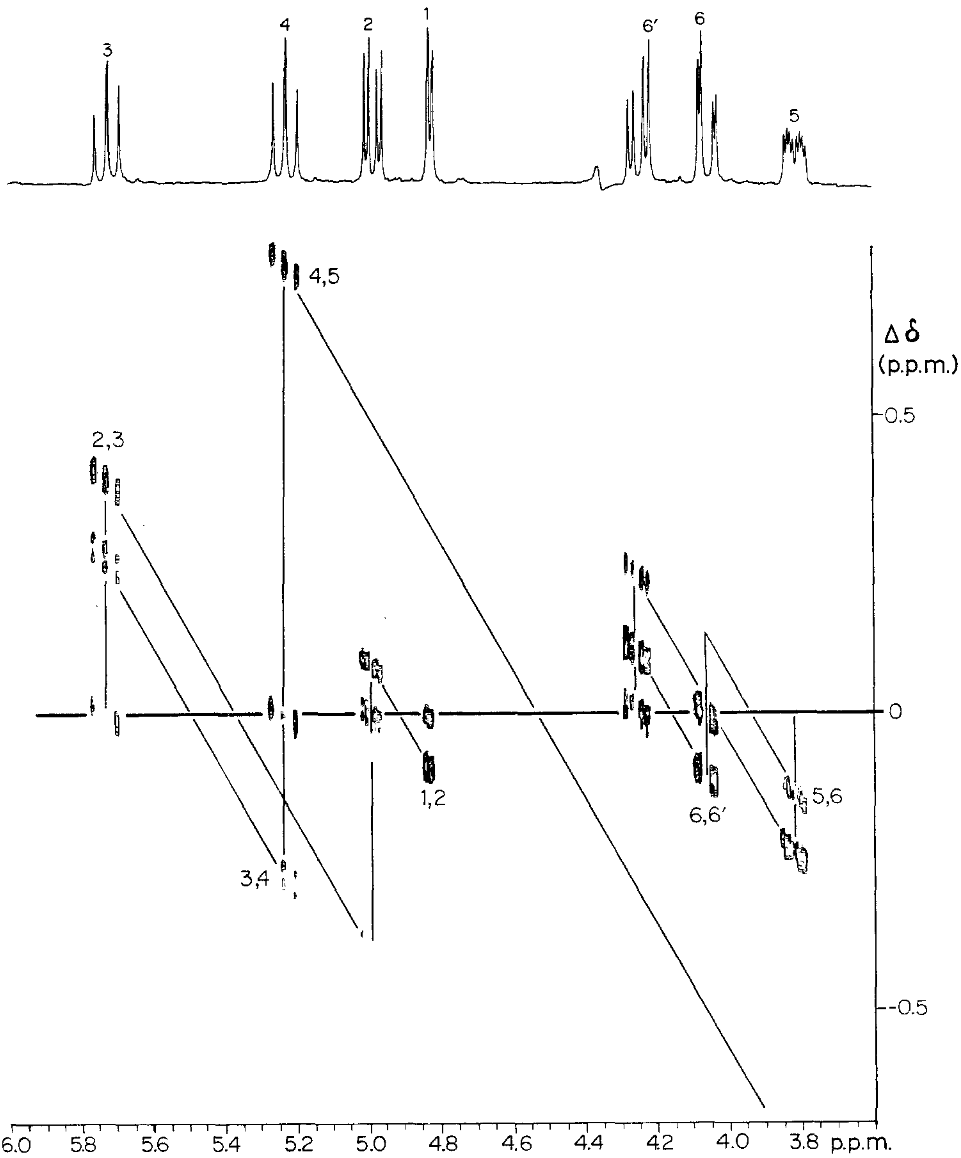


Fig. 3. SECSY spectrum of 1 (0.1M in C_6D_6). SW_{F_2} = 750 Hz and SW_{F_1} = ±0.8 p.p.m. (432 Hz; $1/2\Delta t_1$ = 1.157 ms). The smaller SW_{F_1} allowed a smaller data-matrix to suffice: 128 × 1024. Other instrumental settings were the same as those described for Fig. 2. The total time for the experiment was ~31 min. The final digital-resolution was 3.4 Hz/pt in F_1 and 1.5 Hz/pt in F_2.

for the COSY experiment. Consider, for example, Fig. 3; the H-1 and H-2 resonances each gives a response along the $\Delta\delta = 0$ axis at the appropriate, F_2 value (chemical shift). A single off-axis response is observed for H-1 at $\Delta\delta = 1/2(\delta_{H\text{-}1} - \delta_{H\text{-}2})$. An equivalent response is observed at $\Delta\delta = 1/2(\delta_{H\text{-}2} - \delta_{H\text{-}1})$ for the H-2 resonance; the second off-axis resonance for H-2 corresponds to its scalar coupling with H-3. As depicted in Fig. 3, it is a relatively simple matter to trace the connectivity from one proton to its next neighbor(s).

Having illustrated the potential of these methods, it is appropriate to mention some of the limitations. As with all proton n.m.r. experiments, more-complicated responses are found[5] for spins that are tightly coupled in the J/δ sense. However, accidental degeneracies leading to tight coupling can frequently be entirely eliminated by change of solvent, considering that induced changes can be 0.1–1.0 p.p.m., which, for a superconducting spectrometer, is equivalent to 25–500 Hz. Even for weakly coupled spins, cross-correlation responses located close to the diagonal (or principal axis) are sometimes obscured by the direct responses, which are often rather broad; this is one reason why heavy resolution-enhancement is important. It should also be noted that, occasionally, some intensity asymmetry may be observed, in that one of the pair of correlation responses is below the level of detection as plotted; however, in none of the systems that we have studied so far have *both* of the responses "disappeared".

We consider that both of the experiments described here will greatly facilitate the assignment of complex, proton spectra. Both the data accumulation and the processing are fast [~30 min (0.1M solution), and 1 h, respectively] and contour plots with four intensity levels can be obtained in <10 min. We note that the "symmetrization" techniques[10—12] recently developed should improve effective signal-to-note, and eliminate ambiguities that arise from unequal intensity within pairs of correlation responses; this, and a number of other variants[7,9,13] of the basic COSY experiment that show great promise, already exist.

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