

## Investigation of $^{13}\text{C}$ - $^{13}\text{C}$ Couplings in Natural Abundance Samples: The Strong Coupling Case

Carbon-13 nuclear magnetic resonance spectra from samples with natural abundance exhibit weak satellite responses which carry information about  $^{13}\text{C}$ - $^{13}\text{C}$  coupling, but these lines may be obscured by the 200 times stronger central resonance from the molecules which contain only isolated  $^{13}\text{C}$  spins. The problem is particularly severe in the case of long-range  $^{13}\text{C}$ - $^{13}\text{C}$  couplings because the corresponding satellites are often on the steeply rising flanks of the strong central line and may be confused with spinning sidebands or spurious modulation effects caused by poor field/frequency regulation or incomplete proton decoupling. Some recent experiments (1, 2) have demonstrated the feasibility of suppressing this strong central peak while retaining the interesting satellite lines. The method relies on the momentary conversion of magnetization from coupled spins into double-quantum coherence, followed essentially immediately by reconversion into detectable transverse magnetization. Discrimination from all other signals is achieved through the characteristic phase properties of the double-quantum coherence (3-5). The result is a clean spectrum of the  $^{13}\text{C}$  satellites showing direct and long-range  $^{13}\text{C}$ - $^{13}\text{C}$  couplings. Because of the low natural abundance (1%), the spectra of interest are always of the AX or AB type, molecules with three or more  $^{13}\text{C}$  spins being extremely rare.

The pulse sequence employed to separate out the satellite spectrum may be written

$$90^\circ(X) - \tau - 180^\circ(\pm Y) - \tau - 90^\circ(X) - \Delta - 90^\circ(\Phi) - \text{Acquisition}(\Psi),$$

where  $\Delta$  is a very short fixed delay (typically 10  $\mu\text{sec}$ ) and the phase angles  $\Phi$  and  $\Psi$  are cycled in a prescribed fashion (1, 2). In the limit of first-order spin-spin coupling (AX) there is a very simple expression for the condition for optimum conversion into double-quantum coherence

$$\tau = (2n + 1)/(4J_{CC}); \quad n = 0, 1, 2, \dots \quad [1]$$

This timing parameter must therefore be set according to the estimated value of  $J_{CC}$  for optimum sensitivity, although the condition is not very critical for  $n = 0$ . It is quite feasible to satisfy this condition for two or more different couplings by judicious choice of  $n$  and  $\tau$ .

In practice quite a few of these  $^{13}\text{C}$ - $^{13}\text{C}$  spectra are strongly coupled AB systems and then Eq. [1] is not strictly applicable. It is therefore important to know how to set the pulse timing for good sensitivity when there are strongly coupled pairs of  $^{13}\text{C}$  spins; this is the purpose of the present communication.

### THEORY

After the pulse sequence  $90^\circ(X) - \tau - 180^\circ(Y) - \tau - 90^\circ(X)$  the density matrix for the system can be written

$$\begin{aligned} \sigma' = & R_X(-\pi/2) \exp(-i\mathcal{H}\tau) R_Y(-\pi) \exp(-i\mathcal{H}\tau) R_X(-\pi/2) \\ & \times \sigma_{\text{eq}} R_X(\pi/2) \exp(i\mathcal{H}\tau) R_Y(\pi) \exp(i\mathcal{H}\tau) R_X(\pi/2), \quad [2] \end{aligned}$$

where the rotation operator (3) is given by  $R_X(\alpha) = \exp(-i\alpha F_X)$ . A further  $90^\circ$  pulse with phase angle  $\Phi$  converts this into detectable magnetization given by

$$\text{Tr}(F_Y \sigma_{\text{det}}), \quad \text{where } \sigma_{\text{det}} = R_\Phi(-\pi/2) \sigma' R_\Phi(\pi/2). \quad [3]$$

If the four transition frequencies observed in the conventional AB spectrum are written  $\omega_{12}$ ,  $\omega_{34}$ ,  $\omega_{13}$ , and  $\omega_{24}$  in order of increasing frequency, and if  $\tan 2\theta = J/\delta$ , then the total intensity is proportional to

$$\begin{aligned} I = & u^2 s \sin [(\omega_{13} - \omega_{34})\tau] - uv c \sin [(\omega_{34} - \omega_{12})\tau] \\ & - uv c \sin [(\omega_{24} - \omega_{13})\tau] + v^2 s \sin [(\omega_{24} - \omega_{12})\tau], \quad [4] \end{aligned}$$

where  $s = \sin 2\theta$ ,  $c = \cos 2\theta$ ,  $u = \sin \theta + \cos \theta$ ,  $v = \sin \theta - \cos \theta$ . The second and third terms oscillate at the same frequency ( $2\pi J_{\text{CC}}\tau$ ) and may therefore be combined; these are the only significant terms in the weakly coupled limit.

$$\begin{aligned} I = & \sin 2\theta(1 + \sin 2\theta) \sin [(\omega_{13} - \omega_{34})\tau] - 2 \cos^2 2\theta \sin [(\omega_{34} - \omega_{12})\tau] \\ & + \sin 2\theta(1 - \sin 2\theta) \sin [(\omega_{24} - \omega_{12})\tau]. \quad [5] \end{aligned}$$

This result can be verified experimentally by making  $t_1 = 2\tau$  a variable and transforming the resulting data matrix  $S(t_1, t_2)$  to give a two-dimensional spectrum  $S(F_1, F_2)$  (2). A cross section through this spectrum parallel to the  $F_1$  axis and at the frequency of one of the inner satellite lines in the  $F_2$  dimension generates a one-dimensional spectrum consisting of six lines, three with positive frequencies and three with equal negative frequencies, having the relative intensities indicated by Eq. [5]. The methyl and quaternary carbons in adamantane provide a suitable example for testing this theory, since the chemical shift is 92.3 Hz and the direct coupling is 37.0 Hz, giving  $2\theta = 0.38$  radians. The experimental spectrum  $S(F_1)$  is illustrated in Fig. 1a and is compared with the predicted stick spectrum calculated from Eq. [5], showing good agreement. The observed frequencies and relative intensities are set out in Table 1. It is interesting to note that identical expressions have been calculated for the frequencies and relative intensities in a "J spectrum" obtained by Fourier transformation of the modulation of the peaks of the spin echoes from an AB spin system (6). The only difference is a trivial sign change in the first term of Eq. [5] attributable to the phase inversion which occurs in the present experiment when a line appears at a negative frequency.

These experiments were performed on a Varian XL-200 spectrometer operating at 50 MHz for  $^{13}\text{C}$ , with continuous broadband proton decoupling throughout the experiment.

#### CONCLUSIONS

The theory predicts that the *relative* intensities within an AB spectrum remain exactly the same when detected via double-quantum coherence. The *overall* intensity, however, is a function of  $J_{\text{CC}}\tau$  and the strength of the coupling  $\delta/J_{\text{CC}}$ .

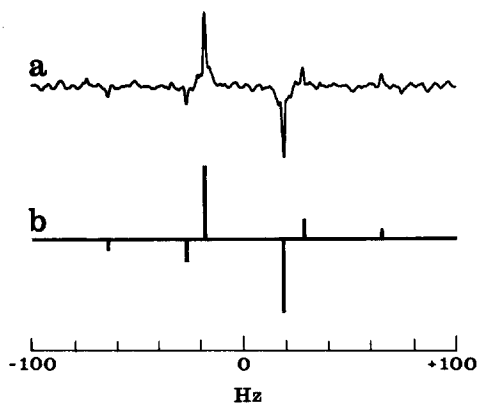


FIG. 1. (a) A cross section through the two-dimensional  $^{13}\text{C}$  spectrum of the AB spin system in tetramethyladamantane. The strong lines at  $\pm 18.5$  Hz ( $\pm(1/2)J$ ) arise from the second term in Eq. [5], and the pair of lines at  $\pm 27.5$  Hz have the same separation as the inner lines of the AB quartet, while the pair of lines at  $\pm 64.6$  Hz match the separation of the outer lines of the AB pattern. The predicted spectrum (b) is calculated from Eq. [5] using the measured values of  $\delta$  and  $J$ .

Since Eq. [5] contains three terms oscillating at different frequencies, it is not possible to give a simple prescription for setting  $\tau$  for optimum signal intensity.

The third term (oscillating at the frequency separation of the outer lines of the AB quartet) may nearly always be neglected in comparison with the other terms; consequently it is the interplay between these first two terms which effectively determines the optimum setting of  $\tau$ . At the condition  $\delta/J = 3^{1/2}$  the inner lines of the AB pattern are separated by exactly  $J$ . For all more strongly coupled cases, the first term of Eq. [5] dominates, but the second term oscillates at a higher frequency and there is a "local maximum" of signal intensity close to the condition of Eq. [1]. It is therefore possible to relate the optimum setting of  $\tau$  to  $J$  rather than the separation of the inner lines of the AB quartet.

The intensity near this local maximum may not be very high, and for cases where the coupling is stronger than the condition  $\delta/J = 2.8$  there can be a significant advantage in setting  $J\tau = 3/4$  rather than the simpler condition  $J\tau = 1/4$ . This is most easily appreciated by a graphical presentation based on numerical evalua-

TABLE I  
CALCULATED AND OBSERVED RELATIVE INTENSITIES  
IN THE  $F_1$  SPECTRUM OF THE AB SYSTEM  
IN TETRAMETHYLADAMANTANE

Line frequency	Calculated intensity	Observed intensity
$(\omega_{34} - \omega_{12})/2\pi; \pm 18.5$ Hz	1.00 <sup>a</sup>	1.00 <sup>a</sup>
$(\omega_{13} - \omega_{34})/2\pi; \pm 27.5$ Hz	0.29	0.27
$(\omega_{24} - \omega_{12})/2\pi; \pm 64.6$ Hz	0.14	0.16

<sup>a</sup> Normalized.

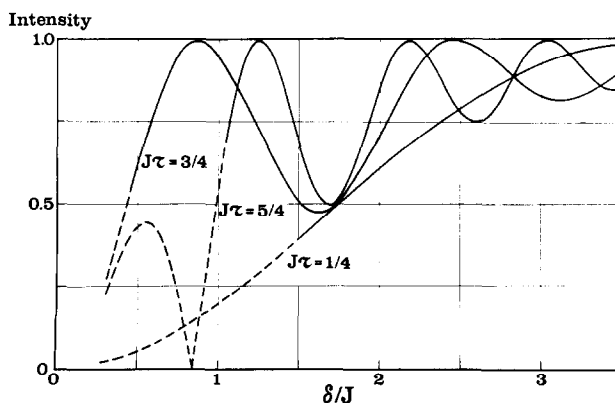


FIG. 2. Calculated intensities for the  $^{13}\text{C}$ - $^{13}\text{C}$  satellite spectra as a function of the strength of the coupling in an AB spin system. For weak coupling,  $J\tau = 1/4$  is an optimum setting and the overall intensity is high. For strong coupling with  $\delta/J < 2.8$ , there is an advantage in setting  $J\tau = 3/4$  or even  $5/4$ . Where the curves are represented by a broken line the timing condition shown deviates significantly from the optimum. For extremely strong coupling, where  $\delta/J < 0.5$ , a different criterion should be adopted for choosing  $\tau$ .

tion of Eq. [5] as illustrated in Fig. 2. Several general conclusions can be drawn from this graph. For all weakly coupled systems  $J\tau = 1/4$  is the best choice, and it remains reasonably good down to the condition  $\delta/J = 3^{1/2}$ . For strong coupling better signal intensity can be achieved with the condition  $J\tau = 3/4$ , although this is three times as sensitive to mismatch. In some limited regions of the graph the condition  $J\tau = 5/4$  (or even higher multiples) can give stronger signals. The curves for the three conditions  $J\tau = 1/4, 3/4, 5/4$  are plotted in Fig. 2, the broken lines indicating that in these regions there may not be a suitable "local maximum" of signal intensity given approximately by Eq. [1]. Such settings of  $\tau$  should therefore be avoided. Note that  $J\tau = 1/4$  is an unsatisfactory choice for strongly coupled spins. For extremely strong coupling, where  $\delta/J$  is less than about 0.5, a different strategy should be adopted since the first term of Eq. [5] dominates completely. The timing should then be adjusted so that  $(\omega_{13} - \omega_{34})\tau = \pi/2$ , which requires a knowledge of the separation of the inner lines of the AB quartet, that is, an estimate of the chemical shift difference as well as  $J$ .

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