

Carbon-13 Nuclear Magnetic Resonance Spectra with Coherent Proton Decoupling: Peak-Height Distortions within Spin Multiplets

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Contribution from the Physical Chemistry Laboratory, Oxford University, Oxford, England. Received March 13, 1978

Abstract: Experiments on the ^{13}C NMR spectrum of methyl iodide with coherent off-resonance proton decoupling indicate that spatial inhomogeneity of the decoupler field causes a preferential broadening of the outer lines of the quartet. For ^{13}C spectra with poor signal to noise ratio, this effect could lead to misinterpretation—quartets being mistaken for doublets, or triplets being mistaken for single lines. Computer-simulated spectra illustrate how this preferential broadening depends on the proton decoupler offset. Several methods for circumventing this problem are discussed. An experimental procedure is presented for mapping a decoupling field.

Coherent off-resonance proton decoupling is a widely used technique for the assignment of carbon-13 resonances to methyl, methylene, methine, or quaternary carbon sites.

A theoretical analysis predicts that the resulting carbon-13 multiplets have reduced splittings but retain the normal binomial intensity distribution, provided that the proton irradiation is sufficiently intense.² In practice, peak heights with exact binomial distribution are seldom observed for triplets or quartets. The effect is symmetrical with respect to the center of the multiplet, the outer peaks being noticeably weaker than expected from the theoretical 1:2:1 or 1:3:3:1 peak height ratios. This distortion can have serious consequences, leading to misassignment of a triplet as a singlet or a quartet as a doublet³ in carbon-13 spectra of poor signal to noise ratio. An example of a distorted triplet and quartet is shown in Figure 1 where the outer lines of a quartet are almost lost in the noise.

Measurements on methyl iodide indicate that the effect involves a preferential broadening of the outer lines of the carbon-13 quartet rather than any true intensity perturbation. Since the frequencies of the outer lines are more sensitive to the intensity of the decoupling field, this suggests that the phenomenon arises out of the spatial inhomogeneity of the proton decoupler field B_2 . NMR samples are commonly examined in cylindrical tubes where the intensity of the decoupler field varies significantly along the effective length of the sample; this has been demonstrated experimentally. In principle an instability in the level of proton irradiation during time averaging of the carbon-13 free induction signal could cause a similar preferential broadening of the outer lines. In practice there is little reason why a well-designed decoupler should suffer significant level fluctuations over this period as long as temperature changes in the decoupler coil are avoided. Spatial inhomogeneity is therefore believed to be the dominant factor.

The "Quartet Effect"

The intensities and line widths in a carbon-13 quartet under coherent proton decoupling conditions will be considered specifically. Although similar arguments apply to the case of a triplet, that situation is complicated by the existence of symmetric and antisymmetric spin states with different relaxation properties⁴ and the fact that the methylene protons are more likely than methyl protons to form part of a strongly coupled spin system.

A. Line Broadening. The calculation of the broadening of a carbon-13 quartet by the spatial inhomogeneity of the decoupler field is based on the treatment given by Anderson.² The splitting S is a function of the irradiation field $F = \gamma B_2/2\pi$ Hz applied at an offset Δ Hz from resonance

$$S_i = [(\Delta + \frac{1}{2}J)^2 + F^2]^{1/2} - [(\Delta - \frac{1}{2}J)^2 + F^2]^{1/2} \quad (1)$$

and

$$S_o = 3S_i \quad (2)$$

The subscripts i and o refer to the inner and outer lines of the quartet. In practice $F \gg J$ and this expression may be simplified to

$$S_i = J\Delta/(\Delta^2 + F^2)^{1/2} \quad (3)$$

which is useful for calibrating the intensity of B_2 experimentally.

The equations show that if Δ or F varies across a sample, the observed splittings will reflect this distribution and the effect will be three times as great for the outer lines compared to the inner lines. This selective broadening accounts for the anomalous peak height ratios observed in coherent off-resonance decoupling experiments. When broadening by this effect is dominant, an upper limit for the peak height ratio of 1:9:9:1 may be anticipated. When broadening by this effect is small and natural line widths dominate, the expected 1:3:3:1 ratio of peak heights is restored.

A detailed calculation of the carbon-13 line shapes in a double resonance experiment, where the resonance condition depends on the intensities of both the static field B_0 and the decoupler field B_2 , requires a knowledge of the distribution of both of these fields and how they correlate in space. (Experimentally this may lead to an asymmetric broadening of the quartets as both the residual splitting and the carbon-13 shift vary along the sample.) Since the distribution of the B_0 field is subject to gross changes from several sources (for example, thermal distortion of the magnet) such a calculation would not be particularly useful in practice. A simpler and more general result is obtained by assuming that the effects of B_0 inhomogeneity are outweighed by the natural line width of the carbon-13 resonances, on which the decoupler imposes a further broadening, dependent on the offset from exact proton resonance.

The greatest variation of B_2 field intensity is along the length of the sample, and the effect of radial gradients has been neglected in this calculation. It was possible to map out the variation of B_2 as a function of height above and below the center of the receiver coil, using a small spherical sample of methyl iodide. The residual splitting S was used as a measure of F at small offsets Δ such that $\Delta \ll F$ and $S = J\Delta/F$ Hz. The form of this measured field distribution is shown in Figure 2a. In the same experiment the relative strength of the carbon-13 signal as a function of sample position was also measured, reflecting the distribution of the intensity of the observing radio-frequency field B_1 (Figure 2b).

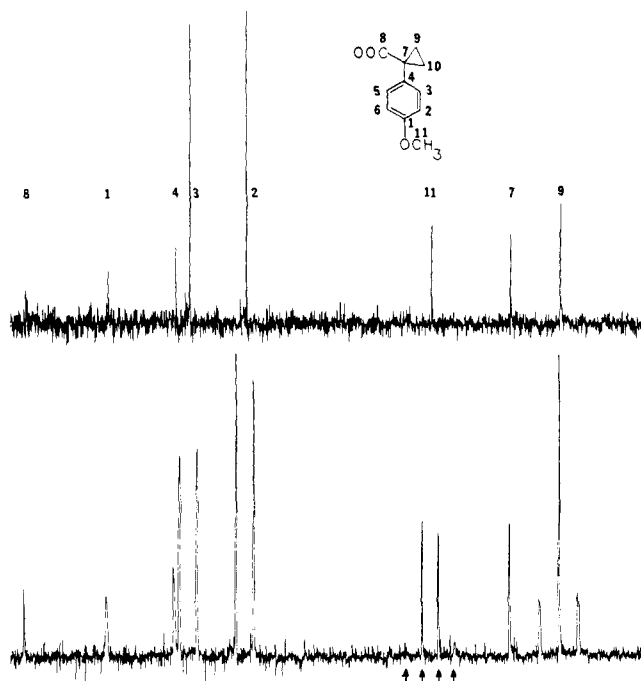


Figure 1. Part of the carbon-13 NMR spectrum of 1-*p*-anisylcyclopropylcarboxylate in D₂O with proton noise decoupling (upper trace) and coherent off-resonance decoupling (lower trace). Note the weak outer lines of the methoxy group quartet, indicated by the arrows, and of the methylene group triplet.

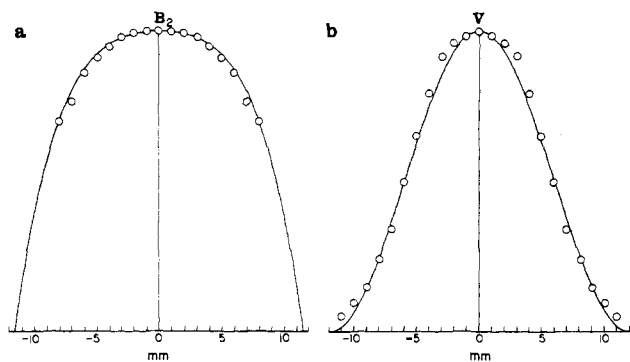


Figure 2. (a) Variation of the proton decoupler field intensity B_2 (arbitrary units) as a function of the height (x mm) above or below the center of the receiver coil in a CFT-20 spectrometer. The experimental measurements (circles) were made with a small bulb sample of methyl iodide and have been fitted to the curve $B_2 = 1 - ax^2 - bx^4$, where $a = 0.0023$, $b = 0.000045$. (b) The corresponding variation of relative signal intensity V with sample height, fitted to a curve $V = \cos^2 \alpha$ where $\alpha = 7.5^\circ$.

For the purposes of this calculation, the B_2 distribution may be represented by a parabolic function with a small fourth-order term, and the signal strength distribution by a cosine squared function. A combination of these two curves provides a plot of signal intensity as a function of decoupler field strength, a curve with a long tail on one side and an abrupt cutoff at maximum B_2 field (Figure 3). This allows the line profiles of the carbon-13 resonances to be calculated through eq 1 and 2 for a given offset Δ and a given nominal setting of B_2 .

The resulting spectra exhibit distorted line profiles, reflecting the asymmetry of Figure 3. When this is the dominant broadening influence on all lines, the profile of the outer lines is the same as that of the inner lines but scaled by a factor of 3 along the frequency axis, giving a peak height ratio of 1:9:9:1. The introduction of a carbon-13 natural line width by convolution with a Lorentzian goes some way to restoring the normal

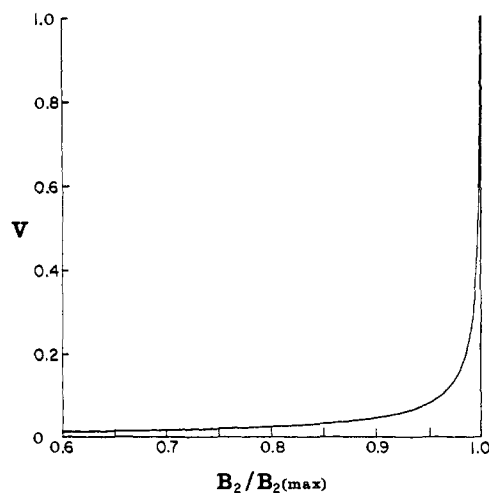


Figure 3. The variation of signal intensity V as a function of proton decoupler field intensity in a CFT-20 spectrometer, calculated from the curves in Figure 2.

1:3:3:1 ratio, since its effect is greater on the narrower lines. The dependence of the decoupler broadening effect on the decoupler offset is most easily appreciated from a "three-dimensional" diagram obtained by stacking spectra for a series of different offsets.⁵ This is shown in Figure 4. Note the gross distortion of the outer lines in the region of decoupler offset of about 1–5 kHz. Even at the largest decoupler offsets used in the calculation (15 kHz) peak height anomalies are still in evidence. Figure 5 shows the calculated ratio of the height of the inner peaks compared with the outer peaks as a function of decoupler offset.

B. Line Intensities. The relative intensities within a quartet during coherent decoupling experiments are in the ratio 1:3:3:1 only in the limit of strong irradiation. This can be seen from the explicit expressions given by Anderson² for the intensities of the A lines of an AX_3 system where X is irradiated. For the purpose of these calculations, spin population effects on the relative intensities are neglected; for example, it might be assumed that both X resonances are completely saturated and that all A transitions experience an identical nuclear Overhauser enhancement. If the irradiation is sufficiently strong that $F \gg J$, a condition easily satisfied in most decoupling experiments, then the ratio of the intensity of the inner lines compared with that of the outer lines is readily shown to be

$$I_i/I_o = 3\{1 + [FJ/(F^2 + \Delta^2)]^4/8\} \quad (4)$$

with higher even-powered terms which can be neglected. Some off-resonance decoupling experiments are carried out with reduced levels of decoupler field B_2 , but even with a setting as low as $F = 1000$ Hz and $J = 200$ Hz, this deviation from the 1:3:3:1 ratio is only 2 parts in 10^4 and therefore cannot account for the observed "quartet effect".

An alternative source of peak height distortion within a carbon-13 multiplet may occur if the carbon-13 nucleus is coupled to two or more groups of protons that are strongly coupled among themselves. The long-range proton-carbon splittings may remain unresolved but can introduce differential broadening of the lines of the carbon-13 multiplet.⁶ This usually leads to an unsymmetrical distortion of the peak heights. Sometimes the situation is aggravated because the protons are more strongly coupled in the presence of the strong irradiation field B_2 than in the unperturbed spectrum.⁷ However, it is quite clear that strong coupling effects cannot explain the symmetrical distortions under consideration here, since the latter occur in systems with only one set of equivalent protons, such as methyl iodide.

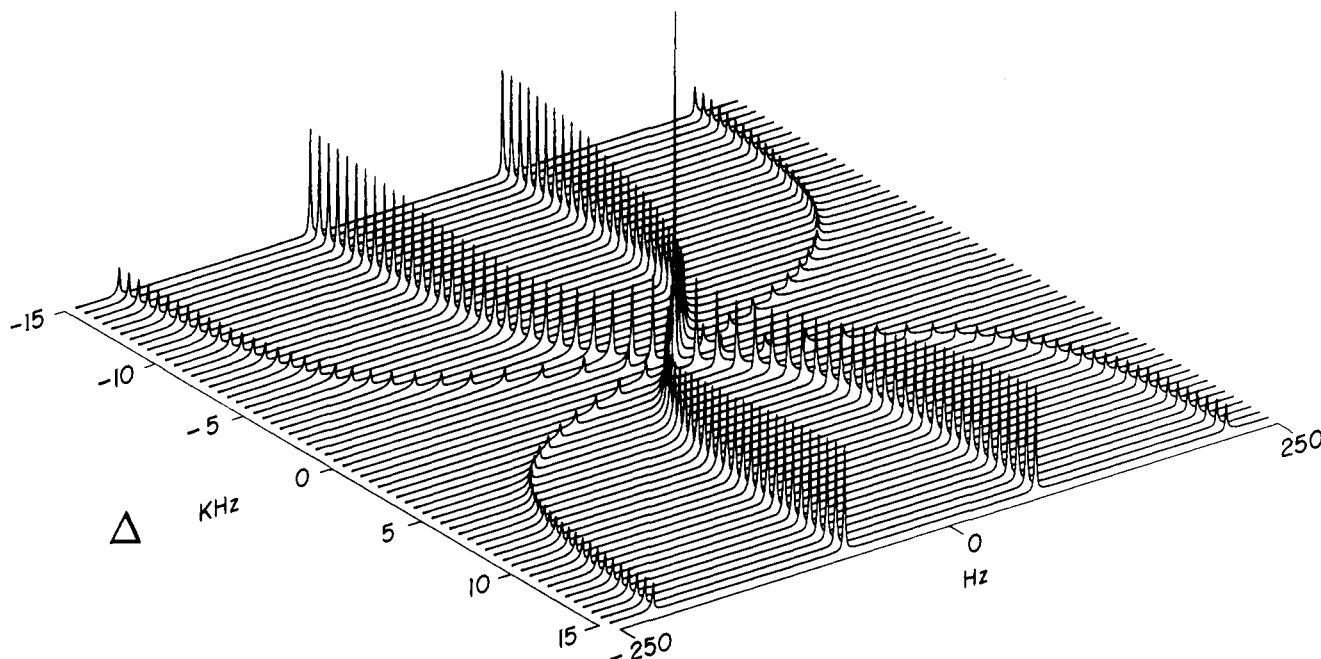


Figure 4. Simulation of the carbon-13 spectrum of a methyl group ($J = 150$ Hz) with coherent proton decoupling ($F_{max} = 4$ kHz) at various offsets Δ . The line widths reflect the distributions of decoupler field intensity and signal intensity shown in Figures 2 and 3, together with a Lorentzian natural line width of 1 Hz. Note the severe broadening and distortion of the outer lines for offsets between about 1 and 5 kHz, leading to a pronounced "quartet effect".

This symmetrical distortion should be also distinguished from spin population effects in which one satellite in the proton spectrum is more strongly saturated than the other, inducing the well-known asymmetric distortion of the relative intensities in the carbon-13 spectrum.⁸ In a Fourier transform spectrometer this type of frequency-selective nuclear Overhauser effect is readily eliminated by preparing the spin system under conditions of wide-band incoherent proton irradiation, switching to coherent off-resonance irradiation just before the carbon-13 excitation pulse. In general the intensity distortions from differential saturation are quite small when the proton decoupler field is strong, and particularly when its displacement from exact resonance is large compared with the appropriate coupling constant.

Experimental Section

Spectra were obtained on a Varian CFT-20 spectrometer which uses a single coil of height 8 mm for excitation and detection of the carbon-13 signal. The proton decoupling field is provided by a pair of rectangular saddle-shaped coils 20 mm high and orthogonal to the carbon-13 coil. Samples were normally examined in cylindrical tubes 10 mm in diameter filled to a depth of about 3 cm. At its maximum setting the decoupler field strength was measured as $F = \gamma B_2 / 2\pi = 6.7$ kHz.

Evidence for distortion of the expected 1:3:3:1 peak height ratio in a quartet was obtained with isotopically enriched methyl iodide samples. With the conventional cylindrical sample a 1:6:6:1 quartet was observed (Figure 6a) but the integrals remained in the ratio 1:3:3:1, confirming that this was a line width effect rather than a true intensity effect. When this experiment was repeated with a Wilmad 35- μL bulb sample, carefully centered in the receiver coil, the peak heights returned to the expected 1:3:3:1 ratio (Figure 6b) since the spatial inhomogeneity of B_2 at this location and over this restricted volume does not broaden the lines appreciably. This establishes that the "quartet effect" originates predominantly from the broadening induced by the spatial inhomogeneity of the decoupler field, with negligible contributions from the other possible sources of distortion considered above. It also rules out the possibility that the relative intensities are significantly perturbed by relaxation through the dipolar mechanism considered by Bain et al.⁸ Further evidence against explanations based on relaxation effects was obtained by reproducing

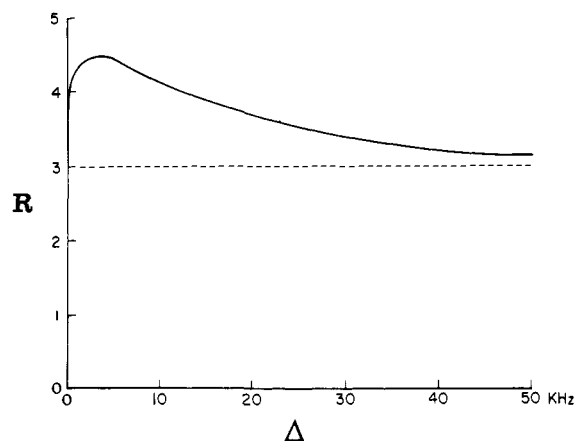


Figure 5. The ratio R of peak heights of inner and outer lines of the quartets of Figure 4 as a function of the decoupler offset Δ , illustrating the difficulty of avoiding a "quartet effect" in practical cases of off-resonance decoupling.

the 1:6:6:1 quartet of Figure 6a with the decoupler switched on only for the acquisition of the free induction signal.

Similar considerations apply to the "triplet effect". Methylene chloride contained in a cylindrical sample tube gives a carbon-13 triplet where the center peak is 3.6 times taller than the outer peaks (Figure 6c). Although for the "quartet effect" there is a maximum distortion of 1:9:9:1, there is no corresponding limiting condition for triplets since the center line is not broadened at all by B_2 inhomogeneity.

Conclusions

The danger of misinterpretation of coherently decoupled carbon-13 spectra recedes once the origin of the peak height distortion is understood. The effect may be eliminated by integrating the component lines of the multiplet, or reduced by imposing a severe sensitivity enhancement function on the free induction decay to swamp the differential broadening caused by B_2 . While it is unlikely to be convenient to use small spherical samples to minimize this effect, restriction of the

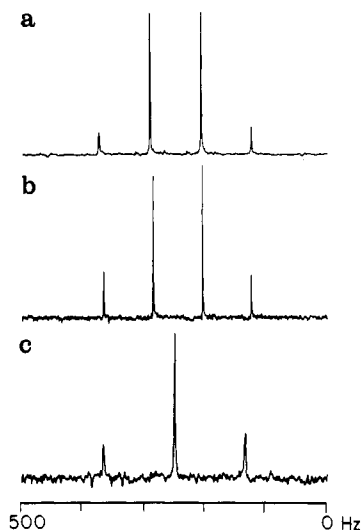


Figure 6. "Quartet" and "triplet effects" observed in carbon-13 spectra with coherent proton decoupling ($\Delta = 4$ kHz, $F_{max} = 6.7$ kHz). (a) Methyl iodide in the usual cylindrical sample tube, 3 cm high, showing a 1:6:6:1 peak height ratio. (b) Methyl iodide in a 35- μ L bulb under the same conditions, showing the expected 1:3:3:1 ratio. (c) Methylene chloride in a cylindrical tube, showing a central peak 3.6 times taller than the outer peaks.

depth of a liquid in a cylindrical tube should reduce the differential broadening to some extent.

The simulations shown in Figures 4 and 5 suggest that, although the magnitude of the "quartet effect" diminishes very close to resonance and very far from resonance, it is nevertheless significant over essentially the entire experimental

range and cannot be completely avoided by a suitable choice of offset. In practice Δ and F must be chosen so as to keep the splittings reasonably small to avoid overlap problems in crowded spectra, and with these settings the broadening by decoupler field inhomogeneity will tend to be large.

This problem provided the impetus for the development of an alternative method for observing the proton-carbon multiplet splittings in carbon-13 spectra, a technique known as "J-scaling".⁹ Since this method is insensitive to the inhomogeneity of the decoupler field it should be particularly useful where off-resonance proton decoupling gives ambiguous results.

Acknowledgments. The authors thank Dr. Geoffrey Bodenhausen for invigorating discussions and discerning criticism. This work was supported by an equipment grant and research studentships (G.A.M. and D.L.T.) from the Science Research Council. The authors' interest in the "quartet effect" was stimulated by discussions with Professor F. A. L. Anet.

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Heat Capacity and Structure in Liquids. Application to the Structure of Water

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Received February 10, 1978

Abstract: Hydrogen-bonded molecules and long-chain molecules have recently been shown to have abnormally high liquid heat capacities. It is now further shown that these abnormal values can only be accounted for by postulating an isomerization reaction. An analysis is made of the heat capacity contribution $\Delta C_{p(\text{isom})}$ of a simple isomerization reaction $A \rightleftharpoons B$ and it is shown that $C_{p(\text{isom})}$ can amount to as much as 8-10 cal/mol·K depending on the thermochemistry of the isomerization. Application is made to H_2O and it is shown that $\Delta H = 2.2 \pm 0.2$, $\Delta S = 8.0 \pm 1$ will account for the excess ΔC_p over the range 0-100 °C. These yield mole fractions for X_B of 0.5 at 0 °C and 0.75 at 100 °C. If form A is assumed ice-like then $\Delta V = 3.2$ cm³/mol and setting the coefficient of expansion $\alpha_{H_2O} = 0$ at 4 °C leads to a simple expression for the molar volume of water which reproduces the coefficient of thermal expansion very well in the range 0-100 °C with no additional assumptions. With additional assumptions about the compressibilities of the two forms, the minimum at 45 °C in the compressibility of water can also be reproduced.

Introduction

The molar heat capacity at constant pressure, C_p , increases when a gas is condensed to a liquid at constant temperature. This increase in C_p , $\Delta C_{p(\text{vap})}$, turns out to be nearly a constant¹ for what are termed normal liquids,² namely, those that have Trouton constants ($\Delta H_{\text{vap}}/T_B$) equal to 20 cal/mol·K (eu). For the others, "abnormal" liquids, the departure of $-\Delta C_{p(\text{vap})}$

from an average value of about 12 eu³ at 25 °C can be used to provide quantitative information about their structure and hence also information about their other "abnormal" properties.

Heat Capacity of Liquids

A general quantitative approach to $\Delta C_{p(\text{vap})}$ has been difficult because of the paucity of data on C_p for both liquids and