The Effect of Paramagnetic Relaxation Reagents on ^{15}N Spin Relaxation and the Use of $Gd(dpm)_3$ as a Nitrogen-15 Nuclear Magnetic Resonance Spin Label

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Abstract: Electron-nuclear relaxation times (T_1^e's) for ^{15}N and ^{13}C in natural abundance are measured for a series of amines of a wide range of pK_as using four paramagnetic relaxation reagents that are soluble in organic solutions. $Cr(acac)_3$ and $Cr(dpm)_3$ are seen to affect the nuclear spin predominantly via an outer sphere mechanism, or through normal translational diffusion when no interactions occur. $Gd(dpm)_3$ and $Gd(acac)_3$ are observed to be specific for the basic sites in substrate molecules where relaxation rate enhancement is seen to be strongly dependent on the availability of the lone electron pair. Variable concentration and temperature studies are used to separate the various contributions to T_1^e. ^{15}N T_1^e's are used as one measure of spin labeling; changes in the negative nuclear Overhauser effect (NOE) of proton-decoupled ^{15}N spectra can also be used in some cases. Examples are presented illustrating spin labeling due to both basicity differences and steric effects.

Introduction

Natural abundance ^{15}N NMR spectroscopy has developed rapidly in recent years despite the severe problems of low natural abundance (0.36%) and low sensitivity (about $^1/_{50}$ that of ^{13}C at constant field).[2] Further problems can be caused by the negative magnetic moment and long spin-lattice relaxation times.[3] When broad-band proton decoupling is employed, the negative magnetic moment results in a negative nuclear Overhauser enhancement factor (NOEF). If this factor, which can range from 0 to −5 depending on the dipolar contribution to relaxation (or on molecular dynamics for very large molecular systems), has a value between 0 and −2, loss of signal intensity will result. In addition, long spin-lattice relaxation times may require long waiting periods between pulses or use of small excitation pulse angles. However, early predictions of long ^{15}N T_1's have proven to be premature, at least in part.[4]

The ^{15}N-^1H NOE can be quenched and the spin-lattice relaxation rate can be greatly enhanced by the addition of paramagnetic compounds. This technique has been successfully exploited for ^{13}C,[5] ^{29}Si,[6] and ^{15}N[7] NMR spectroscopy. For all of these nuclei, the increased relaxation rate permits the use of faster pulse repetition rates in FT NMR and can thus increase the effective sensitivity obtainable in a given period of time. Of course, quenching the NOE is not always desirable but is very helpful in the case of nulled signals or sometimes for quantitative analyses.[8] The NOE can also be quenched by gating the proton decoupler.[9]

There are several requirements that paramagnetic relaxation reagents (PARR) must additionally satisfy. They should not cause significant shifting of resonances and they should not cause excessive line broadening. The former requirement distinguishes the PARRs from the lanthanide induced shift (LIS) reagents and necessitates the use of a metal complex with an isotropic ground state. The line broadening requirement is essential to prevent loss of sensitivity and resolution upon addition of PARRs.

PARRs may be divided into two classes: those that are nonspecific and those that are specific in their interaction with substrates. For the purpose of quantitative analysis, it is necessary that all carbon nuclei, for example, be affected as uniformly as possible. This requires a nonspecific PARR. A reagent that meets this requirement reasonably well in organic solvent systems is tris(dipivaloylmethanato)chromium(III) [$Cr(dpm)_3$].[10]

Tris(acetylacetonato)chromium(III), $Cr(acac)_3$, has also been studied extensively.[5,10-13] It has been shown to act as a specific PARR for acidic protons in chlorinated hydrocarbons such as $CHCl_3$[10,11] and $CHCl_2CHCl_2$[12] and alcohols such as 1-propanol[12] and *tert*-butyl alcohol[11] as well as for phenol.[12] The interaction has been explained in terms of the formation of short-lived H-bonded complexes between acidic substrate hydrogens and the carbonyls of the acetylacetonate ligand.[11] Also, steric factors and polarization effects can prevent a uniform influence on T_1 as has been shown for *n*-butylbenzene and benzonitrile.[13]

A specific PARR is required when a spin-label experiment is desired. Spin labeling is achieved when the PARR selectively enhances the relaxation rate of nuclei at or close to a specific site in the molecule. This relaxation enhancement must proceed via a mechanism involving bonding of the spin-label at that particular site. The effect can be observed by comparing relaxation rates of different nuclei within one molecule or, if several species are present in solution, in different compounds. The important requirement for the spin-label is specificity for a desired structural feature or characteristic. In the Cr(III) complexes, the metal is coordinatively saturated and, as has been described, the only form of chemical interaction with a substrate molecule is hydrogen bonding through the ligand carbonyls. Therefore, $Cr(acac)_3$ represents a weak spin-label which is specific for acidic protons.

An octahedral lanthanide metal complex is capable of coordinating to a basic position on substrate molecules owing to the ease with which the lanthanide can expand its coordination sphere. Tris(dipivaloylmethanato)gadolinium(III) [$Gd(dpm)_3$] has been used in ^{13}C NMR as an organic-soluble "shiftless" relaxation agent.[14] $Gd(acac)_3$ should behave similarly.

^{15}N NMR should be especially sensitive for spin-label experiments since nitrogen frequently possesses a free lone pair of electrons which can be the site of Lewis acid-base interactions. The spin-labeling effect can be monitored in two ways: from changes in ^{15}N relaxation times and also from preferential quenching of the ^{15}N-^1H NOE. By determining the electron-nuclear relaxation times, a *quantitative* measure of spin-labeling is obtained. Alternatively, *under certain conditions* (vide infra) the quenching of the NOE for a resonance can be used to identify the site of spin-labeling. Line widths are not a satisfactory measure of spin-labeling owing to possible large scalar contributions to T_2.[15]

In this paper, the results of our study of the interaction of the above-mentioned specific and nonspecific PARRs with amines of a wide range of basicity using both ^{15}N and ^{13}C

0002-7863/78/1500-2308$01.00/0

NMR are presented. Concentration and temperature dependence studies of the electron-nuclear relaxation times provide the basis for a quantitative discussion of the relaxation mechanism. Gd(dpm)$_3$ is then further studied for its spin-labeling ability with other nitrogen functional groups. The effects of both basicity and stereochemistry on ^{15}N spin-labeling are explored.

Experimental Section

Solvents and liquid compounds of natural isotopic abundance were dried and distilled by standard methods[16] prior to use. ^{15}N-Enriched pyridine and all solid compounds were used without further purification. Paramagnetic compounds were commercially obtained except for Cr(dpm)$_3$, which was synthesized by the following method. 2,2,6,6-Tetramethyl-3,5-heptanedione (6 g) was added to 20 g of urea and 3 g of $CrCl_3 \cdot 6H_2O$ in a 3:1 ethanol solution. The mixture was refluxed for 1 day, cooled, and filtered. The filtrate was dried and vacuum sublimed at 180 °C. Yield was 3.5 g (50% based on Cr(III)).

Proton-decoupled ^{15}N and ^{13}C NMR spectra were obtained at a field of 6.34 T (27.4 MHz for ^{15}N and 67.9 MHz for ^{13}C) on a Bruker HX 270 spectrometer. Spin-lattice relaxation times were measured using the fast inversion-recovery (FIRFT) pulse sequence[17] and a nonlinear least-squares curve fitting procedure using three adjustable parameters.[18] Measurements were made using 15-mm sample tubes with acetone-d_6 contained in a coaxial 5-mm capillary for field/frequency control. Unless specified, measurements were made at 26 °C. Viscosities were measured using Cannon-Manning semimicro-viscometers.

The electron-nuclear relaxation rate ($1/T_1^e$) was obtained as the difference between the relaxation rates measured in the presence and absence of the paramagnetic reagent, $(T_1^e)^{-1} = (T_1^{PARA})^{-1} - (T_1^{DIA})^{-1}$.

Theory

Electron-nuclear relaxation is due to interaction of the nuclear spin with the spin of an unpaired electron. This type of relaxation may result from several mechanisms[19] which shall be briefly described here.

A. Spin-Dipolar Interaction Modulated by Rotational Reorientation of the PARR-Substrate Complex. The relaxation rate for spin I due to a dipolar interaction with spin S is given by Solomon[20] and Bloembergen[21] and is shown in the equation

$$\frac{1}{T_1^e} = \frac{2S(S+1)\gamma_I^2\gamma_S^2\hbar^2}{15r^6}\left(\frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{7\tau_c}{1+\omega_S^2\tau_c^2}\right) \quad (1)$$

Equation 1 applies when a substrate molecule is bound to a paramagnetic metal ion and is the solution for the case where $\omega_S \gg \omega_I$. γ_I and γ_S are the magnetogyric ratios of spins I and S, ω_I and ω_S are the Larmor frequencies, and r is the distance between the nucleus I and the metal ion. τ_c is a correlation time determined by three distinct times[19] as is shown in the equation

$$\tau_c^{-1} = \tau_R^{-1} + \tau_S^{-1} + \tau_M^{-1} \quad (2)$$

Here, τ_R is the rotational correlation time of the metal-substrate complex, τ_S is the electron spin relaxation time, and τ_M is the mean lifetime of the nucleus in the bound state.

The form of eq 1 may be simplified by a few assumptions regarding the magnitude and nature of τ_c. Assuming that $\omega_I^2\tau_c^2 \ll 1 \ll \omega_S^2\tau_c^2$ (we will find motivation for this assumption below) we obtain

$$\frac{1}{T_1^e} = \frac{2S(S+1)\gamma_I^2\gamma_S^2\hbar^2}{5r^6}\tau_c \quad (3)$$

Since the electron spin relaxation time for Cr(III) and Gd(III) is relatively long[22] and there is no evidence for rapid exchange (on a time scale comparable with τ_R), we may further assume that τ_c is dominated by the rotational reorientation time of the substrate-PARR complex, τ_R. Then it is possible to relate τ_c to the microviscosity corrected Stokes-Einstein model with "sticking" boundary conditions.[23]

$$\tau_R = \frac{\eta V_m}{f_r kT} \quad (4)$$

η is macroscopic viscosity, V_m is the molecular volume, k is the Boltzmann constant, and T is the absolute temperature. f_r is the microviscosity factor for which we adopt a value of $^1/_{12}$ as suggested by Glasel.[24] Introduction of the Stokes-Einstein model specifies that the electron-nuclear relaxation rate as given by this mechanism should be proportional to $\gamma_I^2\eta$.

B. Scalar Interaction Modulated by Rotational Reorientation of the PARR-Substrate Complex. Equation 5 describes the relaxation rate due to this mechanism.[19,21]

$$\frac{1}{T_1^e} = \frac{2S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2\left(\frac{\tau_e}{1+\omega_S^2\tau_e^2}\right) \quad (5)$$

A is the hyperfine coupling constant for nucleus I and τ_e is given by the equation

$$\tau_e^{-1} = \tau_S^{-1} + \tau_M^{-1} \quad (6)$$

Again, it is assumed that $\tau_S < \tau_M$. Since $\omega_s^2\tau_e^2$ is extremely large, it is usually assumed that this relaxation mechanism is not very efficient for cases such as those presented here.

On the other hand, as is shown in the equation

$$\frac{1}{T_2^e} = \frac{S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2\left[\tau_e + \frac{\tau_e}{1+\omega_S^2\tau_e^2}\right] \quad (7)$$

the contribution of this mechanism to T_2 is several orders of magnitude more efficient. Previous workers[15] have noted that great care should be taken in using NMR line width information to evaluate spin-labeling.

C. Outer Sphere Relaxation. Unlike the previous two mechanisms, this one does not require direct metal-ligand complexation. In the simplest case of no ligand-PARR interaction of any type, this mechanism may appropriately be denoted as spin-dipolar interaction modulated by translational diffusion. Although a more detailed theory has been given[25] we adopt here a simple formulation by Abragam[26] as shown in the equation

$$\frac{1}{T_1^e} = \frac{16\pi^2}{15}N_S S(S+1)\gamma_I^2\gamma_S^2\hbar^2\frac{\eta}{kT} \quad (8)$$

N_S is the number of paramagnetic ions per unit volume of the solution. As was true for the case of spin-dipolar interaction modulated by rotational reorientation, the relaxation rate here is expected to be proportional to $\gamma_I^2\eta$.

The concept of outer sphere relaxation may also denote a variety of situations corresponding to different weak interactions between the PARR and the substrate.[11] The specificity of the relaxation rate enhancement due to these effects is, however, limited by the fact that distances to the paramagnetic center from the nuclei in this second coordination sphere (solvation sphere) are large. For the purpose of qualitative discussion, eq 8 (and possible deviations from it) may therefore still be useful.

D. Identification of Operant T_1^e Processes. Observed electron-nuclear relaxation times [T_1^e(obsd)] are, of course, due to a combination of all mechanisms. The expression for the observed electron-nuclear relaxation rate is given by Swift and Connick[27] and Luz and Meiboom.[28] This is shown in the equation

$$\frac{1}{T_1^e(\text{obsd})} = \frac{qp}{\tau_M + T_1^e(c)} + \frac{1}{T_1^e(t)} \quad (9)$$

The number of substrate ligands in the coordination sphere of the metal is given by q and p is the ratio of the concentration of the paramagnetic ion to the concentration of the ligand.

Table I. Viscosities, pK_a, and Diamagnetic T_1 and NOEF Data for Selected Amines

Molecule	pK_a	η, cP	^{15}N		^{13}C T_1, s		
			T_1, s	NOEF	C_2	C_3	C_4
Pyrrolidine	11.3	0.798	58	−4.6	11.3	13.3	
n-Butylamine	10.6	0.550	70	−3.9	12.8	12.9	13.3
Imidazole[a,b]	6.95	5.12	7.6	−4.7	1.7		1.7
Pyridine	5.25	0.903	85	−0.4	16.0	16.4	16.0
Aniline	4.63	3.71	13	−5.0	16.0	3.9	3.3
Pyrrole	−0.27	1.18	40	−4.3	11.2	9.8	
Indole[a]	−2.7	11.5	3.0	−4.7	0.73	0.79	

[a] 8 M Me_2SO solution. [b] C_2 is located between the two nitrogens; C_4 represents the averaged signal of the other two carbons.

T_1^e(c) is determinated by both mechanisms A and **B**, while T_1^e(t) is the "outer sphere" relaxation time. τ_M is the mean residence time of the substrate in the first coordination sphere of the metal and can serve to limit the efficiency of the observed relaxation due to the bound state. The degree to which each of the different mechanisms contribute to the observed T_1^e can be determined by the concentration and temperature dependence of T_1^e(obsd) (vide infra).

Results and Discussion

1. Amines. Natural abundance ^{15}N and ^{13}C NMR spin-lattice relaxation data obtained in diamagnetic solutions are presented in Table I. The headings C_2, C_3, and C_4 are used to denote carbons successively removed from the nitrogen. Included in Table I are ^{15}N nuclear Overhauser enhancement factors (NOEF), amine pK_a values, and the solution viscosities. The NOEFs indicate that the nitrogens possessing a covalently bound hydrogen are largely dipolar relaxed. The dipolar T_1 (T_1^{dd}) is seen to be (qualitatively) proportional to the inverse of the solution viscosity. Pyridine, with no proton, is relaxed mainly by chemical shift anisotropy.[29] The NOEFs for protonated carbons are full and therefore are not included in Table I.

It is possible to predict the ratio of the dipolar NT_1 (^{15}N) to $N'T_1$ (^{13}C) where N and N' are the number of covalently bound protons, respectively. Using the "extreme spectral narrowing" condition, the different magnetogyric ratios, and the different average bond lengths to the proton, a factor of 4 is predicted.[4] This factor is observed for indole and pyrrole. For aniline and *n*-butylamine a factor of 7 is found which may be explained by some internal rotation or inversion of the amino groups. For pyrrolidine a ratio of 2.6 is found. This reduction in the ratio might result from hydrogen bond formation with other pyrrolidine molecules (hydrogen bonding is not as likely for pyrrole or indole owing to the absence of an available electron lone pair).

For imidazole, rapid tautomerism results in the observation of a single nitrogen resonance. The tautomerism has been described in terms of a hydrogen-bonded oligomer structure.[30] The observed dipolar T_1 ratio of nearly five is significantly shorter than expected for the averaging of an NH and nonprotonated nitrogen. The shorter ^{15}N T_1 of imidazole might result from the extensive solution hydrogen bonding; a more through investigation of the T_1 process for imidazole is in progress.

Direct comparison of electron-nuclear relaxation times does not show obvious trends for different nuclear species obtained in solutions of different viscosities. However, as is shown in eq 3, 4, and 8, the relaxation rates should be proportional to $\gamma_I^2\eta$. This makes it possible to "normalize" the T_1^e values to the magnetogyric ratio of ^{15}N and to a viscosity of 1 cP. All T_1^e values presented *in this section* (including Tables II-V) are normalized, i.e., are the products of measured T_1^e's and $\eta\gamma_I^2/\gamma_{^{15}N}^2$ (the units are cP s).

Table II. Normalized Electron-Nuclear Relaxation Times in Solutions Containing 0.05 M $Cr(acac)_3$ (cP s)

Molecule	^{15}N	$^{13}C_2$	$^{13}C_3$	$^{13}C_4$
Pyrrolidine	2.7	3.1	3.4	
n-Butylamine	1.7	3.1	3.7	4.1
Imidazole[a]	3.6	4.4		4.7
Pyridine	3.7	3.8	3.4	3.4
Aniline	3.0	3.4	3.9	4.6
Pyrrole	1.8	2.7	3.4	
Indole[a,b]	2.1	3.1	4.6	

[a] 8 M Me_2SO solution. [b] ^{13}C data obtained from 0.01 M $Cr(acac)_3$ solution due to short T_1 and divided by five.

Table III. Normalized Electron-Nuclear Relaxation Times for Selected Amines[a] Containing 0.05 M $Cr(dpm)_3$ (cP s)

Molecule	^{15}N	$^{13}C_2$	$^{13}C_3$	$^{13}C_4$
Pyrrolidine	5.4	5.9	5.4	
n-Butylamine	5.1	6.1	6.1	5.8
Pyridine	5.2	5.3	5.1	5.0
Pyrrole[b]	4.1	5.2	4.5	

[a] $Cr(dpm)_3$ is insoluble in Me_2SO and aniline. [b] T_1's obtained in 0.02 M $Cr(dpm)_3$ solution due to low solubility of $Cr(dpm)_3$ and multiplied by 2.5.

Table IV. Normalized Electron-Nuclear Relaxation Times in Solutions Containing 5×10^{-4} M $Gd(dpm)_3$ (cP s)

Molecule	^{15}N	$^{13}C_2$	$^{13}C_3$	$^{13}C_4$
Pyrrolidine	3.2	29	54	
n-Butylamine	2.5	10	33	62
Imidazole[a]	2.8	7.6		16
Pyridine	8.6	19	53	61
Aniline	18	53	114	200
Pyrrole	38	54	63	
Indole[a,b]	95	>50	>50	>50

[a] 8 M Me_2SO solution. [b] ^{13}C T_1's in presence and absence of $Gd(dpm)_3$ are identical within experimental error.

Table II contains the normalized relaxation data for solutions containing 0.05 M $Cr(acac)_3$. All of the values are roughly comparable except for the ^{15}N T_1^e values of *n*-butylamine, pyrrole, and indole, which are shorter. The rough equivalence of the T_1^e's for $Cr(acac)_3$ (which is expected to induce relaxation primarily via an outer sphere mechanism) further justifies the normalization procedure. The ^{15}N T_1^e's of pyrrole, indole, and *n*-butylamine show the presence of a specific interaction such as hydrogen bonding to the ligand carbonyls.

If acetylacetone is replaced by the bulkier dipivaloylmethane ligand, the efficiency of relaxation is decreased for all cases studied. Table III contains the T_1^e results using 0.05 M $Cr(dpm)_3$ as PARR (the limited solubility of $Cr(dpm)_3$ in Me_2SO and aniline precluded a more extensive comparison with $Cr(acac)_3$). The normalized T_1^e's are about twice as long for $Cr(dpm)_3$ as for $Cr(acac)_3$ probably owing to an increased minimum distance between the substrate and the paramagnetic center. The ^{15}N T_1^e's for *n*-butylamine, indole, and pyrrole compare well with the ^{13}C T_1^e's indicating that specific interactions to $Cr(dpm)_3$ are either absent or of minor significance.

Table IV contains the normalized T_1^e's for solutions containing 5×10^{-4} M $Gd(dpm)_3$. It is obvious that the situation is quite different here than it was for the Cr(III) PARRs. First, the normalized ^{15}N T_1^e's cover a wide range of values. Short ^{15}N T_1^e's are observed for the more basic amines listed at the

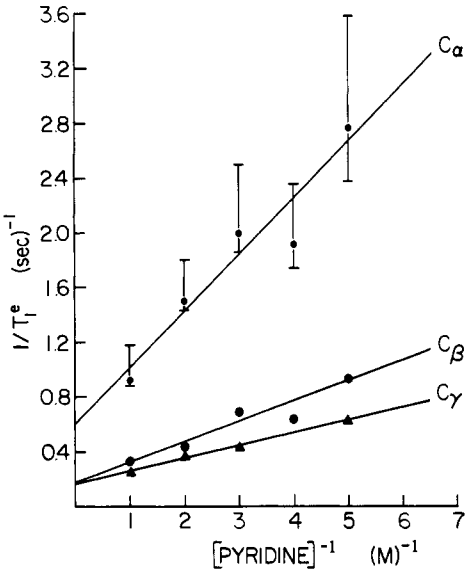


Figure 1. Plot of ^{13}C $1/T_1^e$ values for C_α, C_β, and C_γ of pyridine as a function of inverse pyridine concentration.

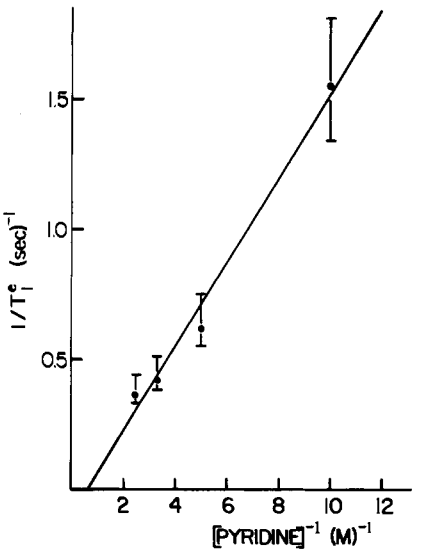


Figure 2. Plot of ^{15}N $1/T_1^e$ values for pyridine as a function of inverse pyridine concentration.

Table V. Normalized Electron-Nuclear Relaxation Times in Solutions Containing 5×10^{-4} M Gd(acac)$_3$ (cP s)

Molecule	^{15}N	$^{13}C_2$	$^{13}C_3$	$^{13}C_4$
Pyrrolidine	6.9	63	130	
n-Butylamine	3.7	20	43	66
Pyridine	7.4	31	65	75
Aniline	24	120	230	>300
Pyrrole	66	91	120	

top of the table (see Table I for the actual pK_a values). Note that for these basic substrates, the ^{15}N T_1^e is much shorter than T_1^e for C_2, which in turn is shorter than T_1^e for C_3 or C_4. Both of these trends definitely indicate that a complex is formed between the substrate and the PARR. For the very weakly basic pyrrole, a trend is found that is qualitatively similar to that for the Cr(III) PARRs.

Table V contains the T_1^e's obtained for solutions containing 5×10^{-4} M Gd(acac)$_3$. The trends observed are the same as for Gd(dpm)$_3$. Gd(dpm)$_3$ appears to be slightly more efficient as far as the ^{15}N T_1^e's are concerned. On the other hand, the T_1^e ratios of $^{13}C_2$ to ^{15}N are attenuated less for Gd(acac)$_3$ indicating a lower specificity for basic substrates.

According to eq 8, it should be possible to estimate the translational diffusion contribution to T_1^e (obsd) by correcting the Cr(III) data for the differences in spin and concentration between Cr(III) and Gd(III). However, a comparison of the results in Tables II and III indicates that such a procedure is tenuous. Evidently, the approximations inherent in eq 8 preclude its use for quantitative comparison between relaxation agents. Qualitatively, the corrected Gd(dpm)$_3$ T_1^e's for carbons β to the nitrogen compare well with the T_1^e's for Cr(acac)$_3$.

2. Variable Concentration and Temperature Studies. The different contributions to T_1^e(obsd) as shown in eq 9 can be evaluated by variable concentration and variable temperature studies. Pyridine was chosen as the substrate for these studies because of its intermediate base strength, the commercial availability of the ^{15}N-enriched compound, and the existence of an x-ray study of the Eu(dpm)$_3$(py)$_2$ complex.[31] Gd(dpm)$_3$ was used as the PARR, cyclohexane as the solvent, and benzene was added as an inert reference.

Assuming that Gd(dpm)$_3$(py)$_2$ is the only complex present, that it has a moderately large stability constant, and that a large excess of pyridine is present, eq 9 can be written as shown in the equation

$$\frac{1}{T_1^e(\text{obsd})} = \frac{2[\text{PARR}]}{[\text{substrate}]} \frac{1}{\tau_M + T_1^e(c)} + \frac{[\text{PARR}]}{T_1^{e\prime}(t)} \qquad (10)$$

The dependence of the translational diffusion contribution on the concentration of PARR has been separated explicitly. Thus a study of T_1^e(obsd) as a function of substrate concentration should separate out the outer sphere contribution.

Figure 1 shows a plot of $1/T_1^e$(obsd) vs. [pyridine]$^{-1}$ for C_α, C_β, and C_γ of pyridine with a constant PARR concentration of 5×10^{-4} M. Linear behavior is observed (estimates of error are shown for C_α). The intercepts indicate that T_1^e(t) is about 2 s for C_α and about 5 s for C_β and C_γ. This difference indicates a lack of reliability in this method of determining T_1^e(t). A better estimate of T_1^e(t) is given by the T_1^e of benzene, which is about 10 s.

Figure 2 shows a plot of $1/T_1^e$(obsd) vs. [pyridine]$^{-1}$ for the nitrogen of pyridine with a constant PARR concentration of 1×10^{-4} M. Again, linear behavior is observed. The intercept in Figure 2 is negative; therefore, a value of T_1^e(t) cannot be determined. However, a value for T_1^e(t) can be calculated using the value for carbon and correcting for the different magnetogyric ratios. This yields an estimated value of 60 s for T_1^e(t) for ^{15}N.

The importance of τ_M in determining $1/T_1^e$(obsd) can be determined from a variable temperature study. The possible effects of temperature on $1/T_1^e$(obsd) have been described elsewhere.[19] Figure 3 shows a semilogarithmic plot of $1/T_1^e$(obsd) for C_α of pyridine vs. the inverse of the absolute temperature. A straight line can be drawn with a slope indicating an activation energy of 7 kcal mol^{-1}. This type of temperature dependence where τ_c is essentially τ_R (and the extreme narrowing condition holds) clearly indicates a dipolar process or domination by T_1^e(c) over τ_M.

It is now possible to use the slopes in Figures 1 and 2 to calculate the value of T_1^e(c) for each nucleus using eq 10. While the intercepts of Figures 1 and 2 were not precise enough for accurate determination of T_1^e(t), the slopes of these lines are defined more accurately. These values are listed in Table VI. Using these values for T_1^e(c) and the values estimated above for T_1^e(t) for ^{13}C and ^{15}N, it is possible to calculate a value of T_1^e for neat pyridine. These values are listed in Table VI and the experimentally determined times are also included. Reasonable agreement is found except for C_α, which is cal-

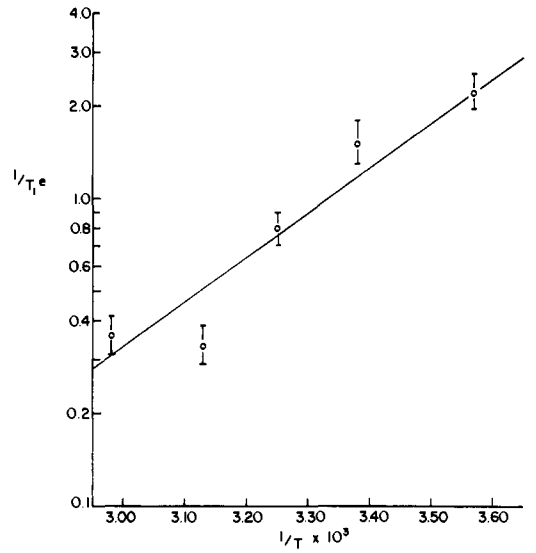


Figure 3. Semilogarithmic plot of ^{13}C $1/T_1^e$ values for C_α of pyridine as a function of inverse absolute temperature.

culated to be longer than is found. This may be due to effects not considered by the simple model presented. However, it is clear that C_β and C_γ appear to be dominated by the outer sphere type contribution to T_1^e while N and to a lesser extent C_α are dominated by T_1^e(c).

It is possible to use the structural information available from the crystal structure of $Eu(dpm)_3(py)_2$ and the values of T_1^e(c) in Table VI to calculate τ_c using eq 1. These values of τ_c are presented at the bottom of Table VI and agree extremely well, again except for C_α. Note that since τ_c is about 2×10^{-11} s, the inequality which led to eq 3 is satisfied.

As a test of the assumption that τ_c is dominated by τ_R, it is possible to use the Stokes-Einstein model[23] to determine whether τ_c is consistent with the size of the complex. By applying eq 4 with $f_r = 1/12$[24] and $\tau_c = 2 \times 10^{-11}$ s a molecular radius of 6.4 Å is calculated. This is certainly consistent with the known crystal structure of the europium complex.

Finally, the conclusion that $Cr(dpm)_3$ affected T_1^e via an outer sphere mechanism can be tested by a variable concentration study. Equation 8 shows that T_1^e(t) should be independent of the substrate contribution. Thus a plot of $1/T_1^e$(obsd) vs. [pyridine]$^{-1}$ for a constant concentration of Cr(dpm) should yield a horizontal straight line. It was found for the nitrogen of pyridine that through a tenfold change in pyridine concentration, the relaxation rate changed by less than 30%, confirming the outer sphere mechanism.

3. Spin Labeling. As was described above, spin labeling may be achieved when the PARR selectively enhances the relaxation rate of nuclei at or close to a specific site. The effect of amine basicity on the efficiency of a spin-label experiment is clear from Table IV. Additional nitrogen functional groups have been investigated and these results are presented in Table VII.

The diamagnetic relaxation of these compounds follows expected patterns. *N*-Methylformamide contains a proton-bearing nitrogen and ^{15}N in this molecule is fully relaxed by ^{15}N-^1H dipolar interactions. In the two oximes dipolar relaxation is of importance, while this mechanism is negligible for nitrobenzene and acetonitrile. As should be expected, the T_1^e results for the first two compounds in Table VII do not indicate a significant spin-labeling effect. In *N*-methylformamide a weak complex may be present, probably bonded through the oxygen atom. The most interesting results in Table VII are the T_1^e values for the aldoximes.

Aldoximes can exist in two isomeric forms—syn and anti as is shown below for acetaldoxime. The percent of each isomer

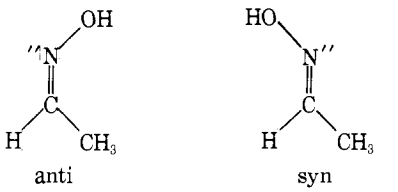
Table VI. Summary of the Results of Variable Concentration Study for the Pyridi-Gd(dpm)$_3$ System

	^{15}N	$^{13}C_\alpha$	$^{13}C_\beta$	$^{13}C_\gamma$
T_1^e(c)	1.2×10^{-3}	2.4×10^{-3}	7.4×10^{-3}	11×10^{-3}
T_1^e-(calcd)[a]	12	7.5	9.0	9.3
T_1^e(exp)	9.6	3.5	9.5	10.9
r, Å[b]	2.65	3.48	4.85	5.40
τ_c	1.8×10^{-11}	0.7×10^{-11}	1.8×10^{-11}	2.3×10^{-11}

[a] Neat pyridine. [b] Reference 31.

Table VII. Relaxation Rate Enhancement for Nitrogen-15 in Various Functional Groups Due to the Presence of 5×10^{-4} M Gd(dpm)$_3$

Compd	η,cP	Diamagnetic T_1, s	Paramagnetic T_1, s	Paramagnetic T_1^e, s
Nitrobenzene	2.03	135	41	59
Acetonitrile	0.34	90	53	129
N-Methylformamide	1.77	23	11	21
Acetaldoxime	2.28			
syn- (38%)		35	9.0	12
anti- (62%)		54	2.9	3.1
Propionaldoxime	2.47			
syn- (57%)		37	16	11
anti- (43%)		31	3.1	2.8



is known[32] for the two aldoximes studied here and these were used to assign the ^{15}N resonances. The T_1^e's clearly indicate a spin-label effect specific for the anti isomer of both oximes. The specificity here is presumably due to easier access in the anti isomer, and thus it would be a steric effect.

For the case of two (or more) competing sites in a solution the ratio of T_1^e's may be used as a measure of spin-labeling efficiency. It is, however, not always necessary to measure T_1^e's in order to observe a spin-labeling effect. Under favorable conditions, the negative NOE for the proton-decoupled ^{15}N nucleus may be utilized. When the paramagnetic reagent is added the NOE is reduced. For the case of two nitrogen sites with different affinities to the spin-label, one of the signals may be nulled or phase inverted compared to the diamagnetic solution, while the other signal is affected to a lesser extent. One obvious requirement for using the NOE technique is that the diamagnetic NOE should be as full as possible; this allows maximum dynamic range for measuring spin-label effects. A second requirement becomes clear upon inspection of the relaxation between the nuclear Overhauser enhancement in the presence of the spin-label, NOEF(para), T_1^e, and the relaxation parameters for the diamagnetic case (we assume for simplicity that the relaxation in the diamagnetic solution is fully dipolar), as given by the equation

$$\text{NOEF(para)} = \text{NOEF(max)} \frac{T_1^e}{T_1^e + T_1^{dd}} \qquad (11)$$

If the dipolar relaxation at one site is much slower than at the other one, the NOEFs will be affected very differently, even if the T_1^e ratio is close to unity. Thus, the use of NOEFs alone to identify spin-labeling is restricted to only certain types of molecular systems.

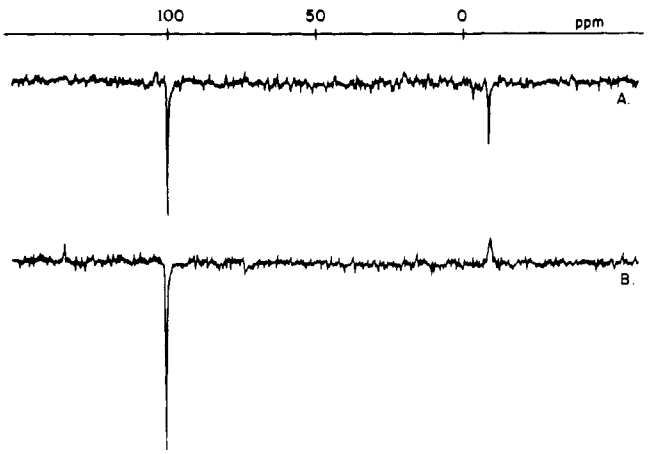
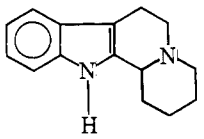


Figure 4. 27.4-MHz ^{15}N NMR spectra of 3:2 pyrrole–pyrrolidine mixture. Shifts are referred to Me_4Nl. The downfield resonance is due to pyrrole. (A) Diamagnetic solution, 100 transients; (B) solution containing 1×10^{-3} M Gd(dpm)$_3$, 400 transients.

An obvious test case which meets these requirements is an equimolar mixture of pyrrole and pyrrolidine. From Table I, it can be seen that both have diamagnetic NOEFs that are nearly full and the dipolar T_1's are comparable (47 and 63 s, respectively). Figure 4 shows the spectrum of the diamagnetic solution at the top and the spectrum of the solution containing 1×10^{-3} M Gd(dpm)$_3$ at the bottom. The high-field resonance due to pyrrolidine is phase inverted for the paramagnetic solution while the phase of the low resonance due to pyrrole remains unchanged. The specificity for pyrrolidine is due to its higher base strength.

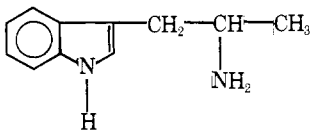
An example which illustrates the need for caution when interpreting spin-label experiments using the NOE is the 15-carbon indolo[2,3-*a*]quinolizidine alkaloid (**1**) shown below.



1

The diamagnetic NOEFs for the two nitrogens are essentially full; however, the dipolar T_1's are 3.5 s for the indole nitrogen and 25 s for the tertiary amine nitrogen.[4] Figure 5 shows the ^{15}N NMR spectra for the diamagnetic sample (1 M in $CHCl_3$) and for solutions containing 2×10^{-4} and 1×10^{-3} M Gd(dpm)$_3$. The low-field resonance is that of the indole nitrogen. Although a casual visual inspection would indicate spin-labeling at the site of the tertiary amine nitrogen, use of eq 11 indicates otherwise. The ratios of $T_1^e(1)$ to $T_1^e(2)$ (indole:tertiary nitrogen) are estimated to be 0.8 for 2×10^{-4} M and 0.6 for 1×10^{-3} M PARR. Within experimental error these ratios indicate no significant spin-labeling.

A clear case of spin-labeling is observed for α-methyltryptamine (**2**). Here, both nitrogens should have nearly full NOEs



2

and similar T_1's as judged from viscosity corrected dipolar T_1's for *n*-butylamine (49 s) and indole (37 s). Figure 6A shows the spectrum of a 4 M solution of **2** in Me_2SO with the NOE. The low-field resonance is due to the indole nitrogen. In the presence of 5×10^{-5} M Gd(dpm)$_3$, the aliphatic amine resonance is nulled (Figure 6B). Addition of more Gd(dpm)$_3$ causes the phase inversion of the aliphatic amine resonance but also produces significant line broadening.

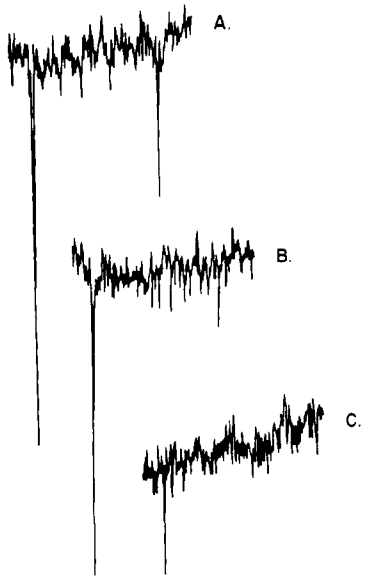


Figure 5. ^{15}N NMR spectra of 1 M **1** in $CHCl_3$. The downfield resonance is that of the indole nitrogen. (A) Diamagnetic solution, 1000 transients; (B) 2×10^{-4} M Gd(dpm)$_3$; (C) 1×10^{-3} M Gd(dpm)$_3$.

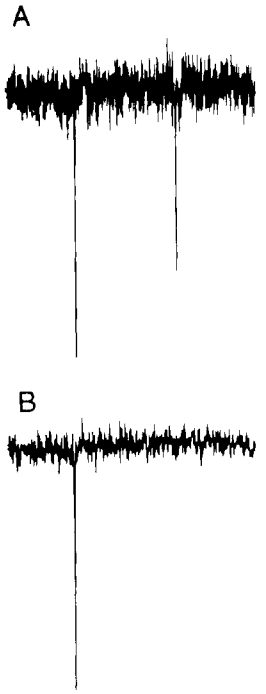


Figure 6. ^{15}N NMR spectra of 4 M α-methyltryptamine in Me_2SO. The downfield resonance is that of the indole nitrogen. The plot width is 220 ppm, the pulse angle is 45°, and the repetition rate is 10 s. (A) Diamagnetic solution with continuous decoupling, 80 transients; (B) 5×10^{-5} M Gd(dpm)$_3$, 200 transients.

Conclusions

The general, qualitative picture of the relaxation mechanisms presented by the single concentration, natural abundance results shown in Tables II–V is fully consistent with the more quantitative approach applied for the pyridine-Gd(dpm)$_3$ system. In particular, for the case of Gd(III) complexes we can explain the increasing normalized T_1^e's across the rows of Tables IV and V by the decreasing efficiency of the dipole-

dipole electron-nuclear relaxation in complexed molecules. For the case of Cr(III) chelates we can see that the simple approach to the translational diffusion and outer sphere mechanisms, using eq 8, is reasonable as far as a specific PARR and molecules of similar size are concerned.

The paramagnetic relaxation reagents under investigation in this study seem to be suitable for applications in ^{15}N NMR spectroscopy using organic solvent systems. If a uniform decrease in ^{15}N spin-lattice relaxation times is required, both $Cr(acac)_3$ and $Cr(dpm)_3$ seem to be a good choice, the latter one being preferable if the compound studied contains acidic hydrogens. On the other hand, the Gd(III) chelates offer an interesting possibility of selective relaxation rate enhancement for basic and sterically accessible sites. Efficiency of the spin-labeling for the case of competing sites is conveniently defined in terms of the ratio of T_1^e's. Under favorable conditions (predominantly dipolar T_1's in diamagnetic solution, similar T_1^{dd} for the different sites of interest) spin-labeling effects can be easily monitored utilizing the differential quenching of the nuclear Overhauser enhancement.

Acknowledgments. We gratefully acknowledge helpful discussions with Professors C. E. Holloway and Robert Lichter. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the U.S. Environmental Protection Agency for support of this research. A grant-in-aid was also provided by Eli Lilly and Co. Jozef Kowalewski expresses his gratitude to the Sweden-America Foundation and the Swedish Natural Science Research Council for providing funds for a 6-month stay at the Florida State University.

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Banana and Nonbanana Bonds of the Carbonyl Group

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Abstract: Using substituted aldehydes, the effect on the form of the C=O repulsion-localized MOs of the presence of oxygen lone pairs and π conjugating substituents (CH_2^+, BH_2, CH_3, NH_2, OH, F) is examined. Generally, the banana model is correct, although for NH_2 and CH_2^+ substituents nonbanana results are obtained. Oxygen protonation, coupled with NH_2 and OH conjugation, also yields nonbanana bonds. The CO_π, O_{lp} repulsion integral is very sensitive to polarization of the CO π orbital and, more than any other factor, forecasts the form of the final LMOs.

Introduction

The carbonyl group continues to attract theoretical attention because of its prominence in enzyme bioprocesses as a ligating group for metal ions and as the site of nucleophilic attack in amino acid hydrolysis[1] and for its utility in 1,2-addition reactions with organometals.[2] Polarizability plays a key role in the chemistry of the C=O function and is particularly dependent on the nature of the carbon substituents. As coordination of a Lewis acid to an oxygen lone pair is an important aspect of enhancing the reactivity of C=O, this too has justifiably received considerable experimental and theoretical attention.

With regard to conceptualization of the electronic structure of the carbonyl group and the implications thereof for reaction properties of that group, previous descriptions have invoked simple Lewis structures to reflect π_{CO} polarization or relied on canonical σ and π MO descriptors. Extensive π_{CO} polarization implies extensive, if not dominant, lone-pair character

0002-7863/78/1500-2314$01.00/0