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Relative Signs of NMR Spin Coupling Constants by Two-Dimensional Fourier Transform Spectroscopy

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Conventional high-resolution NMR spectra yield the magnitudes of the spin coupling constants but not their signs. When relative sign information is required it is necessary to set up a further experiment employing selective double resonance (*1–3*) or proceed to a detailed analysis of a strongly coupled spectrum (*4*). In certain circumstances, relative sign information can be used as a diagnostic tool, for example, in the recognition of geminal and vicinal proton–proton couplings, which normally have opposite signs. The present work describes a simple two-dimensional Fourier transform experiment (*5, 6*) in which relative signs of coupling constants are obtained directly by inspection (*7*).

The basic requirement for any relative sign determination is a system of three coupled nonequivalent spins, for example, an AMX system. It is useful for this discussion to introduce the concept of a "passive" spin, say, the M spin in this case. The role of the M spin is limited to the creation of two AX subspectra, one corresponding to M in an α spin state, the other to M in a β spin state. Apart from introducing the two couplings J_{AM} and J_{MX}, the passive spin M is not involved and its resonance need not be observed. Double resonance provides a method for recognizing which A and X lines belong to a given AX subspectrum, and it is well known that if the upfield A doublet is associated with the upfield X doublet, then J_{AM} and J_{MX} have like signs (*1–3*).

A new approach to relative sign determination is provided by magnetization transfer experiments studied by two-dimensional Fourier transformation (*5, 6*). The magnetization of the A spin is labeled in terms of the characteristic precession frequencies by allowing transverse A magnetization to evolve during a variable interval t_1. A second pulse (known as a "mixing pulse") converts this back into Z magnetization, equivalent to a disturbance of the energy level populations. These population changes affect the intensities of the X signals when the

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latter are excited by a "read" pulse. In this way magnetization can be said to be transferred from A to X. In fact it is always a differential transfer, the low-field line of a given multiplet decreasing in intensity (say) while the upfield line increases; there is no net transfer of magnetization. The signal from the X nuclei is detected as a function of a second time parameter, t_2, and the data matrix $S(t_1, t_2)$ is

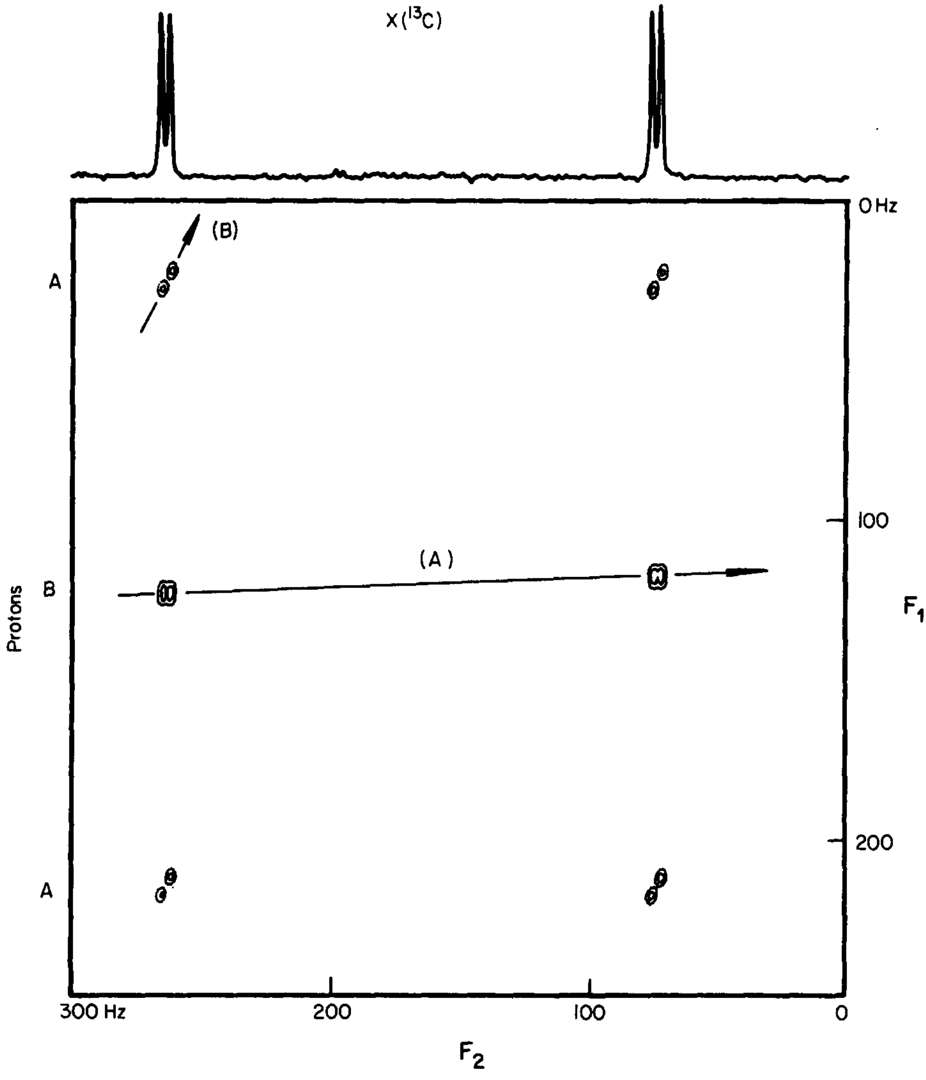

FIG. 1. Two-dimensional spectrum obtained by transferring magnetization from protons to carbon-13 in a sample of maleic anhydride. Each two-dimensional multiplet consists of four lines in a square pattern of side J_{CH} Hz, further split into two four-line patterns through the influence of the "passive" spin (shown in parentheses). For example, for transfer from A to X there are two large square patterns (J_{AX} = 190 Hz) displaced in the direction of the arrow marked (B). This indicates that J_{AB} and J_{BX} have like signs. Transfer from B to X involves the small coupling J_{BX} = 3 Hz, giving two poorly resolved squares displaced in the direction of the arrow (A), indicating like signs for J_{AB} and J_{AX}. The absolute-value mode is used.

converted to a spectrum $S(F_1, F_2)$ by two-dimensional Fourier transformation (*8*–*10*). For this simple AX case the spectrum consists of just four lines in a square pattern. The center of the square has the coordinates δ_A, δ_X and the sides are J_{AX} Hz in length.

The introduction of a third spin M splits this two-dimensional spectrum into subspectra. In the general case the mixing pulse transfers magnetization from all A transitions to all X transitions, giving rise to *four* subspectra, but if the mixing pulse has only a small flip angle (*6*, *11*) then M remains truly "passive" and does not change its spin state when magnetization is transferred from A to X. The transfer is then selective and there are only *two* subspectra of appreciable intensity, giving rise to two square patterns of four lines each. The displacement of one such square with respect to the other reflects the influence of the M spin.

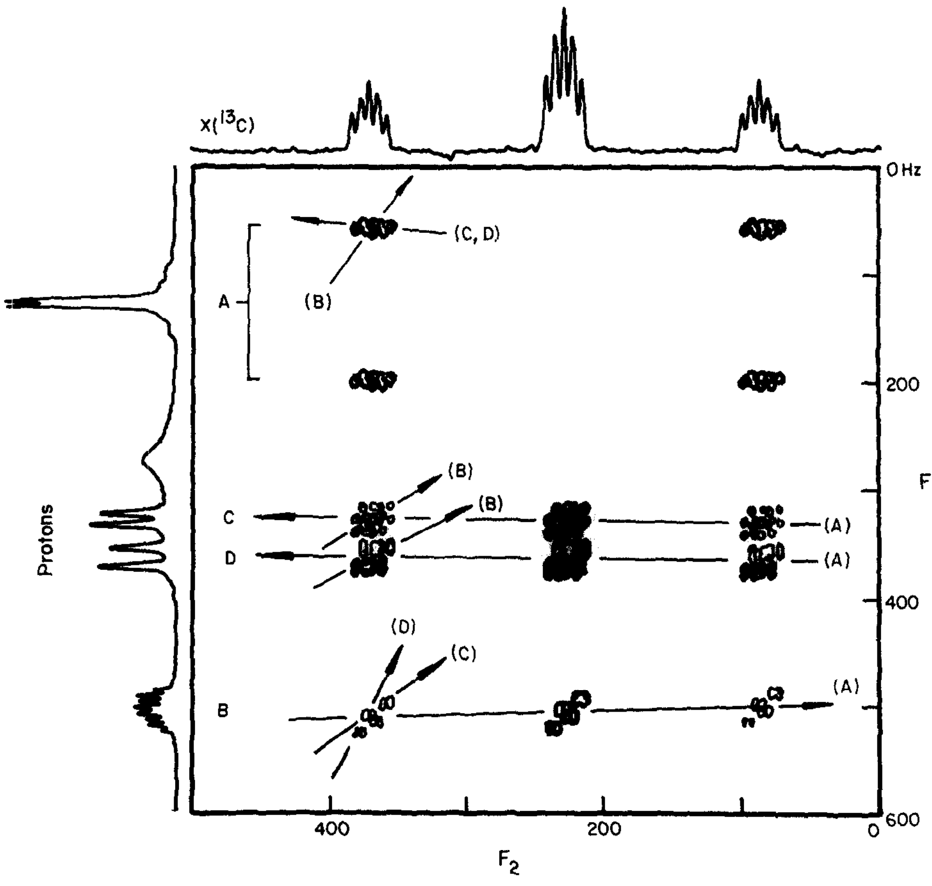

FIG. 2. Two-dimensional spectrum of allyl alcohol obtained by transferring magnetization from protons to the methylene carbon site. The conventional proton spectrum is shown in the left-hand margin and the carbon-13 multiplet is shown at the top of the diagram. The various tilts within a given two-dimensional multiplet are indicated by the arrows, labeled according to the "passive" spins associated with that particular transfer (in parentheses). The coupling J_{CD} is too small to produce a detectable tilt, and at the top left of the diagram the tilt due to the small couplings J_{AC} and J_{AD} is only apparent because they have a cumulative effect.

When this displacement is in the same sense along F_1 and F_2, then J_{AM} and J_{MX} have like signs. A line joining the centers of the two squares has a positive slope for like signs and a negative slope for opposite signs. A zero or infinite slope indicates that either J_{AM} or J_{MX} is vanishingly small. The analogy with selective double irradiation is clear.

Two-dimensional spectra were recorded on a Varian XL-200 spectrometer by transferring proton magnetization to naturally abundant carbon-13. The pulse sequence normally used for this experiment was only slightly modified, reducing the flip angle of the second pulse:

$$90°(\mathrm{H})\text{–}t_1\text{–}45°(\mathrm{H})90°(\mathrm{C})\ \text{Acquire}\ (t_2).$$

In practice 45° turns out to be a suitable compromise between the conflicting requirements for a small flip angle and good sensitivity. Lineshapes in both dimensions were improved by a combination of convolution difference (*12*) and Gaussian weighting of the time-domain signals.

The ABX spectrum of maleic anhydride provides a convenient test case for the method. The carbon-13 spin is X, and J_{AX} is the single-bond proton–carbon coupling (~190 Hz). Figure 1 shows the two-dimensional spectrum with the passive spins indicated in parentheses. Magnetization transferred from A to X shows two large square patterns displaced as indicated by the arrow marked (B). Since this has a positive slope, J_{AB} and J_{BX} have like signs. The long-range coupling J_{BX} is much smaller (~3 Hz), giving rise to incompletely resolved square patterns joined by the long arrow. This too has a positive slope, indicating that J_{AB} and J_{AX} also have like signs. Thus J_{HH}, J_{CH}, and J_{CCH} are all of the same sign in this molecule, presumably positive.

In more complicated systems of coupled spins, there may be more than one passive spin splitting the basic square pattern. The two-dimensional multiplets are correspondingly more complicated, but yield more information about relative signs so that cross-checks become possible.

One of the isotopomers of allyl alcohol illustrates such a case. Only the magnetization transferred from protons to a methylene carbon-13 nucleus is studied, and the spins are therefore labeled

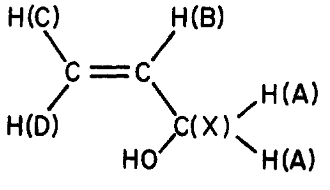

Figure 2 shows the resulting two-dimensional spectrum with passive spins indicated in parentheses. In general, transfer of magnetization between two given spins involves *three* passive spins and hence determines the relative signs of *three* pairs of spin coupling constants. In practice, slightly less information can be gleaned from this spectrum because J_{CD} is too small to give resolvable splittings on this frequency scale. Consider the two-dimensional spin multiplet at the bottom of Fig. 2, arising from magnetization transferred from proton B to X. The basic square pattern is split by coupling to A_2, C, and D, three nonequivalent spins

TABLE 1

SIGNS[a] AND APPROXIMATE MAGNITUDES (Hz) OF COUPLING CONSTANTS IN ALLYL ALCOHOL

Proton–proton		Proton–carbon-13
$J_{AB} = +4.9$	$J_{BC} = +10.5$	$J_{AX} = +142$
$J_{AC} = -1.6$	$J_{BD} = +17.3$	$J_{BX} = +6$
$J_{AD} = -2.0$	$J_{CD} = \pm 2.1$[b]	$J_{CX} = +13$
		$J_{DX} = +6$

[a] Assuming the single-bond J_{CH} is positive.

[b] Sign not determined because the splittings are not resolved.

or groups of spins (A_2 simply introduces a triplet structure). The corresponding displacements (indicated by the arrows) are all of positive slope, demonstrating that J_{AB} and J_{AX} have like signs, J_{BC} and J_{CX} have like signs, and J_{BD} and J_{DX} have like signs. The other multiplets in this spectrum, representing transfer between A and X, C and X, and D and X, provide further information on relative signs, summarized in Table 1.

A wealth of information about the relative signs of spin coupling constants is therefore available by inspection of two-dimensional spectra of this kind, simply by reducing the flip angle of the mixing pulse in a magnetization transfer sequence. This opens up the possibility of a more widespread use of sign information as a diagnostic tool.

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