NATURAL ABUNDANCE ^{15}N NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

by

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CONTENTS

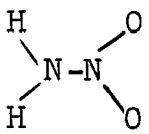
TABLES

ILLUSTRATIONS

CHAPTER I

INTRODUCTION

The occurrence of nitrogen in organic and inorganic compounds makes its potential high as a probe for molecular and electronic structure. Indeed, one of the first applications of nuclear magnetic resonance spectroscopy to structure elucidation utilized ^{14}N chemical shift measurements. In 1957, Ray and Ogg (1) used proton and ^{14}N nmr to establish the solution structure of nitramide as the amide of nitric acid (I) rather than as its structural isomer, hyponitrous acid (II).



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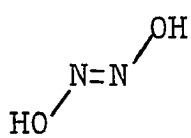


II

Comparison of ^{14}N with ^{15}N

Although the rate of development of nitrogen nmr spectroscopy has been slightly slower than that of carbon, nmr investigations of nitrogen nuclei in organic compounds have been far fewer relative to proton, fluorine and phosphorus (2,3). This can be explained in terms of the increased experimental difficulties encountered with both isotopes of nitrogen. Nitrogen-14, the more abundant isotope, has a sensitivity only 0.000343 that of an equal

number of protons in the same magnetic field. Sensitivity (4) in a magnetic resonance experiment is directly proportional to the cube of the magnetogyric ratio (which is a function of the magnetic moment and spin quantum number of a nucleus).

$$S \propto \frac{n B_o^2 \gamma^3}{T} \qquad (1)$$

Here n is the total number of nuclei and B_o is the field strength. Therefore, the smaller magnetogyric ratios of both ^{14}N and ^{15}N compared with that of ^{1}H (Table 1) result in decreased sensitivity of the former nuclei relative to the latter. Additionally, because it has a spin quantum number of one, the ^{14}N nucleus has an associated nuclear quadrupole moment. The resulting increase in the nuclear relaxation rate which arises from interaction between the quadrupole moment and a nonsymmetrical electric field, may be evidenced by linewidths on the order of 50-1000 Hz, with consequent substantial errors in chemical shift measurements. When spectra of these compounds are obtained either as dilute solutions in solvents of low viscosity or at higher temperatures, the line broadening can be decreased somewhat. Under these conditions, rotational motions of the molecules, which contribute to the molecular correlation times, are increased. Because of the direct relationship between correlation times and relaxation rates, the nuclear relaxation rates are slowed and the resonances sharpen. In some instances, the electric field around the

TABLE 1

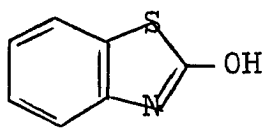
NMR PROPERTIES OF NITROGEN NUCLEI

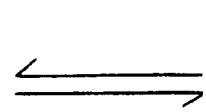
	^{14}N	^{15}N
Natural abundance	99.635%	0.365%
Spin	1	1/2
Sensitivity relative to ^{1}H	0.000343	0.00101
Frequency at 23.5 kG	7.225 MHz	10.134 MHz
Electric quadrupole moment	1.54×10^{-2}	0
Magnetogyric ratio	$1.934 \times 10^{3} \frac{\text{rad}}{\text{sec gauss}}$	$-2.712 \times 10^{3} \frac{\text{rad}}{\text{sec gauss}}$

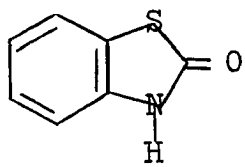
nitrogen is sufficiently symmetrical, as in some ammonium ions and isonitriles, to reduce the effects of quadrupolar relaxation and narrow the resonance lines.

Since the range of nitrogen chemical shifts for organic molecules is large (> 500 ppm), ^{14}N measurements have been important in determining structures for grossly different chemical environments but can obscure solvent effects and consequences of subtle changes in molecular structure. Nitrogen-14 spectroscopy has been very useful in determing preferred tautomeric structures when there are significant differences in the electronic environment around nitrogen (5). An example of this is found in the 2-hydroxy substituted benzthiazoles (III) and quinolines (VII) and their respective 4- and 8-isomers (IV and IX) where only the 2-substituted isomers can exist in the amide form (Figure 1). The absorptions of 4-hydroxybenzthiazole and 8-hydroxyquinoline are found in the range for the parent molecules (VI and X) whereas the high field resonances of the 2-substituted isomers is indicative of the predominance of the amide tautomers (IV and VIII).

The ^{15}N isotope with a spin of 1/2 does not have a quadrupole moment and, hence, is suitable for high resolution studies. However, this isotope of nitrogen only occurs in natural abundance at 0.365 % and has a sensitivity only 0.00101 that of an equal number of protons in the same magnetic field. To circumvent this difficulty, various methods have been employed.

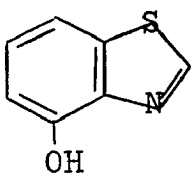






-243.0

III IV



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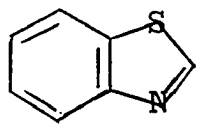

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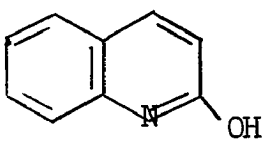
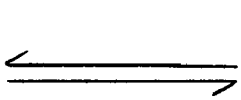
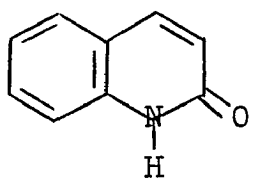
V VI

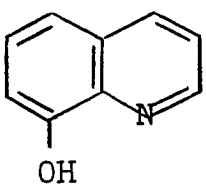






-238+2

VII VIII



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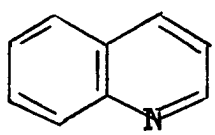


- 72+2

IX X

Figure 1. Nitrogen chemical shifts of hydroxy substituted benzthiazole and quinoline, upfield from nitromethane.

Brief Survey of Experimental Methods

The Continuous Wave and INDOR Techniques

Nitrogen-15 enrichment, which is often tedious and always expensive, was widely used for the direct measurement of ^{15}N chemical shifts and coupling constants (6) by the continuous wave (CW) technique, in which spectra are determined by sweeping the frequency (or field) through the nmr region of absorption such that nonequivalent nuclei are sequentially brought into resonance. In this mode of excitation, only a very narrow band of frequencies contributes a signal at any one time. Indirect detection of ^{15}N resonances is also possible in enriched compounds where a proton is coupled to a ^{15}N nucleus. The proton signal is continuously observed while the nitrogen frequency is varied until the proton resonance is decoupled. Alternatively, indirect measurement of ^{14}N and ^{15}N chemical shifts can be effected using the INDOR (internuclear double resonance) technique (7). Here the observing (proton) frequency is fixed on a resonance line while the decoupling frequency is varied with a voltage controlled oscillator coupled to the instrument recorder. The output often resembles the usual ^{15}N absorption spectrum.

Intensity Enhancement through Proton Irradiation

At the natural abundance level, ^{15}N nmr determinations in the CW mode require time-averaging devices. Spectral accumulation time may be decreased by taking advantage

of proton irradiation, which may produce a twofold effect. Irradiation with a noise-modulated frequency (8) will effect complete proton decoupling, collapse the nitrogen resonance multiplets and increase signal intensity. In addition to the elimination of the multiplicity from N-H coupling, the intensity of the decoupled signal might be greater than that expected from simple collapse of the multiplet. This intensity change, the nuclear Overhauser effect (NOE) (9), arises in general when an observed nucleus relaxes via dipole-dipole interactions with another nucleus, commonly proton, whose energy level populations are saturated. The observed nuclei respond to this saturation by changing their own population distribution. The maximum obtainable NOE is given by:

$$\frac{I}{I_o} = 1 + 1/2\ (\gamma_H/\gamma_X) \qquad (2)$$

where I_o is the intensity without proton irradiation and I is the intensity with proton irradiation.

For ^{13}C, the maximum intensity enhancement from the NOE is threefold. Since the magnetogyric ratio of ^{15}N is negative, saturation of the proton transitions results in an equilibrium excess of nuclei in the upper ^{15}N energy level relative to that required for a Boltzmann distribution. Experimentally, this means that the maximum observable NOE for ^{15}N is -3.93 and inverted resonances are produced. However, other relaxation mechanisms can reduce the NOE and the spectral accumulation time required to

reach a specified signal-to-noise ratio is increased. The intensity range for ^{15}N nuclei is from +1 to -3.93. Since this range encompasses both positive and negative values, the NOE may lead to a decrease in signal intensity. In some cases, opposing relaxation mechanisms reduce the NOE to such an extent that observation of the ^{15}N resonance is eliminated altogether (10).

The Pulsed Fourier Transform Mode

The use of pulsed Fourier transform techniques (11) to improve sensitivity in ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy is well known, but has only been applied to ^{15}N measurements within the past four years (12). In the pulsed FT mode, application of a strong radio frequency pulse excites nuclear spins over the entire range of precession frequencies simultaneously. After each pulse, the free induction decay of the resulting magnetization is stored in a computer and converted to an absorption mode spectrum via the mathematical technique of Fourier transformation. The main advantage of Fourier transform methods lies in the faster repetition rate (i.e. the time between successive pulses) relative to the continuous wave method. Time averaging devices increase sensitivity directly as the square root of the number of scans. Thus, a much shorter time is required to reach a given signal-to-noise ratio compared with that in the CW mode. This time advantage can be lost if the observed nuclei have long T_1's and the longitudinal components of the magnetization do not reattain

their equilibrium values between successive pulses. A variety of techniques has been employed to overcome this problem, so that significant time savings are generally always realized. These include the DEFT (driven equilibrium Fourier transform) pulse sequence or the less complex approach of decreasing the flip angle and increasing the time delay between successive pulses.

A Description of the Chemical Shielding Tensor

Before discussing the experimental chemical shift values for nitrogen, a qualitative description of the factors affecting the nitrogen shielding tensor is appropriate. A quantitative discussion will be deferred until a later section. The chemical shielding tensor consists of two main terms: a diamagnetic term involving mainly s electrons, containing the contribution from the local circulation of electrons on an isolated atom; and a paramagnetic term which is described by the interaction between ground and excited electronic states of the atom upon application of the external magnetic field. Since shielding of the nitrogen nucleus by s electrons is not reflected by large variations in the diamagnetic term, this term has often been neglected. Experimental trends very often can be correlated with calculated changes in the paramagnetic term which usually is assumed to dominate.

$$\sigma^{N} \sim \sigma_{p} \propto (\Delta E_{av})^{-1} \left\langle r_{2p}^{-3} \right\rangle \sum_{B} Q_{AB} \qquad (3)$$

In the above equation, ΔE represents an average of the

excitation energies from the mixing of ground and excited electronic states by the applied field, $\langle r_{2p}^{-3} \rangle$ is the inverse cube of the distance from the nitrogen nucleus to a 2p electron and the Q terms are functions of bond orders representing electronic interactions between nitrogen and the other atoms in the molecule.

The paramagnetic term arises from states which possess orbital angular momentum, i.e. states that are lacking in spherical symmetry, and acts in a direction opposite to that of the diamagnetic term. The larger the asymmetry of the electron distribution, the greater the contribution of the paramagnetic term and the further downfield the nucleus resonates. Thus, in the case of the ammonium ion, which has spherical symmetry and presumably a spherical electronic environment about the nitrogen atom, the resonance is far upfield. In contrast, azo compounds resonate about 400 ppm downfield from the ammonium ion and, hence, are assumed to have considerable electron asymmetry at the nitrogen atom.

Nitrogen Chemical Shifts

Nitrogen chemical shifts of organic compounds can be divided into regions roughly characteristic of the type of nitrogen in a compound (13) (Figure 2). Since there is no discernible isotope effect (14), ^{14}N and ^{15}N chemical shifts can be compared directly. However, the larger experimental error inherent in the ^{14}N chemical shifts should not be overlooked. In Table 2, the nitrogen chemical shifts

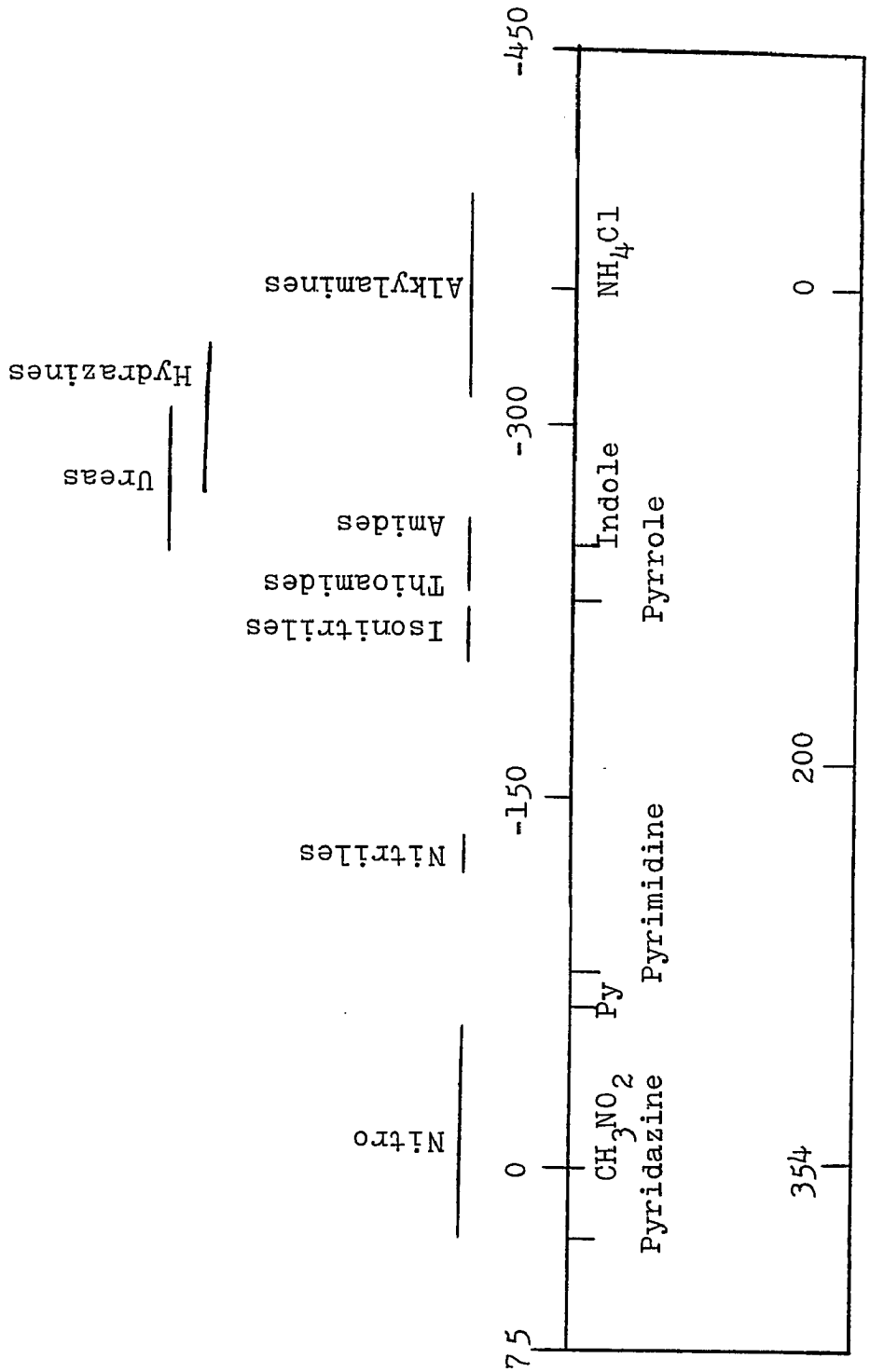


Figure 2. Nitrogen chemical shift range in organic compounds.

TABLE 2

NITROGEN CHEMICAL SHIFTS OF SOME REPRESENTATIVE COMPOUNDS

Compound	δ_N, ppm
Amines	
NH_3	-383
$MeNH_2$	-378
$EtNH_2$	-355
$n\text{-}PrNH_2$	-359
aniline	-317.0
o-toluidine	-319.3
m-toluidine	-317.9
p-toluidine	-319.6
Hydrazines	
H_2NNH_2	-328.7
$(CH_3)_2NN(CH_3)_2$	-271.5
Isonitriles	
CH_3NC	-218
CH_3CH_2NC	-203
Nitriles	
CH_3CN	-137
CH_3CH_2CN	-137
$C_6H_5CH_2CN$	-133
Nitro compounds	
$C_6H_5NO_2$	- 8
CH_3NO_2	0

TABLE 2---continued

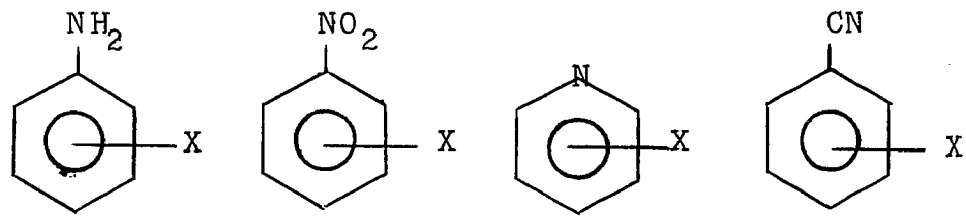
Compound	δ_N, ppm
$C(NO_2)_4$	48
Heterocycles	
s-triazine	- 98
pyrimidine	- 78
quinoline	- 72
pyridine	- 62.2
pyrazine	- 41
tetrazine	5
pyridazine	31

SOURCE: references 13, 16

of some representative classes of compounds are listed. Amines display regular changes in chemical shift with changes in structure. Alkyl substitution at the alpha position produces small downfield shifts, larger shifts (ca. 18 ppm) beta to the nitrogen and small upfield shifts at the gamma position. In the methylhydrazines, the alpha-beta effect is maintained. There is no observable systematic substituent effect in the ^{14}N results for the thioamides but this might be a reflection of the experimental techniques. The more precise ^{15}N data might be more revealing. Nitrile ^{14}N chemical shifts appear to be less sensitive to substitution than amines and span a small region; in contrast, isonitriles resonate 60-85 ppm to higher field. Since the ^{14}N linewidths of the isonitriles are much narrower than those of the corresponding nitriles, a more symmetrical electronic distribution around the former nitrogen is suggested. The consequent expected reduction in the paramagnetic term would lead to an upfield shift, as is observed. Nitro compounds resonate in the low field region of the normal nitrogen chemical shift range; beta alkyl substituents induce downfield shifts and electronegative substituents high field shifts. Thus, the nitrogen nuclei in nitro compounds respond to substituents similarly as the amine compounds.

As noted above, the large error inherent in the measurement of ^{14}N chemical shifts can obscure the effects of substituents on the nitrogen resonance position. The

experimental error often encompasses the expected range of absorption for a particular substituent so that it is obvious that the ^{14}N chemical shift measurements can only account for gross structural changes. This is the case for a substantial number of aromatic nitrogen compounds; small differences for a given class of substituents have remained undetected. Because it is experimentally feasible to determine ^{15}N chemical shifts in natural abundance, it is appropriate to examine these compounds in more precise detail. In particular, we may pose the question: "How do the various types of aromatic nitrogen atoms exemplified in XI-XIV respond to position, number and type of substituent?"



XI XII XIII XIV

Aniline Derivatives

The most extensively investigated of these compounds has been the aniline derivatives in which the nitrogen resonances lie in a region 309-351 ppm upfield from nitromethane. Substitution of the amino protons with aryl groups induces downfield shifts in the nitrogen resonance position relative to aniline itself. Reasonable linear correlations have been reported between the ^{14}N chemical shifts of aniline derivatives (15) and Hammett substituent constants.

However, these results were obtained with strongly interacting substituents making a correlation more likely. It was not until the more precise ^{15}N data were available that the subtle effect of methyl substituents on the nitrogen resonances of the anilines was apparent (16). Thus, while ^{14}N chemical shifts of toluidines and xylidines were well within experimental error of each other (13), ^{15}N chemical shift determinations revealed that ortho and para methyl substitution induced comparable additive ($\sim$2.5 ppm) upfield shifts while meta substitution leads to a small (0.8 ppm) but still upfield change. This was correlated with changes in sigma and total electron densities at nitrogen (16).

Nitrobenzene Derivatives

Both ^{14}N (17) and ^{15}N (18) measurements on the nitrobenzenes indicated a correlation of the chemical shifts with the Hammett substituent constants σ_p and σ_m. The influences of meta and para substituents were found to be nearly equal, although the correlation with σ_m seemed to be slightly better than that with σ_p. Correlations of nitrogen electron densities and π bond orders with the ^{15}N chemical shifts showed strong deviations from linearity. As with the methylanilines, ^{15}N measurements reveal the more subtle interactions of the methyl group on the nitrogen resonance positions than the ^{14}N measurements; a large steric component appears to be an important aspect of the ortho substituent effect (17, 19).

Benzonitrile Derivatives

Presumably because of the expected long T_1's of the cyano nitrogen (20), there have been no definitive studies of the dependence of the nitrogen chemical shifts of benzonitriles on the position and nature of substituents. The ^{14}N chemical shifts of benzonitrile and phenylacetonitrile are, respectively, -124 and -133 ppm upfield from nitromethane. Since nitriles absorb over a region of only 20 ppm as discussed above, one would expect substituted benzonitriles to span a similarly small range.

Pyridine Derivatives

The effect of substituents on the free bases

The pyridine type nitrogen is different from those in the other aromatic compounds XI, XII and XIV in that this nitrogen atom is a constituent of the ring with the lone-pair electrons oriented in the plane of the ring. Conjugative interactions of the lone-pair electrons with the π system are thereby eliminated. Compared to substituent effects on the ^{14}N resonance positions of other aromatic nitrogen compounds, those on pyridines have been extensively investigated (13). With electron-releasing substituents (OH, OMe, NH_2) upfield shifts are induced while electron-withdrawing substituents (CN, NO_2, COR) give downfield shifts relative to pyridine.

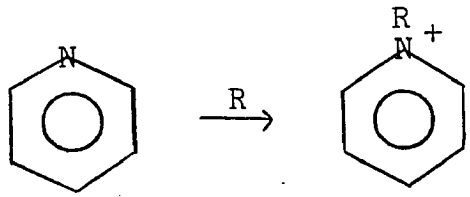
Correlations of pyridine nitrogen resonance positions with molecular orbital parameters

Huckel molecular orbital calculations were carried

out by Hensen and Messer (21) on twelve mono- and disubstituted halogen derivatives of pyridine and electron densities were compared with the measured ^{14}N chemical shifts. Apparently there was a good linear correlation. However, the measured chemical shifts and calculated nitrogen π electron densities spanned a large range (54 ppm and 160 $\times 10^{-3}$, respectively) thereby making an apparent correlation more likely. Although a crude correlation has also been noted between some azine ^{14}N nuclear shieldings and calculated electron densities (13), there is no systematic trend between position of either electron-withdrawing or electron-releasing substituent and nitrogen chemical shift. Extensive investigations have not been made on substituent effects in diazines, triazines and tetrazine (26).

The effect of substituents on the nitrogen chemical shifts of the pyridinium ions

When the nitrogen atom of pyridine is protonated, the lone-pair electrons are removed and the nitrogen resonance signal moves upfield by ca. 100 ppm. This strikingly large change compared with that of aniline (ca. 5 ppm) has been the subject of numerous investigations. A discussion of the protonation shift will be deferred until a later section. Nitrogen-14 measurements within a series of pyridinium ion derivatives have established that a low field shift occurs in the sequence R=H, Me, Et. The magnitude of this change (50 ppm) (3) is comparable with that found for aliphatic amines (28 ppm) (2, 22).



The effect of both electron-withdrawing and electron-releasing substituents on the nitrogen resonance positions of pyridinium ion derivatives parallels that found on the free bases (13).

Very recently, the ^{15}N resonance position of pyridinium ion has been shown to be solvent dependent (23). This emphasizes the necessity of comparing only those ^{14}N and ^{15}N chemical shift data which were obtained in the same solvent at equal molar concentrations.

Object of this Investigation

From the above discussion, it is clear that in order to obtain more insight into the electronic factors affecting the chemical shifts of nitrogen nuclei in aromatic systems measurement of the more precise ^{15}N chemical shifts is warranted. In particular, it was of interest to compare the effect of ring substitution on the resonance position of the pyridine nitrogen, with its lone-pair orthogonal to the π system, with that on the resonance position of the aniline nitrogen, whose lone-pair interacts with the π system. While the effect of conjugatively interacting substituents on the pyridine resonance positions could be qualitatively anticipated from the ^{14}N data, the influence of methyl

substitution could not be estimated in advance. Hence, the purpose here was to obtain accurate data reflecting as much as possible the electronic effects alone, and to attempt to develop a theoretically sound correlation with molecular structure. At the same time, the values could be used to assess the applicability of currently available semi-empirical methods for calculating chemical shifts.

Experimental aspects of natural abundance ^{15}N chemical shift measurements are presented first. A discussion of the substituent effects on the pyridine ^{15}N resonance position is given in Chapter III followed by a theoretical interpretation of the nitrogen nuclear shielding.

CHAPTER II

THE USE OF RELAXATION REAGENTS IN ^{15}N NMR

Several experimental difficulties were encountered in the measurement of the ^{15}N chemical shifts of the pyridines at the natural abundance level. Because there is no directly bonded proton, the nitrogen nuclei are not expected to experience a maximum NOE when the sample is proton decoupled. In pyridine itself, the nitrogen nucleus has a spin-lattice relaxation time, T_1, of 50 seconds (24). This necessitates the use of short pulses and slow repetition rates, resulting in the loss of some of the enhancement gained from the pulse FT method. For example, in order to obtain a resonance signal from neat pyridine an overnight run was needed to collect over 5500 transients at a repetition rate of 9.1 seconds over a five kHz range. To facilitate chemical shift measurements, paramagnetic compounds have been used to shorten T_1 (25), ostensibly leaving the chemical shift unaffected. What is required of a relaxation reagent is that the compound be soluble and kinetically inert in organic solvents, not cause contact shifts and reduce T_1 values but not affect T_2 values enough to cause significant line broadening. These requirements are satisfied, to varying degrees, by the metal ions Mn(II), Fe(III), Cr(III) and Gd(III). Although many metal complexes of these paramagnetic ions are soluble in organic solvents, none are

as inert to substitution as the compound tris(acetylacetonato) chromium(III), $Cr(acac)_3$.

Optimization of Experimental Conditions

To optimize experimental conditions, pulse width and concentration of the paramagnetic relaxation reagent (PRR) $Cr(acac)_3$ were varied in a sample of pyridine and signal-to-noise ratios in the spectra were measured. Signal-to-noise ratio (S/N) is defined as (11):

$$S/N = \frac{5}{2} \frac{\text{signal intensity}}{\text{peak-to-peak noise intensity}} \quad (4)$$

As indicated in Table 3 and Figure 3, S/N increased with the concentration of the paramagnetic additive. However, the ^{15}N resonance in a solution containing 0.200 M $Cr(acac)_3$ was apparently shifted upfield 1.9 ppm from the value obtained in neat pyridine. The latter chemical shift was obtained on a sample of pyridine containing no PRR. Similar concentration studies were carried out on 4-picoline and 2,5-lutidine with parallel results (Figure 4). Because of this upfield shift it was necessary to sacrifice some loss in S/N and use a lower concentration of PRR to obtain chemical shift values held to reflect structural influences. The ^{15}N chemical shifts of the picolines, lutidines, chloro- and bromopyridines were then obtained at a concentration of 0.0500 M $Cr(acac)_3$. Because pyridines are known to be hygroscopic, the samples were stored over KOH pellets. Since the nitrogen resonances of nearly half of the

TABLE 3

OPTIMIZATION OF EXPERIMENTAL CONDITIONS FOR PYRIDINE

S/N	$(Cr(acac)_3)$ (M)	Pulse width (usec)	Repetition rate (sec)	δ^{15}_N, ppm
3.8	0.0250	7	1.1	293.3
3.3	0.0250	11	1.1	293.3
3.2	0.0250	11	2.1	293.3
2.6	0.0250	15	1.1	293.3
...	0.0250	22	1.1	...
6.5	0.0500	7	1.1	292.8
4.3	0.0500	11	1.1	292.7
6.1	0.0750	7	1.1	292.6
3.9	0.0750	11	1.1	292.7
7.6	0.100	7	1.1	292.5
10.8	0.100	11	1.1	292.6
7.5	0.100	22	1.1	292.5
9.2	0.200	11	1.1	291.4
4.2	0.200	15	1.1	291.6
8.0	0.200	22	1.1	291.6

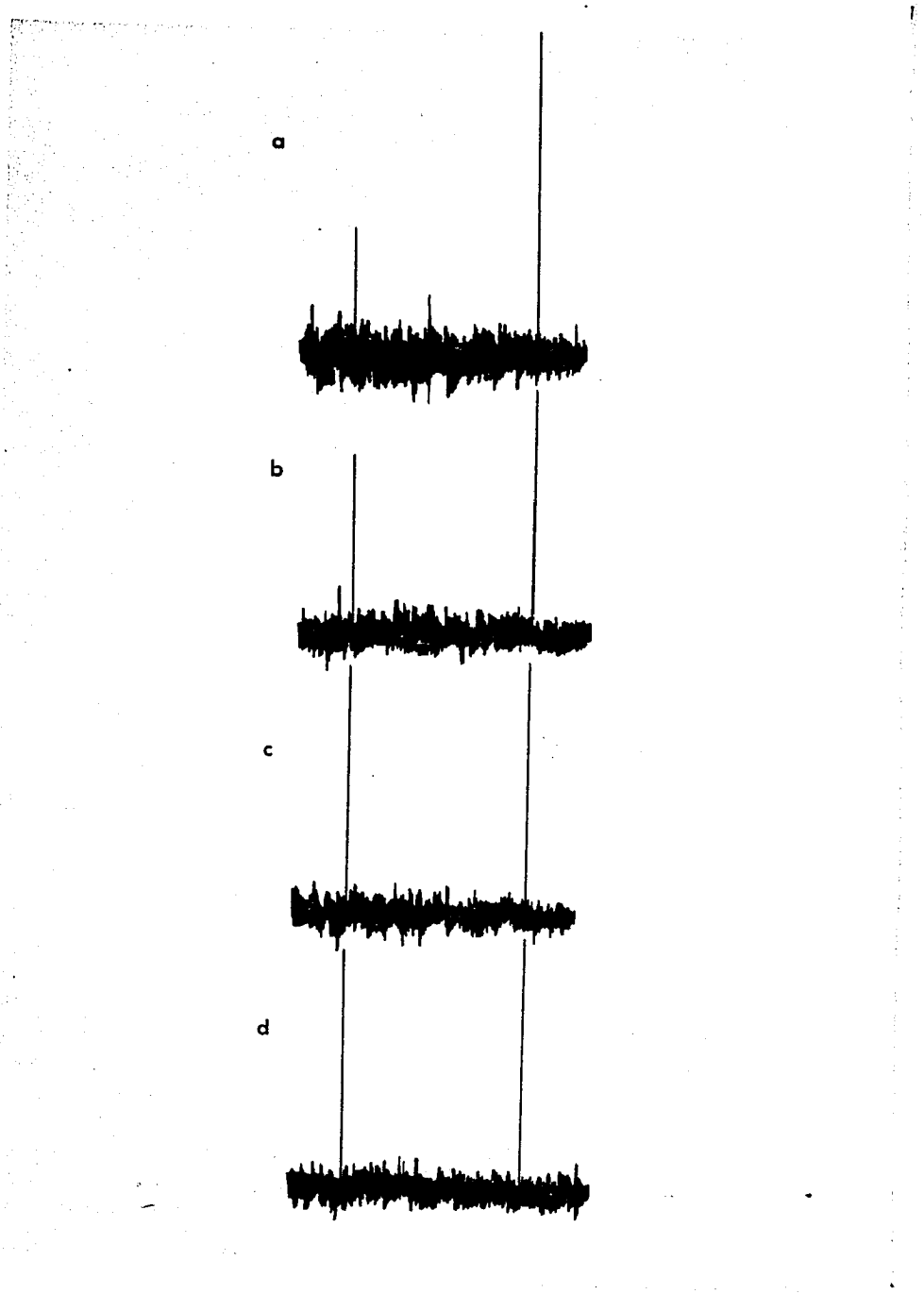


Figure. 3. The effect of $Cr(acac)_3$ concentration on the pyridine nitrogen resonance intensity. (a) 0.0125 M $Cr(acac)_3$, (b) 0.0250 M $Cr(acac)_3$, (c) 0.0500 M $Cr(acac)_3$, (d) 0.100 M $Cr(acac)_3$. The pyridine resonance position was measured downfield from a capillary containing enriched, aqueous ammonium chloride (2 M).

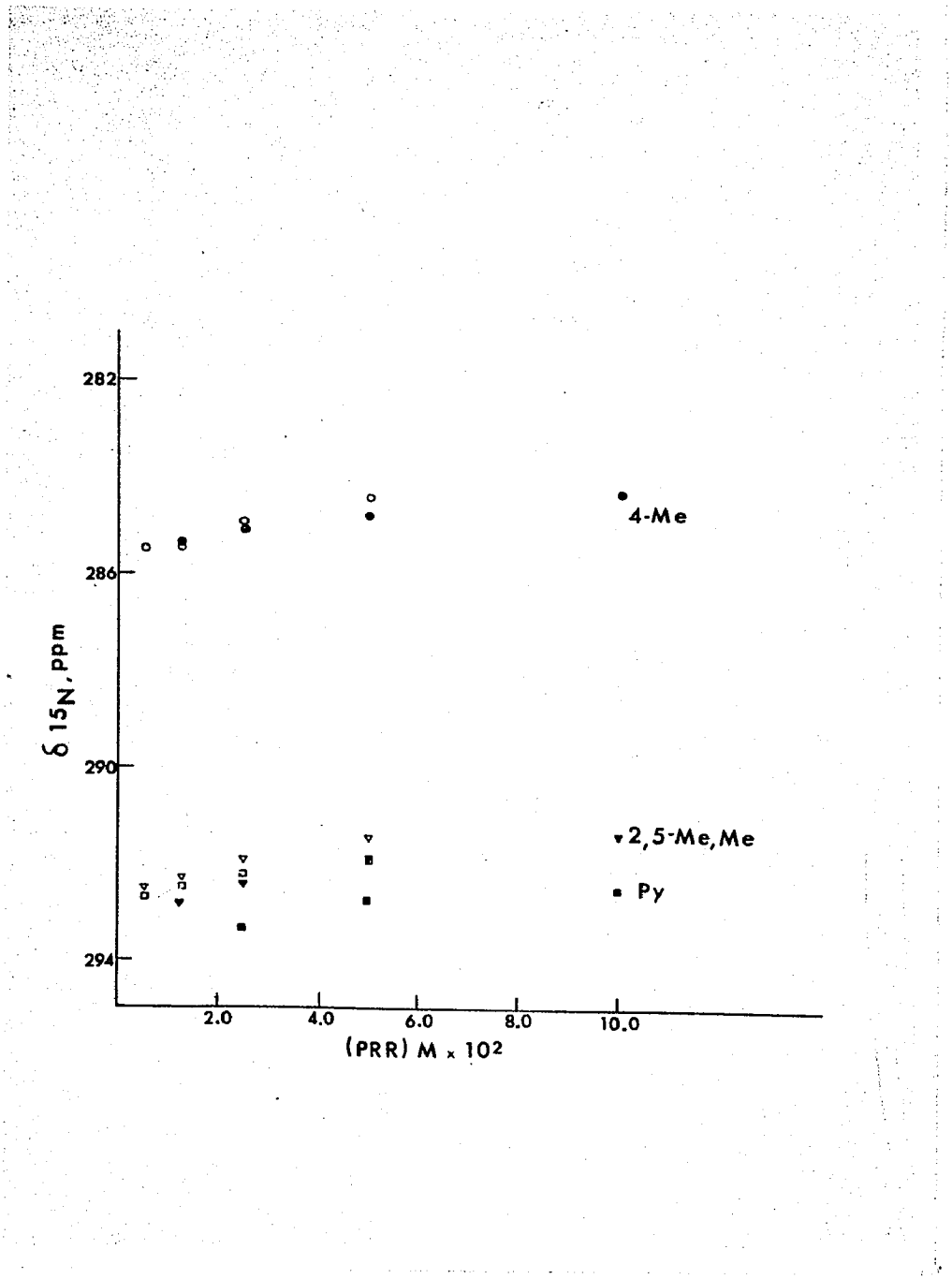


Figure. 4. The effects of $Fe(acac)_3$ and $Cr(acac)_3$ on the nitrogen resonance positions of pyridine, 4-picoline and 2,5-lutidine. $Fe(acac)_3$ o □ ▽, $Cr(acac)_3$ ● ■ ▼.

substrates fell within one ppm of the pyridine resonance and could not readily be rationalized in terms of substituent electronic effects, the possibility that impurities might be obscuring the substituent effects had to be considered. Samples were distilled in addition to being stored over KOH prior to use, but because the boiling points of 3-picoline, 4-picoline and 2,6-lutidine differ by less than one degree, they cannot be separated by ordinary distillation procedures. To determine if the resonance positions would be affected by minor azine impurities, the ^{15}N chemical shift of 4-picoline was obtained from a sample containing 10% v/v 2,6-lutidine; there was no observable shift from the value of the neat liquid as seen in Table 4. Since the chemical shift measurements were obtained on samples which were open to the atmosphere, it was advisable to determine the effect of possible absorption of atmospheric moisture on the resonance positions. The ^{15}N chemical shift of pyridine was measured in a solution containing 10% v/v water. Compared with the value of the neat liquid (Table 4) there was no observable effect on the nitrogen nuclear shielding.

In an attempt to further minimize any interactions with the PRR, a concentration of 0.0125 M $Cr(acac)_3$ was used in the determination of the ^{15}N chemical shifts of the pyridine series. The order of the nuclear shieldings relative to pyridine obtained from the distilled samples was significantly different for several of the substrates.

TABLE 4

THE EFFECT OF IMPURITIES ON NITROGEN NUCLEAR SHIELDINGS

	δ^{15}_{N}, ppm	
	neat	10 vol % (impurity)
Pyridine	293.3	293.3(water)
4-picoline	285.4	285.5(2,6-lutidine)

For example, the ^{15}N resonance positions of 3-picoline and 3,5-lutidine moved downfield of the pyridine resonance whereas the resonance positions of 4-picoline and 2,6-lutidine moved further upfield by nearly one ppm relative to that measured in the undistilled samples.

However, there remained the apparent non-systematic, non-additive effects of methyl substitution. At this point, redetermination of the concentration effect of $Cr(acac)_3$ on the nitrogen resonance position was warranted. This was especially appropriate because of recent reports (26) that ^{13}C chemical shifts can be affected by PRR's to a greater degree than previously considered. Hence, a more detailed investigation of the influence of $Cr(acac)_3$ on pyridine, 4-picoline and 2,5-lutidine was undertaken. The Fe(III) complex with acetylacetone was also investigated since it was reported that this PRR appears to be more inert to substitution than the chromium complex (27).

PRR Concentration Effects

From the results in Tables 5 and 6, it appears that the paramagnetic additives induced shifts from 0.7 to 1.3ppm over the concentration ranges studied. The directions of the shifts are, in all cases, upfield but the magnitudes differ from substrate to substrate. A qualitative observation indicated that at the lowest levels of PRR concentration, ^{15}N signals could be obtained in approximately 1/3 of the time when the iron derivative was used instead of the chromium derivative. That is, the Fe(III) complex is a

TABLE 5

THE EFFECT OF $CR(ACAC)_3$ CONCENTRATION ON $\delta 15_N$

Sample	Memory Address $^{15}NH_4Cl$	Memory Address Sample	$\delta 15_N$ (ppm)	$(Cr(acac)_3)$ (M)
Pyridine	3549	1127	293.3	0.0250
	3546	1129	292.7	0.0500
	3541	1126	292.5	0.100
Range	8	3	0.8	
4-Picoline	3714	1356	285.3	0.0125
	3712	1356	285.0	0.0250
	3710	1356	284.8	0.0500
	3705	1355	284.3	0.100
Range	9	1	1.0	
2,5-Lutidine	3714	1294	292.8	0.0125
	3712	1295	292.4	0.0250
	3710	1297	291.9	0.0500
	3706	1297	291.4	0.100
Range	8	3	1.3	

TABLE 6

THE EFFECT OF $FE(ACAC)_3$ CONCENTRATION ON $\delta 15_N$

Sample	Memory Address $^{15}NH_4Cl$	Memory Address Sample	$\delta 15_N$ (ppm)	$(Fe(acac)_3)$ (M)
Pyridine	3713	1294	292.7	0.00500
	3711	1294	292.4	0.0125
	3708	1293	292.2	0.0250
	3702	1289	291.9	0.0500
Range	11	5	0.7	
4-Picoline	3714	1355	285.4	0.00500
	3712	1353	285.4	0.0125
	3708	1353	284.9	0.0250
	3703	1352	284.4	0.0500
Range	11	3	1.0	
2,5-Lutidine	3713	1295	292.5	0.00500
	3711	1295	292.3	0.0125
	3708	1295	291.9	0.0250
	3703	1294	291.4	0.0500
Range	10	1	1.1	

more efficient relaxation reagent than the Cr(III) complex. This agrees qualitatively with the results of Levy and Cargioli (27) in their investigation of ^{13}C nuclear relaxation rates. Both the iron and chromium acetylacetonates are high-spin octahedral complexes, but iron(III) with a d^5 electronic configuration has a larger magnetic moment (assuming insignificant spin-orbit contributions) than chromium(III) with a d^3 configuration. A larger magnetic moment implies greater efficiency as a relaxation reagent. A serious disadvantage of tris(acetylacetonato) iron(III) is the apparent tendency of this compound to absorb atmospheric moisture. Upon standing in air, the $Fe(acac)_3$ changed in both physical appearance and in melting point. Therefore, the chromium complex was the reagent of choice for subsequent experiments.

Possible Interactions of the PRR

A bulk susceptibility effect

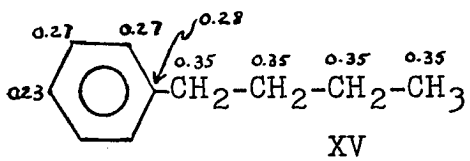
If the data in Tables 5 and 6 are examined more closely, it is evident that the absolute memory address of the ammonium chloride reference changes more than the experimental error of one address, while that of the sample stays relatively constant as the concentration of the paramagnetic additive is increased. Hence, the apparent chemical shift variation results, in fact, from a change in the resonance position of the reference, not the sample. Use of an external reference excludes the possibility of direct

interaction with the relaxation reagent so that the apparent shift must arise from a change in the absolute magnetic field experienced by the reference. Two possibilities can be considered: a bulk susceptibility effect or a preferred interaction with the lock substance (deuteriobenzene in these particular samples). If the former effect dominates, then the same effect (28) should be observed in other systems where an external ammonium chloride reference was used in a sample containing both deuteriobenzene and a paramagnetic additive. Other work in this laboratory (29) has shown that under the same experimental conditions the chemical shifts of nitrobenzene and nitromethane do not depend on concentration of added $Cr(acac)_3$. Moreover, at high concentrations of $Cr(acac)_3$ the nitrogen chemical shift is no longer affected (Figure 4), so that bulk susceptibility is probably not a major influence.

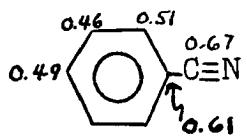
An orientation effect

The other possibility to be considered was interaction of the PRR with the deuteriobenzene. Although PRR's are chemically inert, physical interactions between substrate molecules and paramagnetic additives are feasible. Electrostatic or dipolar interactions can result in preferential orientation of molecules toward the paramagnetic complexes. Because the electron-nuclear dipole-dipole relaxation rates do not exhibit an angular dependence (25e), measurement of the T_1 values can provide information on the relative distances between the substrate nuclei and paramagnetic

metal in the substrate-PRR complex. However, equilibrium constants for these complexes have not been measured, so that qualitative rather than quantitative results are obtained. The T_1 value obtained for a particular nucleus in the substrate depends on the time-averaged internuclear distance between the paramagnetic metal ion and that nucleus. Hence, the shortest T_1 values will be found for those nuclei which are closest to the site of complexation. This is evidenced by the T_1 values measured by Levy and Komoroski (25e) who report a preferential orientation of the phenyl ring of alkylbenzenes (XV) and benzonitrile (XVI) toward iron and chromium acetylacetonates.



XV neat, 6.74×10^{-2} M $Fe(acac)_3$



XVI neat, 5.73×10^{-2} M $Cr(acac)_3$

On this basis a similar interaction with benzene-d_6 is not unreasonable. If the lock substance is affected by the paramagnetic compound competitively with the substrate, this would be reflected in the resonance position of the ammonium chloride reference. Such a pronounced interaction between PRR and five volume per cent of the bulk solution might occur if complexation between PRR and benzene-d_6 is proportionally greater than that between PRR and substrate. Indeed, Frankel, Langford and Stengle (30) have used the effect of paramagnetic solutes on the ^1H T_2 values of solvent nuclei to demonstrate preferential solvation of the

PRR. Their results indicate that a $CHCl_3$-CCl_4 solvent mixture, as low as ten volume per cent in $CHCl_3$, shows a preferential solvation of $Cr(acac)_3$ by $CHCl_3$. If the most favorable complex is formed by the orientation of the PRR over the ring of the substrate, then increasing the number of substituents on the ring should decrease PRR-substrate complexation. One would, therefore, expect a larger interaction of PRR with deuteriobenzene in a substituted pyridine than in pyridine itself. Tables 5 and 6 reveal that the ^{15}N resonance positions are affected in the order pyridine < 4-picoline < 2,5-lutidine. However, the chemical shift variations among the substrates are small and the observed order may be fortuitous.

To test the possibility that the concentration dependence arises because of interaction between the PRR and the deuteriobenzene, the lock substance was contained in an external capillary and nitromethane ($\delta\ ^{15}N = 354.1$) was used as an internal reference. Initial experiments indicated that 20% v/v nitromethane was required to obtain a ^{15}N signal in a reasonable amount of time (less than 2 1/2 hours). Table 7 presents the results, where it is apparent that for the three substrates examined the shifts relative to nitromethane remained constant over the concentration range of PRR used.

TABLE 7

THE EFFECT OF $CR(ACAC)_3$ CONCENTRATION ON $\delta\,15_N$ USING INTERNAL NITROMETHANE AS REFERENCE

	$\delta\,15_N$, ppm		
$(Cr(acac)_3)$(M)	Pyridine	4-Picoline	2,5-Lutidine
0.0125	-62.2	-70.2	-62.8
0.0250	-62.2	-70.2	-62.7
0.0500	-62.2	-70.2	-62.7
0.100	...	-70.2	-62.9

Steric inhibition of the interaction between PRR and substrate

Two additional experiments were carried out in order to gain further qualitative insight into the effects of the paramagnetic metal complexes. Levy (31) has shown that the bulky complex chromium tris(2,2,6,6-tetramethyl-3,5-heptanedionate), $Cr(thd)_3$, is less efficient in shortening ^{13}C T_1 values than $Cr(acac)_3$. Presumably, the larger molecular size inhibits close approach to the substrate and, thus, complex formation. In our case, a steric inhibition of this type would be expected to affect the chemical shifts to a lesser degree with respect to the acetylacetonate complex because of a reduced interaction with the lock compound, with the substrate or with both. At the same time, any bulk susceptibility effect would be maintained because its origin should be primarily in the molar quantity of paramagnetic Cr(III) added irrespective of the ligand. Accordingly, samples of pyridine, 4-picoline and 2,5-lutidine containing internal deuteriobenzene as the lock substance and varying molar quantities of $Cr(thd)_3$ were examined using an external ammonium chloride reference.

The results, given in Table 8, indicate that at concentrations below 0.0500 M the chemical shifts do not change outside of experimental error. However, at the next concentration examined, 0.100 M, the resonance position changed abruptly, spanning a range of 0.5-0.9 ppm. To unambiguously eliminate bulk susceptibility as the origin of this effect, the ^{15}N chemical shift of pyridine in a

TABLE 8

THE EFFECT OF CR(THD)$_3$ CONCENTRATION ON $\delta^{15}N$

Sample	Memory Address $^{15}NH_4Cl$	Memory Address Sample	$\delta^{15}N$ (ppm)	(Cr(thd)$_3$) (M)
Pyridine	3545	1130	292.2	0.0125
	3544	1131	291.9	0.0250
	3542	1131	291.7	0.0500
	3537	1132	291.0	0.100
Range	8	2	1.2	
4-Picoline	....	....	.....	0.0125
	3545	1193	284.5	0.0250
	3544	1193	284.4	0.0500
	3538	1195	283.5	0.100
Range	7	2	1.0	
2,5-Lutidine	....	....	.....	0.0250
	3543	1133	291.6	0.0500
	3539	1133	291.1	0.100
Range	4	0	0.5	

sample 0.100 M in $Cr(thd)_3$ was measured with respect to $^{15}NH_4Cl$ contained in a capillary with 80% v/v benzene-d_6. The resonance position fell within the region of low $Cr(thd)_3$ concentration. This suggests that preferential solvation of $Cr(thd)_3$ by deuteriobenzene does occur but at a higher concentration compared with that found for $Cr(acac)_3$. Presumably, the attraction of Cr(III) for benzene-d_6 molecules is inhibited by the bulky t-butyl groups of the ligand requiring a high concentration of $Cr(thd)_3$ before an orientation interaction can occur.

Operationally, the absence of significant chemical shift variations at low concentrations of $Cr(thd)_3$ would seem to represent an advantage of this reagent over $Cr(acac)_3$ since one would not have to consider interactions between lock substance and PRR. However, longer spectral accumulation times were required in order to obtain S/N ratios comparable to those obtained with $Cr(acac)_3$. Furthermore, use of deuteriobenzene as internal lock substance in this family of compounds is questionable. That the ^{15}N resonance position of pyridine is solvent sensitive was demonstrated by Lichter and Roberts (32). A 17.6 ppm upfield shift was measured when neat pyridine was diluted to ca. 30 volume percent in methanol. Unreported results on nicotine (33) indicate that the nitrogen resonance position of the pyridine moiety is also solvent dependent.

Initial investigations into the effect of solvent on the ^{15}N resonance position of some substituted pyridines indicated that 50% dilution with benzene induced upfield

shifts on the order of 2 ppm (Table 9). If this represents an aromatic solvent induced shift (ASIS) (34), then comparable shift variations could be anticipated with deuteriobenzene as the solvent. Assuming a linear relationship between amount of benzene and the solvent shift, one can crudely approximate the effect of 10% benzene-d_6 as lock substance to be about 0.4 ppm. However, the possibility of a solute-solvent induced interaction between lock substance and substrate, even though only on the order of several tenths of a ppm, would be significant in the methylpyridines series where the chemical shift differences are small. In this case, it is worthwhile to have an internal ^{15}N reference and external lock. The effect of nitromethane on the ^{15}N resonance positions of pyridine and other representative nitrogen containing compounds has been shown to be negligibly small (35). Since 20% nitromethane in solutions containing $Cr(acac)_3$ requires 2 1/2 hours spectral accumulation time to observe a nitrogen resonance, the less molar efficient $Cr(thd)_3$ would require even longer runs. This additional use of spectrometer time is clearly unwarranted if nothing is to be learned.

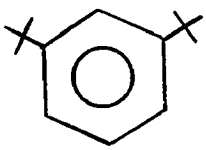
If complexation at pyridine is an important aspect of the effect of the relaxation reagents, then inhibiting this by increasing the steric bulk of the pyridine substrate should minimize or remove dependence of the chemical shift on concentration of $Cr(acac)_3$. For the same reason, the effective reduction of T_1 may be smaller. For these

TABLE 9

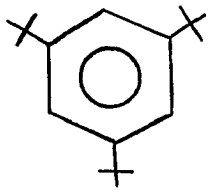
THE SOLVENT DEPENDENCE OF $\delta\,15_N$ OF SEVERAL METHYLPYRIDINES

	$\delta\,15_N$, ppm	
	neat	50% C_6H_6
2-Picoline	291.1	289.8
3-Picoline	293.7	291.9
2,6-Lutidine	293.0	290.9
3,4-Lutidine	285.2	287.6

studies, tert-butylpyridines serve as appropriate models (XVII, XVIII).



XVII



XVIII

The substitution pattern in these compounds is expected to inhibit interaction with both the ring and the nitrogen, although to the extent that the former is important, the effect of complexation should be smaller in XVIII than in XVII.

Qualitatively, the ^{15}N relaxation times of the t-butylpyridines appeared to be considerably longer than that of 2,6-lutidine. Nitrogen-15 resonances could be obtained only using 15^{o} pulse angles at a repetition rate of 16 second, requiring spectral accumulation times of at least eighteen hours. In contrast, a useful spectrum of 2,6-lutidine was obtained in a two hour run employing a 30^{o} flip angle at a repetition rate of 2 seconds.

If the interaction of the PRR with the lock substance predominates, it should be even more apparent in this system because interaction with the t-butylpyridine should be reduced. If it is a bulk susceptibility effect that is being observed, the chemical shift differences should remain the same. Because of the long spin-lattice relaxation times involved, it was not possible to observe

^{15}N resonances below a concentration of 0.0500 M $Cr(acac)_3$. On the other hand, the effect of $Cr(acac)_3$ at higher concentrations levels off (Figure 4). Hence, useful information would not be obtained from a concentration study under the present limits of instrument sensitivity.

Summary

Theoretical interpretations of nuclear relaxation in solutions containing paramagnetic species have been given and agreement with experimentally determined parameters has been demonstrated (36). For example, the use of ^{13}C spectra for quantitative analysis requires that observed intensities indicate the relative numbers of carbon nuclei in the sample. In practice different nuclei experience different NOE's so that this enhancement must be quenched for purposes of quantitative analysis. Quenching of the NOE is accomplished by introducing sufficient amounts of PRR into the sample so that the T_1 process is dominated by the unpaired electron spins and the relative contribution of carbon-proton dipolar relaxation is diminished. A quantitative formulation of the effect of adding paramagnetic ions on the ^{13}C NOE has been presented by Natusch (37), while the quenching technique has been thoroughly investigated by LaMar (38).

Characterization of the interaction between paramagnetic relaxation reagents and substrates has also been actively pursued by several investigators (25e, 39, 40). While it is agreed that the nature of the interaction is

complex, there is no general agreement on either the site of interaction between PRR and substrate or whether the unpaired electron spin is delocalized into the σ or π electronic systems. Morishima suggested (39a,e) that there is a direct complexation at the nitrogen nuclei in several unsaturated and saturated nitrogen heterocycles. The basis of this hypothesis was the linear relationship found between concentration of added PRR and ^{13}C chemical shifts of piperidine and some of its methyl derivatives. However, in solutions containing more than 0.4 mole % PRR, deviations from linearity occurred. This deviation was explained as a possible deformation of the piperidine ring due to complex formation. In view of the non-linear relationship found between the ^{15}N chemical shifts of the rigid pyridine ring and concentration of PRR (Figure 4), Morishima's hypothesis becomes somewhat tenuous. It was noted earlier that Levy (25e) argues for an ordering or orientation effect between PRR and substrate rather than a true complex formation based on measurement of ^{13}C T_1 values.

However, it was not the focus of this study to elucidate the mechanism of interaction between PRR and substrate but rather to determine the experimental conditions under which one can easily obtain reliable ^{15}N data on molecules with long T_1's. For the pyridines, use of $Cr(acac)_3$ as the T_1 reagent, nitromethane as an internal reference and deuteriobenzene as the external lock appears to satisfy this requirement.

CHAPTER III

SUBSTITUENT EFFECTS ON THE ^{15}N CHEMICAL SHIFTS OF PYRIDINES

The Effects of Electron-withdrawing Substituents

As discussed above, it was now feasible to reliably measure ^{15}N chemical shifts at the natural abundance level using the paramagnetic relaxation $Cr(acac)_3$. The ^{15}N chemical shifts of several series of substituted pyridines are given in Table 10. Literature values of ^{14}N shifts are included for comparison. On the basis of the effects on aromatic ^{13}C resonance positions (41), one would expect the conjugatively electron-withdrawing cyano group and the inductively electron-withdrawing halogens, in non-conjugative positions relative to the particular ring atoms, to deshield the nitrogen nucleus. This is supported by the experimental chemical shifts for 4-cyanopyridine and the 3-halopyridines. However, substitution of cyano at the 2- and 3-positions shifts the nitrogen resonance upfield several tenths of a ppm relative to pyridine.

The difference in the effects of the cyano group at the 2- and 4-positions is worthy of comment. Consideration of simple conjugative interactions suggest that the order of shielding of the pyridine nitrogen nuclei ought to be opposite that of the nitrile nitrogen:

TABLE 10

NITROGEN CHEMICAL SHIFTS OF SOME SUBSTITUTED PYRIDINES

	Solvent	$\delta 15_N$ Py-N [a]	$\delta 15_N$ other [a]	$\Delta \delta 15_N$ [b]	$\delta 14_N$ [c]	$\delta 15_N$ [d]
Pyridine	neat	- 62.2		0.0	-63+2	291.9
2-Me	neat	- 62.6		- 0.4	-72+2	291.5
3-Me	neat	- 61.9		0.3	-68+3	292.2
4-Me	neat	- 70.2		- 8.0	-74+3	283.9
2,3-Me,Me	neat	- 62.3		- 0.1		291.8
2,4-Me,Me	neat	- 71.0		- 8.8	-74	283.1
2,5-Me,Me	neat	- 62.7		- 0.5		291.4
2,6-Me,Me	neat	- 62.4		- 0.2	-73	291.7
3,4-Me,Me	neat	- 68.8		- 6.6		285.3
3,5-Me,Me	neat	- 61.7		0.5		292.4
-N-ox	neat	- 87.5		-25.3		266.6
2-Me,N-ox	neat	- 89.6		-27.4		264.5
3-Me,N-ox	neat	- 87.5		-25.3		266.6
4-Me,N-ox	H_2O	-110.2		-48.0		243.9
2-F [e]	neat	-103.3		-41.1	-106+5	250.8
3-F [e]	neat	- 54.7		7.5	-59+5	299.4
2,6-F,F [e]	neat	-133.8		-71.6		220.3
2-Cl	neat	- 70.9		- 8.7	-67+3	283.3
3-Cl	neat	- 56.4		5.8	-59+3	297.7
4-Cl	CH_2Cl_2	- 67.5		- 5.3		286.6
2-Br	neat	- 62.7		- 0.5	-61+3	291.4
3-Br	neat	- 55.7		6.5	-55+3	298.4

TABLE 10---continued

	Solvent	$\delta 15_N$ Py-N[a]	$\delta 15_N$ other[a]	$\Delta\delta 15_N$[b]	$\delta 14_N$[c]	$\delta 15_N$[d]
4-Br	CH_2Cl_2	- 68.6		- 6.4		285.5
2-NH_2	DMSO	-114.2	-251.7	-52.0		239.9
3-NH_2	DMSO	- 51.8	-166.4	10.4		302.3
4-NH_2	DMSO	-105.5	-246.4	-43.3		248.6
2-CN	CH_3NO_2	- 62.6	-127.8	- 0.4		291.5
3-CN	CH_3NO_2	- 62.5	-118.3	- 0.3		291.6
4-CN	CH_3NO_2	- 51.1	-117.5	11.1		303.0
2-OH	DMSO	-120.5		-58.3		233.6
4-OH	DMSO	-112.2		-50.0		241.9
2-OMe	neat	-110.9		-48.7		243.2
2,6-(t-butyl)$_2$	neat	- 70.4		- 8.2		283.7
2,4,6-(t-butyl)$_3$	C_6H_6	- 76.1		-13.9		278.0

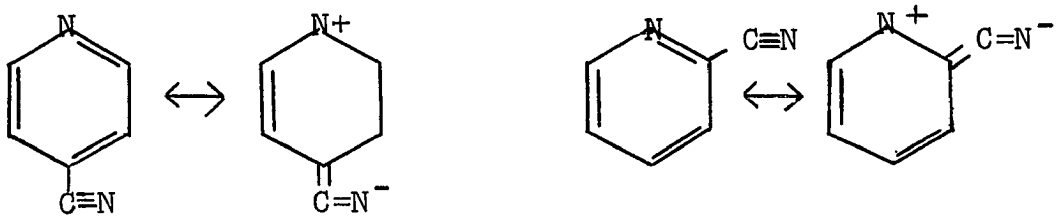
[a]Chemical shifts are reported in ppm from internal CH_3NO_2; excluding the pyridine-N-oxides, all samples contained 0.0250 M $Cr(acac)_3$.

[b]$\Delta\delta 15_N$, ppm relative to pyridine

[c]reference 13

[d] $\delta 15_N$, ppm relative to $^{15}NH_4Cl$

[e]measured by R.L.Lichter



XIX XX XXI XXII

That the nitrile nitrogen of 2-cyanopyridine lies at higher field than that of the 4-isomer is consistent with the possible greater importance of XXII relative to XX, for electrostatic reasons. On this basis, however, the pyridine nitrogen resonance of XXI is expected to lie at lower field than that of XIX; this is not observed. Conceivably, the well-known anisotropy of the cyano group may exert an opposing influence, but if this were so, the corresponding resonances of C-3 and C-5 in XIX and C-3 in XXI ought to be similarly affected. Table 11 indicates that this does not obtain---these resonances all lie to lower field than the corresponding resonances in pyridine. Hence, as is discussed below, simple considerations of electron density do not apply here.

Conjugatively Electron-releasing Substituent Effects

Substituents which are capable of conjugative electron release to the ring (OH, NH_2, OCH_3, halogen) shield the nitrogen nucleus relative to pyridine. Halogen substitution at C-2 shifts the nitrogen resonance upfield in the order Br < Cl < F. This can be rationalized in terms of the greater similarity in 2p orbital size between carbon and

TABLE 11

^{13}C CHEMICAL SHIFTS OF SOME SUBSTITUTED PYRIDINES

	$\delta\,^{13}C$, ppm				
	C-2	C-3	C-4	C-5	C-6
Pyridine	150.6	124.5	136.4	124.5	150.6
2-F	<u>164.6</u>	110.5	141.8	122.1	148.3
4-F	152.5	111.8	<u>168.7</u>	111.8	152.5
2-Cl	<u>152.0</u>	124.8	139.4	123.3	150.6
2-Br	<u>143.2</u>	128.9	139.7	123.9	151.0
4-Br	151.8	127.8	<u>133.4</u>	127.8	151.8
$2\text{-}NH_2$	<u>160.4</u>	109.5	138.1	114.0	149.0
$4\text{-}NH_2$	149.6	109.8	<u>156.3</u>	109.8	149.6
2-OH	<u>164.7</u>	107.2	136.8	121.3	156.6
4-OH[a]	147.3	116.5	<u>154.3</u>	116.5	147.3
2-OMe	<u>164.8</u>	111.4	138.2	116.9	148.0
2-CN	<u>134.3</u>	129.2	138.4	127.8	151.7
4-CN	151.5	126.7	<u>120.9</u>	126.7	151.5

SOURCE: reference 41, ppm from TMS

NOTE: Underscorings indicate substituted carbons.

[a]this work

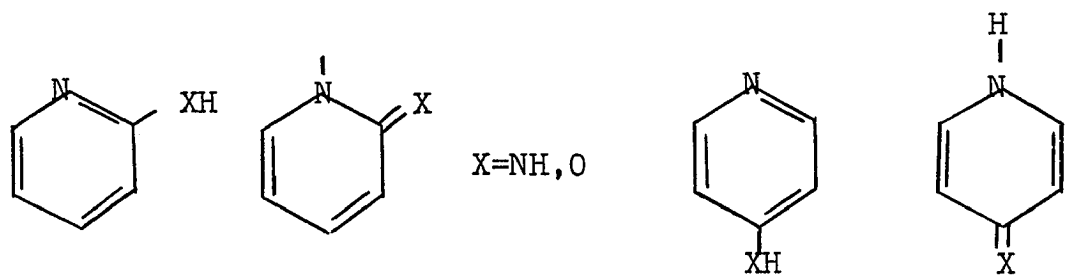
fluorine, which permits more extensive overlap than with chlorine and bromine, respectively, and provides larger back donation of electrons from the fluorine atom. In the amino-, hydroxy- and halopyridines substitution at the 2-position induces a greater upfield nitrogen shift compared with substitution at the 4-position. Because C-2 is adjacent to the electronegative nitrogen nucleus, it is expected to have a smaller electron density than C-4. Hence, with strongly conjugatively interacting substituents, π donation at C-2 is enhanced relative to that at C-4. That is, the ^{13}C resonances of the ring carbon bonded to the substituent should not be shifted as far downfield with respect to pyridine in the 2-derivatives relative to the 4-derivatives. The interactions of these substituents is supported by the ^{13}C chemical shift data as seen in Table 11. Thus, carbon-2 of the chloro-, amino-, hydroxy- and methoxy-2-substituted pyridines resonates downfield of its position in pyridine while C-4 of the corresponding 4-substituted isomers resonates even further downfield. 2-Bromopyridine appears to be an anomaly. Possibly, one might attempt an explanation in terms of predominance of the electronegativity effects as a result of the limited ability of a bromine nucleus to conjugate with the π system. At C-4, the electronegativity effect is negligible because of distance, and π donation dominates.

The opposing effects of conjugation and induction are clearly evident in the ^{15}N chemical shift of 2-fluoro-

pyridine compared with those of 2-amino- and 2-methoxypyridine. These values parallel the relative conjugative abilities of the substituents but are not apparent from the ^{13}C nuclear shieldings which do not differ widely for these molecules.

Pyridine-Pyridone Tautomerization

The 2- and 4-amino and hydroxy derivatives may exist as tautomers (XXIII-XXVI). Since the tautomeric forms XXIV



XXIII XXIV XXV XXVI

and XXVI represent a change from an azine to an amide type nitrogen, the corresponding nitrogen resonances would be expected at much higher fields (Figure 2). For example, the chemical shift of N-methyl-2-pyridone which may be taken as a model for X=O is 212.5 ppm upfield of nitromethane (42). In dimethyl sulfoxide solution, the experimental chemical shift values indicate that there is no appreciable isomerization to forms XXIV and XXVI for either the amino or hydroxypyridine derivatives. This is in contrast with the ^{14}N results for the hydroxy derivatives in acetone solution which indicate the predominance of the pyridone forms (Table 10) (13). However, the ^{15}N results for 2- and 4-hydroxypyridine are consistent with the general linear

relation found by Witanowski (13) between $\delta\,14_N$ of 3-OH- and 2-, 3- and 4-aminopyridine and π charge densities. The δ values for 2- and 4-hydroxypyridine calculated on the basis of this relationship are in excellent agreement with the ^{15}N data. This result must be regarded to some extent as fortuitous but still provides the absorption range for these resonances. Support of the ^{15}N results can also be found in the large upfield ^{15}N chemical shift of 2-methoxypyridine where conjugative interactions are expected to be similar to that of a hydroxy substituent.

The hydroxypyridine-pyridone tautomerization has been extensively investigated using various spectroscopic techniques. Ultraviolet absorption (43) studies have demonstrated that in the vapor phase the 2- and 4-hydroxypyridines exist as such whereas in solution the pyridone tautomers predominate. This has been confirmed by IR measurements and proton nmr (43). However, ^{13}C nmr investigations which used DMSO as the solvent were ambiguous (44). Hence, a specific solute-solvent interaction might prevent isomerization to the pyridone form. Possibly, DMSO acts in one or a combination of the following ways: (1) interruption of pyridine-pyridine association, (2) interaction at the hydroxy proton to eliminate proton migration to the nitrogen or (3) interaction at the nitrogen with the same result as in (2). To test this possibility the ^{15}N chemical shifts of 2- and 4-hydroxypyridine were obtained in solutions containing equal volumes of acetone and water. The results

were comparable with the ^{14}N data in acetone solution (Figure 5). The sensitivity of this tautomerization process to the presence of water in the sample solution is evidenced by the 60.4 ppm upfield shift measured in a solution of 2-hydroxypyridine in DMSO which had not been distilled and dried prior to use. It is noteworthy that the ^{15}N results for 2- and 4-hydroxypyridine in DMSO are the first observations of the predominance of the pyridine tautomer in solution.

Comparison with ^{14}N Chemical Shift Data

The ^{14}N chemical shifts for all the compounds listed in Table 10 have been reported. Since the nitrogen resonance position is solvent sensitive, only those ^{14}N chemical shifts which were obtained as neat liquids with internal nitromethane as reference were included in the table for comparison. The agreement is good for pyridine and the halopyridines but less satisfactory for the methylpyridines.

The Effect of Methyl Substitution on $\delta\ ^{15}N$ of Pyridine

The electronic effect of methyl substitution on the pyridine nitrogen resonance requires a separate analysis. Methyl substitution on the pyridine ring results in a very small perturbation on the ^{15}N resonance except when the substituent is on the para carbon. Although the ^{15}N chemical shifts of the dimethylpyridines are not strictly additive, the general trend is reasonable for the observed

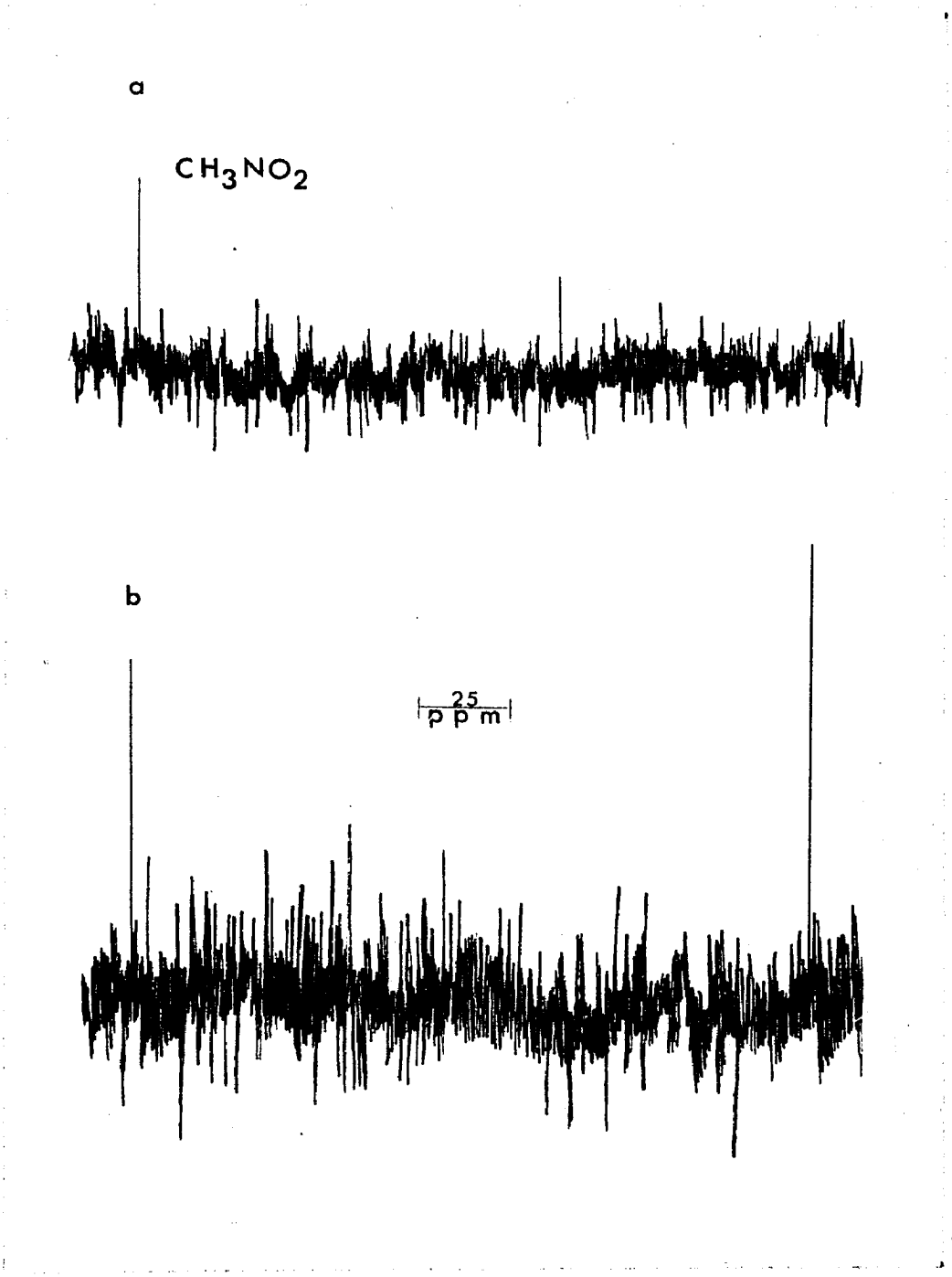


Figure 5. Natural abundance ^{15}N spectra of 2-hydroxypyridine 2 M in (a) DMSO, (b) acetone/water. Measured relative to internal nitromethane.

chemical shift range. Thus, the small ortho and meta effects remain small, while para substitution consistently induces much larger changes in resonance positions. Current theory does not readily explain the large para upfield shift. However, it is of interest to note that in mono- and disubstituted benzenes, the polarity of the substituent is reflected in the shielding of the para carbon so that an approximate correlation exists between ^{13}C para chemical shifts and Hammett σ_{para} values. The same trend is observed for the para proton and fluorine chemical shifts in substituted benzenes and fluorobenzenes (41). In the 4-substituted methylpyridines (4-picoline, 2,4-lutidine, 3,4-lutidine), the nitrogen nucleus is analogous to the para carbon in the methylbenzenes. Moreover, in both the methylbenzenes and the methylpyridines, the ^{13}C chemical shift data indicate that the methyl groups deshield the carbons to which they are bonded and shield the para nuclei, while the ortho carbons are slightly deshielded relative to those in the parent compounds. An apparent exception is the 1.1 ppm shielding for C-3 of 2-picoline.

Libit and Hoffman (45) have discussed the charge transfer and polarization effects of methyl substitution on polyenes, including the benzene ring. Their model predicts electron donor properties for the methyl group and the buildup of electron density at the para nucleus by electron polarization. However, only interactions between orbitals of π symmetry were considered based on the assumption

that charge polarization occurs primarily in the π system. This assumption is not generally applicable (Chapter IV, Table 15). Even though separation of the molecular electronic distribution into σ and π contributions is in itself an approximation, exclusion of one of these in a discussion of substituent electronic effects, while useful in some instances, is generally overly simplistic.

The methyl group may be considered to interact conjugatively via the π system and inductively via the σ system. Since there are no lone-pair electron orbitals on the methyl group, the molecular orbitals of π symmetry interact with the π system. This interaction has been called hyperconjugation (46). This scheme may be used to rationalize the experimental ^{15}N chemical shifts of the methylpyridines. Hyperconjugation should have the largest influence at carbons 2 and 4 whereas inductive effects should be operative at all positions but should attenuate with distance. Substitution at C-2 should have a larger inductive (deshielding) effect on the nitrogen nucleus relative to 4-substitution because of the proximity of C-2 to the electron rich nitrogen. Conjugative effects should, therefore, dominate the shieldings of nitrogen nuclei in 4-substituted derivatives and, indeed, large upfield shifts are observed. At C-3 inductive effects dominate and the nitrogen resonance position shifts downfield relative to pyridine.

The high field shifts found for 2,6-di-t-butyl-

pyridine (-70.4 ppm) and 2,4,6-tri-t-butylpyridine(-76.1ppm) are consistent with the γ effect observed in alkylamines (47). Presumably, the shielding is sterically induced since additional electronic interactions of the t-butyl with the aromatic ring compared with that of a methyl group are unlikely.

It has been established (41) that a methyl substituent exerts an additive influence on the ^{13}C chemical shifts of polymethylbenzenes. However, in contrast with the xylidines and toluidines, the methyl substituent effect on the pyridine nitrogen resonance is apparently non-additive. Conceivably, the incorporation of the nitrogen atom into the aromatic ring structure, with the concurrent change in lone-pair orientation with respect to the ring, is a major factor in determining the response of the nitrogen chemical shift to methyl substitution. However, it could be expected that more strongly interacting substituents could produce additive effects on the nitrogen nuclear shielding.

The Pyridinium Ions

Origin of the Protonation Shift

The resonance positions of the nitrogen nuclei in the pyridine-N-oxides and pyridinium ions lie at higher field relative to the free bases. It is interesting that upon protonation the chemical shifts of C-2 in pyridine (41) and the fluorine atom in 2-fluoropyridine (48) move in the same direction as the nitrogen chemical shift, although by a

smaller amount (7.8 and 18.7 ppm vs. 100 ppm). This has been the subject of frequent discussions in the literature (49). Two possibilities can be considered: (1) The ΔE term in σ_p contains contributions from all possible electronic transitions, including $\sigma \rightarrow \sigma^*$ and $\sigma \rightarrow \pi^*$, but is probably most heavily influenced in the free base by the $n \rightarrow \pi^*$ transition involving the nitrogen lone-pair. Increasing lone-pair σ delocalization may also lead to smaller values of ΔE affecting nuclei adjacent to the nitrogen atom. Removal of the $n \rightarrow \pi^*$ transition in the protonated species would decrease σ delocalization and increase the relative importance of the higher energy $\sigma \rightarrow \sigma^*$ and $\sigma \rightarrow \pi^*$ transitions. Hence, upfield shifts would be induced for both the nitrogen and adjacent nuclei. (2) Molecular orbital calculations indicate that protonation decreases the N-C π bond order. A decrease in bond order is associated with a shielding effect. (41). Intuitively, one would expect the electron density of the nitrogen nucleus of pyridine to become more positive upon protonation. However, an upfield shift of more than 100 ppm compared with the free base is observed.

Emsley (49g) has suggested that variations in Q (Chapter I) are insufficient to account for the large upfield nitrogen shift; rather, this should be attributed to changes in the ΔE value of the paramagnetic term in the approximate chemical shift expression. Since contributions to the paramagnetic term are inversely proportional to the average

excitation energy, compounds with low excitation energies have large paramagnetic shifts, e.g. the pyridines. Estimations of relative excitation energies for a series of compounds can be obtained from ultraviolet absorptions and, more recently, from photoelectron spectroscopy. Protonation or oxide formation removes the lone-pair electrons on nitrogen and removes the contribution of the low energy $n \longrightarrow \pi^*$ transition to the overall average excitation energy. The increase in the average excitation energy results in an upfield shift. This argument has been previously used to explain the proton (49b) and carbon (50) chemical shifts in the pyridinium ions. Estimates of the relative contributions of (1) and (2) made be Adam, Grimison and Rodriguez (49e) indicate that although bond order variations do contribute to differences in the paramagnetic term, changes in excitation energy appear to dominate.

The Pyridine-N-oxides

Listed in Table 10 are the nitrogen chemical shifts of pyridine-N-oxide and the picoline-N-oxides. The trend observed in the free bases is maintained in this series with the 4-substituted derivative displaying the largest upfield shift. It is interesting that oxide formation decreased the relaxation rate of the nitrogen nuclei, thus eliminating the need for paramagnetic additives. This probably arises because of an increase in the molecular correlation times associated with these polar molecules (Chapter I).

Discussion of Protonation Conditions

The effect of substituents on the nitrogen resonance position of the pyridinium ion would be useful in elucidating the extent of lone-pair interaction as a factor in determining the nuclear screening of the pyridine nitrogen atom. For an initial investigation, the ^{15}N chemical shifts of the hydrochlorides of the methyl- and halopyridines were obtained in dichloromethane solution. The methyl derivatives displayed a chemical shift range of 31 ppm, which was larger than expected. Since the substituted pyridine hydrochlorides are not equally soluble in dichloromethane, the possibility of a solvent effect superimposed on the protonation effect was considered. Therefore, the pyridinium ions were formed directly in trifluoroacetic acid (TFA) where the mole ratio of substrate to TFA could be maintained constant for the entire series.

The pKa's of the methylpyridines (51) lie between 5.67 and 6.85, while that of TFA is less than one. Hence, protonation at a 1:1 mole ratio of pyridine:TFA is expected to be complete. This is supported by the proton and carbon chemical shift data of Pugmire and Grant (48f). Since a 1:1 mole ratio produced solid pyridine trifluoroacetates for half of the substrates under investigation (the other samples remained liquids), a one mole excess of TFA was used as the solvent. The ^{15}N chemical shifts obtained are listed in Table 12. Because the nitrogen nuclear shieldings could not be rationalized in terms of

TABLE 12

PROTONATION SHIFTS

	$\delta\ ^{15}N$, ppm									
	Substrate:TFA:$CHCl_3$ (mole ratio)									
	1:1:1	1:2	1:2:2	(δpy-δpy^+)	1:3:3	1:4:4	1:5:5	1:6:6	1:10:10	1:2:5
Pyridine	...	183.2	179.9	0.0	...	...	...	...	...	...
2-Me	...	184.3	179.5	- 0.4	...	...	...	...	...	...
3-Me	195.0	173.4	179.6	- 0.3	...	...	...	...	...	...
4-Me	...	170.3	173.1	- 6.8	171.6	169.1	168.2	167.7	...	180.9
2,3-Me,Me	192.0	178.7	178.9	- 1.0	...	...	...	...	...	...
2,4-Me,Me	194.3	172.8	173.0	- 6.9	...	...	...	...	...	...
2,5-Me,Me	204.9	183.2	179.8	- 0.1	...	...	...	...	...	...
2,6-Me,Me	...	180.1	179.3	- 0.6	...	...	...	...	...	...
3,4-Me,Me	...	176.2	173.1	- 6.8	171.8	169.7	168.5	167.7	166.5	176.9
3,5-Me,Me	...	177.7	178.7	- 1.2	...	...	...	...	...	...
2-Br	...	223.6	215.2	35.3	198.7	...	...	...	...	...

TABLE 12---continued

3-Br	...	184.9	190.4	10.5	...	...	...	...	...	...
2-Cl	...	203.0	212.9	33.0	...	...	...	...	...	...
3-Cl	...	229.9	189.0	9.1	184.4	...	...	...	...	...
2-NH_2	...	...	135.1	-44.8	...	...	...	...	...	...
3-NH_2	...	...	179.4	- 0.5	...	...	...	...	...	...
4-NH_2	...	...	139.2	-40.7	...	...	...	...	...	...
2,6-NH_2,NH_2	...	...	118.1	-61.8	...	...	...	...	...	...
2-OH	...	...	150.0	-29.9	...	...	...	...	...	...
3-OH	...	...	178.1	- 1.8	...	...	...	...	...	...
4-OH	...	...	157.4	-22.5	...	...	...	...	...	...

substituent electronic effects, it was necessary to consider the possibility that the excess TFA as solvent might be influencing the chemical shifts. Therefore, chloroform was chosen as solvent because of its relative inertness and the high solubilities of the substituted pyridine trifluoroacetates. The nitrogen chemical shifts for several of the substrates in solutions containing 1:1:1 mole ratios of pyridine:TFA:chloroform clearly do not reflect complete protonation. In order to determine the mole ratios of TFA and chloroform to be used, the ^{15}N chemical shifts of 4-picoline and 3,4-lutidine were obtained by increasing the (TFA, chloroform):substrate ratio while maintaining the TFA:chloroform ratio. This had the effect of diluting the substrate and produced upfield shifts which did not level off up to a ratio of 1:6:6. This ratio represents the upper practical time limit for observation of an ^{15}N resonance. At a concentration of 1:10:10, overnight spectral accumulation was required to obtain a nitrogen resonance signal. The ^{15}N chemical shifts of 4-picoline and 3,4-lutidine were also obtained from solutions containing mole ratios of 1:2:5 substrate:TFA:chloroform. This concentration was chosen to assess which factor was contributing to the upfield shift, a solvent effect from excess TFA or dilution of substrate. As indicated in Table 12, a downfield shift was produced relative to the 1:2:2 concentration ratio. If the upfield shifts were the result of dilution only, then the ^{15}N chemical shifts observed in the

1:2:5 solution should fall between the 1:3:3 and 1:4:4 concentration ratios. When both protonated and unprotonated species are present in solution, the nitrogen chemical shift obtained would be an average of the two contributing forms and would be observed downfield of the full protonated species. Hence, partial dissociation may be a reasonable explanation for the downfield shift in the 1:2:5 solutions, although a more subtle solvent effect cannot be excluded. Possibly, ion-pair formation may be important, and the extent of this clearly would depend on concentration. In this context, Roberts (52) has noted that the protonation shift of pyridine itself varies over a 20 ppm range depending on solvent, although no explanation was suggested. Based on the assumption that the upfield shift is induced primarily by the TFA, the concentration which would minimize the effect of excess TFA and of dissociation is the 1:2:2 mole ratio.

Substituent Effects

Except for the β methyl substituted pyridinium ions, the substituent effects parallel those found in the free bases. This might be explainable in terms of the greater demand for electrons at the positive nitrogen nucleus, which results in a polarization of electrons toward nitrogen even at positions on the ring where induction is favored. In pyridinium ion itself, the β carbon resonance is downfield of its position in pyridine, which may indicate charge polarization toward nitrogen (48f). For the observed

chemical shift range, additivity of the methyl substituent effect is satisfactory, with incremental shifts of ca. 0.3 ppm for ortho and meta methyls, and ca. 6 ppm for para methyl. Discussion of the remaining pyridines is hampered by the large variations in pKa (51) values as well as by complex equilibria involving amino and hydroxypyridines. It is apparent, however, that fairly extensive protonation occurs at the pyridine nitrogen of the aminopyridines. A comparison of the trends exhibited by the methylpyridinium ions and the methylpyridines suggests that while the lone-pair determines the region of chemical shift absorption, it does not exert a major influence on the methyl substituent effect.

Correlation of $\delta\ ^{15}N$ with Other Experimental Methods

To facilitate an understanding of the nature of proton, carbon and fluorine chemical shifts it has often been helpful to correlate the nuclear shieldings with other spectroscopic and thermodynamic data which have been more extensively investigated. Similar correlations were sought for the substituted pyridines with UV absorption data, pKa (and K) and Hammett σ values. Since the energies of the highest transitions observed in UV spectra (which may or may not correspond to transition involving the lone-pair electrons; see Chapter IV) vary very little with position of substitution in the pyridine ring, they do not reflect the effect of para substituents on the ^{15}N chemical shifts (Table 13). Likewise, there is no apparent correlation of

TABLE 13

COMPARISON OF HIGHEST ENERGY UV TRANSITION WITH $\delta 15_N$

	Solvent	λ(mu) $\pi \rightarrow \pi^*$		$\Delta\delta 15_N$, ppm
Pyridine	cyclohexane	251	0	0.0
	water	253	0	
2-Me	water	262	9	- 0.4
	isooctane	262		
3-Me	water	263	10	0.3
	isooctane	258		
4-Me	water	255	2	- 8.0
	isooctane	256		
2-F	alcohol	258		-41.1
3-F	alcohol	262		7.5
2-Cl	isooctane	265	14	- 8.7
3-Cl	isooctane	268	17	5.8
2-Br	isooctane	266	15	- 0.5
3-Br	isooctane	258	7	6.5
2-CN	cyclohexane	265	14	- 0.4
3-CN	cyclohexane	265	14	- 0.3
4-CN	cyclohexane	271	20	11.1
2-OMe	water	269	16	-48.9

SOURCE: H.H. Jaffe and M. Orchin, Ch.14, Theory and Applications of Ultraviolet Spectroscopy, J. Wiley and Sons, Inc., 1970

nitrogen nuclear shieldings with basicity of the compound (Figure 6). However, a very crude correlation can be noted between Hammett σ_{para} values and $\delta\,15_N$ (Figure7).

Pyridinium ions are isoelectronic with the substituted benzenes, hence, if similar factors dominate both the carbon and nitrogen chemical shifts a correlation between the chemical shifts of analogous carbon and nitrogen nuclei is expected. The absence of such a relationship suggests that additional factors operate in the pyridinium ions which are not found in the benzenes. The most obvious is polarization of the ring carbons because of the greater charge and, hence, electronegativity of nitrogen relative to carbon. A correlation has been noted between the ^{14}N chemical shifts of a series of ortho substituted N-ethylpyridinium ions and the ^{13}C chemical shifts of analogously substituted benzenes (53). However, the nitrogen chemical shifts spanned a range of about 30 ppm which not only makes a correlation more likely but suggests that different electronic factors may influence the shifts than in the substituted pyridinium ions discussed here. This question could be resolved by determining the chemical shifts of the corresponding N-methylpyridinium ions, in which the extent of protonation is not a factor.

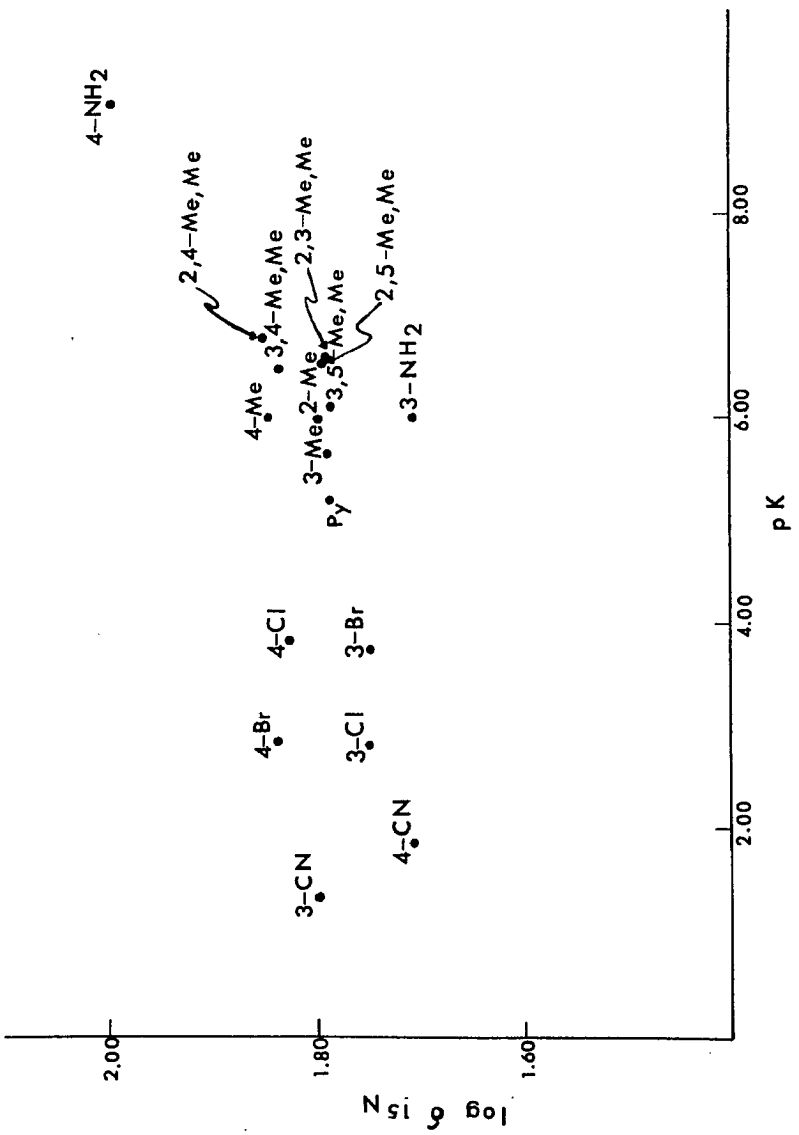


Figure 6. Relationship between basicity of some substituted pyridines and $\delta^{15}N$.

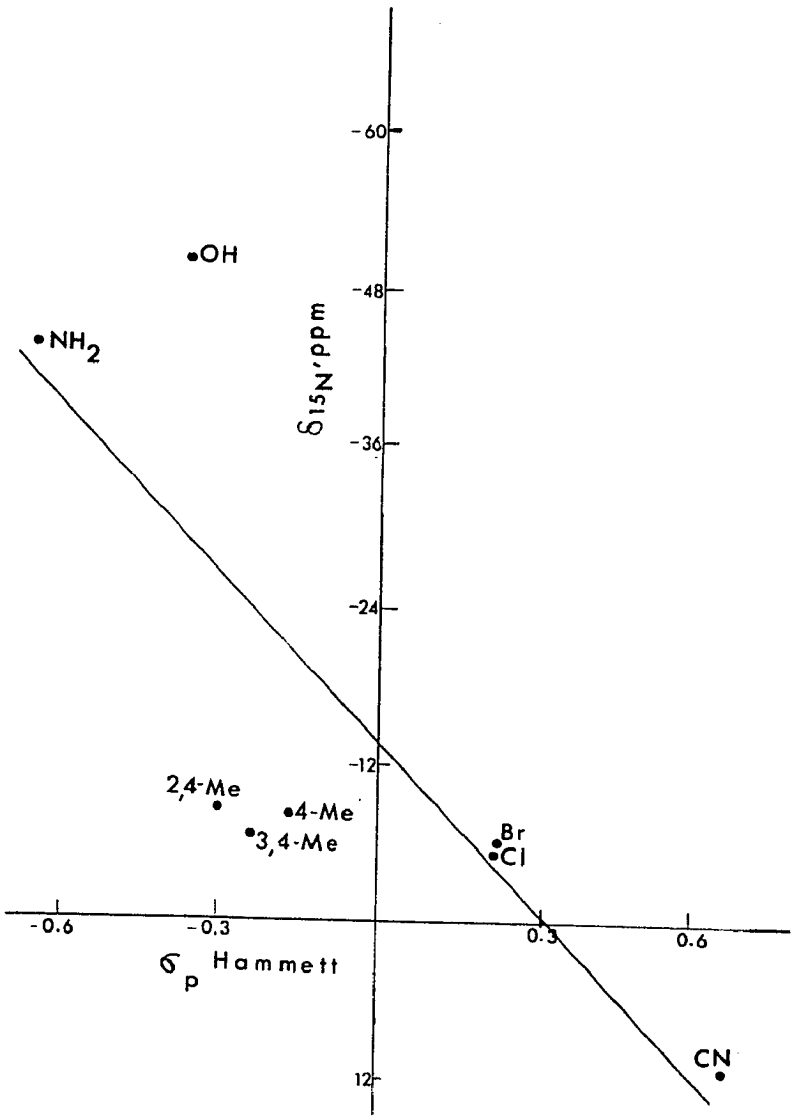


Figure 7. Relationship between the Hammett substituent constant σ_{para} and the ^{15}N chemical shifts of some substituted pyridines.

CHAPTER IV

THEORETICAL SECTION

Introduction

An important objective of this investigation was to explore the extent to which the measured ^{15}N chemical shifts could be used to discern the features of molecular electronic distribution. The merit of this approach lies in the ability to reproduce experimental chemical shifts by calculation, then to identify changes in experimental values as a function of structure with changes in relevant calculated indices. These may include electron densities, bond orders, excitation energies, dipole moments and the like. Empirically derived formulas can be useful aids in predicting chemical shifts, but these suffer from their limited range of applicability and usually rest on tenuous theoretical bases. Hence, understanding the nature of chemical shifts requires a reliable theoretical framework which can be directly related to experimental quantities. Over the years, there has been considerable activity in relating the experimental nuclear shieldings of ^{1}H (54), ^{13}C (48e, 54, 55), ^{19}F (54, 56) and N (48c, 57) to parameters calculated from

various molecular orbital theories. As computer capabilities have increased, the range of usable molecular orbital methods has expanded from pi-electron-only approaches (the simple Huckel method and the approximate self-consistent field theory of Salem and Murrell) (58) to the all-valence-shell calculations of self-consistent field semi-empirical CNDO (59) and INDO (60) methods. Most recently, an ab initio finite perturbation method (61) has been applied. Not surprisingly, the less approximate all-valence electron and ab initio calculations have been more useful than pi-only methods in accounting for observed chemical shifts of organic molecules.

Bloor and Breen (55b, c), in the first study of polyatomics larger than AB_3 which included both pi and sigma electrons, used CNDO/2 parameterization to calculate electron densities of some monosubstituted benzenes, several azines and five-membered N,O heterocycles. Even though the nature of the theory was very approximate, a definite relationship was apparent when the experimental ^{13}C chemical shifts (spanning a range of 59 ppm) were compared with calculated excess total electron densities. Since the substituted pyridines are structurally similar to those molecules examined in the Bloor and Breen investigation, a comparison between ^{15}N chemical shifts and calculated electron densities seemed worthwhile. Both INDO and CNDO programs have been implemented in this department, but since the INDO approach is less approximate (monatomic differential over-

lap is retained in one-center integrals) than the CNDO approach (differential overlap is neglected in all integrals), the former was the method of choice. Emphasis was placed on the methylpyridines because interaction of the methyl group with the ring is expected to be more subtle than that of substituents capable of conjugative interactions.

Experimental geometrical parameters of pyridine, listed in Table 14, were used for the calculations. The geometry of the ring was taken to be that of pyridine for the entire series. Any deviation from this geometry upon methyl substitution was assumed to be constant throughout the series and not to affect trends. A preliminary set of calculations on the methylbenzenes indicated that rotation of ortho methyl groups perturbed the electron densities of both the ring carbon directly bonded to the methyl group and the methyl carbon itself. Rotation of the methyl groups was treated in the calculations as successive 90° variations of the dihedral angle between a methyl C-H bond and the plane of the ring. When substituents were ortho to each other and bonded to the alpha carbon, it was then necessary to consider the effect of methyl conformation on the excess electron densities in the methylpyridines.

Discussion

Electron Density Calculations

The calculated electron densities (Table 15) of the

TABLE 14

INPUT GEOMETRICAL PARAMETERS FOR INDO CALCULATIONS

Bond lengths Å		Bond angles deg	
N-C	1.3402	C_2HC_3	118.058888
$C_2=C_3$	1.3945	HC_3C_2	120.133333
$C_3=C_4$	1.3944	HC_4C_3	120.833333
C_2-H	1.0843	HC_3C_4	121.300000
C_3-H	1.0805	HCH (methyl)	109.47125
C_4-H	1.0773		
C-CH_3	1.51		
C-H (methyl)	1.09		

SOURCE: L. E. Sutton, ed., Tables of Interatomic Distances and Configurations in Molecules and Ions, The Chemical Society, London, 1958

TABLE 15

ELECTRON DENSITIES OF THE METHYLPYRIDINES CALCULATED WITHIN THE INDO APPROXIMATION

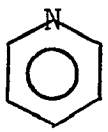
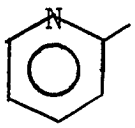
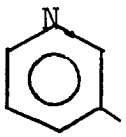
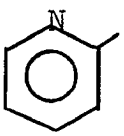
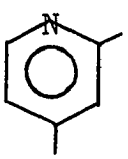
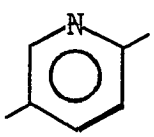
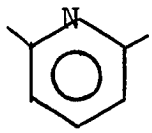
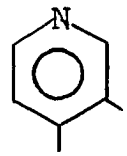
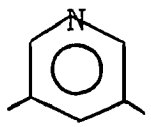
		N	C-2	C-3	C-4	C-5	C-6
	Δq^{tot}	-0.187	0.155	-0.027	0.070	-0.030	0.155
	Δq^{π}	-0.075	0.042	-0.030	0.050	-0.030	0.042
	Δq^{σ}	-0.112	0.113	0.002	0.020	0.001	0.114
	Δq^{tot}	-0.205	o.160	-0.040	0.072	-0.035	0.158
	Δq^{π}	-0.094	0.059	-0.047	0.057	-0.040	0.051
	Δq^{σ}	-0.111	0.101	0.007	0.015	0.006	0.107
	Δq^{tot}	-0.185	0.141	-0.009	0.056	-0.023	0.148
	Δq^{π}	-0.066	0.024	-0.008	0.032	-0.021	0.031
	Δq^{σ}	-0.119	0.118	-0.001	0.024	-0.002	0.117
	Δq^{tot}	-0.194	0.158	-0.042	0.081	-0.042	0.158
	Δq^{π}	-0.087	0.050	-0.048	0.069	-0.048	0.050
	Δq^{σ}	-0.107	0.109	0.007	0.013	0.007	0.108
	Δq^{tot}	-0.203	0.136	-0.022	0.058	-0.030	0.151
	Δq^{π}	-0.084	0.041	-0.025	0.049	-0.029	0.040
	Δq^{σ}	-0.118	0.106	0.004	0.019	-0.001	0.110
	Δq^{tot}	-0.214	0.162	-0.054	0.084	-0.048	0.161
	Δq^{π}	-0.107	0.067	-0.065	0.075	-0.059	0.058
	Δq^{σ}	-0.107	0.010	0.012	0.008	0.010	0.103
	Δq^{tot}	-0.202	0.153	-0.036	0.058	-0.017	0.145
	Δq^{π}	-0.084	0.049	-0.038	0.039	-0.019	0.033
	Δq^{σ}	-0.117	0.104	0.002	0.019	0.002	0.111

TABLE 15---continued

		N	C-2	C-3	C-4	C-5	C-6
	Δq^{tot}	-0.224	0.161	-0.046	0.075	-0.046	0.162
	Δq^{π}	-0.114	0.067	-0.057	0.064	-0.057	0.067
	Δq^{σ}	-0.109	0.095	0.011	0.011	0.011	0.095
	Δq^{tot}	-0.192	0.145	-0.024	0.067	-0.037	0.152
	Δq^{π}	-0.077	0.032	-0.027	0.051	-0.040	0.040
	Δq^{σ}	-0.114	0.113	0.003	0.016	0.002	0.111
	Δq^{tot}	-0.181	0.134	-0.006	0.041	-0.006	0.135
	Δq^{π}	-0.055	0.014	-0.001	0.013	-0.001	0.014
	Δq^{σ}	-0.126	0.121	-0.006	0.027	-0.006	0.121

nitrogen and ring carbons were insensitive to methyl conformation. Although rotation of the methyl groups is reflected in variations in the methyl proton and carbon electron densities, there is no obvious relationship with the ^{15}N chemical shifts. Plots of $\delta\,^{15}N$ vs. Δq^{total}, Δq^{π} and Δq^{σ} are shown in Figures 8, 9 and 10; there are no apparent correlations. For pyridine, 3-picoline and 3,5-lutidine, the directions and magnitudes of the calculated excess total and π electron densities compare favorably with the experimental ^{15}N chemical shifts. However, the order of the nuclear shieldings for the other methylpyridines is not consistent with the calculated electron densities. The most obvious discrepancy is noted for 2,6-lutidine in which the largest total and π electron densities in the were calculated, but for which a chemical shift only 0.4 ppm upfield of pyridine is observed.

Previous reports indicate that linear relationships do exist between calculated electron densities and chemical shifts of azine (62) and halopyridine (21) nitrogens. However, the observed ranges (118 ppm and 54 ppm, respectively) of chemical shifts in these systems are large compared with that of the methylpyridines which makes an apparent correlation with electron densities easier to establish. Adler and Lichter (16b) recently reported a dependence of the ^{15}N chemical shifts of the methylanilines on total electron densities calculated at the INDO level of approximation. That a similar correlation does not exist for the methyl-

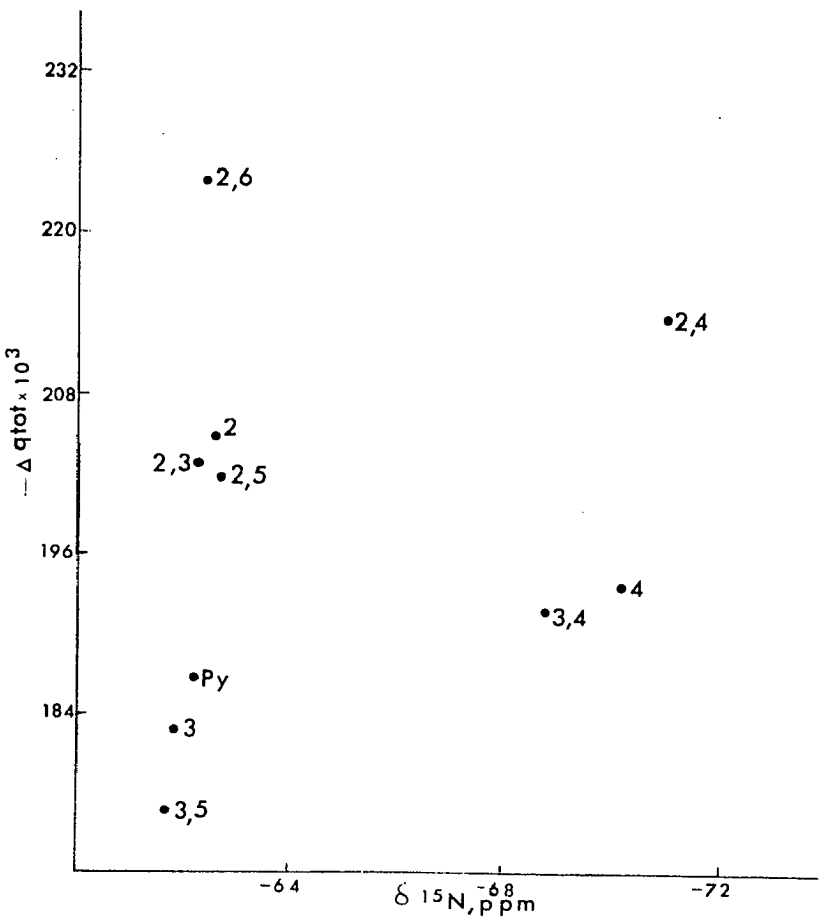


Figure 8. Relationship between the calculated total excess electron densities on nitrogen and $\delta\ 15_N$.

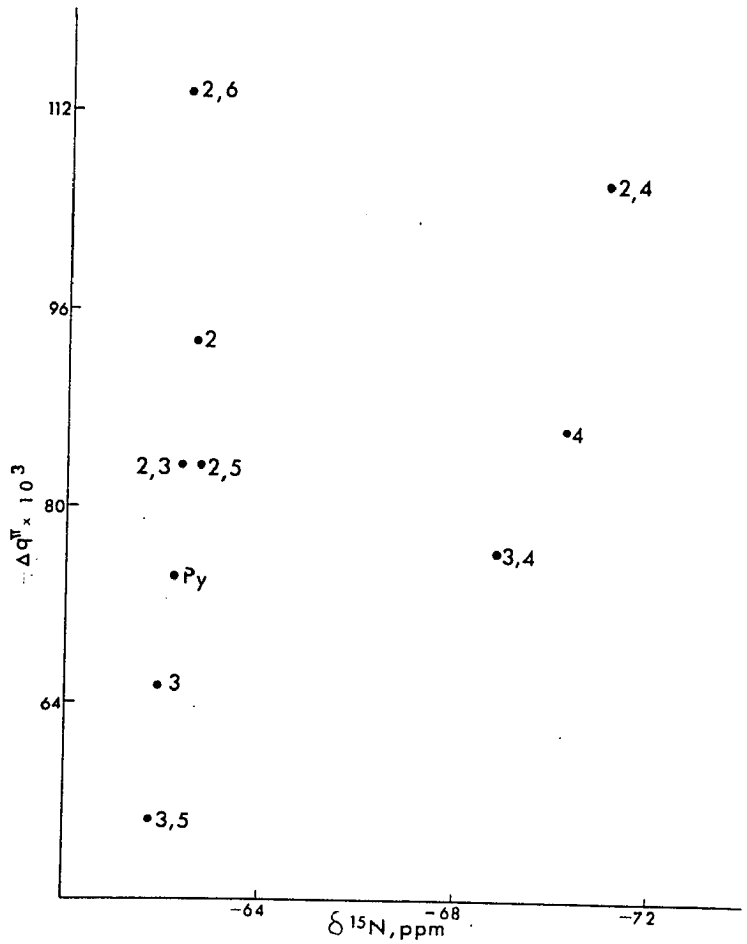


Figure 9. Relationship between the calculated electron densities on nitrogen and $\delta\,15_N$.

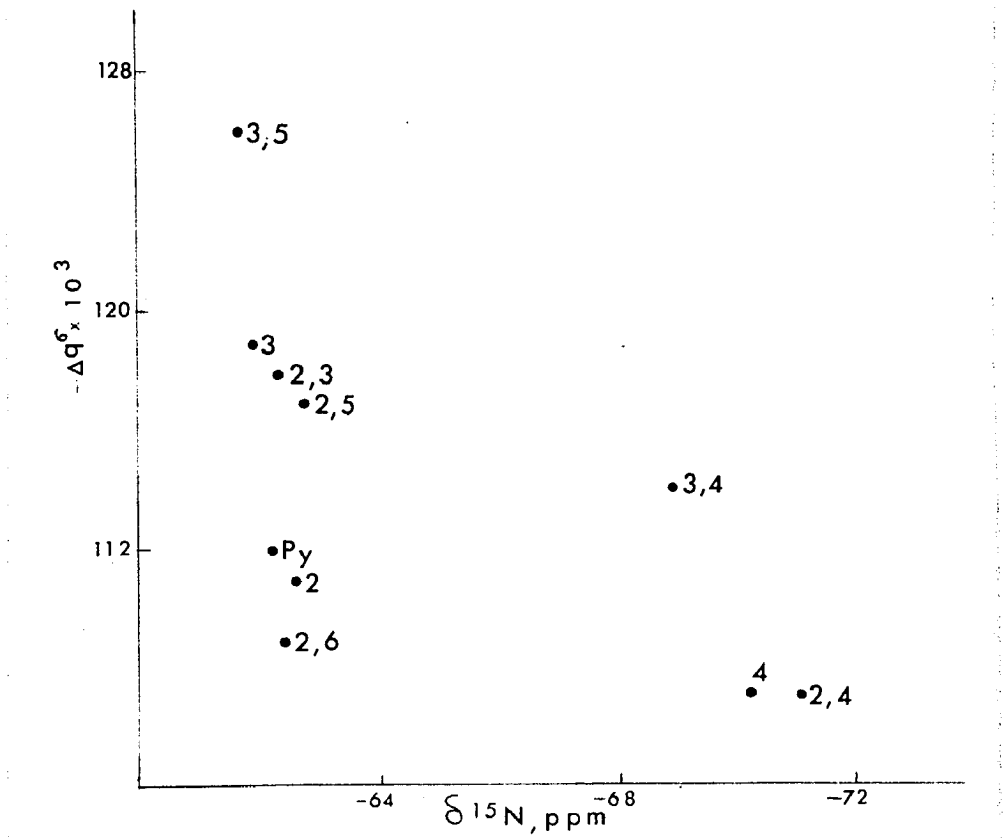


Figure 10. Relationship between the calculated electron densities on nitrogen and $\delta^{15}N$.

pyridines might be attributable to the difference between the nature of the nitrogen lone-pair in the two systems. In the anilines, the lone-pair has a large component perpendicular to the ring and is capable of conjugative interactions with the π system. In the pyridines, the lone-pair is in the plane of the ring and is part of the σ system with which it can interact. Moreover, the nitrogen atom of the pyridines is a constituent atom of the ring and not a substituent, hence it should not be subject to the same electronic effects as substituent nitrogen atoms. This is evidenced by the stricter additivity of the methyl effect in the methylanilines compared with that in the methylpyridines. That apparent correlations of chemical shifts with calculated electron densities have been obtained might be fortuitous results. Nuclear screening is proportional to the inverse cube of the electron density (equation 10, below) so that the expectation of a linear relationship rests on questionable grounds.

Chemical Shift Calculations

Having demonstrated that electron density alone is not the dominant influence on the shift we turned to direct calculations of the chemical shifts. Early in the development of nuclear magnetic resonance spectroscopy, Ramsey (63) formulated an expression to describe the magnetic shielding of a nucleus in an applied magnetic field. It was this theory which formed the basis for all later theoretical expressions of chemical shift. The magnetic shielding

tensor, σ, is defined as the second derivative of the energy of a nucleus of magnetic moment μ_n in an applied field, H, with respect to the magnetic moment and the field.

$$\sigma = \frac{\partial^2 E}{\partial \mu_n \partial \underset{\sim}{H}} \tag{5}$$

In order to calculate σ one must first be able to calculate the energy. Second-order perturbation theory readily yields an expression for the energy:

$$E^{(2)} = \frac{e^2}{2mc^2} \left\langle \psi_o \middle| \sum_k \frac{\mu}{r_k^3} \cdot \left(r_k^2 \underset{\sim}{I} - \underset{\sim}{r}_k \cdot \underset{\sim}{r}_k \right) \cdot \underset{\sim}{H} \middle| \psi_o \right\rangle \tag{6}$$

$$- \frac{e^2}{m^2c^2} \sum \frac{\left\langle \psi_o \middle| \sum_k \left(\frac{\mu_3}{r_k^3} \right) \cdot \underset{\sim}{M}_k \middle| \psi_n \right\rangle \left\langle \psi_n \middle| \sum_k \underset{\sim}{M}_k \cdot \underset{\sim}{H} \middle| \psi_o \right\rangle}{E_n - E_o}$$

Here r_k is the distance between the nucleus and the k_{th} electron, $\underset{\sim}{I}$ is the unit dyadic and $\underset{\sim}{M}_k$ is the angular momentum operator. However, the energy equation contains contributions from excited state wavefunctions and energies, information about which is not available for most molecules. To obtain the second-order perturbation expansion in closed form, the closure theorem of matrix multiplication is used to eliminate integrals over excited state wavefunctions, and the sum-over-excited-state energies is replaced by an average excitation energy. This simpler approach of Ramsey became known as the average excitation energy approximation (AEEA):

$$E^{(2)} = \frac{e^2}{2mc^2} \langle \psi_o | \sum_k \frac{\underset{\sim}{u}_k}{r_k^3} \left(r_k^2 \underset{\sim}{I} - \underset{\sim}{r}_k \underset{\sim}{r}_k \right) \cdot \underset{\sim}{H} \quad | \psi_o \rangle \qquad (7)$$

$$- \frac{e^2}{m^2c^2} \left(\Delta E_{av} \right)^{-1} \langle \psi_o | \sum_{kk} \frac{\underset{\sim}{u}}{r_k^3} \cdot \underset{\sim}{M}_k \underset{\sim}{M}_k \cdot \underset{\sim}{H} \quad | \psi_o \rangle$$

The Karplus-Pople formalism

A modification of the Ramsey equation for ^{13}C chemical shifts was derived by Karplus and Pople (55a)(equations 8-13). Following the analysis of the fluorine chemical shift by Saika and Slichter (56), they divided the chemical shielding tensor into three parts.

$$\sigma = \sigma_d + \sigma_p + \sigma' \qquad (8)$$

σ' is a nonlocal term whose origin is in contributions from other atoms in the molecule not bonded to the atom of interest and is usually negligibly small except for proton. The diamagnetic term σ_d represents the shielding arising from the local atomic electrons circulating as if they were free:

$$\sigma_d = \frac{e^2}{3mc^2} \sum_i \left\langle r_i^{-1} \right\rangle \qquad (9)$$

Here $\left\langle r_i^{-1} \right\rangle$ is the distance from the nucleus to the i_{th} molecular orbital. The paramagnetic term σ_p is a correction term to account for the fact that molecular bonding inhibits the free electronic circulation described by σ_d. As described above (equation 6), its description by a

second-order perturbation theory approach involves a mixing of ground and excited states by the applied magnetic field. Within the framework of the average energy approximation, σ_p is given by:

$$\sigma_p = -\frac{2}{3}\frac{e^2h^2}{m^2c^2}\,(\Delta E_{av})^{-1}\left\langle r_{2p}^{-3}\right\rangle \sum_B Q_{AB} \qquad (10)$$

Here $\left\langle r_{2p}^{-3}\right\rangle$ is an orbital expansion term representing the mean inverse cube of the distance from nucleus A to a 2p orbital. The term $\sum_B Q_{AB}$ properly includes all atoms B in the molecule; however, because they are negligibly small, contributions from atoms not bonded to the atom under investigation A are usually not considered. By examining the local magnetic field at the nuclear position, an expression for Q_{AB} was derived by Pople (64):

$$Q_{AB}=\frac{4}{3}\delta_{AB}(P_{xAxB}+P_{yAyB}+P_{zAzB})-\frac{2}{3}(P_{xAxB}P_{yAyB}+P_{xAxB}P_{zAzB}+P_{yAyB}P_{zAzB})+\frac{2}{3}(P_{xAyB}P_{xByA}+P_{xAzB}P_{xBzA}+P_{yAzB}P_{yBzA}) \qquad (11)$$

The terms of the form P_{uv} are elements of the charge density bond order matrix:

$$P_{uv}= 2\sum_i^{occ} c_{iu}c_{iv} \qquad (12)$$

The orbital expansion term is evaluated as:

$$\left\langle r_{2p}^{-3}\right\rangle = \frac{Z^*}{24a_o^3} = \frac{(3.90 - 0.35(\rho - 5))^3}{24\,a_o^3} \qquad (13)$$

The nuclear charge Z^* is chosen according to Slater's rules; a_o is the Bohr radius; ρ is the total electron density at nitrogen. It has been suggested (55a) that since adding a 2p electron to a carbon (or nitrogen) atom changes the diamagnetic term only slightly, this term cannot account for the range of observed carbon (or nitrogen) chemical shifts and can be neglected in calculations on a similar series of molecules. Changes in the paramagnetic term are usually considered to be the dominant factors.

Following the above formalism, we have calculated the paramagnetic contribution to the chemical shifts of the methylpyridines. The results are given in Table 16. While numerical agreement is not satisfactory, the range of calculated shifts is the same order of magnitude as that of the experimental values, and the direction of the changes is reproduced. However, a detailed interpretation of the calculations is not warranted.

The Witanowski formalism

Witanowski et al (65) have reported reasonable agreement between the experimental ^{14}N chemical shifts of several azines (pyridine, the diazines, s-triazine and s-tetrazine) and nuclear screening constant evaluated from electron densities calculated at the INDO level of approximation. The method which they used for calculating the nitrogen nuclear screening constant has the following form:

$$\sigma_d^N = 17.7501 \sum_i P_{ii} Z_i n_i^{-2} \qquad (14)$$

TABLE 16

CHEMICAL SHIFT CALCULATIONS USING KARPLUS-POPLE THEORY

	$-\sigma^{para} \times 10^6$	$\Delta\sigma^{para}$, ppm	$\Delta\delta^{15}_N$, ppm
Pyridine	483.3	0.0	0.0
2-Me	476.1	- 7.3	-0.4
3-Me	486.9	3.5	0.3
4-Me	479.3	- 4.1	-8.0
2,4-Me,Me	469.8	-13.6	-8.8
2,5-Me,Me	479.8	- 3.6	-0.5
2,6-Me,Me	461.6	-21.8	-0.2
3,4-Me,Me	482.2	- 1.2	-6.7
3,5-Me,Me	490.9	7.5	0.5

$$\sigma^{N}_{p} = -30.1885\ (\Delta E_{av})^{-1}\ Z_{2p}^{3} \sum_{B} Q_{AB} \qquad (15)$$

Equations 14 and 15 were evaluated using the quantum mechanical averages of reciprocal orbital radii as:

$$\langle r_i^{-1} \rangle = Z_i / n_i^2 a_o \qquad (16)$$

$$\langle r_{2p}^{-3} \rangle = \frac{1}{3} (Z_{2p} / 2a_o)^3 \qquad (17)$$

The Z_i terms are approximated by Burns' rules (66) for Slater type orbitals as:

$$Z_{1s} = 6.6 - 0.1(P_{2s2s} + P_{2p_x2p_x} + P_{2p_y2p_y} + P_{2p_z2p_z}) \qquad (18)$$

$$Z_{2s} = 5.6 - 0.4P_{2s2s} - 0.35(P_{2p_x2p_x} + P_{2p_y2p_y} + P_{2p_z2p_z}) \qquad (19)$$

$$Z_{2p} = 5.35 - 0.5P_{2s2s} - 0.35(P_{2p_x2p_x} + P_{2p_y2p_y} + P_{2p_z2p_z}) \qquad (20)$$

where the first terms in equations 18-20 are the screening constants S of the nuclear charge Z. Burns' rules differ from Slater's rules to calculate S in the following ways: (1) A hydrogenic form of the wavefunction is used (i.e. the function contains nodes) compared with the nodeless exponential hydrogenlike form employed by Slater. (2) The screening constant was obtained by comparison of the expectation values of the hydrogenic function with that of Hartree-Fock wavefunctions, whereas Slater's method calculated wavefunctions by minimizing the energy of the system. This distinction is important in wavefunctions that have nodes (2s and beyond) but is not apparent in the simpler hydrogen-

like wavefunctions.

A comparison of the theoretical values obtained from the Witanowski modification of the Karplus-Pople equation is given in Table 17. Although uniformly smaller in magnitude, the observed trend of the calculated chemical shifts parallels that found with the Karplus-Pople approach. The essential difference between this approach and that of Karplus and Pople lies in the evaluation of the effective nuclear charge which enters into the orbital expansion term of the paramagnetic contribution to the chemical shift. Using Slater's rules, the Karplus-Pople method would calculate the effective nuclear charge for a nitrogen 2p electron as 3.90, then attempt to compensate for fractional electron populations by including the term $(-0.35\ (\rho - 5))$, where ρ is the total electron density calculated at nitrogen (equation 13). Values of ρ greater than five induce additional screening of the nitrogen nucleus. Witanowski's calculation of Z^* differs from that of Karplus and Pople in two ways. First, as noted above, Burns' rules are used to provide the screening constant for electron groups. Secondly, as indicated in equations 18-20, fractional calculated electron densities at nitrogen are used in the determination of the effective nuclear charge. Witanowski's Z^* values thus indicate that the nitrogen nuclei are more screened by the intervening electrons than the Karplus-Pople Z^* values. Consequently, the calculated chemical shifts using the Witanowski modification are smaller in magnitude.

TABLE 17

CHEMICAL SHIFT CALCULATIONS USING WITANOWSKI'S METHOD

	$\Delta\sigma^{dia}$, ppm	$\Delta\delta^{para}$, ppm	δ, ppm	$\Delta\delta^{15}_{N}$, ppm
Pyridine	0.0	0.0	0.0	0.0
2-Me	-0.6	- 4.7	- 5.3	- 0.4
3-Me	0.0	1.2	1.2	0.3
4-Me	0.1	- 1.8	- 1.7	- 8.0
2,4-Me,Me	-0.8	- 7.1	- 7.9	- 8.8
2,5-Me,Me	-0.9	- 1.1	- 2.0	- 0.5
2,6-Me,Me	-1.2	-10.5	-11.7	- 0.2
3,4-Me,Me	0.1	- 0.5	- 0.4	- 6.7
3,5-Me,Me	-0.2	2.9	2.7	0.5

It is noteworthy that the latter method demonstrates that the paramagnetic term does indeed dominate the calculated chemical shifts. Considering the drastic assumptions involved in the molecular orbital theories and in the derivations of the nuclear shielding equations, the relative order of magnitude of the calculated nuclear shieldings is more significant than the absolute values in assessing the usefulness of the calculation.

The Average Excitation Energy Approximation

The dangers inherent in the use of the average excitation energy approximation have been frequently discussed in the literature (48c, e, 54, 55a, 62). In particular, two important limitations should be pointed out. First, in 1960 McLachlan (67) noted a possible result of the mathematics employed in simplifying the second-order perturbation sum. As a consequence of using the closure theorem, the possibility exists that the sign of the perturbation sum in equation 6 may not be correctly predicted unless the product of integrals over ground and excited states has the same sign for every excited state. This would lead to a smaller value of the paramagnetic term than expected. Second, experimental determinations of ΔE are usually not available, and the value of 10 ev suggested by Pople (64) for homocyclic systems has been widely used by others in calculations on dissimilar systems. It should be noted that the ΔE value of 10 ev does not represent a weighted average of the contributions from the different excited states and

should, therefore, be considered an arbitrary rather than an approximate value.

If chemical shift calculations are performed on a series of closely related molecules where the excited state energies could be expected to correspond to the same transitions, then by using an arbitray ΔE one might expect to obtain calculated nuclear shieldings which maintain the order in the experimentally observed chemical shifts. When the highest occupied molecular orbital (HOMO) is not the same for each molecule in the series, then the average excitation energies will contain unequally weighted contributions from the various excited states. Support for the use of the same ΔE becomes even more tenuous in this instance. The photoelectron spectroscopic results of Heilbronner et al (68) indicate that methyl substitution in the pyridine ring in some cases can raise the energy of the π_A orbital above the nonbonding orbital. Hence, the HOMO varies within the methylpyridine series. The forms of equations 10 and 15 might lead one to suspect a linear relationship between the chemical shift and the inverse of the excitation energy. Although it is expected that the lowest energy excited state will make the major contribution to the average excitation energy of a molecule, the inclusion of other excited states in the AEE might not be negligible. For example, a ΔE value of 10.1 ev produces a change in the calculated chemical shift of 2,4-lutidine of 2.3 ppm. In the substituted pyridines, there are three low

energy bands lying between approximately 9 and 11 ev. The problem then remains as to what percentage of these other contributions should be included to more accurately represent the excitation energy of a molecule.

Alternative Molecular Orbital Methods

An alternative approach to the calculation of chemical shifts which does not incorporate the AEEA, makes use of Hartree-Fock finite perturbation theory. The applicability of this method for ^{13}C chemical shifts has been investigated by Ellis, Maciel and McIver (69) and by Ditchfield et al (70) at different levels of approximation. The former group employed the semi-empirical INDO wavefunctions, while the latter group used ab initio wavefunctions calculated with various minimal and extended basis sets. While both groups report good agreement between the calculated and experimental ^{13}C chemical shifts, the gauge invariant ab initio calculations which use a slightly extended basis set result in the best agreement. Serious limitations of this type of calculation are computer time and expense, both of which increase substantially with the size of the model system. However, further investigations are warranted to determine if ab initio finite perturbation theory can produce sufficient quantitative accuracy to be more useful than semi-empirical methods in the interpretation of carbon and nitrogen chemical shifts.

SUMMARY

Changes in the nitrogen resonance position of pyridine are dependent on the nature and position of the substituent. Substitution para to the nitrogen or with strongly interacting substituents induces large variations in the chemical shift whereas methyl substitution only slightly perturbs the nitrogen nuclear shielding. That the nitrogen chemical shift of pyridine is influenced by steric effects is apparent in the large upfield shift induced by ortho t-butyl substitution.

Removal of the lone-pair electrons via protonation has little effect on the manner in which substituents influence the nitrogen shielding, suggesting that the lone-pair does not dominate substituent interactions with the aromatic ring. However, lone-pair electrons determine the region of nuclear absorption.

While paramagnetic relaxation reagents are expected to find increased application in the detection of natural abundance ^{15}N resonance signals in compounds which have long T_1's, extreme caution must be exercised in the use of these T_1 reagents. Chemical shift variations are tolerable in cases where gross structural identification is sought, however, more subtle effects of substituents and solvent require more precise experimental conditions. Preliminary concentration studies on each model system are necessary to investigate the influence of the PRR on the substrate,

reference and lock compound before reliable chemical shift determinations can be carried out. In the pyridines, this was accomplished by using an internal nitrogen reference and an external capillary to contain the lock substance. An alternative approach would employ a ^{15}N enriched deuterated compound (e.g. $CD_3{}^{15}NO_2$) in an external capillary thereby eliminating interactions of PRR with both the reference and lock substance.

Calculations of nitrogen chemical shifts employing semi-empirical wavefunctions to obtain the molecular electronic distributions reproduce the directions of the experimental nitrogen shieldings but neither the magnitude nor the order relative to pyridine. The less approximate ab initio finite perturbation theory method might be more useful in providing a more realistic picture of electronic distribution.

In conclusion, the determination of ^{15}N chemical shifts at the natural abundance level, although not quite routine, can provide additional information about molecular structure not available from other spectroscopic methods. This is evidenced in the pyridines by (1) the characterization of the methyl substituent effect, (2) the apparent influences of opposing conjugative and inductive interactions in the fluoropyridines and (3) the observation of the predominance of the pyridine tautomers of 2- and 4-hydroxypyridine.

EXPERIMENTAL SECTION

The ^{15}N spectra were obtained at a frequency of 10.09 MHz on a JEOL PS/PFT-100 spectrometer equipped with a JEOL EC-100 data system. The ^{15}N probe was fitted with a receiver insert modified to improve sensitivity. The free induction decays were accumulated over a 5 kHz range using 8 K words of memory (1.22 Hz/address). Exponential multiplication of the FID prior to Fourier transformation induced a calculated line broadening of 0.9 Hz. The resonance position of fresh samples of pyridine was checked periodically; reproducibility was within 0.1 ppm.

Samples were contained in 10 mm o.d. tubes. For the free bases, a coaxial 3 mm capillary of deuteriobenzene provided the external field/frequency lock. Twenty percent by volume nitromethane was used as the internal reference. The normal operating conditions employed a 30° flip angle and a pulse delay of 2 seconds. With these conditions useful spectra could be obtained on neat liquids within 2 1/2 hours. The hydroxy- and aminopyridines were run as solutions in DMSO; narrower flip angles and longer pulse delays were needed, requiring overnight runs in some cases.

For the pyridinium ions, a coaxial 2 mm capillary of 2.9 M aqueous ^{15}N enriched ammonium chloride in 1 M HCl provided the reference. Ten percent by volume deuteriobenzene was used as the internal lock substance. Spectra

were obtained within one hour using a 30^{o} flip angle at a repetition rate of 1.1 seconds.

The pyridine derivatives used in this study were all commercially available (Reilly Tar and Chemical Co. and Aldrich Chemical Co.). Except where noted the liquid samples were distilled from and dried by storage over solid potassium hydroxide. All solvents were distilled prior to use.

The chloro- and bromopyridines are known to dimerize when heated and, therefore, were not distilled but were stored over KOH pellets. Carbon-13 spectra of these compounds did not indicate the presence of more than two percent organic impurity, as determined by adding to the samples 2% chloroform and running the solution until a resonance signal was observed in the spectrum. 4-Chloro- and 4-bromopyridine were obtained from the hydrochlorides by extraction from a strongly alkaline aqueous solution with dichloromethane. After drying, this solution was used directly to obtain the ^{15}N spectra.

The substituted pyridine hydrochlorides were obtained by passing HCl gas through ethereal solutions of the free bases. The solid hydrochlorides were collected by vacuum filtration.

The substituted pyridine trifluoroacetates were made directly in the sample tube by volumetric addition of TFA to the free base in chloroform.

The mono-aminopyridines had a specified purity of

99+% (Aldrich Chemical Co.) and were used without further purification. 2,6-diaminopyridine was recrystallized from water.

The relaxation reagents $Cr(acac)_3$ and $Fe(acac)_3$ were used without further purification. The melting points of the compounds were checked periodically. $Cr(thd)_3$ was prepared by the following method. Urea (20 g) and 5 g of 2,2,6,6-tetramethyl-3,5-heptanedione were added to 3 g of $CrCl_3 \cdot 6\ H_2O$ dissolved in 25 ml of H_2O and 65 ml of ethanol. After refluxing for 24 hours, 100 ml of water was added and the solution cooled in an ice bath. $Cr(thd)_3$ was separated by vacuum filtration and dried in vacuo. M.P. 223.2°, Yield: 90%.

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