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Simultaneous Measurement of ^{13}C Multiplicities and ^{1}H and ^{13}C Chemical Shifts

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^{13}C NMR is a well-recognized tool for the investigation of structural and dynamic properties of organic molecules (*1, 2*). ^{13}C chemical shifts and coupling constants are very sensitive to bond geometries and hybridization (*3*), and carbon relaxation rates provide a sensitive probe of motional spectral density functions (*4–6*). The major limitations associated with applying ^{13}C NMR, namely low sensitivity and low natural abundance, have been largely circumvented with elegant pulse schemes which make use of ^{13}C–^{1}H heteronuclear multiple-quantum coherence (*1, 2, 7*). Figure 1a indicates one of several such sequences, developed by Bax and co-workers (*7*). The sequence, as normally executed, generates a ^{13}C–^{1}H correlation map where proton-decoupled ^{13}C chemical-shift information is obtained during t_1 and ^{13}C-decoupled ^{1}H magnetization is acquired during t_2.

Decoupling, although an integral part of these experiments, unfortunately results in a loss of information concerning ^{13}C multiplicities. This is not simply a matter of choice. The 180° ^{1}H pulse applied in the middle of the t_1 period, which removes ^{1}H chemical shifts from the t_1 domain, also generates ^{1}H decoupling during this period. In the absence of this pulse peaks are generated which, in addition to being modulated by both ^{1}H and ^{13}C chemical shifts in ω_1, have the undesirable feature of being in the phase-twisted mode (*7*). ^{1}H–^{13}C coupling could be retained in the t_2 domain by the simple omission of ^{13}C noise decoupling during acquisition; however, the more useful information concerning ^{13}C multiplicities would not be retrieved.

In this communication we provide a simple extension of the sequence of Fig. 1a which allows for the determination of ^{13}C multiplicities in addition to ^{13}C and ^{1}H chemical shifts. Figure 1b illustrates the proposed sequence. The effects of this sequence on an isolated heteronuclear AX spin system can be understood qualitatively in the following way. Two-spin heteronuclear multiple-quantum coherence is generated by the

$$\begin{array}{ll} ^{1}H: & 90°—\tau/2—180°—\tau/2— \\ ^{13}C: & \qquad\qquad 180° \qquad\quad 90° \end{array}$$

portion of the sequence, with $\tau/2 \sim 1/(4J_{AX})$, where J_{AX} is the geminal ^{13}C–^{1}H coupling constant. ^{13}C magnetization is then allowed to evolve for t_1, with ^{1}H mag-

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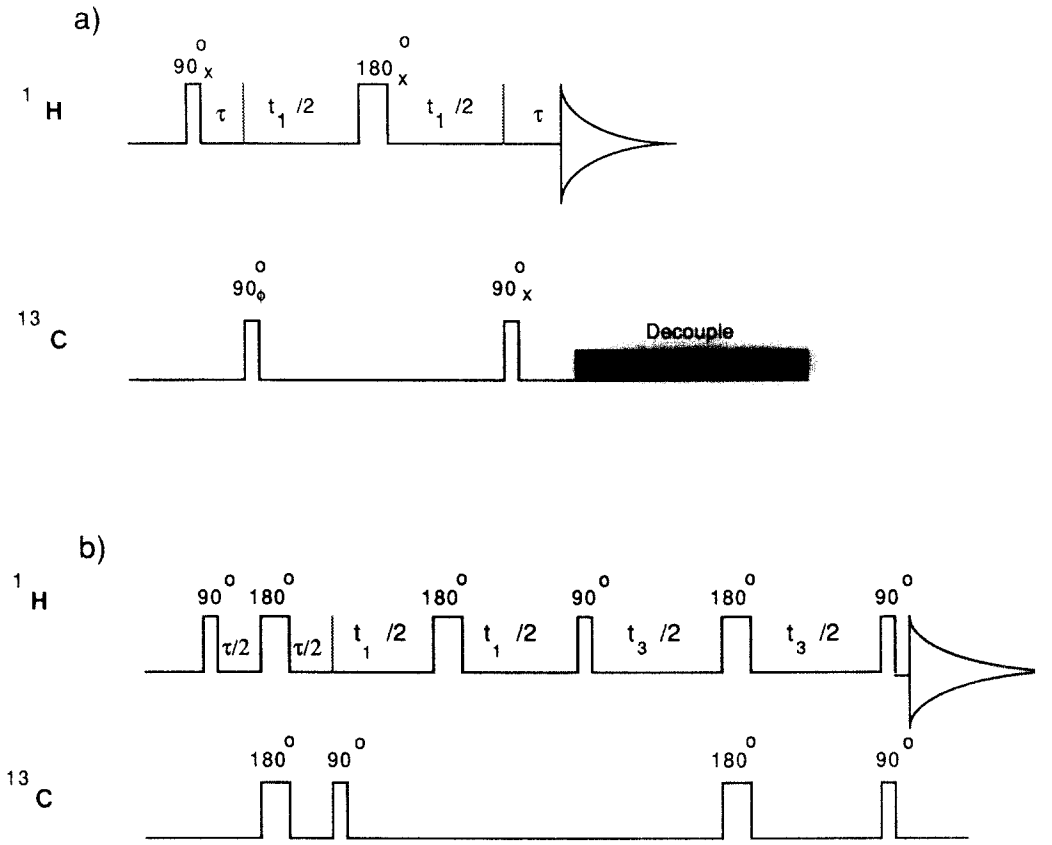

FIG. 1. Pulse schemes for correlating ^{13}C and ^{1}H chemical shifts. The sequence of (a), proposed by Bax and co-workers, results in a decoupled spectrum in both ω_1 and ω_2. The sequence of (b) allows a determination of ^{13}C multiplicities, in addition to ^{13}C and ^{1}H chemical shifts.

netization and heteronuclear scalar coupling effects refocused through application of the π pulse to the ^{1}H spin in the middle of the t_1 period. Double-quantum coherence is converted to ^{13}C single-quantum magnetization by application of the next 90° ^{1}H pulse. ^{13}C magnetization evolves for an additional evolution period, t_3, under the effects of J modulation only and is finally reconverted to ^{1}H magnetization for observation during t_2 by application of the final 90° $\{^1$H, ^{13}C$\}$ pulses. In all applications considered so far, t_3 was set equal to t_1 so that a two-dimensional data set was generated with both ^{13}C–^{1}H geminal couplings and ^{13}C chemical-shift information recorded in t_1. In principle, however, magnetization could be collected as a function of t_1, t_2, and t_3 to generate a three-dimensional profile (*8, 9*). ^{13}C decoupling would normally begin at $\sim\tau$ after application of the final pulses to allow for refocusing of the antiphase ^{1}H components. However, we have not implemented ^{13}C decoupling in the experiments presented.

In ^{1}H-observe, ^{1}H–X nucleus correlation experiments, interfering signals arising from protons not coupled to the X nucleus must be removed. In the sequence of Fig.

1b, uncoupled ^1H magnetization is suppressed by alternating the phase of the final 90° ^{13}C pulse with each acquisition (*10*). Further suppression can be achieved by a scheme proposed by Bax and co-workers (*11*) which effectively saturates ^1H magnetization not directly coupled to the X spin at the outset of the experiment.

Phase-sensitive spectra can be generated with the sequence of Fig. 1b using either the method of States *et al.* (*12*) or TPPI (*13*). Table 1 shows the phase-cycling scheme employed. In general, $t_{1,max}$ should be kept as short as possible so that ^1H–^1H scalar modulations do not interfere with acquisition of the data in the phase-sensitive mode (*7*).

The advantages of this sequence for structural analyses from ^{13}C, ^1H NMR data parallel those of the original sequence (*7*) in that magnetization originates on the sensitive ^1H nucleus and is detected through ^1H's as well. A comparison to the sequence of Fig. 1a shows that the sequence of Fig. 1b, with ^{13}C decoupling during t_2, is at least a factor of 2 less sensitive since ^{13}C multiplicities are not suppressed in ω_1. The increased number of delays and pulses tend to decrease the relative sensitivity further. However, the sensitivity of the proposed sequence is superior to ^{13}C-observe methods which provide information concerning ^{13}C multiplicities, and ^1H and ^{13}C chemical shifts (*14*).

Table 2 indicates the ^{13}C cross-peak multiplicities generated by this sequence for various spin systems. Of particular interest is the fact that the ^{13}C multiplicity pattern for an AX_3 spin system is in the ratio 3:1:1:3, and not in the ratio 1:3:3:1 which might have been expected. Insight into why this is the case can be obtained from a product operator description (*15*) of the effects of the sequence of Fig. 1b on an AX_3 spin system. A simple calculation, including the effects of the phase-cycling scheme depicted

TABLE 1

Phase Cycling for the Sequence of Fig. 1b

^1H:	90°	180°		180°	90°	180°	90°	Receiver
	x	x		x	y	x	x	x
	x	x		x	y	x	x	$-x$
	x	$-x$		$-x$	y	$-x$	x	x
	x	$-x$		$-x$	y	$-x$	x	$-x$
	x	x		x	$-y$	x	x	x
	x	x		x	$-y$	x	x	$-x$
	x	$-x$		$-x$	$-y$	$-x$	x	x
	x	$-x$		$-x$	$-y$	$-x$	x	$-x$
^{13}C:		180°	90°			180°	90°	
		x	x			x	x	
		x	x			x	$-x$	
		$-x$	x			$-x$	x	
		$-x$	x			$-x$	$-x$	
		x	$-x$			x	x	
		x	$-x$			x	$-x$	
		$-x$	$-x$			$-x$	x	
		$-x$	$-x$			$-x$	$-x$	

TABLE 2

^{13}C Multiplicity Patterns for Several Spin Systems

Spin system	^{13}C multiplicity	Separation of multiplet components
AX_3	3:1:1:3	J_{AX}
AX_2	1:1	$2J_{AX}$
AMX[a]	1:1	$J_{AX} + J_{MX}$
AX	1:1	J_{AX}

Note. A = carbon spin; X, M = proton spins.

[a] This assumes that $J_{AX} \sim J_{MX}$. A product operator calculation shows that in the most general case, a quartet is expected with a separation between the outer two lines of $J_{AX} + J_{MX}$ and a separation between the inner two lines of $|J_{AX} - J_{MX}|$. The intensities predicted are proportional to $|\sin \pi J_{AX}\tau + \sin \pi J_{MX}\tau|$ for the outer two lines and $|\sin \pi J_{AX}\tau - \sin \pi J_{MX}\tau|$ for the inner two lines.

in Table 1 shows that, in the absence of relaxation, ^{13}C magnetization is modulated in t_1 according to

$$\exp(i\omega_A t_1) \cdot [\cos^3(\pi J_{AX} t_1) - 2 \sin^2(\pi J_{AX} t_1)\cos(\pi J_{AX} t_1)], \quad [1]$$

where ω_A is the frequency of the ^{13}C nucleus and J_{AX} is the A–X scalar coupling constant. After some algebra, Eq. [1] becomes

$$(1/8) \cdot [\exp\{i(\omega_A \pm \pi J_{AX})t_1\} + 3 \exp\{i(\omega_A \pm 3\pi J_{AX})t_1\}]. \quad [2]$$

This shows clearly that multiplet components are observed with a 3:1:1:3 intensity pattern.

It is important to note also that both methylene and methine fragments produce doublets. However, CH_2 and CH fragments are easily distinguished since the doublet separation is $2J_{AX}$ in the former case and only J_{AX} in the latter case.

The sequence of Fig. 1b was tested using a sample consisting of 150 m*M* alanine and 150 m*M* glycine dissolved in D_2O at 303 K. $Cr(AcAc)_3$ was added to the solution, giving a final concentration of 1.5 m*M*, to act as a source of random fields. This greatly shortens the ^1H T_1 of both alanine and glycine and hence also the experimental time. The data were acquired on a homebuilt 490 MHz spectrometer operating in the Fourier transform mode. Quadrature in ω_1 was obtained using TPPI (*13*). Three hundred experiments, consisting of 1 K complex points per experiment, were acquired with a t_1 increment of 83 μs to give a $t_{1,max}$ of 25 ms. A 3-s relaxation delay was used. The total measuring time was 2.9 h. The data were processed on a VAX 11/750 computer equipped with a CSPI Mini-map array processor using software written by Dr. D. Hare. The first 600 points in t_2 were processed using a pure sine-bell function and then zero-filled to 2K. In t_1 a cosine-bell function was applied to all 300 points and the data were zero-filled to 2K. Figure 2 shows column cross sections indicating the multiplet patterns for AX (αCH of alanine), AX_2 (αCH_2 of glycine), and AX_3 (βCH_3 of alanine) spin systems which are in excellent agreement with the results in Table 2.

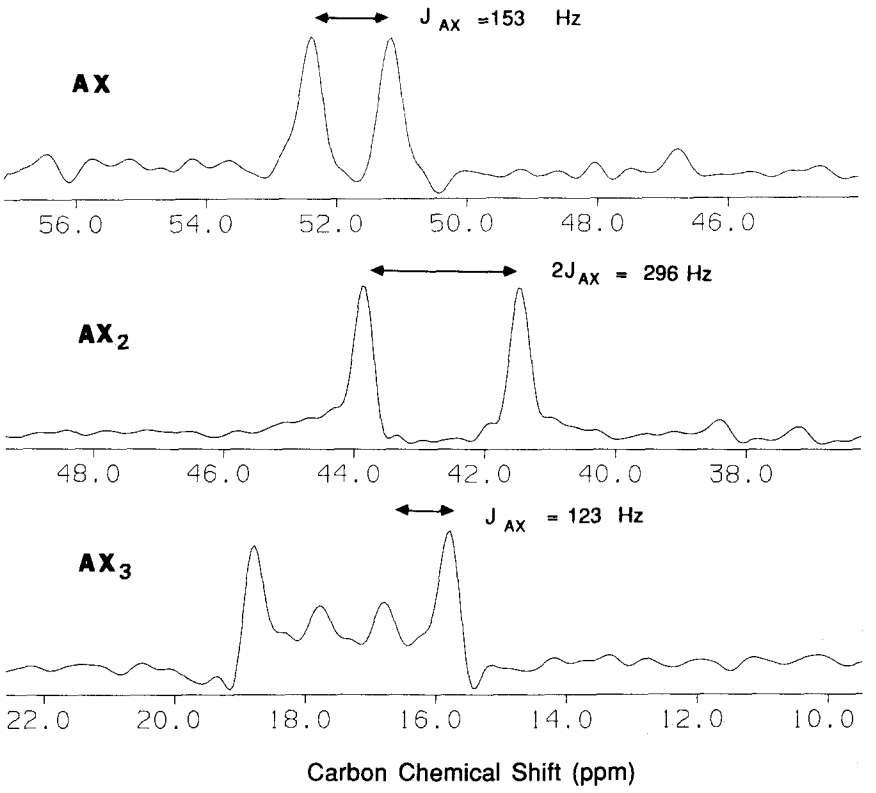

FIG. 2. Column cross sections through satellite peaks of ^1H resonances centered at δ = 3.78 ppm (αCH ala, AX), δ = 3.57 ppm (αCH_2 gly, AX_2), and δ = 1.49 ppm (βCH_3 ala, AX_3). Proton chemical shifts are relative to H_2O (δ = 4.72) while ^{13}C chemical shifts are relative to TSP.

In summary, the sequence that we have described provides a simple way of simultaneously measuring the multiplicity of ^{13}C resonances as well as ^1H and ^{13}C chemical shifts. Substantial improvements in sensitivity are obtained over schemes which provide similar information via direct detection of ^{13}C magnetization (*14*).

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