

Investigation of ^{13}C - ^{13}C Long-Range Couplings in Natural-Abundance Samples

A recent communication (1) has suggested a method of studying ^{13}C - ^{13}C couplings in samples with the natural ^{13}C abundance by suppressing the strong central resonance from molecules containing a single isolated ^{13}C nucleus, revealing the weak satellite signals from the coupled ^{13}C - ^{13}C systems. Excellent suppression ratios were achieved by momentary conversion of the magnetization from coupled spins into double-quantum coherence (2, 3), exploiting the characteristic phase properties of the latter. The principal restriction on the generality of this technique arises from the condition for optimum transfer into double-quantum coherence (4, 5). For the case of weakly coupled spins, this condition is

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

where τ is the delay in the pulse sequence

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

(The delay Δ remains fixed at 10 μsec throughout.) Optimum transfer requires either a good estimate of J_{CC} or the trial-and-error adjustment of the τ delay. A more serious problem occurs when there are several different ^{13}C - ^{13}C couplings to be observed in the same spectrum. This can be achieved by a judicious choice of τ with suitable different values of n , aiming at a set of compromise conditions near the optima predicted by Eq. [1]. For the routine application to problems where no a priori estimates of the couplings have been made, a more general method would clearly be useful.

A method is now proposed which utilizes the concept of two-dimensional spectroscopy (3, 6) to allow τ to become a variable parameter in this experiment. In principle this should exhibit a sensitivity comparable with that of the one-dimensional experiment with optimized τ delays. The period 2τ becomes the well-known evolution period t_1 , and two-dimensional Fourier transformation creates a spectrum $S(F_1, F_2)$ where the F_2 dimension carries the conventional ^{13}C spectral information (but with the strong central signals suppressed), while F_1 carries resonances at $\pm(1/2)J_{CC}$ corresponding to the transform of the time domain signal,

$$S(t_1) = \sin(\pi J_{CC} t_1) \exp(-t_1/T). \quad [2]$$

Because of the refocusing effect of the 180° pulse, the decay time constant T of this signal can approach the spin-spin relaxation time T_2 under favorable conditions, and the resolution in the F_1 dimension may be considerably enhanced in comparison with conventional experiments. Thus, although the ^{13}C - ^{13}C splittings appear in both frequency dimensions, there is a marked advantage in observing them in the F_1 dimension. This is conveniently achieved by selecting the appropriate traces from the complete two-dimensional spectrum, the equivalent of

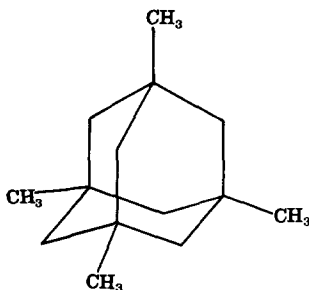


FIG. 1. Tetramethyladamantane.

taking cross sections through $S(F_1, F_2)$ at fixed values of F_2 . In general it is not necessary to plot the two-dimensional spectrum.

In order to illustrate the technique, an investigation was made of the long-range couplings in tetramethyladamantane, chosen partly because the alternative approach, specific isotopic enrichment, is particularly difficult in ring compounds. As can be appreciated from Fig. 1, symmetry considerations limit the observable long-range C-C couplings to four:

$${}^2J(\text{CH}_3\text{-C-CH}_2), \quad {}^3J(\text{CH}_3\text{-C-CH}_2\text{-C}), \quad {}^3J(\text{CH}_2\text{-C-CH}_2\text{-C}), \\ {}^4J(\text{CH}_3\text{-C-CH}_2\text{-C-CH}_2).$$

Three of these couplings turn out to be less than 1 Hz, making this a stringent test of the method.

The measurements were made at 50 MHz on a Varian XL-200 spectrometer using the pulse sequence described above. Broadband proton decoupling is used throughout the experiment. Several precautions are necessary to achieve good suppression of the central signal from molecules with isolated ^{13}C spins. The phase angles Φ and Ψ are rotated in 90° steps in opposite senses, so that the receiver reference phase follows the phase shifts of the signal derived from double-quantum coherence. The phase of the 180° pulse is alternated to reduce the effects of pulse imperfections. Finally, all the radiofrequency pulse phases and the receiver phase are cycled in 90° steps, giving a complete sequence of 32 steps. Since sensitivity considerations make it inadvisable to wait for complete recovery of the equilibrium signal through spin-lattice relaxation, each new section of the experiment is started with a 90° pulse followed by the standard delay time ($T_d \sim T_1$) to ensure that the signals in each step of the cycle are essentially identical, giving precise cancellation of the strong unwanted signals. Careful calibration of the 90 and 180° pulse lengths has also been found to be important. Suppression ratios in excess of 1000:1 can be achieved.

The number of increments in the t_1 dimension is a compromise between the requirement for adequate digitization in the F_1 dimension and the desire to minimize the overall length of the experiment; in practice 32 increments were used. The overall data matrix consisted of 32×4096 words. The signals (t_2) were weighted with a Gaussian function to give a linewidth in the F_2 dimension of 0.35 Hz. Thus although in principle each splitting should appear on a different

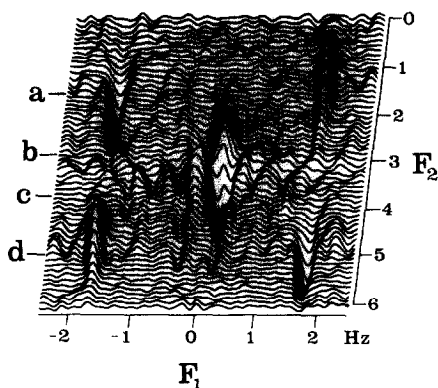


FIG. 2. A small section of the two-dimensional ^{13}C spectrum of tetramethyladamantane, approximately centered on the methyl carbon chemical shift frequency. Note the suppression of the strong signal from isolated ^{13}C molecules. Traces (a) and (d) carry splittings due to $^3J(\text{CH}_3\text{-C-CH}_2\text{-C})$, while traces (b) and (c) carry splittings due to both $^2J(\text{CH}_3\text{-C-CH}_2)$ and $^4J(\text{CH}_3\text{-C-CH}_2\text{-C-CH}_2)$ which are not resolved in this diagram.

horizontal (F_1) trace, in practice as many as three splittings appeared together, because of overlap of the signals in the F_2 dimension. The same splitting information occurs on both sides of the ^{13}C chemical shift frequency, the spectra having antiphase intensities. This can be seen in Fig. 2, which shows a small section taken from the two-dimensional spectrum encompassing the resonances from the

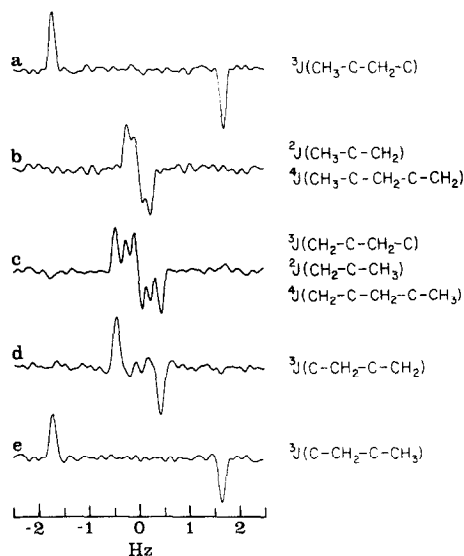


FIG. 3. Spectra showing the long-range $^{13}\text{C}\text{-}^{13}\text{C}$ couplings in tetramethyladamantane obtained as sections through the two-dimensional spectrum parallel to the F_1 axis. A resolution-enhancement function has been used. Traces (a) and (b) originate from the methyl groups, (c) from the methylene groups, and (d) and (e) from the quaternary sites. A small artifact near the center of trace (d) is attributable to the failure to allow complete spin-lattice relaxation between sections of the experiment.

TABLE 1
CARBON-CARBON COUPLINGS IN TETRA-
METHYLADAMANTANE (Hz)

$^1J(\text{C}-\text{CH}_3) = 37.00 \pm 0.05$
$^1J(\text{C}-\text{CH}_2) = 32.23 \pm 0.05$
$^2J(\text{CH}_3-\text{C}-\text{CH}_2) = 0.45 \pm 0.03^a$
$^3J(\text{CH}_3-\text{C}-\text{CH}_2-\text{C}) = 3.38 \pm 0.03$
$^3J(\text{CH}_2-\text{C}-\text{CH}_2-\text{C}) = 0.89 \pm 0.03$
$^4J(\text{CH}_3-\text{C}-\text{CH}_2-\text{C}-\text{CH}_2) = 0.14 \pm 0.03^a$

^a Assigned on the assumption that $|^2J| > |^4J|$.

methyl carbon sites. Trace (a) shows an antiphase doublet due to $^3J(\text{C}-\text{CH}_2-\text{C}-\text{CH}_3)$ while trace (d) carries a similar doublet with the intensities inverted. Consequently traces (a) and (d) were combined in antiphase to give a resultant spectrum with improved signal-to-noise. Similarly trace (b) was inverted and then combined with trace (c) to give a spectrum illustrating the smaller splittings $^2J(\text{CH}_3-\text{C}-\text{CH}_2)$ and $^4J(\text{CH}_3-\text{C}-\text{CH}_2-\text{C}-\text{CH}_2)$ which are not quite resolved in this spectrum.

In this way five critical spectra were extracted from the two-dimensional data; they are set out in Fig. 3. The sensitivity achieved by an overnight run was sufficient to permit the application of a resolution enhancement function in the t_1 dimension, reducing the linewidth in F_1 from 0.2 to 0.1 Hz. This allowed all four long-range splittings to be resolved. Each splitting appears twice in these spectra, which helps to confirm their assignment, the only ambiguity being between $^2J(\text{CH}_2-\text{C}-\text{CH}_3)$ and $^4J(\text{CH}_2-\text{C}-\text{CH}_2-\text{C}-\text{CH}_3)$; these were tentatively assigned on the assumption that $|^2J| > |^4J|$. The results are set out in Table 1, along with the two direct couplings observed in a separate experiment.

By removing the necessity of estimating τ values, the proposed extension of the original $^{13}\text{C}-^{13}\text{C}$ coupling experiment makes the application to unknown long-range couplings quite general. The attainable sensitivity is expected to be comparable with the one-dimensional experiment, particularly since it is possible to combine pairs of traces carrying the same information. The resolution may be significantly improved through the refocusing effect of the 180° pulse at the midpoint of the evolution period.

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