tion. Though the dielectric constant of the solvent influences the extent of association of a given salt, it is clear that ion size and solvent coordinating ability are important factors which can determine the *nature* of the associated species in solution.

Appendix

Elemental analysis was used to verify the identities of new compounds as well as those of compounds whose melting points disagreed with literature values. These data are presented in Table XV.

Nuclear Magnetic Resonance Studies of Mercury(II) Interaction with Nucleosides in Dimethyl Sulfoxide[1]

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Abstract: By using a nuclear magnetic resonance method, the formation constants of the 1:1 mercury(II) complexes with cytidine, adenosine, and guanosine in dimethyl sulfoxide at 36° were found to be 33.9, 7.2, and 5.9 l./mol, respectively. In dimethyl sulfoxide solutions containing 0.1 *M* cytidine and $HgCl_2$ concentration above 0.2 *M*, two peaks were observed for the amino group, indicating nonequivalence of the amino protons due to insufficiently fast rotation of the amino group. The mercuration leads to hindered rotation about the 4-C–N bond in cytidine. As the $HgCl_2$ concentration is decreased, the lines broaden, and coalesce into one broad peak at 0.1 *M* $HgCl_2$. As the concentration of $HgCl_2$ decreases below this value, the single peak sharpens, indicating fast rotation of the amino group. Starting with a dimethyl sulfoxide solution containing 0.1 *M* cytidine and 1 *M* $HgCl_2$, with increasing temperature the lines broaden and then also coalesce to a single peak. Mercury does not bind uridine, and forms a weaker complex with purine than with guanosine. Unlike zinc, mercury(II) does not form ternary complexes.

In 1952, Katz[2] added mercuric chloride to sodium thymonucleate (DNA) and discovered a remarkable decrease in the viscosity, as well as changes in other properties, indicating some kind of configurational rearrangement. Upon addition of excess chloride or cyanide ions, which strongly complex mercury(II), the solution regains its original viscosity. This discovery of the interaction of mercury(II) with DNA was followed by a demonstration of Thomas[3] that the mercury is bonded to the bases and led to further studies on interaction of mercury with the nucleosides.[4–7] Thomas has suggested that since the original size and shape of the DNA molecule is restored after the mercuric chloride is removed, a sufficient number of hydrogen bonds must remain in contact in the presence of mercuric chloride to provide a "skeleton" to guide the re-formation of the molecule when the reaction is reversed.

This work was motivated by the thought that useful information on the nature of interaction of mercury with nucleic acids can be obtained by a nuclear magnetic resonance (nmr) study of mercury(II) complexes of the nucleosides and purine. Dimethyl sulfoxide was used as solvent, as has been done in three previous papers from this laboratory,[8–10] because it provides adequate solubility without proton transfer, whereas the use of water as solvent causes complications in that the bond-forming hydrogens exchange rapidly with water protons. Although dimethyl sulfoxide is a hydrogen acceptor, we can take as reference state the one in which nucleoside is hydrogen-bonded to the solvent *via* the protons attached to nucleoside nitrogens, and look for further shifts of the NH, NH_2, and CH proton signals as an increasing amount of $HgCl_2$ is added.

Experimental Section

Materials. Purine and the nucleosides were obtained from Sigma Chemical Co. Anhydrous mercuric chloride was reagent grade. Dimethyl sulfoxide was purified by vacuum distillation after drying over potassium amide.

Nmr Measurements. Spectra were obtained with a Varian A-60 or a Perkin-Elmer R-20 spectrometer operated at 60 MHz. The latter instrument was operated at 36 ± 1°. With the A-60 spectrometer, a V-6031 variable temperature probe was used, and the V-6040 variable temperature controller was calibrated to ±1° by using the known chemical shifts of the CH_2 and OH proton resonances in ethylene glycol and the temperature calibration chart of Van Geet.[11] Sample preparation and the technique of chemical shift measurements were the same as described previously.[8–10] Frequencies of sharp peaks were accurate to ±0.2 Hz.

Results

(A) $HgCl_2$–Nucleoside Complexes in Dimethyl Sulfoxide (DMSO). In Figure 1 are reproduced the low-field portions of the nmr spectra of cytidine (0.1 *M*) in the absence and presence of 0.5 *M* $HgCl_2$. Plots of frequency and line width of the cytidine (C)–NH_2 signal in DMSO solution containing 0.1 *M* cytidine and

(1) This investigation was supported by National Science Foundation Grant No. GB 8237 and by Public Health Service Grant No. GM 10539-07.

(2) S. Katz, *J. Amer. Chem. Soc.*, 74, 2238 (1952).
(3) C. A. Thomas, *ibid.*, 76, 6032 (1954).
(4) T. Yamane and N. Davidson, *ibid.*, **83**, 2599 (1961).
(5) R. Ferreira, E. Benzvi, T. Yamane, J. Vasilevskis, and N. Davidson, "Advances in the Chemistry of the Coordination Compounds," S. Kirschner, Ed., Macmillan, New York, N. Y., 1961, p 457.
(6) G. L. Eichhorn and P. Clark, *J. Amer. Chem. Soc.*, **85**, 4020 (1963).
(7) R. B. Simpson, *ibid.*, **86**, 2059 (1964).
(8) S. M. Wang and N. C. Li, *ibid.*, **88**, 4592 (1966).
(9) S. M. Wang and N. C. Li, *ibid.*, **90**, 5069 (1968).
(10) L. S. Kan and N. C. Li, *ibid.*, **92**, 281 (1970).
(11) A. L. Van Geet, *Anal. Chem.*, **40**, 2227 (1968).

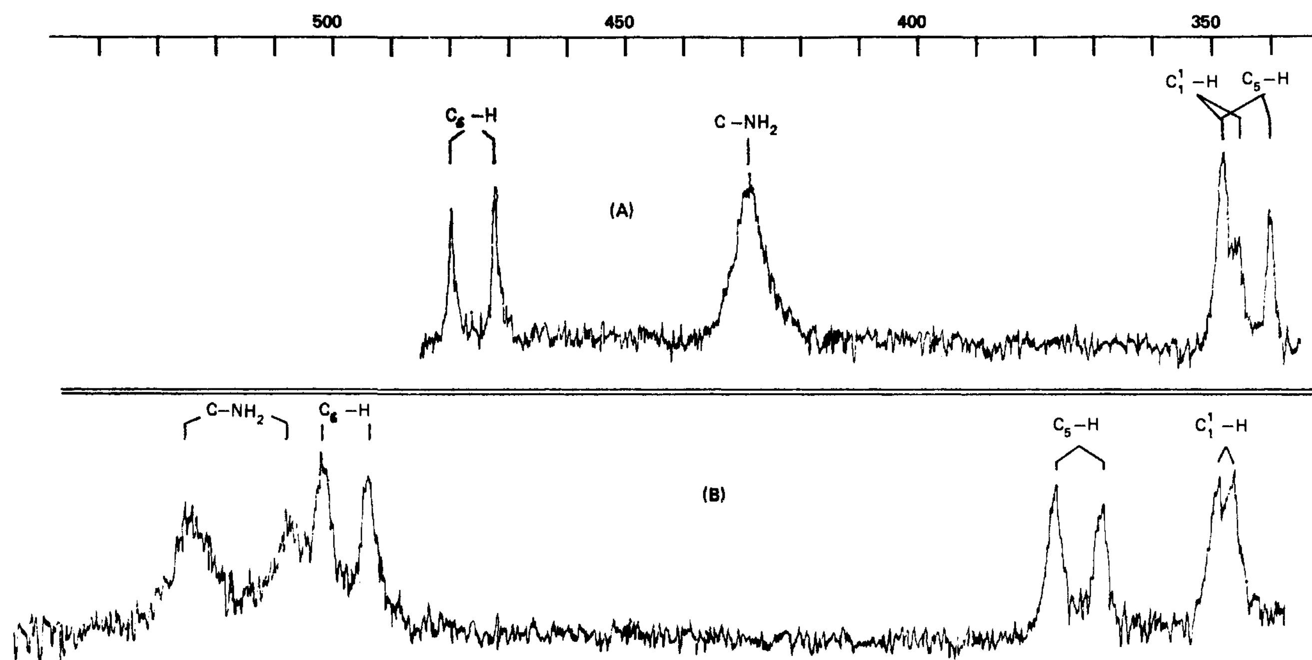


Figure 1. The low-field portions of the nmr spectra of cytidine in DMSO at 36°: (A) 0.1 M cytidine; (B) 0.1 M cytidine, 0.5 M $HgCl_2$. Numbering is according to II.

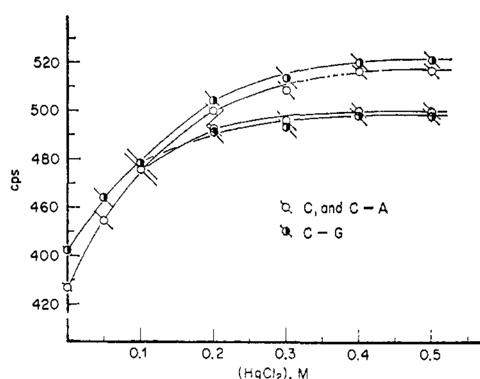


Figure 2. Nmr frequencies of cytidine amino protons *vs.* varying concentrations of $HgCl_2$ in DMSO at 36°: O, 0.1 M C and 0.1 M C, 0.1 M A; ◑, 0.1 M C, 0.1 M G; ⌒ ⌣, refers to the full line width at half-height.

varying concentrations of $HgCl_2$ at 36°, in the absence and presence of 0.1 M adenosine (A) or 0.1 M guanosine (G), are shown in Figure 2. Figures 3 and 4, respectively, give plots of frequencies of guanosine and of adenosine protons *vs.* varying concentrations of $HgCl_2$ in DMSO, in the absence and presence of one of the other nucleosides.

The formation constant of 1:1 mercury–cytidine complex, K_c, was evaluated by the application of eq 4

$$\frac{M_0}{\nu - \nu_f} = \frac{1}{\nu_c - \nu_f}(B_0 + M_0 - (MB)) + \frac{1}{K_c(\nu_c - \nu_f)} \quad (1)$$

of Wang and Li[9] in which M_0 and B_0 are the initial concentrations of metal and nucleoside, respectively, (MB) is the equilibrium concentration of the metal complex, ν is the observed frequency of a nucleoside proton, and ν_f and ν_c are the characteristic frequencies of the free and complexed molecules, respectively. Self-association of cytidine at a total concentration of 0.1 M in DMSO has been shown to be negligible,[9] so that ν_f is taken to be the frequency of 0.1 M cytidine in the absence of $HgCl_2$. Although the observed frequency of 0.1 M cytidine increases on the addition of $HgCl_2$, the value becomes almost constant at $(HgCl_2) > 0.4$ M, and we have taken ν_c to be the frequency of 0.1 M cytidine in the presence of 1 M $HgCl_2$ (for the two peaks for the amino group, the center is taken). The value of (MB) is then calculated from eq 1 of Wang and Li[9]

$$(MB) = \frac{\nu - \nu_f}{\nu_c - \nu_f}B_0 \quad (2)$$

with $B_0 = 0.1$ M. Plots of $M_0/(\nu - \nu_f)$ *vs.* $M_0 + B_0 -$

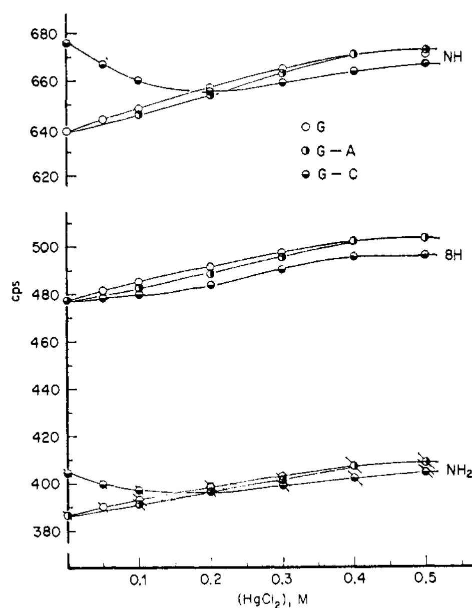


Figure 3. Nmr frequencies of guanosine protons *vs.* varying concentrations of $HgCl_2$ in DMSO at 36°: O, 0.1 M G; ◑, 0.1 M G, 0.1 M A; ●, 0.1 M G, 0.1 M C.

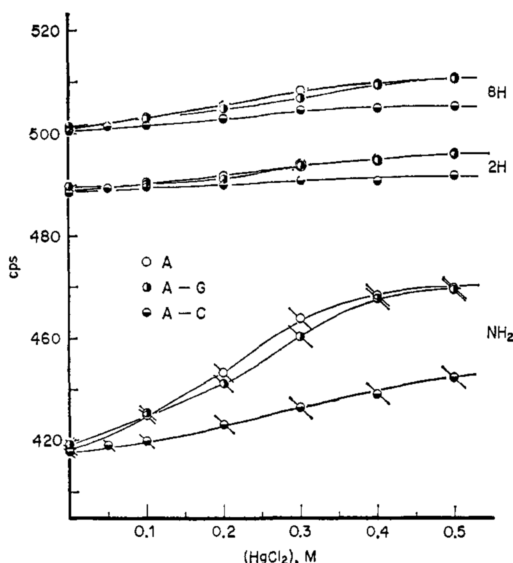


Figure 4. Nmr frequencies of adenosine protons *vs.* varying concentrations of $HgCl_2$ in DMSO at 36°: ○, 0.1 *M* A; ◑, 0.1 *M* A, 0.1 *M* G; ⊖, 0.1 *M* A, 0.1 *M* C.

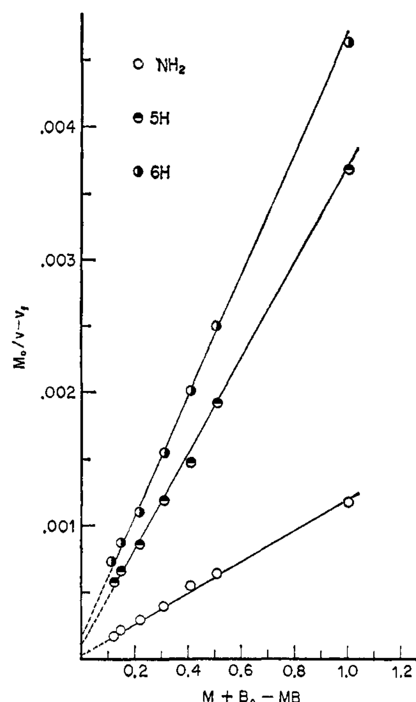


Figure 5. Plots of eq 1 for mercury–cytidine complex, using data on cytidine proton frequencies: ○, NH_2; ⊖, 5-H; ◑, 6-H.

(*MB*) according to eq 1 are given in Figure 5, and the values of K_c obtained from plots using 5-H, 6-H, and C-NH_2 frequencies are 32.9, 35.3, and 33.4 l./mol, respectively. It is very gratifying to note the excellent agreement in the K_c values, using nmr data from different protons in the molecule, since heretofore formation constants of metal complexes have been calculated from data on one proton only.

The formation constant of 1:1 $HgCl_2$–guanosine and $HgCl_2$–adenosine complexes were evaluated from the data of Figures 3 and 4 for DMSO solutions containing 0.1 *M* guanosine and 0.1 *M* adenosine, respectively, and varying concentrations of $HgCl_2$. Using the data on G–NH_2, G–NH, and G–8H, the values of K_c for $HgCl_2$–guanosine are 5.9, 6.3, and 5.5 l./mol, respectively. Using the data on A–NH_2 and A–8H, the values of K_c for $HgCl_2$–adenosine are 7.0 and 7.4, respectively. The formation constant of $HgCl_2$–cytidine ($K_c = 33.9 \pm 1.0$) is therefore 5–6 times larger than the values for the $HgCl_2$ complexes of guanosine and adenosine. It is interesting to note that the formation constant of zinc–cytidine complex is 4–5 times larger than the values for the zinc complexes of guanosine and adenosine, also in DMSO.[9]

Figure 3 shows that in the absence of $HgCl_2$, the G–NH and G–NH_2 signals in G–C mixture are shifted downfield from those in G alone and the frequencies in G alone are identical with those in G–A mixture. This is easily explained because of the strong G–C pairing, whereas G–A pairing in DMSO is slight.[12] The G–8H frequency has the same value in solutions containing G, G and C, and G and A, in the absence of $HgCl_2$. The G–8H therefore is at a distance away from the hydrogen-bonding sites, and this can be seen from the structure of the Watson–Crick G–C pair.[12]

In DMSO solutions containing G only and in the G–A mixture all the G signals go downfield on adding $HgCl_2$, whereas in the G–C mixture (below 0.2 *M* $HgCl_2$) the G–NH and G–NH_2 signals go upfield and only the G–8H signal goes downfield. In G–C pairing, the G–NH and G–NH_2 protons act as hydrogen donors to 3-N and the oxygen atom in cytidine, respectively. On adding $HgCl_2$, the metal binds at the 3-N of cytidine, and the hydrogen bonds are therefore partially disrupted resulting in an upfield shift of the G–NH and G–NH_2 proton signals. In the case of G–A mixture, no such pairing occurs. It will be noticed that in the G–C mixture, the rate of upfield shift of G–NH is about twice that of G–NH_2. This is understandable because of the two NH_2 protons, only one is involved in hydrogen bonding with C. The other proton is hydrogen bonded to DMSO. When the $HgCl_2$ concentration is increased beyond 0.2 *M*, all the G-proton signals in the G–C mixture now are shifted downfield. This is understandable because it has been shown that mercury binds to 7-N and the nitrogen atoms of NH and NH_2 in guanosine.[6,7]

Figure 4 shows that in A–C mixture, the A-proton signals remain unchanged in position up to 0.1 *M* $HgCl_2$. This is because $HgCl_2$ is preferentially bound to C (K_c for the cytidine complex is larger than for the adenosine complex). Only after an excess of $HgCl_2$ is added are the A-proton signals shifted slightly downfield.

In DMSO solutions containing 0.1 *M* A and 0.1 *M* G, in the presence of $HgCl_2$, the A proton signals are generally upfield from the corresponding signals in DMSO solutions containing 0.1 *M* A (G absent). This indicates that in A–G mixture, some $HgCl_2$ binds to guanosine so that the amount of $HgCl_2$ available for binding to A becomes less.

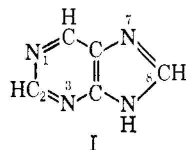
Simpson[7] has shown that mercury binds to the amino group of adenosine with loss of one proton. Our nmr data do not show the presence of an imino group; however, mercury can still bind to the amino group without loss of a proton. Because of the shift of the A–8H signal on mercuration, 7-N is also a binding site. Wang and Li[9] postulate that zinc binds to adenosine

(12) R. A. Newmark and C. R. Cantor, *J. Amer. Chem. Soc.*, 90, 5010 (1968).

in the form of a chelate involving 7-N and the amino group, and the structure may hold for the mercury complex. Although mercury tends to form collinear sp bonds,[4] Eichhorn and Clark[6] have pointed out that the tetrahedral configuration has been established for many mercury complexes and that chelate stabilization does occur with mercury.

On adding $HgCl_2$ (up to 0.5 *M*) to 0.1 *M* uridine solution in DMSO, the frequencies of 5-H, 6-H, and NH remain unchanged. This finding indicates that mercury does not form a complex with uridine in DMSO, and is in line with the finding that zinc does not bind uridine,[9] and copper(II) does not bind thymidine.[13] Eichhorn and Clark[6] and Simpson[7] have shown that in aqueous medium mercury is bound to 3-N in uridine with loss of the 3-NH proton. Our experiments were conducted in DMSO medium, and no loss of proton occurs.

(B) $HgCl_2$–Purine in DMSO. The spectrum of 0.1 *M* purine (I) in DMSO consists of three resonance peaks at 8.60, 8.91, and 9.12 ppm, which are assigned correctly[14] to 8-H, 2-H, and 6-H, respectively. When

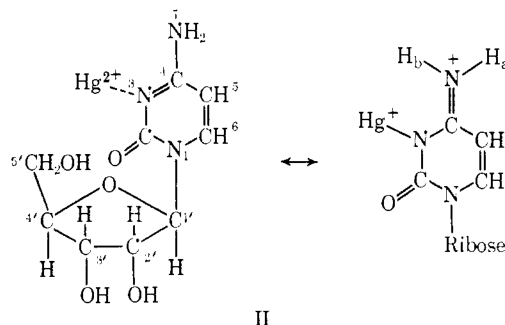


the DMSO solution contained 0.1 *M* purine and 0.5 *M* $HgCl_2$, the signals of 2-H, 6-H, and 8-H were shifted downfield by 8, 12, and 13 Hz, respectively, from those in the absence of $HgCl_2$. The effects of charge densities on nmr shifts have been treated in terms of a field effect,[14] and the changes in charge densities are generally greater at the carbon atom which is closest to the binding site. Since among the C protons, the 2-H frequency is influenced the least by the addition of $HgCl_2$, the indication is 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 site may be ruled out leaving 7-N to be the preferred binding site. It is interesting to note that Wang and Li[9] have shown that zinc and copper(II) also preferentially bind to 7-N.

(C) Remarks on Stability of Mercury Complexes in DMSO. In equimolar mixture of a pair of nucleosides, Wang and Li[9] have demonstrated the existence of ternary zinc complexes: Zn–A–C and Zn–A–G. Under the same condition, no ternary mercury(II) complexes are formed. The order of stability of binary mercury(II) complexes is: $C > A \sim G$.

In equimolar mixture of purine and C, G, or A, again no ternary mercury–purine–nucleoside complex is formed. By taking the spectra of the purine protons in DMSO solutions containing 0.1 *M* purine in the absence and presence of 0.1 *M* A, 0.1 *M* C, or 0.1 *M* G, and varying concentrations of $HgCl_2$, similar to the experiments described in Figures 2, 3, and 4, we have arrived at the conclusion that of the four ligands, the binary mercury(II)–purine complex is the least stable.

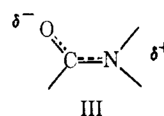
Discussion on Hindered Rotation of the Amino Group. Figures 1 and 2 show that the extent of the downfield shift of the cytidine amino signal and changes in the line shape on adding $HgCl_2$ are extraordinary, and the following resonance structures may be written



At 36°, for $HgCl_2$ concentrations greater than 0.2 *M*, two peaks are observed for the amino group, indicating nonequivalence of the amino protons. Apparently, mercuration leads to hindered rotation about the 4-C–N bond. As the $HgCl_2$ concentration is decreased, the lines broaden, and coalesce into one broad peak at 0.1 *M* $HgCl_2$. As the concentration of $HgCl_2$ decreases below this value, the single peak sharpens, indicating fast rotation of the amino group. This is an interesting finding, because it is the first report that metal binding leads to increased hindrance to internal rotation.

In principle, these data may be interpreted in terms of exchange rates for internal rotation of the amino group. Although limitations in the data do not permit us to carry out a detailed study of these rate processes, nevertheless it is possible to make some rough estimates of rates from some of the data of Figure 2, using the treatment outlined by Pople, *et al.*[15] At 36°, the mean lifetime of the NH_2 group of cytidine in a given conformation increases from 20 to 40 msec when the $HgCl_2$ concentration increases from 0.2 to 1 *M*.

For 1,3-dimethylcytosine hydrochloride in liquid SO_2 at −60°, Becker, *et al.*,[16] also observed two lines for the amino protons. With increasing temperature, the lines broaden and then coalesce to a single peak with concomitant frequency shifts. From the temperature dependence of the line shapes, they made a rough estimate of an activation energy of about 4–5 kcal/mol. We have observed similar temperature dependence of the line shapes for the amino lines in a DMSO solution containing 0.1 *M* cytidine and 1 *M* $HgCl_2$ in the temperature range 36–60°. A rough estimate gives an activation energy of about 6 kcal/mol, which is higher than the estimated value for 1,3-dimethylcytosine hydrochloride in SO_2. Part of the difference may be due to the use of DMSO as solvent, which serves as a very strong hydrogen acceptor to the amino protons. Rotation about the 4-C–N bond in cytidine and cytosine is thus very much less restricted than rotation about the central C–N bond in the amide functional group



(13) G. L. Eichhorn, P. Clark, and E. D. Becker, *Biochemistry*, **5**, 245 (1966).

(14) M. P. Schweizer, S. I. Chan, G. K. Helmkamp, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, **86**, 696 (1964).

(15) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, N. Y., 1959, Chapter 10.

(16) E. D. Becker, H. T. Miles, and R. B. Bradley, *J. Amer. Chem. Soc.*, **87**, 5575 (1965).

since the activation energy is about 20 kcal/mol for N,N-dimethylacetamide.[17] The severe restricted rotation in the amide functional group is due to electron delocalization (III) and this property imparts rigidity to proteins and polypeptides.[18]

On ^{15}N substitution in the amino group of 1-methylcytosine hydrochloride,[16] the amino proton peaks at 7.42 and 7.82 ppm are each split into doublets of 92.6 and 91.4 Hz, respectively. The lines of the high-field doublet show a splitting of 0.6 Hz, ascribed to the geminal amino coupling, while those of the low-field doublet are broadened appreciably. Becker, *et al.*,[16] suggested that the geminal amino coupling in the low-field pair is obscured because of the *trans* 6-H, 7-H coupling and therefore assigned the 7.82-ppm signal to H_b and the 7.42-ppm signal to H_a. By analogy in Figure 2, for $HgCl_2$ concentrations greater than 0.2 *M*, we assign the low-field signal to H_b, and the high-field signal to H_a. This assignment is reasonable because H_b is obviously closer to the coordinated mercury (see II) than H_a, and one would expect therefore that the H_b signal would be downfield from the H_a signal.

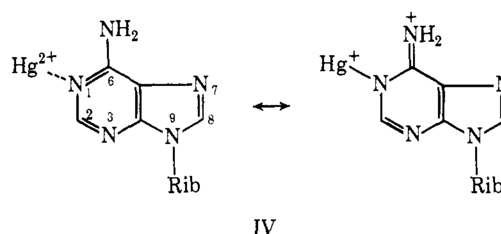
In II, we have postulated that mercury is bound to 3-N. Although several investigators[6,7] have shown that mercury is also bound to the amino group, they have carried out experiments in the aqueous medium and shown that the amino group loses a proton and becomes an imino group as a consequence of the mercuration. In the present research with DMSO as solvent, our nmr spectra show that the amino signals are still due to two protons in the amino group in the presence of mercury and that no imino group is formed. While some mercury may still be bound to the amino group, we think that the preferred site is 3-N. It is interesting to note that Eichhorn, *et al.*,[13] found that the NH_2 signal in deoxycytidine is relatively unaffected by addition of copper(II), so that 3-N, rather than the amino group, is the preferred binding site. Wang and Li[8,9] have also reported that zinc binds to 3-N of cytosine and cytidine in DMSO.

(17) R. C. Neuman, Jr., W. R. Woolfenden, and V. Jones, *J. Phys. Chem.*, 73, 3177 (1969).

(18) (a) H. R. Mahler and E. H. Cordes, "Biological Chemistry," Harper and Row, New York, N. Y., 1966; (b) L. A. LaPlanche, H. B. Thompson, and M. T. Rogers, *J. Phys. Chem.*, **69**, 1482 (1965).

For DMSO solutions containing 0.1 *M* cytidine and 0.1 *M* guanosine, Figure 2 shows that the C–NH_2 peak (for $(HgCl_2) \geq 0.4$ *M*, consider the low-field peak, ascribed to H_b in II) always lies at lower field from the peak observed for solutions of the same composition except that no guanosine is added. This is as expected, since it is known that[12] the cytidine amino signal is shifted downfield in the presence of guanosine because of strong G–C pairing. On the other hand, since pairing between adenosine and cytidine in DMSO is slight,[19] one might expect that the C–NH_2 frequency in 0.1 *M* cytidine would be independent of whether 0.1 *M* adenosine is present or not, and this is as observed.

The broadening of the A–NH_2 signal (Figure 4) indicates that $HgCl_2$ may be bound to adenosine in a manner similar to II. However, the binding with A is



IV

not as strong as with C, so that the NH_2 signal in A is not separated into two peaks.

Wang and Li[9] have reported broadening of the C–NH_2 signal in 0.1 *M* cytidine in DMSO on adding $ZnCl_2$, but did not observe separate peaks for the amino protons. In view of the present results, the broadening may be ascribed to an increase in hindrance to rotation of the amino group on addition of $ZnCl_2$. The formation constant of zinc–cytidine complex however is only 7.49 l./mol, so that even at the highest concentration of $ZnCl_2$ used, 0.5 *M*, the hindrance to rotation is not sufficiently great to cause the appearance of two peaks.

Acknowledgment. We wish to thank Professor A. A. Bothner-By for permission to use the R-20 spectrometer at Carnegie-Mellon University.

(19) R. R. Shoup, H. T. Miles, and E. D. Becker, *Biochem. Biophys. Res. Commun.*, **23**, 194 (1966).