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Two-Dimensional Heteronuclear Relayed Coherence Transfer Spectroscopy

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Recently, Bolton and Bodenhausen (*1, 2*) have developed a new experimental 2D technique which can be useful for structure determination and for assignment of resonances. This so-called RELAY experiment transfers coherence in two steps: first magnetization is transferred from one nucleus to another, e.g., one proton to another, and then this transferred magnetization is relayed to a third nucleus, e.g., a carbon-13. As vicinal proton–proton couplings are often well resolved, magnetization can be transferred between those protons, and pairs of adjacent protonated ^{13}C nuclei can then be identified simply by inspection of the 2D spectrum (*1*), analogously to the more versatile but less sensitive two-dimensional INADEQUATE experiment (*3–5*). The RELAY experiment appears to be most valuable in cases where the resonances in the proton spectrum cannot be assigned because of severe overlap.

The original RELAY experiments (*1, 2*) require a 16-step sequence, each step with different phases of the rf pulses. On some spectrometers a 16-step experiment is not easily implemented, and we therefore propose a simplified version of this experiment which requires only a 4-step sequence and also improves the sensitivity by a factor of $\sqrt{2}$.

The pulse sequence of the four-step RELAY experiment is set out in Fig. 1. The phases ϕ_1 and ϕ_2 are cycled according to Table 1 in the four steps of the experiment. For completeness, the way the magnetization is transferred will be discussed briefly. Consider an AMX spin system in which A and M are protons with a coupling J_{AM} and X is a ^{13}C nucleus directly coupled to proton M. The coupling J_{AX} is assumed to be zero. The first 90° proton pulse simply rotates the longitudinal magnetization into the transverse plane (Fig. 2a). The 180° ^{13}C pulse at the midpoint of the t_1 interval serves to eliminate the overall effect of heteronuclear coupling at the end of the evolution period, just before the second proton 90° pulse (Fig. 2b). This 180° pulse serves a similar function as the 180° pulse in the center of the evolution period in the decoupled version of the heteronuclear shift correlation experiment (*6*). The second, nonselective 90° proton pulse can, for convenience, be considered as a cascade of two semiselective 90° proton pulses (*7*), one applied to the A nucleus followed by one applied to the M nucleus. The hypothetical 90° A pulse changes the longitudinal magnetization of the A transitions and therefore the longitudinal magnetizations of the connected M transitions. The hypothetical M pulse, applied along the x axis, rotates the longitudinal M magnetization, which originates from spin A, along the y

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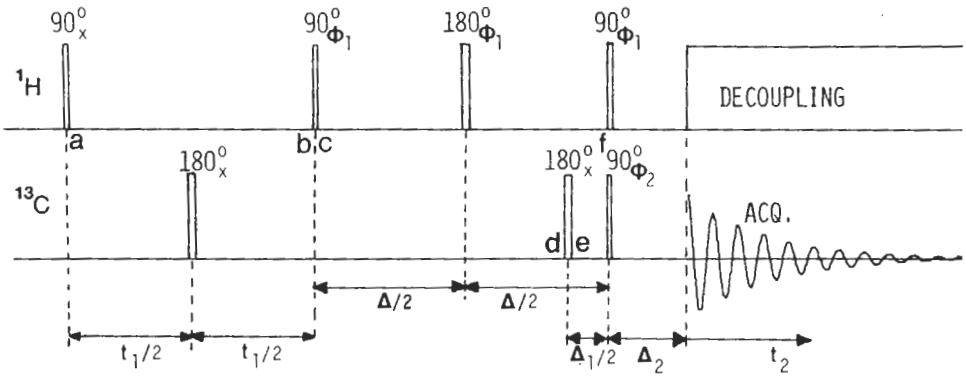

FIG. 1. Pulse scheme of the heteronuclear RELAY coherence transfer experiment. The phases of the rf pulses are cycled as given in Table 1, and all data are co-added with identical receiver phase.

axis. It can be shown that the transferred magnetization components always are in antiphase just after the transfer pulse (*6, 8, 9*), in this case along the $+y$ and $-y$ axes (Fig. 2c), and they will yield no net magnetization.

After another period, Δ, equal to $1/(2J_{AM})$, the transferred M multiplet vectors will be parallel again, and a net M magnetization that originates from nucleus A will be present in the transverse plane. Average precession due to proton chemical shifts and heteronuclear coupling is suppressed during this time, Δ, by the application of a proton 180° pulse at the center of this interval, but precession due to J coupling is not affected. Therefore, proton M magnetization vectors, originating from nucleus A, would be present along either the $+x$ or $-x$ axis at the end of the interval, Δ. However, at a time $\Delta_1/2$, equal to $1/(4J_{MX})$, before the end of the Δ interval, a 180° pulse is applied to the ^{13}C. Just before this 180° pulse, the M multiplet vectors have positions in the transverse plane as shown in Fig. 2d. The 180° ^{13}C pulse applied at this point flips the spin state of the ^{13}C nucleus (Fig. 2e), and therefore the heteronuclear M multiplet vectors will be aligned along the $\pm y$ axis at the end of the interval, Δ (Fig. 2f).

Just as in the INEPT experiment (*10*), a proton $90°_x$ pulse applied simultaneously with a 90° ^{13}C pulse then transfers the M magnetization to the X nucleus. As the transverse M magnetization that is present after the second proton pulse is modulated

TABLE 1

PHASES ϕ_1 AND ϕ_2 OF THE rf PULSES IN FIG. 1 IN THE FOUR STEPS OF THE RELAY EXPERIMENT[a]

Exp. no.	ϕ_1	ϕ_2
1	x	x
2	y	$-y$
3	$-x$	$-x$
4	$-y$	y

[a] All data are co-added with identical receiver reference phase.

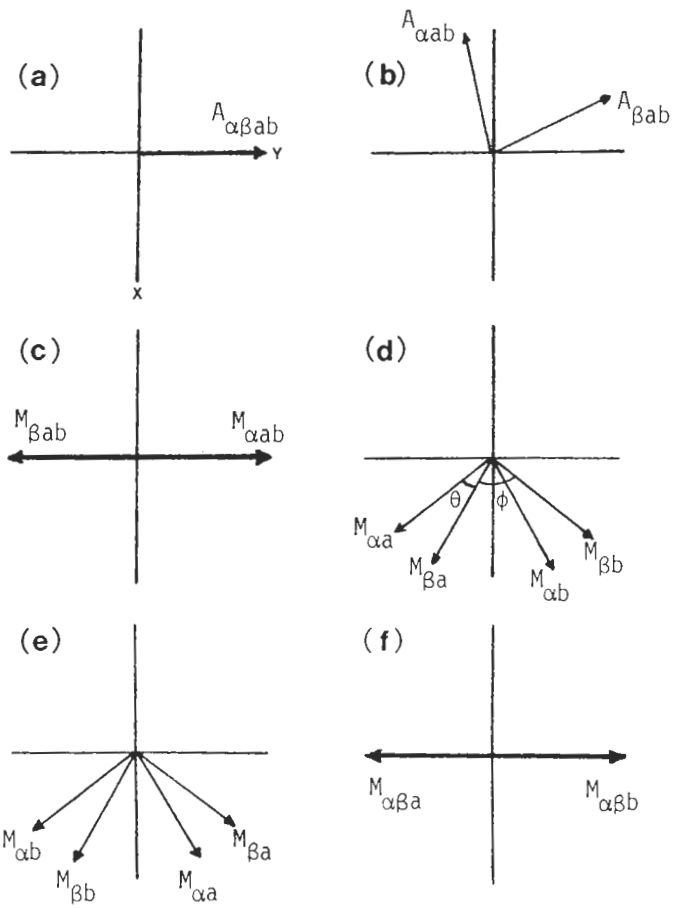

FIG. 2. Proton magnetization vectors during the pulse sequence of Fig. 1. The magnetization vectors of nuclei A and M have indices corresponding to the spin state of the coupled proton (α and β) and the spin state of the ^{13}C nucleus (a and b for the $m = ½$ and $m = -½$ states, respectively). The angles θ and ϕ are equal to $\pi J_{AM}\Delta_1$ and $\pi J_{MX}\Delta_1$, respectively. The diagrams a–f correspond to the times a–f indicated in Fig. 1.

as a function of t_1 with the resonance frequencies of both nuclei A and M, one would expect the ^{13}C magnetization during detection to be modulated with these frequencies. However, just after the second 90° proton pulse, the magnetization due to multiplet components that have not been transferred from A magnetization by the second 90° proton pulse, and which would give rise to the diagonal peaks in the homonuclear proton shift correlation experiment, are aligned parallel along either the $+x$ or $-x$ axis. They then will be antiparallel at a time $1/(2J_{AM})$ later, and X magnetization will not be modulated with the frequency of its directly coupled proton, M. For sensitivity purposes, however, values for the delay Δ will often be selected shorter than $1/(2J_{AM})$, usually of the order of $1/(4J_{AM})$ if J_{AM} is a typical vicinal proton–proton coupling constant.

Phases ϕ_1 and ϕ_2 are cycled in a way similar to the scheme used in the heteronuclear shift correlation experiment with quadrature proton detection (*11*), and are given in

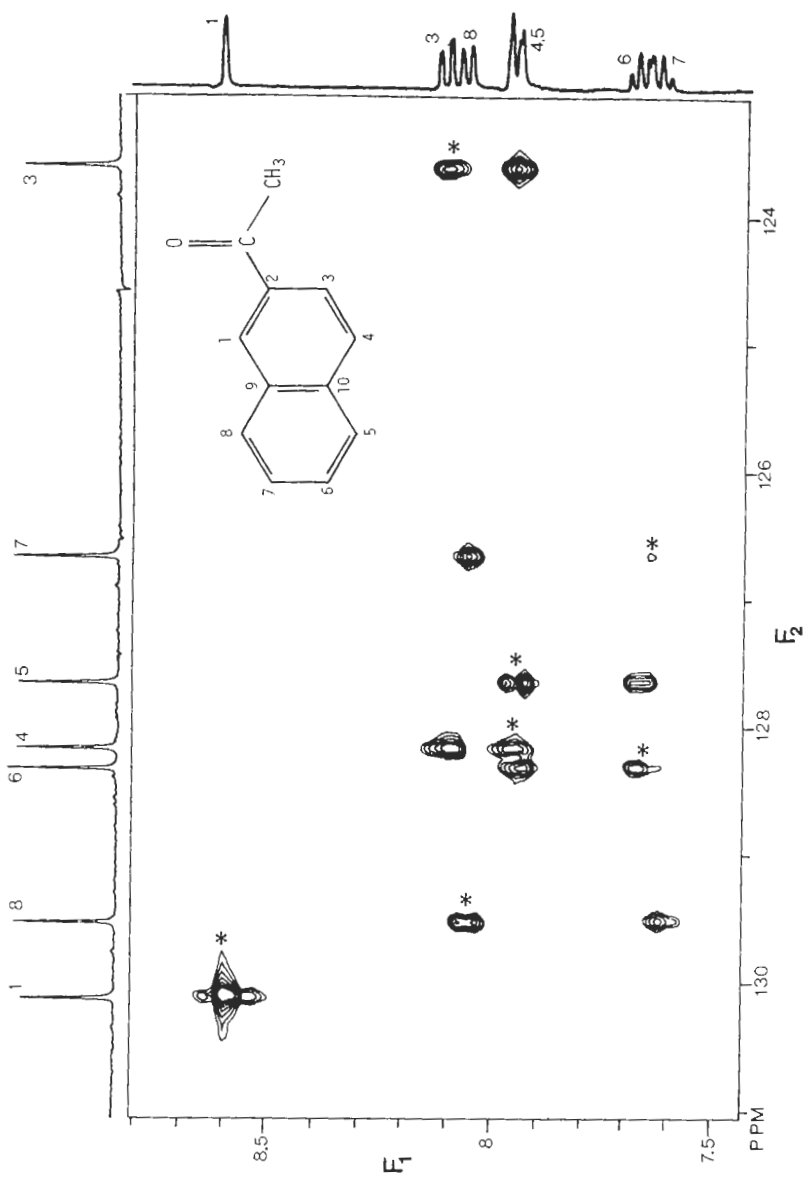

FIG. 3. Contour plot of a 2D RELAY spectrum of the aromatic resonances in a 2 *M* concentration of 2-acetonaphtalene in acetone-*d6*. A 64 × 1024 data matrix was acquired and the total measuring time was 20 min. The peaks which are due to nonrelayed magnetization were identified by comparison with a normal heteronuclear shift correlation spectrum and are marked with asterisks. The spectrum was recorded on a NT-360 spectrometer, operating at 90 MHz ^{13}C frequency.

Table 1. All data are co-added with identical receiver phase, and the coherence transfer echo (*12*) is detected. As the third 90° proton pulse is always applied along an axis which is perpendicular to the difference of the M multiplet magnetization vectors, all four steps in this version of the RELAY experiment provide optimum relayed coherence transfer. This provides an improvement in sensitivity by a factor of $\sqrt{2}$ over the original version of the experiment (*1, 2*).

The experiment is demonstrated with a spectrum of the aromatic resonances of 2-acetonaphtalene, obtained using a Nicolet NT-360 spectrometer, controlled by a 293A′ pulse programmer. A 64 × 1024 data matrix was acquired, and four experiments were performed for each t_1 value with the phases of the rf pulses cycled as indicated in Table 1. The values for the delays Δ, Δ_1, and Δ_2 were set to 50, 3, and 3 msec, respectively. Figure 3 shows a contour plot of the RELAY spectrum with the conventional 1D proton and ^{13}C spectra along its axes. The resonances due to direct, nonrelayed transfer are easily identified by comparison with a conventional heteronuclear shift correlation spectrum and those peaks are indicated with asterisks. Because C7, for example, is directly coupled to the proton to which C8 is indirectly coupled, and vice versa, it follows that C7 and C8 are adjacent nuclei. Similarly it can be seen that the pairs C3,C4 and C5,C6 are adjacent nuclei. The peaks which indicate that C6 and C7 are adjacent have too low an intensity to be observed in this contour plot. It can be seen that, even while the resonances of protons H4 and H5 are completely overlapping, the carbons C4 and C5 can be assigned unambiguously, which would not be possible from a conventional heteronuclear shift correlation spectrum (*6*), in which only direct coupling correlations are observed.

Furthermore, it can be shown that in the case of complicated proton coupling networks a reduction of the flip angle of the second proton pulse to about 60° increases the intensity of the *relayed* magnetization (*8, 9, 13*), and therefore improves the sensitivity. In practice, however, the sensitivity of the RELAY experiment is often a factor of 3 to 10 lower than for the normal heteronuclear shift correlation spectrum.

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REFERENCES

1. P. H. Bolton, *J. Magn. Reson.* **48,** 336 (1982).
2. P. H. Bolton and G. Bodenhausen, *Chem. Phys. Lett.* **89,** 139 (1982).
3. A. Bax, R. Freeman, and T. A. Frenkiel, *J. Am. Chem. Soc.* **103,** 2102 (1981).
4. A. Bax, T. A. Frenkiel, R. Freeman, and M. H. Levitt, *J. Magn. Reson.* **43,** 478 (1981).
5. T. H. Mareci and R. Freeman, *J. Magn. Reson.* **48,** 158 (1982).
6. A. A. Maudsley, L. Muller, and R. R. Ernst, *J. Magn. Reson.* **28,** 463 (1977).
7. G. Bodenhausen and R. Freeman, *J. Magn. Reson.* **36,** 221 (1977).
8. W. P. Aue, E. Bartholdi, and R. R. Ernst, *J. Chem. Phys.* **64,** 2229 (1976).
9. A. Bax, "Two-dimensional Nuclear Magnetic Resonance in Liquids," pp. 69–82, Reidel, Boston, 1982.
10. G. A. Morris and R. Freeman, *J. Am. Chem. Soc.* **101,** 760 (1979).
11. A. Bax and G. A. Morris, *J. Magn. Reson.* **42,** 501 (1981).
12. A. A. Maudsley, A. Wokaun, and R. R. Ernst, *Chem. Phys. Lett.* **69,** 567 (1979).
13. A. Bax and R. Freeman, *J. Magn. Reson.* **44,** 542 (1981).