An Improved Method for Two-Dimensional Heteronuclear Relayed-Coherence-Transfer NMR Spectroscopy

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Recently, a variety of schemes for obtaining 2D heteronuclear RELAY spectra have been proposed (*1–6*). These methods can be quite powerful for structure determination and spectral assignment. However, a recent analysis (*7*) has shown that in many cases the sensitivity of heteronuclear relayed-magnetization-transfer spectroscopy is considerably lower than for heteronuclear chemical-shift correlation (*8–10*). Especially in compounds with saturated aliphatic ring systems, such as steroids and alkaloids, the sensitivity of the heteronuclear RELAY experiment is often very low. Moreover, in all heteronuclear RELAY experiments proposed so far the ^{1}H–^{1}H multiplet components of the (^{1}H–^{13}C) RELAY multiplet are in antiphase relative to one another (analogous to the antiphase nature of multiplet components in a COSY spectrum (*11*)) and the ^{1}H–^{13}C RELAY multiplet is 90° out of phase relative to nonrelayed signal in the 2D spectrum. No pure-absorption 2D spectra can therefore be recorded with the existing RELAY experiments.

We propose a new pulse scheme for heteronuclear RELAY spectroscopy that partly overcomes the problems mentioned above. In the new scheme, net magnetization transfer among protons is achieved via homonuclear Hartmann–Hahn-type cross-polarization. This type of homonuclear magnetization transfer was first discovered (*12*) as an artifact in homonuclear transverse NOE spectroscopy (*13*) and is closely related to the magnetization-transfer mechanism in the TOCSY experiment (*14*). We have recently demonstrated that this type of homonuclear cross-polarization is very powerful for the assignment of complex proton spectra (*15*). The possibility of obtaining net magnetization transfer in coupled ^{1}H spin systems makes this approach very suitable for heteronuclear RELAY spectroscopy.

The pulse scheme of the new method is sketched in Fig. 1. An alternating spin-lock field along the $\pm x$ axis of the proton rotating frame provides the crucial mechanism for homonuclear Hartmann–Hahn-type cross-polarization. Consider first, for reasons of simplicity, a rf field of nominal strength, $\nu = \gamma H_2/2\pi$, that is continuously aligned along the x axis of the proton rotating frame. Two protons, A and B, with offset frequencies Δ_A and Δ_B, experience effective rf field strengths, ν_A and ν_B. Provided that $\nu \gg \Delta_A$, Δ_B, ν_A and ν_B are to a good approximation given by
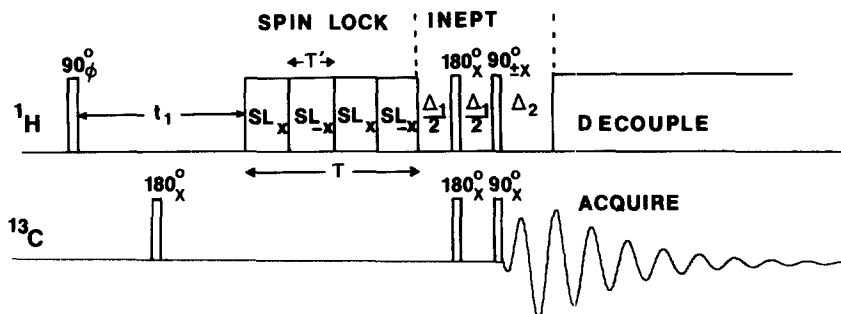
FIG. 1. Pulse scheme of heteronuclear relayed Hartmann–Hahn spectroscopy. The rf phase of the spin-lock field is varied along the $\pm x$ axis. The second part of the mixing period consists of an INEPT-type transfer to ^{13}C. The phases of the rf pulses are cycled according to Table 1.

$$\nu_A = \nu + \Delta_A^2/(2\nu)$$ [1a]

$$\nu_B = \nu + \Delta_B^2/(2\nu).$$ [1b]

If $|\Delta_A| = |\Delta_B|$, the effective rf fields are identical for the two spins, and a perfect Hartmann–Hahn match condition occurs. In this case, oscillatory magnetization exchange between spins A and B will occur, with period $1/J_{AB}$ (15). The situation where Δ_A and Δ_B differ (and thus $\nu_A \neq \nu_B$) has been analyzed for the heteronuclear case by Müller and Ernst (16) and by Chingas et al. (17). Their derivations are readily applied to the homonuclear case (15). We have previously shown (15) that the effect of a mismatch induced by $|\Delta_A| \neq |\Delta_B|$ can to a large extent be compensated for by phase alternation of the spin-lock field, with period, $2/\tau'$, provided that

$$\tau' < 0.25/|\nu_A - \nu_B|.$$ [2]

To minimize the loss of magnetization during the spin-lock time, τ' should not be set to much shorter values than calculated on the basis of Eq. [2]. In practice, we usually employ a total spin-lock time of 25–40 ms, with a τ' value of 3–10 ms and a ^1H rf field strength of 5 kHz (6 W rf power).

As mentioned above, magnetization exchange for a two-spin system under Hartmann–Hahn match conditions occurs at a rate J_{AB}^{-1}, giving an optimum cross-polarization time of $1/(2J_{AB})$. In practice, however, most molecules contain more than two coupled protons and ^1H–^1H magnetization relay will occur for longer spin lock times (14, 15), in a way that shows a close similarity to ^1H–^1H spin diffusion in a solid. To limit the extent of these sometimes confusing homonuclear relay effects, a coherent mixing time, τ, on the order $1/(4J_{AB})$ is usually employed. A more detailed analysis of the way in which magnetization is transferred under spin-locked conditions will be presented elsewhere.

After net magnetization is transferred from proton A to proton B, an INEPT type transfer (18–20) relays the ^1H magnetization to the ^{13}C nucleus attached to proton B. To record the 2D spectrum in the pure-absorption mode, phase cycling according to Müller and Ernst (16) and States et al. (21) is used (Table 1), and data

TABLE 1

Phases of the First 90° ^{1}H Pulse, ϕ, of the Final 90° ^{1}H Pulse
and of the Receiver in the Pulse Sequence of Fig. 1

Step	First 90°	Last 90°	Acq.[a]	
1	x	x	$+$	
2	y	x		$+$
3	$-x$	x	$-$	
4	$-y$	x		$-$
5	x	$-x$	$-$	
6	y	$-x$		$-$
7	$-x$	$-x$	$+$	
8	$-y$	$-x$		$+$

[a] Data acquired in odd- and even-numbered scans are stored in
separate locations (16, 21).

acquisition is started immediately after the 90° ^{13}C pulse (20), before broadband proton decoupling is started.

As an example, the method is demonstrated for a solution of 50 mg quinine in 0.35 ml CDCl$_3$, in a 5 mm sample tube. Experiments were performed on a Nicolet 270 MHz spectrometer. Acquisition times in the t_1 and t_2 dimensions were 70 and 128 ms, respectively. Thirty-two scans were recorded for each t_1 value, and the total measuring time was 2 h. Zero filling prior to Fourier transformation was used in both dimensions to yield a 128 × 2048 data matrix for the absorptive part of the final 2D spectrum displayed in Fig. 2. A total ^{1}H spin-lock time, τ, of 30 ms was used, consisting of six periods, τ', of 5 ms each.

Figure 2 shows the 2D spectrum of the aliphatic carbons in quinine, obtained as described above. It is evident from the spectrum that many of the RELAY peaks have higher intensity than the correlation via direct coupling. For example, the inset in Fig. 2 shows the F_1 section taken at the F_2 frequency of carbon $\underline{b}$, and shows magnetization relay from proton $\underline{a}$, and from the two methylene protons, $\underline{c}$. The two protons, $\underline{c}$, overlap with protons, $\underline{g}$, but since carbon $\underline{c}$ shows relay from proton $\underline{b}$, and carbon g shows relay from protons $\underline{h}$, carbons $\underline{c}$ and g are readily distinguished. This confirms spectral reassignments made by Wenkert et al. (22) and by Moreland et al. (23).

Before recording a heteronuclear RELAY spectrum, a regular heteronuclear chemical-shift correlation spectrum should be obtained. The additional resonances in the 2D RELAY spectrum then indicate directly which carbons are adjacent in the structure of the molecule (1, 2) in a similar but more sensitive way than the 2D INADEQUATE experiment (24–26). We have also attempted to obtain a RELAY spectrum in the conventional way (3, 4), using a mixing time of 30 ms, and 128 scans per t_1 value (8 h experiment). In this spectrum (not shown) all RELAY peaks showed lower signal-to-noise ratio than the corresponding peaks in the spectrum obtained with the new method, despite the four times longer measuring time. In neither of the two types of spectra could magnetization relay to carbon $\underline{d}$ or from
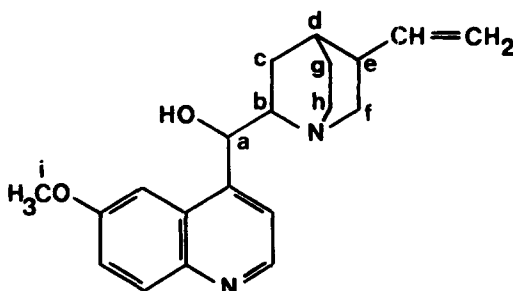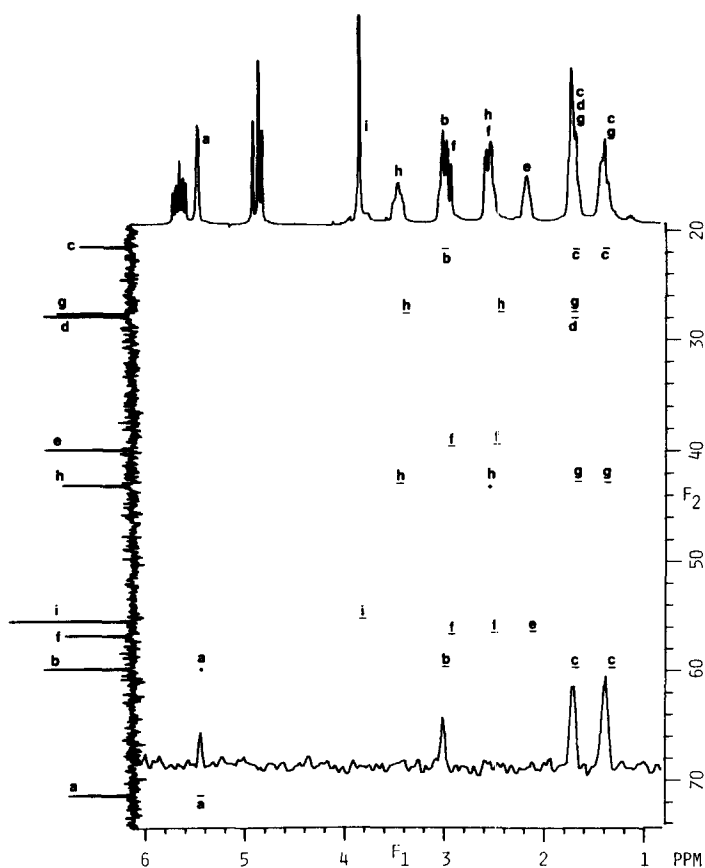
FIG. 2. Heteronuclear RELAY spectrum of quinine, recorded on a 270 MHz spectrometer. The assignment of the carbons is indicated on the spectrum along the vertical axis. In the 2D spectrum it is indicated from which proton the various peaks originate. For example, the peak labeled "b" at the F_2 frequency of carbon "c" indicates magnetization relay. The inset displays a section parallel to the F_1 axis, taken at the F_2 frequency of carbon "b," showing relay connectivity to protons "c" and "a."

proton $\underline{d}$ to adjacent carbons be observed. This is probably due to the complex multiplet structure of proton $\underline{d}$ (32 multiplet components). The sensitivity of the RELAY peaks in the spectrum obtained with the new method has also been

compared with the sensitivity obtained with the absolute-value-mode heteronuclear chemical-shift-correlation method. The signal-to-noise ratio for RELAY peaks in the spectrum shown in Fig. 2 was a factor of 2 to 5 lower than for the resonances in the chemical-shift correlation spectrum obtained in the same measuring time.

We have demonstrated that Hartmann–Hahn-type homonuclear magnetization transfer can be effectively relayed to ^{13}C nuclei. The pure-absorptive-mode spectra obtained with this method provide high resolution in both frequency dimensions. The good resolution and the relatively high sensitivity of the new RELAY method make the experiment an attractive alternative to more conventional approaches.

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