Proton Magnetic Resonance Studies of Metal Complexes of Imidazole, Purine, and Pyrimidine Derivatives[1]

Sung Mao Wang and Norman C. Li

Contribution from the Department of Chemistry, Duquesne University, Pittsburgh, Pennsylvania 15219. *Received May 19, 1966*

Abstract: For the purpose of deciding whether a ternary complex is formed on mixing a metal salt and two ligands, A and B, we have carried out proton magnetic resonance studies on systems in which the metal salt is $ZnCl_2$ and A = imidazole (Im), in dimethyl sulfoxide as medium. For B = purine and cytosine, the bonding schemes in the ternary complexes are: Zn←Im···purine and Im→Zn←cytosine. The binding sites in purine and cytosine toward Zn(II) are 7-N and 3-N, respectively. In the Zn←Im···purine complex, purine is not bonded directly to Zn; instead, the NH moiety of imidazole in Zn←Im forms a hydrogen bond with purine in which 1-N is the preferred hydrogen-acceptor site. The equilibrium constants of the reaction $Zn^{2+} + 4Im = Zn(Im)_4^{2+}$ and Im–Zn–cytosine^{2+} + 3Im = $Zn(Im)_4^{2+}$ + cytosine, in dimethyl sulfoxide as medium, are 1.4×10^6 l.^{4} mole^{-4} and 3×10^4 l.^{2} mole^{-2}, respectively.

The imidazole group of histidine is of prime biological importance in that it is generally responsible for most of the buffering power of proteins in the physiological pH range and plays an important part in the binding of metallic ions to proetins.[2] Koltun, Fried, and Gurd[3] measured the rates of hydrolysis of *p*-nitrophenyl acetate in the presence of imidazole (Im), $CuCl_2$, and glycyl glycinate (GG) and calculated the equilibrium constant for the reaction CuGG + Im = CuGGIm. These authors have pointed out that the metal ion may bridge the N-terminus of a protein polypeptide chain to a side-chain imidazole group, so that studies on ternary metal complexes involving imidazole may serve to some degree as models for the protein complexes. This paper presents the results of proton magnetic resonance (pmr) studies on these complexes. For the other ligands we have chosen purine and cytosine because of the relevance of these substances to nucleic acid structure.

Dimethyl sulfoxide (DMSO) is a highly polar liquid (dielectric constant 48.9 at 20°) and is a versatile and powerful solvent for many aromatic compounds and inorganic salts. Since the use of water as a solvent produces complications in the case of the purine and pyrimidines, we have selected DMSO as solvent because it provides adequate solubility without proton transfer. Moreover, Kokko, *eta l.*,[4] have reported that for pyrimidines and related nucleosides in DMSO solution, the pmr spectra indicate an absence of serious perturbations arising from solute–solvent interactions, so that the spectrum of each substance is approximately typical of the isolated solute molecules.

Calculation of Formation Constants of Metal Complexes. The quantitative nature of the empirical correlation between the pmr shifts in aromatic molecules and heterocyclic aromatic molecules and the local electron density on the carbon atom to which the proton is bonded has been discussed.[5,6] The downfield shifts of heterocyclic aromatic ring proton resonances upon protonation or metal complexation have been observed by several investigators[6,7] and have been ascribed to extensive π-electron redistribution. The ring proton resonance shifts may therefore serve as a measure of the bond strength of the interaction between a metal and a heterocyclic aromatic molecule.

Since the observed frequency of a ring proton of an aromatic molecule in the presence of a metal is a weighted average of the characteristic frequencies of the free and complexed molecule (respectively, ν_f and ν_c), the observed frequency is given by the equation

$$\nu = \frac{B_0 - x(MB_z)}{B_0}\nu_f + \frac{x(MB_z)}{B_0}\nu_c$$

$$= \nu_f + \frac{x(MB_z)}{B_0}(\nu_c - \nu_f) \quad (1)$$

where B_0 is the initial concentration of base, x is the number of base molecules bonded to a metal ion, and (MB_z) is the equilibrium concentration of the metal complex. The equilibrium constant of the reaction

$$M + xB = MB_z \quad (2)$$

is given by the equation

$$K = \frac{(MB_z)}{(M_0 - (MB_z))(B_0 - x(MB_z))^x} \quad (3)$$

where M_0 is the initial concentration of metal ion. When $B_0 \gg M_0$ and thus $B_0 \gg (MB_z)$, eq 3 may be written

$$K = \frac{(MB_z)}{(M_0 - (MB_z))B_0^x} \quad (4)$$

and eq 1 becomes

$$\nu = \nu_f + \frac{xB_0^{x-1}K(\nu_c - \nu_f)M_0}{1 + B_0^xK} \quad (5)$$

From eq 5, a plot of ν *vs.* M_0 would be linear with intercept equal to ν_f and slope, S, equal to

$$S = \frac{xB_0^{x-1}K}{1 + B_0^xK}(\nu_c - \nu_f) \quad (6)$$

(1) This investigation was supported by National Science Foundation Grant No. GB-4065, and Public Health Service Grant No. GM 10539-04.

(2) C. Tanford, *J. Am. Chem. Soc.*, 74, 211 (1952); E. A. Barnard and W. D. Stein, *Advan. Enzymol.*, 20, 51 (1958).

(3) W. L. Koltun, M. Fried, and F. R. N. Gurd, *J. Am. Chem. Soc.*, 82, 233 (1960).

(4) J. P. Kokko, J. H. Goldstein, and L. Mandell, *ibid.*, 83, 2909 (1961).

(5) G. Fraenkel, R. E. Carter, A. McLachlan, and J. H. Richards, *J. Am. Chem. Soc.*, 82, 5846 (1960).

(6) T. Schaefer and W. G. Schneider, *Can. J. Chem.*, 41, 966 (1963).

(7) R. H. Carlson and T. C. Brown, *Inorg. Chem.*, 5, 268 (1966).

For two sets of experiments in which the initial concentrations of base are kept constant at B_i and B_j, respectively, the value of K may be calculated from the ratio of the slopes, S_i/S_j, according to the equation

$$(S_i/S_j) = (B_i/B_j)^{x-1}[(1 + B_j{}^xK)/(1 + B_i{}^xK)] \quad (7)$$

For very large values of B^xK, eq 7 becomes

$$(S_i/S_j) \approx (B_j/B_i) \quad (8)$$

When $B_0 < M_0$, or when only the 1:1 complex MB exists, $x = 1$. For the Zn(purine)$^{2+}$ complex, which is weak, $(MB)^2$ is negligible relative to $(M_0B_0 - (B_0 + M_0)(MB))$, and combination of eq 1 and 3 leads to the equation

$$\frac{1}{\nu - \nu_f} = \frac{1}{\nu_c - \nu_f} + \frac{1 + B_0K}{K(\nu_c - \nu_f)}\frac{1}{M_0} \quad (9)$$

From eq 9, a plot of $1/(\nu - \nu_f)$ *vs.* $1/M_0$ would be linear, from which the values of $(\nu_c - \nu_f)$ and K can be evaluated.

Experimental Section

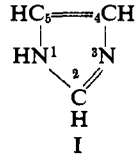
Materials. Imidazole, purine, and cytosine were obtained from Sigma Chemical Co. and used without further purification. DMSO was purified by vacuum distillation after drying over sodium hydroxide. Anhydrous $ZnCl_2$ and $CuCl_2$ were reagent grade.

Pmr Measuremets. All spectra were obtained with a Varian A-60 nmr spectrometer at 36 ± 1°. Because of the marked hygroscopic character of DMSO, solution of the samples was carried out under dry nitrogen gas, although separate experiments had shown that a small quantity of water up to 1 *M* introduced into DMSO solution did not affect the C–H spectra of the base molecules. The solutions were then transferred, again under dry nitrogen, to 5-mm precision nmr tubes and sealed.

The frequencies were measured with respect to tetramethylsilane (TMS) as an internal standard and were calibrated by the usual side-band modulation technique within ±0.2 cps, using an audio-oscillator and an electronic counter.

Results and Discussion

(A) Imidazole Complex. The pmr spectrum of imidazole (I) shows a triplet at 7.64 ppm and a doublet at 7.01 ppm of twice the triplet intensity. The less intense peak is assigned to the 2-CH proton while the other is assigned to the 4- and 5-CH protons. The coupling constants are: $J_{2H-5H} = J_{2H-4H} = 1.0$ cps. Because of the rapid exchange of the 1-N proton,[8,9] 4-H and 5-H become magnetically equivalent, and no further spin–spin splitting by interaction with the 1-N proton was observed.



I

Imidazole undergoes extensive self-association through NH···N bonds in solvents which do not compete effectively for hydrogen bonding. Infrared study of imidazole in CCl_4[10] has shown that linear oligomers are formed. The pmr spectra of imidazole in chloroform-*d*[8,11] show a triplet (2-H) at 7.73 and a doublet (4,5-H) at 7.14 ppm, with $J_{2H-4H} = J_{2H-5H} = 1.0$ cps. For 0.22 *M* imidazole in chloroform-*d*, Mannschreck, *et al.*,[11] report the NH resonance peak at 11.10 ppm, with line width of 10 cps, whereas the NH signal sharpens (line width of 1 cps) and moves downfield to 13.50 ppm when the concentration of imidazole is increased to 2.2 *M*. From this marked concentration dependence of the NH chemical shift, there is no doubt that imidazole is also strongly self-associated in $CHCl_3$. In DMSO, however, no NH signal was observed up to 0.3 *M*, and a broad signal was seen at 9.7 ppm (with half-width of 15 cps) for 0.5 *M* imidazole in DMSO. Since the signals of imidazole in DMSO are all upfield from those in $CHCl_3$, it may be inferred that there is less self-association owing no doubt to some NH bonding to DMSO.

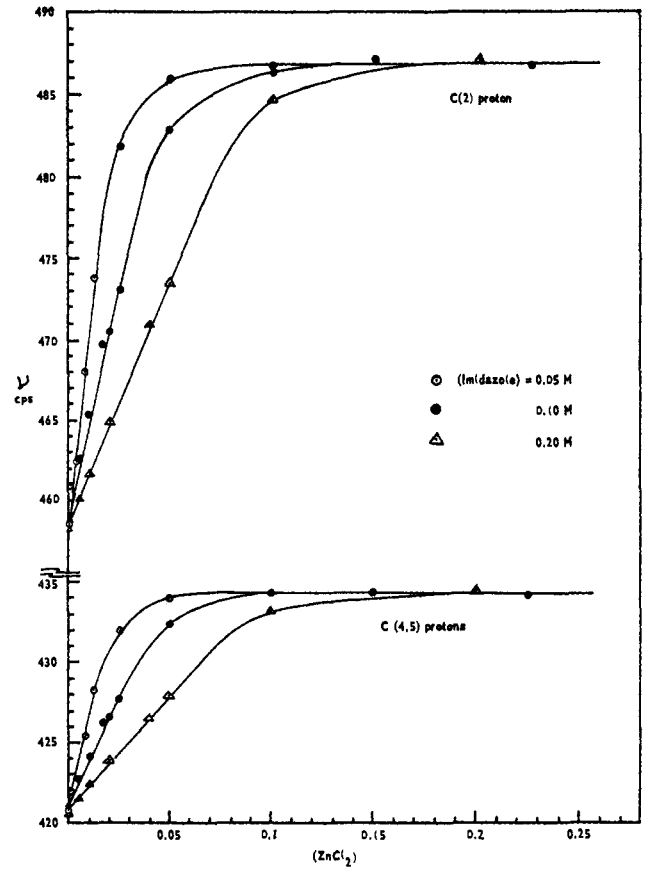


Figure 1. Effects of $ZnCl_2$ on 2-H and 4,5-H frequencies of imidazole in DMSO.

The effects of $ZnCl_2$ on the ring proton signals of imidazole in DMSO are shown in Figure 1. In the presence of excess $ZnCl_2$, the splitting of 2-H and 4,5-H signals by interaction with the 1-N proton was observed. These indicate that a stable Zn–imidazole complex is formed. Figure 1 shows that when (Zn) < (Im), in the region of (Zn):(Im) up to 1:4, a plot of ν *vs.* (Zn) is linear. In this concentration range, we may consider the formation of a 1:4 complex, $Zn(Im)_4{}^{2+}$, as has been done for aqueous medium.[12] When (Zn) increases still further, up to (Zn) = (Im), the frequency shifts further downfield, but the plot is no longer linear. When (Zn) > (Im), the frequency be-

(8) G. S. Reddy, R. T. Hobgood, Jr., and J. H. Goldstein, *J. Am. Chem. Soc.*, **84**, 336 (1962).

(9) H. A. Staab and A. Mannschreck, *Tetrahedron Letters*, 913 (1962).

(10) D. M. W. Anderson, J. L. Duncan, and F. J. C. Rossotti, *J. Chem. Soc.*, 2165 (1961).

(11) A. Mannschreck, W. Seitz, and H. A. Staab, *Ber. Bunsenges. Physik. Chem.*, **67**, 470 (1963).

(12) N. C. Li, J. M. White, and E. Doody, *J. Am. Chem. Soc.*, **76**, 6219 (1954).

Table I. Slopes of Plots in Figure 1

Initial imidazole concn, M, B_0	$B_0/0.05$	For 2-H: Slope, S	$1193/S$	For 4,5-H: Slope, S	$541/S$
0.05		1193		541	
0.10	2.00	573	2.08	276	1.96
0.20	4.00	306	3.90	144	3.76

comes constant, indicating that a 1:1 complex has been formed.

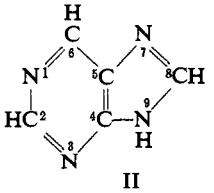
In the region of (Zn) < $^1/_4$(Im), the slopes of the linear plots for 2-H and 4,5-H are obtained from Figure 1 and listed in Table I. The table shows that eq 8 is approximately obeyed for both the 2-H and 4,5-H proton signals. This indicates that the equilibrium constant for eq 2 must be very large, and indeed at 25° in aqueous solution, Edsall, *et al.*,[13] reported that $K = 10^{9.20}$ for the reaction $Zn^{2+} + 4Im = Zn(Im)_4^{2+}$.

In order to estimate K of eq 2 in DMSO for $x = 4$, we proceeded as follows. Rearrangement of eq 1 gives

$$(MB_z) = \frac{(\nu - \nu_f)B_0}{(\nu_c - \nu_f)x} \qquad (10)$$

For 2-H, it is reasonable to assume that ν_c has a value somewhere between 486.5 (the limiting frequency in Figure 1) and 473 cps (the frequency for the solution in which (Zn) = $^1/_4$(Im)). By assuming different values of ν_c in this frequency range together with the data of Figure 1, one can calculate (MB_z) using eq 10, and then K from eq 3. We found that only by assuming ν_c = 478 cps could we obtain a reasonably constant value for K. For the first five experimental points in Figure 1, K was calculated to be 1.4 (± 0.9) × 10^6. This value of K, found in DMSO, is much smaller than the value in aqueous medium, and the decrease in K may be due to competition by DMSO for bonding to metal. Selbin, Bull, and Holmes,[14] and Cotton and Francis[15] have reported the preparation and study of a large number of metal complexes of DMSO.

(B) Purine Complex. The pmr spectrum of 0.1 M purine (II) in DMSO consists of three resonance peaks, at 8.60, 8.91, and 9.12 ppm. The original assignments of the C-protons[16,17] were shown to be incorrect by Matsuura and Goto[18] and by Schweizer, *et al.*[19] The correct assignment is:[19] the highest field peak, 8.60 ppm, to 8-H; the center field peak to 2-H; and the lowest field peak, 9.12 ppm, to 6-H.



II

When the DMSO solution contained 0.1 M purine and 0.1 M $ZnCl_2$, the signals of 2-H, 6-H, and 8-H were shifted downfield by 2.0, 2.7, and 3.7 cps, respectively, from those in the absence of $ZnCl_2$. The effects of charge densities on pmr shifts have been treated in terms of a field effect,[19] and the changes in charge densities are generally greater at the carbon atom which is closest to the binding site. Since 8-H is affected the most, this indicates that the preferred binding site in purine toward Zn is 7-N.

Eichhorn, Clark, and Becker[20] have shown that when Cu(II) forms a complex with a ligand, the protons near the binding site are relaxed by the paramagnetic ions and their pmr lines are thus broadened. The concentration of the copper salt is much less than that of the ligand and rapid exchange causes all ligand molecules to be affected equally. We have used this method in an attempt to determine the preferred binding site of purine to Cu(II). On adding 10^{-3} M $CuCl_2$ to 0.1 M purine in DMSO, the line broadening was found to be greater for 6-H than for 8-H. The width of the 2-H signal, however, was influenced very little by the addition of Cu(II), indicating that 2-H is relatively at a distance away from the binding site. In purine therefore, the likelihood that 1-N or 3-N is the preferred binding site may be ruled out, leaving 7-N to be the preferred binding site for both Zn and Cu(II).

In the concentration range (Zn) > (purine), it is reasonable to assume the existence of a 1:1 complex. For a solution of 0.1 M purine in DMSO at 36°, the frequency of 8-H was found to be 516.0 cps and to shift downfield on adding $ZnCl_2$ (see Figure 2b, dotted curves). Since Chan, *et al.*,[21] have shown that the proton resonances of purine are essentially independent of concentration in DMSO, it is safe to take 516.0 cps as ν_f. Using eq 9, a linear plot of $1/(\nu - \nu_f)$ *vs.* $1/(ZnCl_2)$ was obtained, from the intercept and slope of which the values of $(\nu_c - \nu_f)$ and K were calculated to be 15.8 cps and 4.4 l./mole^{-1}, respectively. K refers to the reaction Zn^{2+} + purine = $Zn(purine)^{2+}$, in DMSO. Although this value of K seems to be too small when compared to the formation constants of the Cu(II)- and Ni(II)-purine complexes (log K_1 = 6.9 and 4.9, respectively) obtained by pH titration in 50% v/v dioxane–water medium at 25°,[22] it must be remembered that in the pH titration experiment, the proton on the 9-N is displaced by metal complexation, so that the ligand is the anion of purine, and not the uncharged purine molecule.

Equations 3 and 10, with $x = 1$, were also programmed in Fortran II, and the computations of (MB) and K, assuming different values of ν_c, were performed using an IBM 1620 computer. It was found that K for the $Zn(purine)^{2+}$ complex became constant (K = 5.2 ± 0.5) only with ν_c = 529.5 ± 0.5 cps. The graphical results using eq 9 (K = 4.4, ν_c = 531.8 cps) therefore are in fair agreement with values obtained by the computer method.

(C) Cytosine Complex. The pmr spectrum of 0.1 M cytosine in DMSO consists of two doublets of

(13) J. T. Edsall, G. Felsenfeld, D. S. Goodman, and F. R. N. Gurd, *J. Am. Chem. Soc.*, 76, 3054 (1954).
(14) J. Selbin, W. E. Bull, and L. H. Holmes, Jr., *J. Inorg. Nucl. Chem.*, 16, 219 (1961).
(15) F. A. Cotton and R. Francis, *J. Am. Chem. Soc.*, 82, 2986 (1960).
(16) C. D. Jardetzky and O. Jardetzky, *ibid.*, 82, 222 (1960).
(17) G. S. Reddy, L. Mandell, and J. H. Goldstein, *J. Chem. Soc.*, 1414 (1963).
(18) S. Matsuura and T. Goto, *Tetrahedron Letters*, No. 22, 1499 (1963).
(19) M. P. Schweizer, S. I. Chan, G. K. Helmkamp, and P. O. P. Ts'o, *J. Am. Chem. Soc.*, 86, 696 (1964).
(20) G. L. Eichhorn, P. Clark and E. D. Becker, *Biochemistry*, 5, 245 (1966).
(21) S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. K. Helmkamp, *J. Am. Chem. Soc.*, 86, 4182 (1964).
(22) G. E. Cheney, H. Frieser, and Q. Fernando, *ibid.*, 81, 2611 (1959).

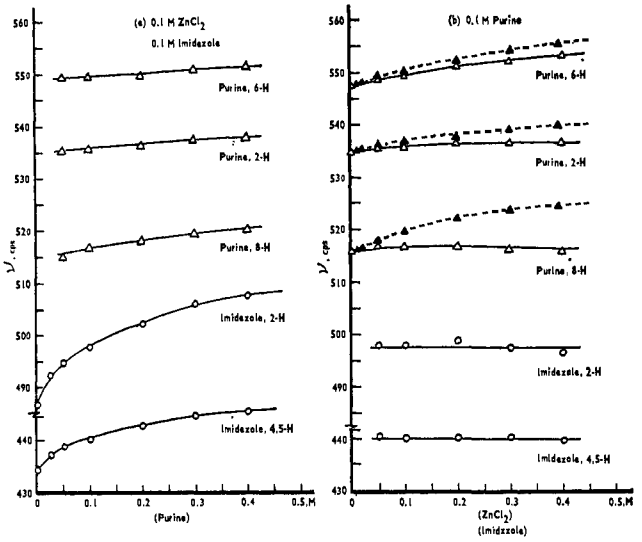


Figure 2. Pmr frequencies of purine and imidazole protons in DMSO solutions containing: (a) 0.1 *M* $ZnCl_2$, 0.1 *M* imidazole, and varying concentrations of purine; (b) 0.1 *M* purine and varying concentrations of $ZnCl_2$ and imidazole (ratio of $ZnCl_2$ to imidazole concentrations = 1.0). Dotted curves indicate the absence of imidazole.

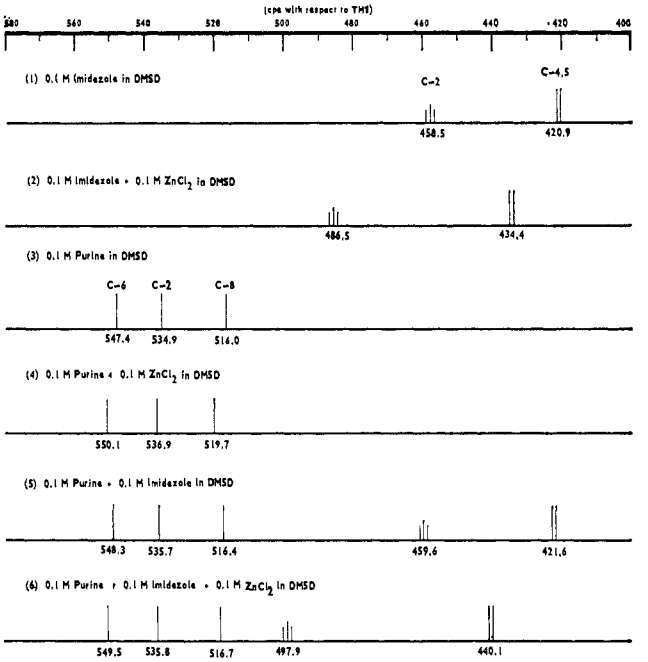
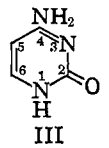


Figure 3. Pmr spectra of DMSO solutions of imidazole and purine, in the absence and presence of $ZnCl_2$.

equal intensity at 5.58 and 7.73 ppm, due to protons at 5-H and 6-H, coupled by 7 cps. The spectrum is identical with that reported by Katritzky and Waring.[23] At this low concentration, the broad peak due to the amino protons was not observable.

Among the possible tautomeric structures of cytosine, Katritzky and Waring[23] and Jardetzky, Pappas, and Wade[24] showed that III is the preferred structure for cytosine in DMSO and that protonation occurs at the 3-N position.



III

On adding $ZnCl_2$ to 0.1 *M* cytosine solution in DMSO the 5-H and 6-H signals are shifted downfield to an equal extent (Figure 4b). On adding 10^{-3} *M* $CuCl_2$ to 0.1 *M* cytosine in DMSO, the line broadening for 5-H is slightly greater than for 6-H. These facts, together with the finding of Eichhorn, Clark, and Becker[20] that the NH_2 line in deoxycytidine is relatively unaffected by addition of Cu(II), lead to the conclusion that the 3-N position is the preferred binding site in cytosine for both Zn and Cu(II).

From the chemical shifts of 6-H of cytosine in the absence and presence of $ZnCl_2$ in DMSO (Figure 4b, dotted curves), computations of (MB) and *K*, assuming ν_f = 439.7 cps and different values of ν_c, were performed using a computer. It was found that *K* for the Zn-(cytosine)$^{2+}$ complex became relatively constant in the $ZnCl_2$ concentration range 0.100 to 0.840 *M* (K = 24.5 ± 3.0), when ν_c is assumed to be 457.5–458.0 cps.

(D) Zn–Imidazole–Purine Complex. Figure 2a gives the frequency of purine and imidazole protons for solutions containing 0.1 *M* $ZnCl_2$, 0.1 *M* imidazole, and varying concentrations of purine in DMSO. It is seen that the effect of adding purine is greatest for the 2-H signal of imidazole. A possible explanation is that the NH of Zn–imidazole serves as a hydrogen donor to purine. The ring proton signals in imidazole and purine move downfield because of mutual ring current effects.

A comparison of Figure 3(1–4) shows that zinc is strongly bonded to imidazole, and only weakly bonded to purine. A mixture of purine and imidazole gives a spectrum, Figure 3(5), in which the imidazole and purine signals are slightly displaced downfield, indicating that there is interaction, probably hydrogen bonding between imidazole and purine. In Figure 3(6), for equimolar mixtures of $ZnCl_2$, imidazole, and purine, the imidazole proton signals are shifted further downfield while the purine proton signals are displaced upfield from those in the presence of $ZnCl_2$ only. The spectrum gives qualitative evidence that a ternary complex is formed.

By comparing Figure 3(3, 5, and 6), it will be seen that of the three peaks in purine, the 6-H proton is most affected by hydrogen bonding with NH of imidazole. This indicates that while 3-N and 7-N may be sites, the preferred acceptor site in purine is 1-N. In this connection it is interesting to note that Read and Goldstein[25] report that their nmr results are consistent with a protonation scheme for purine involving all three basic centers, 1-N, 3-N, and 7-N, with 1-N the principal site of protonation. It will be recalled from the discussion in part B that 7-N is the preferred binding site toward metal ion.

From the data of Figure 2a, using the 2-H frequencies of imidazole, we have obtained a linear plot of $1/(\nu - \nu_f)$ *vs.* 1/(purine). Here ν_f is taken to be the 2-H frequency of imidazole in a solution of 0.1 *M* $ZnCl_2$ and 0.1 *M* imidazole in DMSO, in the absence of purine, since from Figure 1 it is seen that in an equimolar mixture of

(23) A. R. Katritzky and A. J. Waring, *J. Chem. Soc.*, 3046 (1963).
(24) O. Jardetzky, P. Pappas, and N. G. Wade, *J. Am. Chem. Soc.*, **85**, 1657 (1963).
(25) J. M. Read, Jr., and J. H. Goldstein, *ibid.*, **87**, 3440 (1965).

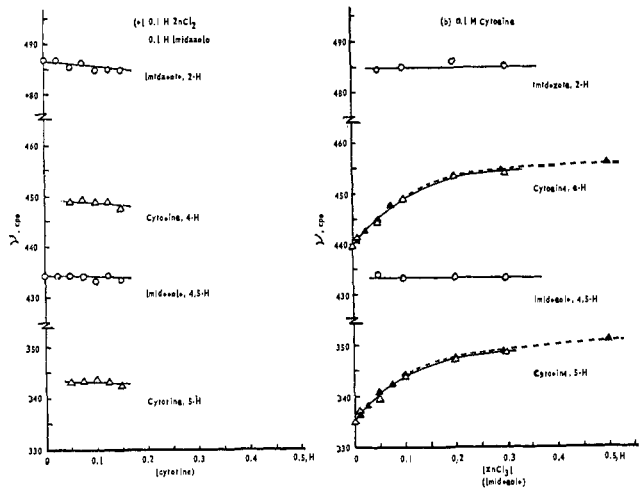


Figure 4. Pmr frequencies of cytosine and imidazole protons in DMSO solutions containing: (a) 0.1 M $ZnCl_2$, 0.1 M imidazole, and varying concentrations of cytosine; (b) 0.1 M cytosine and varying concentrations of $ZnCl_2$ and imidazole (ratio of $ZnCl_2$ to imidazole concentrations = 1.0). Dotted curves indicate the absence of imidazole.

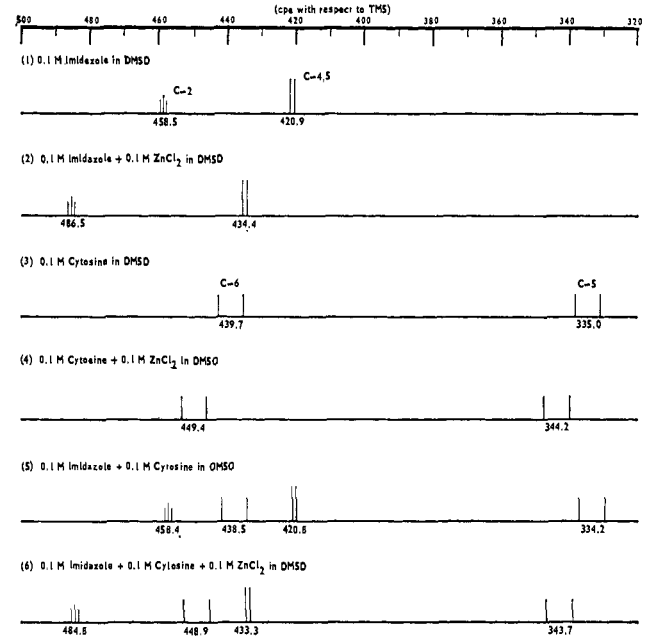


Figure 5. Pmr spectra of DMSO solutions of imidazole and cytosine in the absence and presence of $ZnCl_2$.

Zn and imidazole, a stable 1:1 complex is formed. The values of $(\nu_c - \nu_f)$ and K are obtained from eq 9 and the plot, except that B_0 in eq 9 now refers to the initial concentration of $ZnCl_2$ and imidazole (0.1 M), and M_0 is replaced by (purine). The values of $(\nu_c - \nu_f)$ and K are 29.4 cps and 16.9 l. mole^{-1}, where K refers to the hydrogen-bonding reaction

$$\text{Zn(imidazole)}^{2+} + \text{purine} = \text{Zn(imidazole)}\cdots\text{purine}^{2+} \quad (11)$$

In part B, we reported that the formation constant for Zn–purine complex in DMSO is 4.4, using the plot of eq 9. In view of the larger K for eq 11, obtained also from the plot of eq 9, it is reasonable to postulate that the ternary complex is one in which purine is hydrogen bonded to Zn–imidazole, rather than one in which purine is bonded directly to the metal.

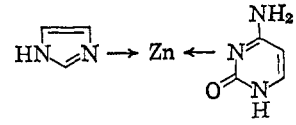
On the basis of the above discussion, a possible structure of the ternary complex may be Zn←imidazole···purine, in which the acceptor site of purine is 1-N, whereas in Zn–purine complex, the binding site is 7-N. Our proposal receives support from the results of Figure 2b, which show that for mixtures of $ZnCl_2$, imidazole, and purine (solid curves) the change of 6-H frequency of purine is the largest, whereas for mixtues containing $ZnCl_2$ and purine only (dotted curves), the change of 8-H frequency is the largest.

(E) **Zinc–Imidazole–Cytosine Complex.** Figure 4a gives frequencies of cytosine and imidazole protons for solutions containing 0.1 M $ZnCl_2$, 0.1 M imidazole, and varying concentrations of cytosine in DMSO. Because of the low solubility of cytosine in DMSO, it was not possible to extend the cytosine concentration beyond 0.15 M. However, comparison of Figure 4a with Figure 2a demonstrates that the bonding schemes in the two ternary complexes must be different.

Figure 4b gives the imidazole and cytosine proton frequencies for solutions containing 0.1 M cytosine and varying concentrations of Zn(imidazole)$^{2+}$ complex in DMSO. It is seen that the imidazole proton signals remain constant and the cytosine proton signals move downfield on increasing the concentration of Zn(imidazole)$^{2+}$ (solid curves). The dotted curves give the frequency of cytosine protons on adding $ZnCl_2$ alone, and it is seen that the cytosine frequency depends only on the concentration of $ZnCl_2$, regardless of whether imidazole is present or not.

Figure 5(5) gives the spectrum of an equimolar mixture of cytosine and imidazole in DMSO. On comparing this with Figure 5(1 and 3), the conclusion is reached that there is no appreciable interaction between imidazole and cytosine, in DMSO. Figure 5(6) shows that in an equimolar mixture of $ZnCl_2$, imidazole, and cytosine, both the imidazole and cytosine signals are in the same positions as those of imidazole and cytosine, each *separately* in the presence of $ZnCl_2$.

The above discussion demonstrates that in a mixture of $ZnCl_2$, imidazole, and cytosine in DMSO, a ternary complex is formed in which imidazole is bonded to Zn, which in turn is bonded to cytosine. From what has already been said regarding the binding sites in cytosine toward metal, a preferred structure for the imidazole–Zn–cytosine ternary complex may be written



It will be recalled that the formation constant of the Zn–purine complex is smaller than the value for the Zn–cytosine complex. Moreover, hydrogen bonding between imidazole and purine is appreciable compared to that between imidazole and cytosine. For these reasons, it is easy to see why the bonding schemes for the ternary complexes Zn–imidazole–purine and imidazole–Zn–cytosine are different.

We have shown above that a ternary complex exists in an equimolar mixture of $ZnCl_2$, imidazole, and cytosine in DMSO. It became of interest to determine what happens when imidazole is present in excess. For this purpose we carried out a series of experiments in which the concentration of $ZnCl_2$ and cytosine was

Table II. Pmr Chemical Shifts of 6-H of Cytosine and 2-H of Imidazole in Solutions Containing 0.1 M $ZnCl_2$, 0.1 M Cytosine, and Varying Concentrations of Imidazole in DMSO, at 36°

Concn of imidazole, M	ν, cps 6-H of cytosine	ν, cps 2-H of imidazole
0	449.4	
0.10	448.5	485.5
0.20	443.8	482.6
0.30	441.9	477.7
0.40	441.8	474.3

kept constant at 0.1 M in DMSO, and the imidazole concentration was varied. Table II gives the results obtained. As the imidazole concentration increases from 0.1 to 0.4 M, the imidazole 2-H signal moves upfield from 485.5 to 474.3 cps, and the cytosine 6-H signal also moves upfield, from 448.5 to 441.8 cps. This is qualitative evidence for the reaction

$$\text{imidazole–Zn–cytosine} + 3\text{Im} = \text{Zn(Im)}_4 + \text{cytosine} \quad (12)$$

since Figure 1 shows that the frequency of imidazole 2-H at (Im)/(Zn) = 4:1 is 473.5 cps, which is upfield from the limiting frequency of 486.5 cps, obtained when the ratio (Im)/(Zn) $\leq$ 1. The upfield shift of cytosine 6-H frequency on increasing imidazole concentration is as expected, since part of the cytosine bound in the ternary complex is becoming free.

The value of K of eq 12 was calculated for a solution of 0.1 M $ZnCl_2$, 0.1 M cytosine, and 0.4 M imidazole in DMSO, as follows: the observed frequency for 6-H in cytosine, ν = 441.8 cps, may be considered to be the weighted average of the free and complexed cytosine

$$\nu = \frac{0.1 - x'}{0.1}\nu_f + \frac{x'}{0.1}\nu_c \quad (13)$$

where x' = concentration of the ternary complex at equilibrium, and ν_f and ν_c are the characteristic frequencies of free and complexed cytosine (taken to be equal to 439.7 and 459.7 cps, respectively). From eq 13, the value of x' was calculated to be 0.01. The equilibrium constant of eq 12 is then given by

$$K = \frac{(0.1 - 0.01)^2}{0.01(0.4 - 0.01 - 4(0.1 - 0.01))^3} = 3 \times 10^4$$

The large value of K of eq 12 is in line with the expectation that $Zn(Im)_4^{2+}$ is much more stable than Im–Zn–cytosine^{2+}.

Proton Nucear Magnetic Resonance Contact Shifts in the Complexes $Co\{OP[N(CH_3)_2]_3\}_2X_2$ and $Co(C_5H_5N)_2X_2$

Bradford B. Wayland[1] **and Russell S. Drago**

Contribution from the William A. Noyes Laboratory, University of Illinois, Urbana, Illinois. *Received December 10, 1965*

Abstract: The proton nmr contact shifts for a series of paramagnetic cobalt(II) complexes of general formula CoL_2X_2 (where L = pyridine or $[(CH_3)_2N]_3PO$ and X^- = Cl^-, Br^-, I^-, or NCS^-) are reported. Mechanisms for spin delocalization are proposed. Evidence for both π- and σ-electron-spin-delocalization mechanisms is found for pyridine. The magnitude of electron spin delocalization to the neutral ligand is found to increase in the order $CoL_2Cl_2 \leq CoL_2Br_2 < CoL_2I_2$. A molecular orbital model is invoked to explain the anion effects.

The present status of the theory for nuclear magnetic resonance contact shifts has been discussed in several recent publications.[2-8] This article reports the anion effects on the neutral ligand proton contact shifts for the series of complexes of the general formula

CoL_2X_2 (X^- = Cl^-, Br^-, I^-, NCS^-; L = HMPA ($[(CH_3)_2N]_3PO$), py (C_5H_5N))

The metal ion and neutral ligand are the same in each series of complexes. Only the coordinated anion is changed. For these series of complexes, it is possible to relate the ligand proton contact shifts directly to the relative unpaired electron density on the neutral ligand.

Experimental Section

Apparatus. The nmr spectra were obtained with Varian Model A-60 and Varian Model DP-60 nmr spectrometers. All nmr spectra were measured relative to tetramethylsilane (TMS) as an internal standard. When very accurate chemical shift measurements were necessary, two internal standards, TMS and cyclohexane or benzene, were used.

Reagents and Solutions. Hexamethylphosphoramide (HMPA) (Fisher) was distilled from barium oxide at reduced pressure. The middle fraction boiling at 127° (20 mm) was collected. The material 2,2-dimethoxypropane was obtained from Dow Chemical Co. and was used without further purification. Reagent grade $CDCl_3$ was used without further purification.

All solutions of hygroscopic materials were prepared in a drybox equipped with an automatic continuous air-flow drying system. When accurate complex or ligand concentrations were necessary, materials were weighed in stoppered volumetric flasks.

(1) Abstracted in part from the Ph.D. thesis of B. Wayland, University of Illinois, 1964; NSF Graduate Fellow, 1964.
(2) B. B. Wayland and R. S. Drago, *J. Am. Chem. Soc.*, 87, 2372 (1965).
(3) D. R. Eaton, A. D. Josey, W. D. Phillips, and R. E. Benson, *J. Chem. Phys.*, 37, 347 (1962).
(4) D. R. Eaton, *J. Am. Chem. Soc.*, 87, 3097 (1965).
(5) R. H. Holm, A. Chakravorty, and G. O. Dudek, *ibid.*, 86, 379 (1964).
(6) R. S. Milner and L. Pratt, *Discussions Faraday Soc.*, 34, 88 (1962).
(7) J. A. Happe and R. L. Ward, *J. Chem. Phys.*, 39, 1211 (1963).
(8) G. N. LaMar, W. D. Horrocks, Jr., and L. C. Allen, *ibid.*, 41, 2126 (1964).