

Nuclear Magnetic Resonance Studies of Cobalt(II) and Zinc Complexation with Nucleosides and Imidazole in Dimethyl Sulfoxide¹

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Abstract: For the purpose of deciding whether a ternary complex is formed on mixing a metal salt and (a) a nucleoside and imidazole or (b) two nucleosides, we have carried out nuclear magnetic resonance studies in which the metal salt is CoCl_2 (paramagnetic) or ZnCl_2 (diamagnetic), in dimethyl sulfoxide medium. Although dimethyl sulfoxide is a hydrogen-bond acceptor, we take as reference state the one in which the imidazole and nucleoside are hydrogen-bonded to the solvent *via* the protons attached to imidazole and nucleoside nitrogens, and look for the shifts of NH, NH_2 , and CH proton signals as increasing amounts of CoCl_2 or ZnCl_2 are added. The following ternary complexes are found: adenosine-Co-guanosine, guanosine-Zn-imidazole, cytidine-Zn-imidazole, and guanosine-Zn-adenosine.

The nucleosides and imidazole are of prime biological importance, and nuclear magnetic resonance studies of these have been reported by a number of investigators.²⁻⁸ Studies of binary and ternary metal complexes involving nucleosides and imidazole may serve to some degree as models for the nucleic acid and protein complexes. For the purpose of deciding whether a ternary complex is formed on mixing a metal salt and (a) a nucleoside and imidazole or (b) two nucleosides, we have carried out extensive nuclear magnetic resonance (nmr) studies on these systems, using CoCl_2 and ZnCl_2 as paramagnetic metal salt and diamagnetic metal salt, respectively, and adenosine (A), guanosine (G), cytidine (C), and uridine (U) as nucleosides. Although Wang and Li⁹ have used the nmr method to study the possible ternary complexes of zinc with pairs of nucleosides, their studies were confined to the use of one concentration of ZnCl_2 (0.1 M) only.

Dimethyl sulfoxide (DMSO) is a highly polar liquid and a good solvent for many aromatic compounds and inorganic salts. Since the use of water as solvent causes complications in that the bond-forming hydrogens exchange rapidly with the water protons and vertical stacking of the bases occurs in aqueous medium,³ we have selected DMSO as solvent. We take as reference state the one in which nucleoside and imidazole are hydrogen bonded to the solvent *via* the protons attached to nucleoside and imidazole nitrogens, and look for the shifts of NH, NH_2 , and CH proton signals as increasing amounts of CoCl_2 or ZnCl_2 are added.

Experimental Section

Materials. Imidazole and the four nucleosides were obtained from Sigma Chemical Co. and used without further purification.

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DMSO was purified by vacuum distillation after drying over sodium hydroxide. Anhydrous CoCl_2 and ZnCl_2 were reagent grade.

Nmr Measurements. All spectra were obtained with a Varian A-60 nmr spectrometer at $35 \pm 1^\circ$. Samples were prepared as described previously.⁷ The frequencies were measured with respect to tetramethylsilane (TMS) as an internal standard and were calibrated by the usual side-band modulation technique, using an audio oscillator and an electronic counter. The frequencies were accurate to about 0.2 cps; however, in the presence of CoCl_2 , broad peaks were obtained and the accuracy in the reported shifts is, of course, lower.

Results and Discussion

(A) Cobalt(II)-Imidazole-Nucleoside Systems. The nmr spectrum of imidazole 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-H proton while the other is assigned to the 4- and 5-H protons with the coupling constants $J_{2\text{H}-4\text{H}} = J_{2\text{H}-5\text{H}} = 1.0$ cps. Because of the rapid exchange of the 1-N proton, 4- and 5-H are magnetically equivalent.

The addition of a very small amount of CoCl_2 to 0.1 M imidazole in DMSO results in a very large downfield shift and broadening of the imidazole proton signals, as shown in Figure 1. The addition of CoCl_2 to 0.1 M adenosine in DMSO also results in a downfield shift and broadening of the adenosine proton signals. However, the extents of downfield shift are much smaller for 0.1 M adenosine than for 0.1 M imidazole, indicating that cobalt(II) complexation with imidazole is much stronger than with adenosine. This is in line with the finding by Wang and Li^{7,9} that the formation constants of the 1:4 zinc-imidazole and 1:1 zinc-adenosine complexes in DMSO are $1.4 \times 10^6 \text{ l.}^4 \text{ mol}^{-4}$ and $1.55 \text{ l.}^4 \text{ mol}^{-4}$, respectively. From Figure 1, the downfield shifts of the 8-H and 2-H signals in adenosine are about equal and there is a broadening of the amino signal. These data indicate that adenosine forms a chelate with cobalt(II) and the binding sites are 7-N and the amino group. The structure of cobalt(II)-adenosine is similar to Zn-adenosine.⁹ It is of interest to note that Eichhorn, *et al.*,⁴ consider that copper(II) binds only 7-N since Cu(II) does not broaden the NH_2 signal.

Addition of CoCl_2 (up to 0.01 M) to 0.1 M adenosine and 0.1 M imidazole in DMSO broadens the imidazole proton signals so much that they become unobservable.

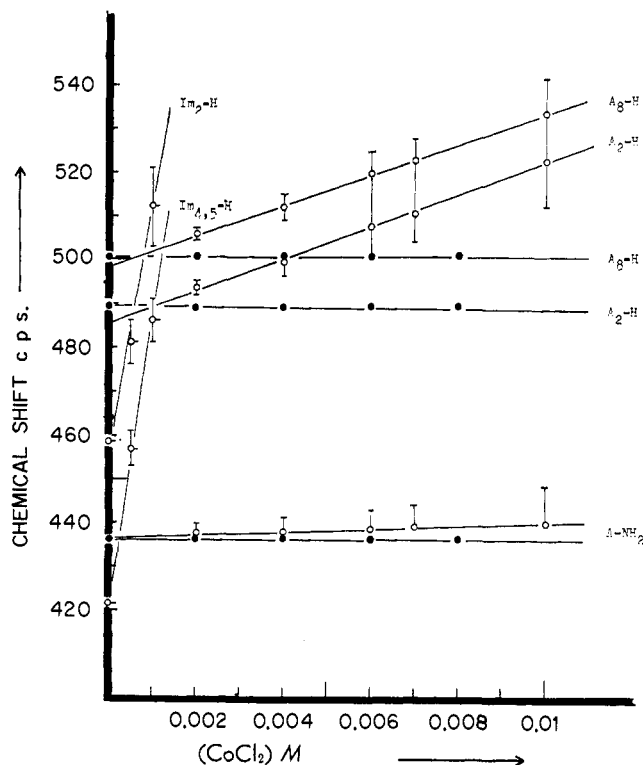


Figure 1. Nmr frequencies of adenosine and imidazole protons in DMSO: \circ , 0.1 M A; \bullet , 0.1 M A, 0.1 M Im; \circ , 0.1 M Im. The symbols and refer to the full-line width and half-line width at half-height, respectively.

The adenosine proton signals, however, are not broadened and remain in the same position as in the absence of CoCl_2 (see Figure 1). Since the total imidazole concentration is 0.1 M and the highest CoCl_2 concentration is only 0.01 M , all of the cobalt(II) is made complex with the stronger electron donor, imidazole, and there is no free cobalt(II) left to complex with adenosine. Therefore, the adenosine proton signals remain the same as in the absence of cobalt(II). Thus in 0.1 M adenosine and 0.1 M imidazole in DMSO, the addition of CoCl_2 up to 0.01 M results only in complexation of cobalt(II) with imidazole, and no cobalt(II)-adenosine or ternary cobalt(II)-adenosine-imidazole complex is formed.

Plots of the imidazole 2-H frequencies vs. concentration of CoCl_2 are also linear for total imidazole concentrations of 0.2 and 0.3 M , with slopes equal to 3.06×10^4 and 2.00×10^4 , respectively. We have ascribed part of the shift to the isotropic nuclear magnetic resonance shifts of a paramagnetic system which require that either or both $1/T_1 \gg A_i$ and $1/T_e \gg A_i$ is fulfilled, where $1/T_1$ is the rate of electron spin relaxation (T_1 is the electron spin relaxation time), $1/T_e$ is the rate of chemical exchange (T_e is the electronic exchange time), and A_i is the unpaired electron spin-nuclear spin coupling constant. When chemical exchange is rapid and the resonance absorptions of complexed and bulk protons are averaged, the contact shift, $\Delta\nu_i$, is given by the equation¹⁰

$$\Delta\nu_i = -\nu A_i \frac{\gamma_e}{\gamma_H} \frac{g\beta S(S+1)}{3kT} \frac{(\text{complexed ligand})}{(\text{total ligand})} \quad (1)$$

where $\gamma_e/\gamma_H = 6.577 \times 10^2$, $g = \mu_{eH}/\sqrt{S(S+1)}$, $\beta =$

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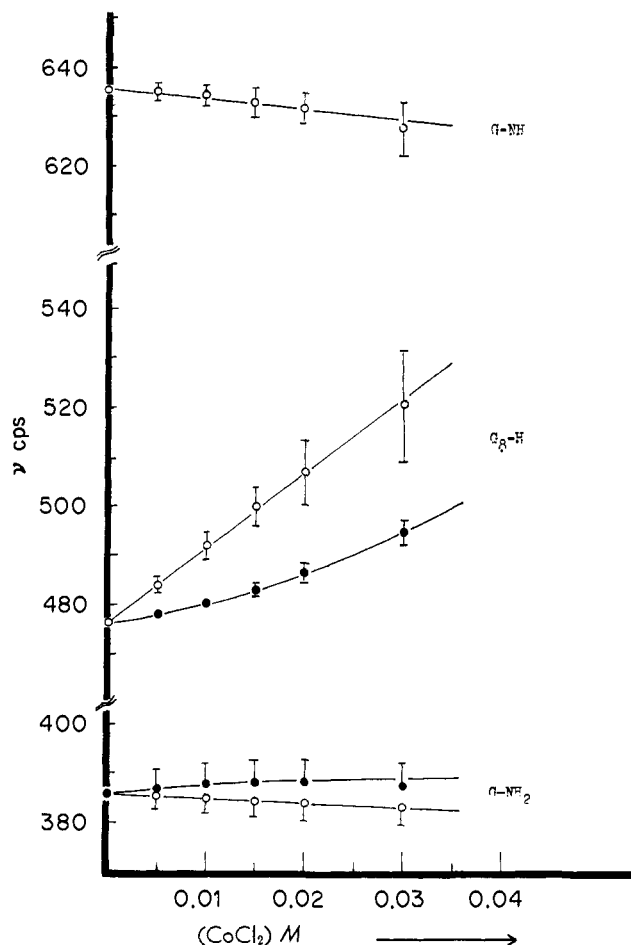


Figure 2. Nmr frequencies of guanosine protons vs. varying concentrations of CoCl_2 in DMSO: \circ , 0.1 M G; \bullet , 0.1 M G, 0.1 M Im. The symbols and (or) represent full-line width and half-line width, respectively.

9.273×10^{-21} erg G^{-1} . A positive hyperfine constant A_i results in a downfield shift of the signal relative to the pure ligand in solution and a negative hyperfine constant results in an upfield shift. Under our experimental conditions, the concentration of the complexed ligand may be taken to be proportional to the total CoCl_2 concentration at constant temperature, and eq 1 predicts that the chemical shift would vary with the concentration of CoCl_2 and inversely with the total concentration of imidazole, since the imidazole is always present in large excess. The slopes of 3.06×10^4 and 2.00×10^4 for total imidazole concentrations of 0.2 and 0.3 M , respectively, in plots of imidazole 2-H frequencies vs. (CoCl_2) , therefore, are in agreement with the prediction of eq 1.

Parts of the isotropic nuclear resonance shifts are probably pseudocontact shifts, which arise from electron spin-nuclear spin dipolar interaction and are dependent on the geometry of a complex and the magnetic anisotropy. However, the equation for the pseudocontact shift would also include the term (coordinated ligand)/(total ligand), so that at constant temperature for a given system, the shifts would also be proportional to $(\text{CoCl}_2)/(\text{total imidazole})$.

Figure 2 shows that the addition of CoCl_2 to 0.1 M guanosine in DMSO results in upfield shifts of the NH and NH_2 and downfield shift of 8-H signals. In the

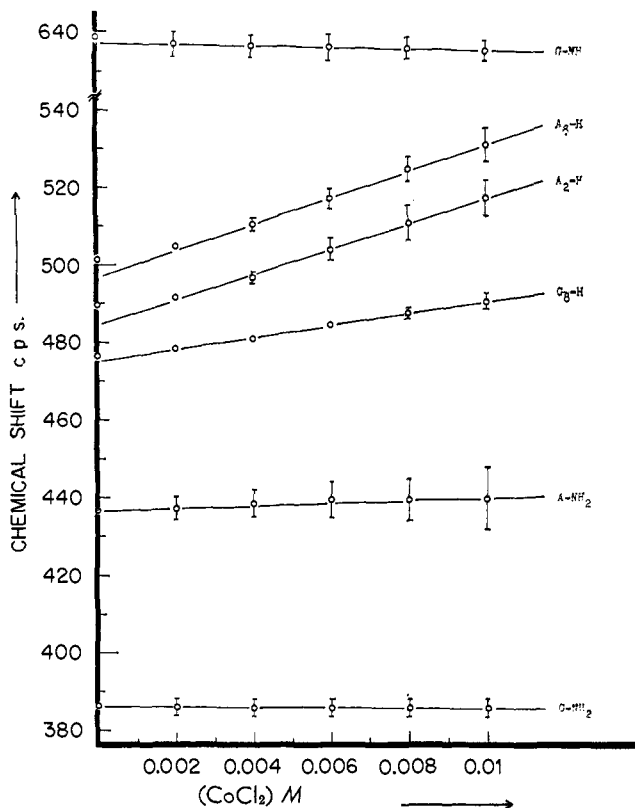


Figure 3. Nmr frequencies of guanosine and adenosine protons in DMSO solutions containing 0.1 *M* G, 0.1 *M* A, and varying concentrations of CoCl₂.

presence of 0.1 *M* imidazole, Figure 2 shows that the addition of CoCl₂ (up to 0.03 *M*) results in a very slight downfield shift of the G-NH₂, and a smaller downfield shift of the G₈-H compared to the effect obtained in the absence of imidazole. The latter is in contrast to the result shown in Figure 1, where the frequency of the A₈-H signal in the presence of 0.1 *M* imidazole is precisely the same regardless of the CoCl₂ concentration. The explanation is simple and is as follows: like nickel(II),¹¹ the highest order cobalt(II) complex of imidazole is probably CoIm₆²⁺. In the experiments shown in Figure 2, the highest CoCl₂ concentration is 0.03 *M*, while the total imidazole concentration is 0.1 *M*. This means that there is not enough imidazole present to complex all of the cobalt(II) to form CoIm₆²⁺, and hence there is an excess of CoCl₂ present which can complex with guanosine. A possible ternary G-CoIm₄²⁺ may be formed. On the other hand, Figure 1 shows that the increased width of the adenosine lines limits us to a maximum concentration of 0.01 *M* CoCl₂ (total imidazole: 0.1 *M*), so that all the cobalt is in the form CoIm₆²⁺, and there is no free cobalt(II) to complex with adenosine.

Figure 3 is similar to Figure 2, except that adenosine replaces imidazole. The addition of CoCl₂ affects both the adenosine and guanosine signals, and data of Figure 3 suggest that a ternary complex is formed in which G is bonded to Co, which in turn is bonded to A (with binding sites 7-N and NH₂).

(B) Zinc-Imidazole-Nucleoside System. Figure 4 gives frequencies of guanosine and imidazole protons

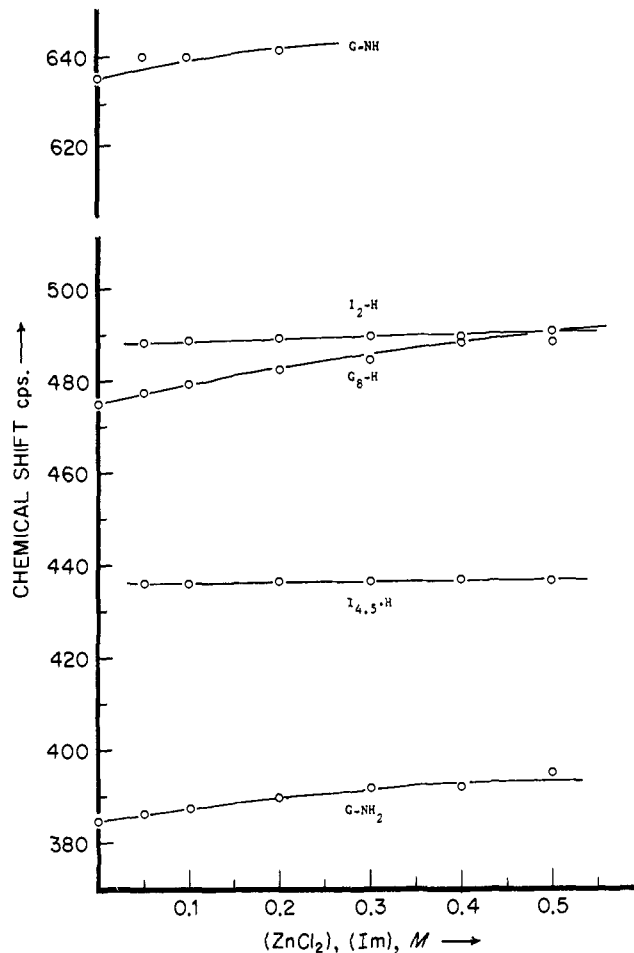


Figure 4. Nmr frequencies of guanosine and imidazole proton in DMSO solutions containing 0.1 *M* guanosine and varying concentrations of ZnCl₂ and imidazole (ratio of ZnCl₂ to imidazole concentrations = 1.0).

for solutions containing 0.1 *M* guanosine and varying concentrations of Zn(imidazole)²⁺ in DMSO. It is seen that the imidazole proton signals remain constant and the guanosine proton signals move downfield on increasing the concentration of Zn(imidazole)²⁺. The frequencies of the guanosine proton signals are the same as those in Figure 6 of Wang and Li⁹ (same as Figure 4 of this paper except that imidazole is absent). The data show that in a mixture of ZnCl₂, guanosine, and imidazole, a ternary complex is formed in which guanosine is bonded directly to Zn, which in turn is bonded to imidazole.

We have carried out similar experiments using cytidine in place of guanosine. In each case the imidazole proton signals remain constant and the nucleoside proton signals move downfield on increasing the concentration of Zn(imidazole)²⁺. Moreover the nucleoside frequency depends on the concentration of ZnCl₂, regardless of whether imidazole is present or not. In mixtures of ZnCl₂, G, and imidazole, and of ZnCl₂, cytidine, and imidazole, therefore, the following ternary complexes are present: G-Zn-Im and C-Zn-Im, respectively.

(C) Zinc-Nucleoside System. Figure 5 gives frequencies of G protons in equimolar G-A and G-C mixtures, as an increasing amount of ZnCl₂ is added. In the G-A mixture all the G signals go downfield, whereas in the G-C mixture, the G-NH and G-NH₂ signals go

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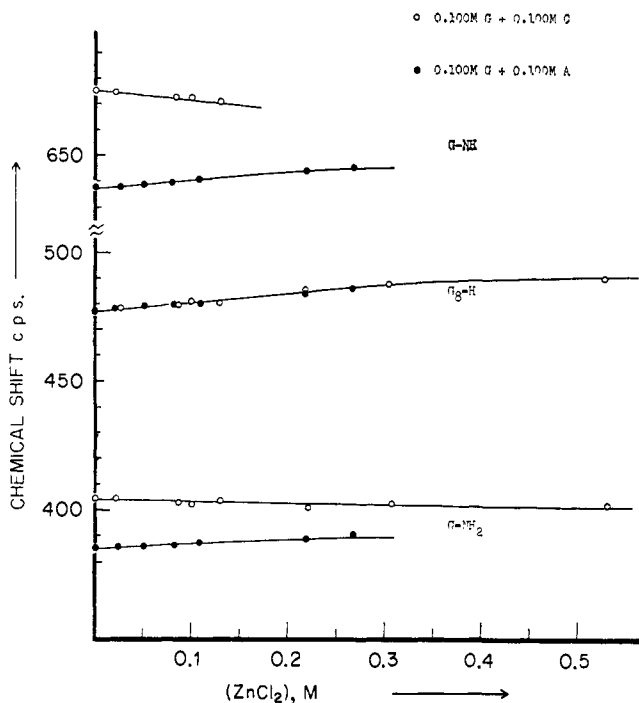
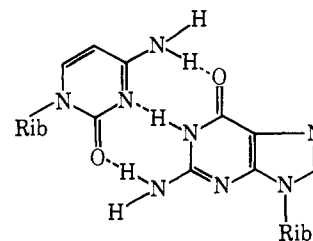


Figure 5. Nmr frequencies of guanosine in DMSO solutions containing 0.1 *M* guanosine and 0.1 *M* cytidine, O, or 0.1 *M* guanosine and 0.1 *M* adenosine, ●, and varying concentrations of ZnCl₂.

upfield and only the G₈-H signal goes downfield. Because of the G-C pairing, the G-NH and G-NH₂ protons participate in hydrogen bonding in the following manner.



On adding ZnCl₂, the metal binds at the 3-N position of cytidine,⁹ and the hydrogen bonds are therefore partially disrupted resulting in an upfield shift of the G-NH and G-NH₂ proton signals. In the case of G-A mixture, no such pairing occurs, and in a mixture of ZnCl₂, G, and A, the ternary complex G-Zn-A is formed. It will be noticed that in the G-C mixture, the rate of upfield shift of G-NH is about twice that of G-NH₂, and this is understandable because of the two NH₂ protons, only one is involved in hydrogen-bonding with C. Wang and Li⁹ have also proposed that ZnCl₂ disrupts the G-C pairing; however, their experiments were done at one ZnCl₂ concentration (0.1 *M*) only and therefore are open to question. The authors have stated: "Additional experiments would have to be carried out before a full interpretation of the effect of ZnCl₂ can be given." The results of such additional experiments are now given in Figure 5.

Wang and Li⁹ have also proposed that in an equimolar mixture of ZnCl₂, G, and U in DMSO, only binary Zn-G complex is formed. We have carried out concentrations studies of Zn, G, and U similar to the others reported and have confirmed the finding that a ternary zinc complex involving G and U is not formed.