Chemical Shift-correlated Two-dimensional Spin Echo N.M.R. Spectroscopy

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Summary An alternate two-dimensional n m r pulse sequence gives the chemical shifts of directly coupled nuclei

TWO-DIMENSIONAL (2D), homonuclear J-spectroscopy[1–4] has been applied to many complex problems in proton n m r spectroscopy[5] Free induction decays (F I D 's) with $2m$ datum points are accumulated over an acquisition time t_2 for a series of evolution times t_1 in the pulse sequence 90°–$t_1/2$–180°–$t_1/2$ F I D Double Fourier transformation of the signal data matrix $S(t_1,t_2)$ gives rise to a 2D spectrum in which signal intensity is plotted as a function of two frequency axes, f_1 and f_2 There is a J-spectrum, parallel to the f_1 axis, for each of the m points along f_2 The projection of the 2D spectrum on to the f_2 axis gives the conventional 1D spectrum In the limit of weak coupling and perfect 180° pulses, the J-spectrum of any transition in f_2 will contain only a single line

Much more information is gained from a 90°–$t_1/2$–90°–$t_1/2$-F I D pulse sequence In general, the spectrum in f_1 (J-spectrum) of a transition in f_2 will contain many lines, owing to the mixing of that transition with every other transition of the same spin or of a directly coupled spin Furthermore, the frequencies of f_1 can arise from both the sum and difference of f_2 frequencies The chemical shift of any one nucleus coupled to another may be found by inspection of the J-spectra of the second nucleus, even if all the transitions of the first nucleus are hidden in the 1D spectrum This technique is a development of a previous 2D experiment[2,6] in which the second delay time was omitted The second delay time ensures that at least some of the line broadening due to magnetic field inhomogeneities is eliminated Furthermore since the J-spectra start at f_1 = 0 Hz a considerable saving in f_1 digitization can be achieved over the original experiment,[2] as has recently been pointed out[7] The technique may be considered as an F1 alternative to the homonuclear INDOR experiment,[8] or selective decoupling

We report here the results of applying the 90°–$t_1/2$–90°–$t_1/2$-F I D pulse sequence to the AB protons in 5-bromofuroic acid Figure 1 shows the 2D spectrum revealing many lines in f_1 for each transition in f_2 Figure 2(A) shows the J-spectrum of transition 2 [$(\omega_A - J/2)$ in f_2] Figure 2(B) shows a computer simulation of this J-spectrum produced on a 3-spin two-dimensional simulation program,‡ based on the superspin formalism[9]

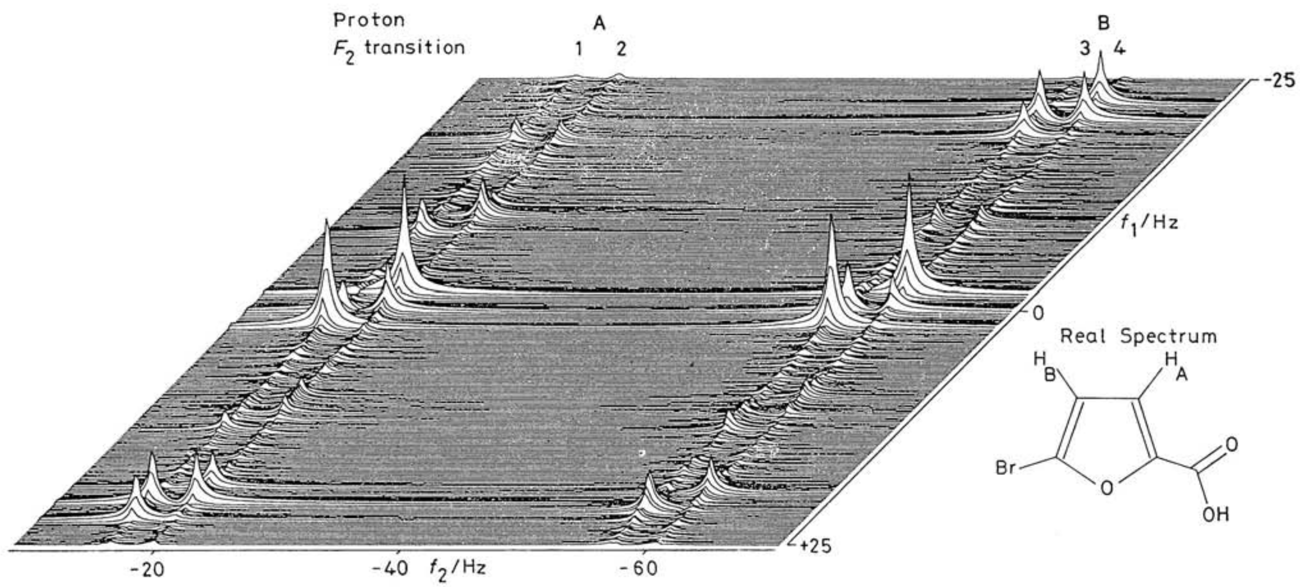

FIGURE 1 The 2D proton n m r spectrum of 5-bromofuroic acid in Me_2SO at 90 MHz F I D 's were accumulated over 2048 datum points with t_1 being incremented by 0 010 s 179 times, from an initial value of 0 001 s sweep width = ±125 Hz delay between pulse sequences 2 s After the first Fourier transform was performed a fixed phase-correction was given to the spectra A magnitude calculation was applied after the second Fourier transformation No line-broadening was used

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‡ A listing of this program is available upon request to Dr A D Bain

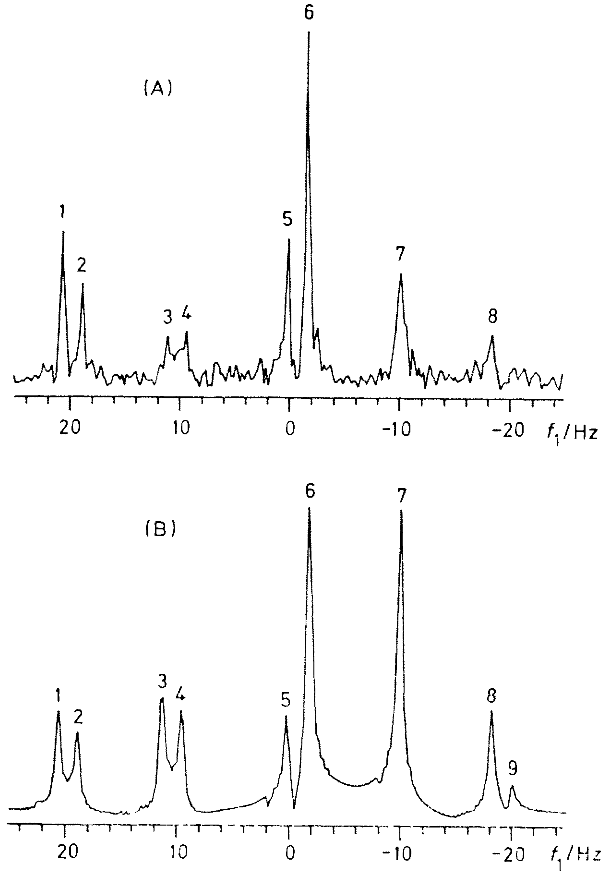

FIGURE 2. (A) The real J-spectrum of transition 2; (B) the simulated J-spectrum of transition number 2. The line intensities depend strongly on the flip angle of the refocussing pulse (120° pulses were used in the simulation). Furthermore B_0 inhomogeneity will have differential effects on line intensities.

The analysis of the J-spectra is quite simple. In this case, each transition in f_2 can be mixed with every other one, since these transitions either belong to coupled spins or they belong to the same spin. Three distinct sets of peaks will arise in f_1 for each transition in f_2. Firstly, subtractive mixing of a transition with others in f_2 will give a spectrum of four peaks starting at $f_1 = 0$ Hz, with the transition mixing with itself $[(\omega_i - \omega_i)/2 = 0 \text{ Hz}]$. Secondly, additive mixing will give a spectrum of four peaks, starting at $f_1 = \omega_i$ Hz (ω_i is the appropriate f_2 transition frequency)§ with a peak due to the mixing of a line with itself $[(\omega_i + \omega_i)/2 = \omega_i \text{ Hz}]$. Thirdly, a phantom[4] peak will arise in f_1 at $\omega_i/2$ Hz. The Table lists the predicted and observed f_1 frequencies of the peaks in transition number 2. It will be noted that all the frequencies in f_1 are reduced by a factor of 2 after mixing, $(\omega_i \pm \omega_j)/2$, as in the 90°–$t_1$/2–180°–$t_1$/2-echo experiment.[1] The analysis given here applies equally well to strongly or weakly coupled systems. For this experiment, the second pulse can only mix lines present in the normal 1D spectrum, or create a phantom.

TABLE. The predicted and observed frequencies of the peaks in the J-spectrum of the transition in f_2 at $\omega_A - J/2$ Hz (transition number 2)[a]

Peak No.	Origin	Predicted freq./Hz	Observed freq. /Hz
5	$(\omega_A - J/2) - (\omega_A - J/2)$	0	+ 0·05
6	$(\omega_A - J/2) - (\omega_A + J/2)$	− 1·79	− 1·79
2	$(\omega_A - J/2) - (\omega_B + J/2)$	+18·70	+18·74
1	$(\omega_A - J/2) - (\omega_B - J/2)$	+20·49	+20·50
9	$(\omega_A - J/2) + (\omega_A - J/2)$	−20·03	c
8	$(\omega_A - J/2) + (\omega_A + J/2)$	−18·26	−18·48
3	$(\omega_A - J/2) + (\omega_B + J/2)$	+11·25[b]	+10·94[b]
4	$(\omega_A - J/2) + (\omega_B - J/2)$	+9·46[b]	+9·24[b]
7	$(\omega_A - J/2)$	−10·02	−10·16

[a] $(\omega_A + J/2) = -16{\cdot}48$, $(\omega_A - J/2) = -20{\cdot}03$, $(\omega_B + J/2) = -57{\cdot}43$, $(\omega_B - J/2) = -61{\cdot}02$ Hz. [b] Folded lines. [c] Not observed, but note particularly low intensity of this line in the simulation.

This technique promises to be valuable for finding the chemical shifts of hidden, coupled spins in n.m.r. spectra. We have used this technique to solve the complicated assignment problems in the 90 MHz ^{1}H n.m.r. spectra of some ribonucleic acids, and will report on this application shortly.¶

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§ All frequencies in f_2 are relative to the carrier frequency at 0 Hz.

¶ After submitting this communication a report on the application of the method to the analysis of the ^{1}H n.m.r. spectrum of a small protein has appeared; see ref. 7.

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