^{15}N Nuclear Magnetic Resonance Spectroscopy. XII. Nuclear Overhauser Enhancement of ^{15}N Resonances[1]

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Abstract: The enhancement of the ^{15}N signal intensity of ammonium chloride by means of the nuclear Overhauser effect (NOE) has been measured as a function of pH. At low pH under conditions of complete proton decoupling, the ratio of the coupled to decoupled signal intensities is about 56% of the theoretical maximum and the signal is inverted. As the pH is raised, the decoupled signal progressively diminishes in intensity, disappears, and reappears upright. The observations are explained in terms of contributions to relaxation from spin–rotation interactions and from chemical modulation of the N–H spin–spin coupling constant. Implications for natural-abundance ^{15}N spectroscopy are discussed.

The advantage of the nuclear Overhauser effect (NOE) for enhancement of natural-abundance ^{13}C resonances has been amply demonstrated.[2] For the resonances of the much less abundant and less easy to detect ^{15}N nucleus a corresponding enhancement would be of great value, especially because natural-abundance ^{15}N spectroscopy is now experimentally feasible.[3] In order to elucidate some of the factors affecting the NOE of ^{15}N resonances, we report here the results of a study of ammonium-^{15}N chloride, together with their theoretical and practical implications.

Experimental Section

Ammonium-^{15}N chloride (95.5% enriched, Isomet Corp.) was dissolved in water, filtered through Dowex 1A chelating resin to remove heavy metal salts, and evaporated to dryness under reduced pressure. Solutions ($\sim$0.5 *M*) for the nmr studies were in triply distilled conductivity water and each was degassed by passing nitrogen through the solution. Spectra were determined on a Varian DFS-60 spectrometer operating at 6.07 MHz, with associated proton-decoupling equipment as previously described.[4,5] The sample temperatures under decoupling conditions were $\sim$50°; in order to minimize effects of temperature changes, spectra taken without proton decoupling were recorded with the decoupler offset to a nonperturbing frequency. Each pair of coupled and decoupled spectra was taken under the same conditions of power level and sweep rate, and were recorded after the same number of accumulated scans. Areas were measured by counting squares; the results are the average of ten independent measurements in 1 *M* hydrochloric acid, and three at the other acid concentrations. The pH was measured at room temperature with a Radiometer Model pH 26 meter using a combined glass electrode. The pH changes were made by adding concentrated hydrochloric acid; volume changes were negligible.

Results

While the proton-coupled ^{15}N spectrum of ammonium chloride in $\sim$1 *M* hydrochloric acid consists of a quintet (J_{NH} = 72.6 Hz), irradiation of the sample at 60 MHz collapses the pattern to a sharp inverted singlet (Figure 1). Comparisons of the areas under the curves show the ratio of the decoupled to coupled intensities, I_d/I_c, to be -2.22 ± 0.25.[6] As the pH is raised, the coupled signal first broadens and disappears because of exchange with the medium,[7] then reappears as a broadened singlet. The inverted decoupled ^{15}N signal decreases in intensity with increasing pH, disappears, and reappears with the same polarity as the nonirradiated signal, but with diminished intensity. These observations are summarized in Table I and illustrated in Figure 2.[8]

Table I. Variation of the Nuclear Overhauser Enhancement of Ammonium-^{15}N Chloride with pH[a]

pH	I_d/I_c[b]
$\sim$0	-2.22
0.08	-1.9
4.58[c]	-1.7
5.31	0
6.41	+0.5
7.26	+0.5

[a] Measured at a concentration of $\sim$0.5 *M*. [b] Ratio of the intensities of the proton-decoupled and proton-coupled signals. [c] Between pH 0.08 and 4.58, I_d/I_c was not measurable because the coupled spectrum was not observable.

Discussion

Figure 3 shows the various relaxation probabilities, W_i, in an AX system in which the sign of the magnetogyric ratio, γ_A, of nucleus A (^{15}N) is opposite to that of γ_X (^{1}H). The effect of such a sign opposition is to change the labeling of the spin states from that in which both γ's have the same sign (*e.g.*, Figure 1 of ref 9a)

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[1] (a) Part XI: R. L. Lichter and J. D. Roberts, *Spectrochim. Acta, Part A*, **26**, 1813 (1970); (b) supported by the Public Health Service, Research Grant No. GM-11072-07 from the Division of General Medical Sciences.

[2] (a) E. G. Paul and D. M. Grant, *J. Amer. Chem. Soc.*, **86**, 2977 (1964); (b) D. E. Dorman and J. D. Roberts, *ibid.*, **92**, 1305 (1970); (c) A. J. Jones, D. M. Grant and K. F. Kuhlmann, *ibid.*, **91**, 5013 (1969).

[3] R. L. Lichter and J. D. Roberts, submitted for publication.

[4] F. J. Weigert and J. D. Roberts, *J. Amer. Chem. Soc.*, **89**, 2967 (1967); **90**, 3543 (1968).

[5] F. J. Weigert, M. Jautelat, and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S.*, **60**, 1152 (1968).

[6] In order to avoid ambiguities of definition, especially in the present case where proton irradiation can lead to a diminution in intensity, we shall refer to the ratio I_d/I_c as the value of the NOE.

[7] (a) M. T. Emerson, E. Grunwald, and R. A. Kromhout, *J. Chem. Phys.*, **33**, 547 (1960); (b) H. Benoît, H. Ottavi, and J. Pommier, *C. R. Acad. Sci., Paris*, **256**, 399 (1963).

[8] At pH values higher than those given in Table I, the nitrogen resonance could not be observed either with or without proton irradiation. This phenomenon, which also has been encountered in a study of ^{15}N-enriched amino acids (R. L. Lichter and J. D. Roberts, unpublished results), is not yet understood.

[9] (a) K. F. Kuhlmann and D. M. Grant, *J. Amer. Chem. Soc.*, **90**, 7355 (1968); (b) K. F. Kuhlmann, D. M. Grant, and R. K. Harris, *J. Chem. Phys.*, **52**, 3439 (1970).

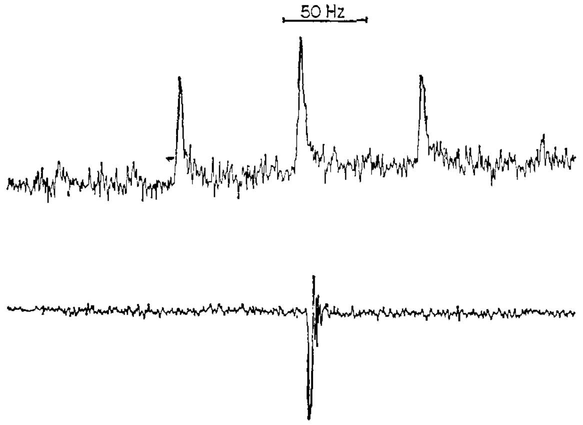

Figure 1. ^{15}N spectrum of ammonium chloride. The upper trace is the proton-coupled spectrum, while the lower trace is that obtained on proton irradiation.

without changing the definitions of the W_i's.[10,11] For an AX_n system ($n \geq 1$) it has been shown[9b,11] that the ratio I_d/I_c is given by eq 1, in which the denominator of

$$I_d/I_c = 1 + \frac{W_2 - W_0}{W_0 + 2W_1^A + W_2}\frac{\gamma_X}{\gamma_A} \qquad (1)$$

the term involving the W_i's is in fact the total relaxation rate of the A nucleus. When dipole–dipole interactions are the dominant relaxation mechanism, the W_i's are related[9,11] as $W_2{:}W_1^A{:}W_0 = 12{:}3{:}2$. Qualitatively, the dominance of the W_2 process (Figure 3) means that saturation of the X transitions repopulates the higher energy $\alpha\alpha$ state at the expense of the lower energy $\beta\alpha$ state. Because the intensity of a nuclear resonance depends on the difference in populations of the states between which the transition occurs, excitation at the A frequency induces a "downward" transition corresponding to an emission of energy, so that the observed signal is inverted.

Quantitatively, eq 1 allows prediction of the maximum NOE. For A = ^{13}C, X = ^1H, $(I_d/I_c)_{max} = 2.988$, which Kuhlmann and Grant have elegantly confirmed[9a] for the AX system formic acid. When the relaxation rate of A is increased by mechanisms other than dipole–dipole interactions, the second term in eq 1 is correspondingly reduced, with a concomitant reduction in the NOE.[12] For ^{15}N with $\gamma_{^1H}/\gamma_{^{15}N} = -9.867$, a maximum NOE of -3.93 is expected. Because the observed value at pH 0 is only 56% of the maximum, some other relaxation mechanism must contribute.

An additional relaxation mechanism available to this small, tetrahedral, freely rotating molecule can arise *via* spin–rotation (SR) interaction.[13] In order to assess the importance of this mechanism we have estimated its associated relaxation rate following the method of Burke and Chan.[14] The spin–rotational relaxation rate, R_{SR}, for this type of molecule, a spherical top, is given by eq 2,[14] where I is the moment of inertia, C is the

$$R_{SR} = \frac{8\pi^2 IkTC^2\tau_1}{\hbar^2} \qquad (2)$$

spin–rotational coupling constant, and τ_1 is the correlation time for reorientation of molecular angular momentum. Calculation of the last quantity depends on the model chosen for molecular motion. If reorientation is assumed to be controlled by isotropic Brownian motion, τ_1 can be expressed[14,15d] as

$$\tau_1 = I/6kT\tau_2 \qquad (3)$$

where τ_2 is the correlation time for reorientation of molecular position. To evaluate τ_2 we can use the Debye expression modified by the microviscosity correction factor of Gierer and Wirtz[15] to account for the fact that the bulk viscosity is not an accurate measure of the solvent–solvent interactions. The resulting expression for τ_2 is given by eq 4, where r is the radius

$$\tau = \frac{4\pi r^3\eta}{3kT}\left[6\frac{r_s}{r} + \left(1 + \frac{r_s}{r}\right)^{-3}\right]^{-1} \qquad (4)$$

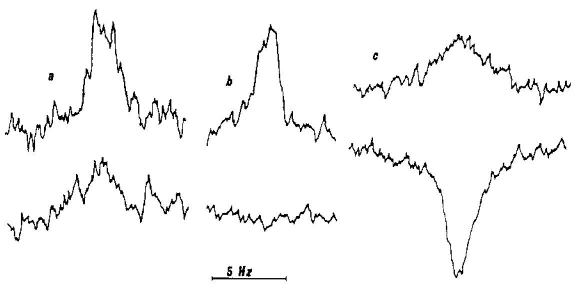

Figure 2. Dependence of ^{15}N signal on pH. The upper traces are the proton-coupled spectra, while the lower are those obtained on proton irradiation: (a) pH 6.41, (b) pH 5.31, (c) pH 4.58.

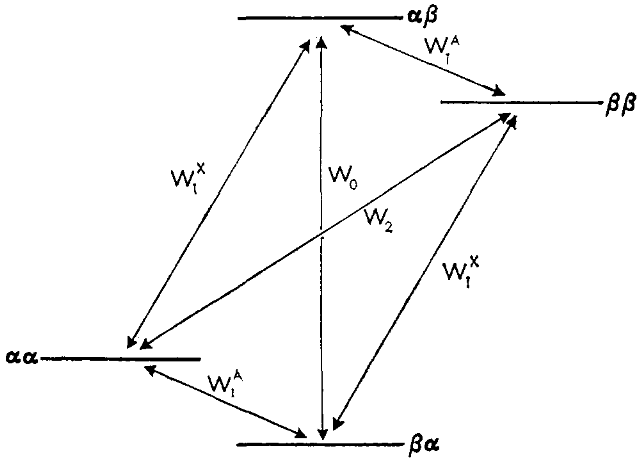

Figure 3. Energy level diagram for an AX system without spin-spin interaction, in which the magnetogyric ratios are of opposite sign. In each pair of spin labels, the first term refers to the A nucleus and the second to the X.

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(10) J. D. Baldeschwieler and E. W. Randall, *Chem. Rev.*, **63**, 81 (1963).

(11) I. Solomon, *Phys. Rev.*, **99**, 559 (1955).

(12) An exception is discussed below.

(13) (a) N. Bloembergen, E. M. Purcell, and R. V. Pound, *Phys. Rev.*, **73**, 679 (1948); (b) H. S. Gutowsky, I. J. Lawrenson, and K. Shimomura, *Phys. Rev. Lett.*, **6**, 349 (1961); (c) J. A. Pople, *Discuss. Faraday Soc.*, **43**, 192 (1967); (d) R. A. Dwek and R. E. Richards, *ibid.*, **43**, 196 (1967).

(14) (a) T. E. Burke and S. I. Chan, *J. Magn. Resonance*, **2**, 120 (1970); (b) see also S. I. Chan and A. S. Dubin, *J. Chem. Phys.*, **46**, 1745 (1967).

(15) (a) A. Gierer and K. Wirtz, *Z. Naturforsch. A*, **8**, 532 (1953); (b) A. Spernol and K. Wirtz, *ibid.*, **8**, 522 (1953); (c) P. S. Hubbard, *Phys. Rev.*, **131**, 1155 (1963); (d) T. E. Burke, Ph.D. Dissertation, California Institute of Technology, 1969; (e) it should be noted that this procedure has been reported to give rather larger τ_2 values than observed experimentally; see H. G. Hertz, *Progr. Nucl. Magn. Resonance Spectrosc.*, **3**, 171 (1967).

of the spherical solute molecule (the N–H bond length plus the hydrogen van der Waals' radius), r_s is the radius of the solvent molecule (1.933 for water), and η is the viscosity of the solvent. On the other hand, for systems in which the motion of the molecule of interest is more or less independent of the molecular environment, the inertial model can be applied, in which case τ_2 is given by[16]

$$\tau_2 = \frac{1}{2}(\pi I/3kT)^{1/2} \qquad (5)$$

Under these conditions, however, eq 3 relating τ_1 to τ_2 does not hold. Nevertheless, substitution of eq 5 into eq 3 yields an expression in which τ_1 is proportional to $(I/kT)^{1/2}$. Previous calculations of ^{19}F[16] and ^{13}C[17] spin–rotational relaxation rates have indicated that a proportionality constant of 1.5 reproduces experimental results reasonably well; accordingly, we shall adopt this factor and assume that τ_1 can be approximated by

$$\tau_1 = 1.5(I/kT)^{1/2} \qquad (6)$$

It should be noted that in fact the two models are limiting cases, so that the experimental situation is undoubtedly best described by some combination of each.

Evaluation of eq 2 also requires knowledge of the SR coupling constant for ammonium ion. This can be obtained from eq 7[14] and the SR coupling constant of some other molecule whose chemical shift with respect to ammonium ion is known. Here, m and M are the electron and proton masses, respectively, μ_N is the nuclear magneton, g_N is the nuclear g factor for the nucleus under consideration (−0.567 for ^{15}N), and the summation is taken over all the principal axes λ of the reference molecule.[18]

$$\Delta\sigma = \frac{e^2h}{6mc^2\mu^2{}_N M g_N}\frac{1}{2}\left[3C^{+NH_4}I^{+NH_4} - \sum_\lambda C_\lambda I_{\lambda\lambda}\right] \qquad (7)$$

For $^{15}N_2$, the values of C and $\Delta\sigma$ are 22 kHz[18] and 334 ppm,[19] respectively. Substitution in eq 7 ($I_{XX}{}^{N_2} = I_{YY}{}^{N_2}$, $I_{ZZ}{}^{N_2} = 0$) yields $C^{+NH_4} =$ 10.1 kHz. From eq 2–4, with $\eta = 0.0055$ P and $T =$ 320°K, R_{SR} is estimated to be 2.55×10^{-4} sec^{-1}; use of eq 2 and 6 (the inertial model) yields a value of 2.39×10^{-2} sec^{-1}. To ascertain the effect of this process on the NOE, the relaxation rate arising from dipole–dipole interactions must be estimated. For an AX system, this is given by eq 8,[11] where R is the A–X distance (the N–H bond length in the present case). With the added assumption that the relaxation rate for an AX$_n$ system is n times that for an AX system,[9b] evaluation of eq 8 based on the Debye model gives $R_D = 2.05 \times 10^{-2}$ sec^{-1}, while with the inertial model a value of 1.0×10^{-3} sec^{-1} is obtained.

$$R_D = \hbar^2\gamma_A{}^2\gamma_X{}^2\tau_2/R^6 \qquad (8)$$

Substitution of these values into eq 1 (by adding R_{SR} to R_D in the denominator) gives $I_d/I_c = -3.87$ and $+0.604$ for the Debye and inertial models, respectively. The range encompassed by the values merely underscores the difficulty in choosing suitable models for describing nuclear relaxation processes,[15d] and particularly in properly estimating τ_1 and τ_2, especially in an aqueous system containing a charged species. Indeed, a value of the NOE in better agreement with experiment (−1.28) can be obtained if the dipolar relaxation rate based on the Debye model, which gives an intuitively more reasonable value, is used with the SR relaxation rate based on the inertial model. Nevertheless, the results suggest that SR interactions may be able to account for the deviation of the NOE from its maximum value.

The pH dependence of the NOE most probably arises because of a contribution to the ^{15}N relaxation *via* exchange modulation of the ^{15}N–H scalar coupling constant.[20–23] This mechanism contributes only to the process characterized by W_0 and depends on the proton exchange rate and the magnitude of J_{NH}. In the limit of $W_0 \gg W_2$, $W_1{}^A$, the theoretical maximum enhancement is +10.87; disappearance and reinversion of the ^{15}N resonance is thus to be expected as the scalar coupling mechanism increases in importance. It should be noted, however, that the observed pH dependence does not demand the proposed explanation, because any relaxation pathway independently available to the ^{15}N nucleus will decrease the NOE. The mechanism proposed is unequivocally indicated only if $I_d/I_c > +1$, which we were unable to achieve experimentally.[8,24] Nevertheless, because the ammonium proton exchange rate is well known to increase with pH,[7] the scalar coupling mechanism seems most likely to occur at the higher pH values. Its unimportance at low pH is suggested by substitution of $\tau_e = 0.16$ sec[7a] and $J_{NH} = 75$ Hz into eq A-11 of ref 25, which gives the corresponding relaxation rate on the order of 10^{-13} sec^{-1}.

Practical Consequences. Perhaps more important than the actual value of the NOE is to note that for an AX$_n$ system whose γ's are of opposite sign, saturation of the transitions of one nucleus does not necessarily lead to enhancement of the signal intensity of the other, even when the dipole–dipole relaxation mechanism makes a substantial contribution to the total A relaxation. This contrasts with the situation exemplified by ^{13}C spectroscopy where, apart from unlikely contributions from the scalar coupling mechanism, $I_d/I_c \geq 1$. The effect in ^{15}N spectroscopy has been noted in other cases. Thus, the proton-decoupled ^{15}N resonance of enriched glycine at pH 5.32 is inverted, while that at pH 6.16 is not.[26] Even more striking is the behavior exhibited in the natural-abundance ^{15}N spectra of some amines.[3] For example, while no signal derived from 1,4-butanediamine could be detected even after several hours of spectral accumulation with complete proton decoupling, a strong signal could be detected after 15 min of scanning from a sample which had been carefully dis-

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(16) W. A. Steele, *J. Chem. Phys.*, **38**, 2404, 2411 (1963).

(17) C. F. Schmidt, private communication.

(18) Equation 7 assumes that the sum of the diamagnetic and the first term of the paramagnetic parts of the chemical shifts as given by Chan and Dubin[14b] are approximately equal for the two molecules.

(19) S. I. Chan, M. R. Baker, and N. F. Ramsey, *Phys. Rev. A*, **136**, 1224 (1964).

(20) I. Solomon and N. Bloembergen, *J. Chem. Phys.*, **25**, 261 (1956).

(21) D. E. O'Reilly and E. M. Peterson, *ibid.*, **49**, 2872 (1968).

(22) (a) T. Fukumi, Y. Arata, and S. Fujiwara, *ibid.*, **49**, 4198 (1968); (b) Y. Arata, T. Fukumi, and S. Fujiwara, *ibid.*, **51**, 859 (1969).

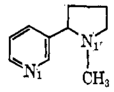
(23) S. L. Gordon and P. P. Yang, Abstracts of Papers, 158th National Meeting of the American Chemical Society, New York, N. Y., 1969, PHYS 8.

(24) Proton irradiation of a sample of wet ^{15}N-enriched ammonia produced no change in the intensity or polarity of the ^{15}N singlet observed. Because the contribution from the scalar coupling mechanism is proportional to $\tau_e/(1 + \Delta\omega^2\tau_e{}^2)$, where τ_e is the exchange lifetime, it is probable that τ_e is so small as to eliminate both this and the intramolecular dipole–dipole mechanisms.

(25) M. R. Baker, C. H. Anderson, and N. F. Ramsey, *Phys. Rev. A*, **133**, 1533 (1964).

(26) R. L. Lichter and J. D. Roberts, unpublished results.

Table II. Effect of Proton Irradiation on the Appearance of Some Natural-Abundance ^{15}N Signals

Compound	δ_N,[a] ppm	Disposition of signal
Hydrazine	328.66	Inverted
Pyridine	56.84	Noninverted
1,1-Dimethylhydrazine	317.59 (N_1)	Noninverted
	275.29 (N_2)	Inverted
	55.80 (N_1)	Inverted
	321.78 (N_1')	Inverted

[a] Measured with respect to external $H^{15}NO_3$, ref 3.

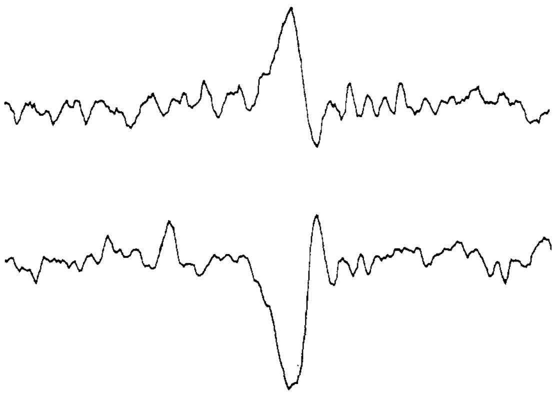


Figure 4. Natural-abundance ^{15}N resonance of anhydrous hydrazine, obtained after 58 scans at a sweep rate of 4 Hz/sec. The upper trace is the nonirradiated signal, while the lower is that obtained on irradiation at the proton resonance frequency. The line widths, determined by the sweep rates, are about 2 Hz.

tilled. Presumably, distillation removed traces of material which catalyzed proton exchange. With the exception described below, no signals from any amines were detectable without proton irradiation, which parallels the behavior of the ammonium resonance between pH 0.08 and 4.58. Thus, a second difference from ^{13}C spectroscopy arises, namely that proton irradiation is required, but is not necessarily sufficient, for the observation of natural-abundance ^{15}N resonances of amines.

Finally, it should be emphasized that the presence of scalar spin–spin coupling is not a requirement for a nuclear Overhauser effect which depends only on the direct dipole–dipole interaction that has no effect upon the splittings observed in high-resolution spectra of liquids. This is perhaps most clearly illustrated in Figure 4, where the upper trace shows the natural-abundance ^{15}N resonance of anhydrous hydrazine without irradiation. The sharp singlet, indicating that spin–spin coupling has been eliminated, is also obtained, but with inversion, when the sample is irradiated at the proton resonance frequency (Figure 4, lower trace). Evidently, nitrogen relaxation occurs to an appreciable extent through the dipole–dipole interaction,[27] and indeed, the existence of an inverted ^{15}N signal on proton irradiation, even when the proton-coupled signal cannot be observed, can be taken as evidence of the importance of such an interaction. Several examples are given in Table II, where it is apparent that some nitrogen nuclei exhibit NOE's even when they bear no protons.[28] Several categories can be identified: (1) nitrogen with directly bonded hydrogen, giving rise to inverted signals (hydrazine, N-2 of 1,1-dimethylhydrazine); (2) nitrogen with no directly bonded hydrogen, giving rise to noninverted signals (pyridine, N-1 of 1,1-dimethylhydrazine); (3) nitrogen with no directly bonded hydrogens, giving rise to inverted signals (nicotine). In the last case, the larger size of the molecule may likely inhibit relaxation *via* SR interaction, so that intramolecular dipole–dipole interactions with adjacent hydrogens, or indeed intermolecular dipole–dipole interactions, become important. Further detailed studies of the NOE in these various classes of compounds can be expected to reveal the nature of the relaxation processes which are important with nitrogen compounds.[29]

Acknowledgment. The authors are indebted to Professors Karl Kuhlmann and David M. Grant for enlightening discussions, and to Professor Sunney Chan and Mr. C. F. Schmidt for invaluable aid in calculating the spin–rotation relaxation rate.

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(27) R. Kaiser, *J. Chem. Phys.*, 42, 1838 (1965).

(28) However, in some cases noninverted signals were obtained after extreme difficulty from samples containing fully substituted nitrogen nuclei, and in some cases no signals could be detected at all.[3]

(29) (a) R. E. Schirmer, J. H. Noggle, J. P. Davis, and P. A. Hart, *J. Amer. Chem. Soc.*, 92, 3266 (1970); (b) R. A. Bell and J. K. Saunders, *Can. J. Chem.*, 48, 1114 (1970).