solvents and solutions. The constants were reproducible to within ±20%. For solvents which were of low volatility, distribution between the two solvents in sealed dessicators (or for solutes of low volatility, distribution between each solvent and decalin) gave solvent activity coefficients agreeing to ±50%. Analysis by vpc of the vapor phase, above solutions of alkyl halides using a gas-circulating pump and a gas-sampling valve, attached to a Perkin-Elmer 880 gas chromatograph, followed by analysis of the liquid phase, was only suitable for very volatile solutes. Agreement between the solvent activity coefficients of the methyl halides by the three methods (Henry's law, distribution, and vpc) was to within ±30%. We used the Henry's law method to measure data in Table III, which are satisfactory for our purposes.

Elimination Reactions. Elimination or substitution products were determined by vpc analysis at 1 half-life and 5 half-lives of reaction. Details are in Table XVI. Reactions of thiophenoxide, for vpc analysis, were performed in the presence of a threefold excess of 2,6-lutidine, because thiophenol adds to alkenes in dipolar aprotic solvents. For reactions of azide ion, the fractions of elumination, as estimated by vpc and by titration of acid produced, were in good agreement. The reactions of thiophenoxide and *p*-nitrothiophenoxide were complicated by oxidation; the vpc analysis of products was considered more reliable than the titration procedures. The reaction of isopropyl bromide with sodium methoxide in methanol is accompanied by methanolysis. The small amount (5.4%) of isopropyl methyl ether, observed by vpc for reaction in methanol at 75°, could be accounted for by methanolysis, but 2.7% of this ether is produced by an SN2 reaction in 80% DMSO–methanol at 25°.

To estimate fractions of elimination in reactions of azide ion with alkyl bromides, the sample was poured into 80% acetone–water and the hydrazoic acid was estimated by titration with NaOMe–MeOH using thymol blue as indicator. Bromphenol blue was then added and the sample was titrated with HCl in 80% acetone–water to estimate the total azide ion present, *i.e.*, unconsumed by the SN2 reaction. The procedure was also used for reactions of thiophenoxide but was less satisfactory. The total (SN2 + E2) reaction was given by bromide ion produced.

Acknowledgment. We thank Mrs. Y. C. Smart and Miss S. H. Tay for technical assistance. This research was supported by a grant from the Australian Research Grants Commission.

Proton Magnetic Resonance Studies of Self-Association and Metal Complexation of Nucleosides in Dimethyl Sulfoxide[1]

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Contribution from the Chemistry Department, Duquesne University, Pittsburgh, Pennsylvania 15219. *Received February 16, 1968*

Abstract: For the purpose of deciding whether a ternary complex is formed on mixing a metal salt and two nucleosides, we have carried out proton magnetic resonance studies in which the metal salt is $ZnCl_2$ and the nucleosides are adenosine (A), guanosine (G), cytidine (C), and uridine (U), in dimethyl sulfoxide medium. Although dimethyl sulfoxide is a hydrogen-bond acceptor, we take as reference state the one in which the nucleoside is bonded to solvent and look for further shifts of NH, NH_2, and CH proton signals at different temperatures as the nucleoside concentration is increased and as an increasing amount of $ZnCl_2$ is added. Formation constants of 1:1 Zn–nucleoside complexes are obtained. Of the four nucleosides studied, the ternary complexes formed are Zn–A–G and Zn–A–C.

The nucleosides are of prime biological importance, and proton magnetic resonance studies of these have been reported by a number of investigators.[2-8] In order to decide whether a ternary complex is formed on mixing a metal salt and two nucleosides, it is desirable to obtain data on self-association of the individual nucleosides and on formation of binary metal–nucleoside complexes. This paper presents the results of proton magnetic resonance (pmr) studies on these systems, using $ZnCl_2$ as metal salt and adenosine (A), guanosine (G), cytidine (C), and uridine (U) as nucleosides.

Dimethyl sulfoxide (DMSO) is a polar liquid and a powerful 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, we have selected DMSO as solvent because it provides adequate solubility without proton transfer. Although DMSO is known to be a hydrogen-bond acceptor,[9,10] 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 at different temperatures as the nucleoside concentration is increased and as an increasing amount of $ZnCl_2$ is added.

Calculation of Formation Constants of Binary Metal Complexes. The downfield shifts of heterocyclic aromatic ring proton resonances upon protonation or metal complexation have been ascribed to extensive π-electron redistribution.[11,12] The ring proton res-

(1) This investigation was supported by Public Health Service Grant No. GM 10539-05 and by National Science Foundation Grant No. GB-4065.

(2) L. Katz and S. Penman, *J. Mol. Biol.*, **15**, 220 (1966).

(3) S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. K. Helmkamp, *J. Am. Chem. Soc.*, **86**, 4182 (1964).

(4) O. Jardetzky, *Biopolymers, Symp.*, [1] 501 (1964).

(5) J. P. Kokko, J. H. Goldstein, and L. Mandell, *J. Am. Chem. Soc.*, **83**, 2909 (1961).

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

(7) L. Gatlin and J. C. Davies, Jr., *J. Am. Chem. Soc.*, **84**, 4464 (1962).

(8) H. T. Miles, *ibid.*, **85**, 1007 (1963).

(9) T. Gramstad, *Spectrochim. Acta*, **19**, 829 (1963).

(10) S. F. Ting, S. M. Wang, and N. C. Li, *Can. J. Chem.*, **45**, 425 (1967).

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

(12) S. M. Wang and N. C. Li, *J. Am. Chem. Soc.*, **88**, 4592 (1966).

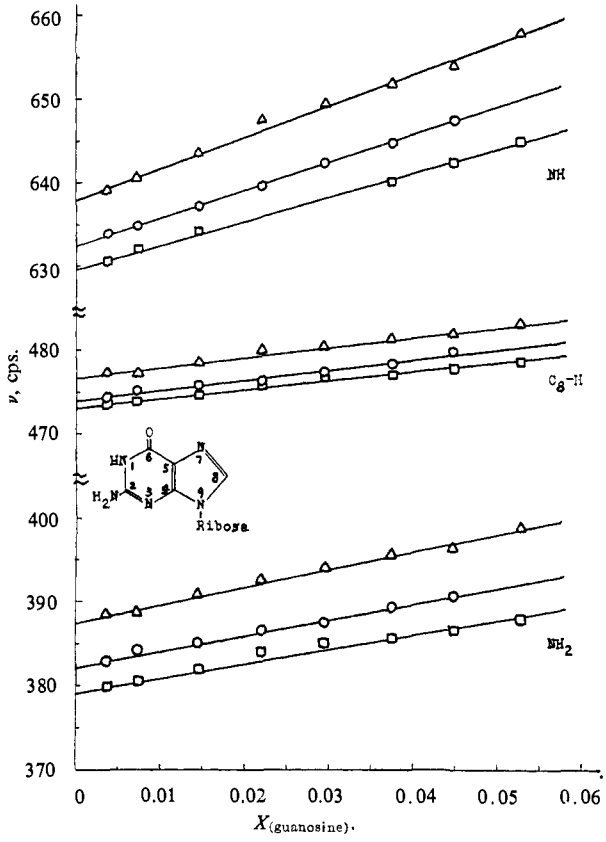


Figure 1. Pmr frequencies of guanosine protons as a function of mole fraction in dimethyl sulfoxide: △, 20.5°; ○, 37°; □, 54°.

onance shifts may therefore serve as a measure of the bond strength of the interaction between a metal and a nucleoside.

If one may neglect self-association, the observed frequency of a ring proton of a nucleoside in the presence of a metal is a weighted average of the characteristic frequencies of the free and complexed molecules (respectively ν_f and ν_c), and the observed frequency is given by the equation

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

where B_0 is the initial concentration of base, (MB) is the equilibrium concentration of the metal complex, ν_f is taken to be the frequency of 0.1 M nucleoside (mole fraction, X, of 0.007) in the absence of metal. The equilibrium constant of the reaction

$$\mathrm{M} + \mathrm{B} = \mathrm{MB} \quad (2)$$

is given by the equation

$$K = \frac{(\mathrm{MB})}{(M_0 - (\mathrm{MB}))(B_0 - (\mathrm{MB}))} \quad (3)$$

where M_0 is the initial concentration of metal ion. Combination of eq 1 and 3 leads to the equation

$$\frac{M_0}{\nu - \nu_f} = \frac{1}{\nu_c - \nu_f}(B_0 + M_0 - (\mathrm{MB})) + \frac{1}{K_c(\nu_c - \nu_f)} \quad (4)$$

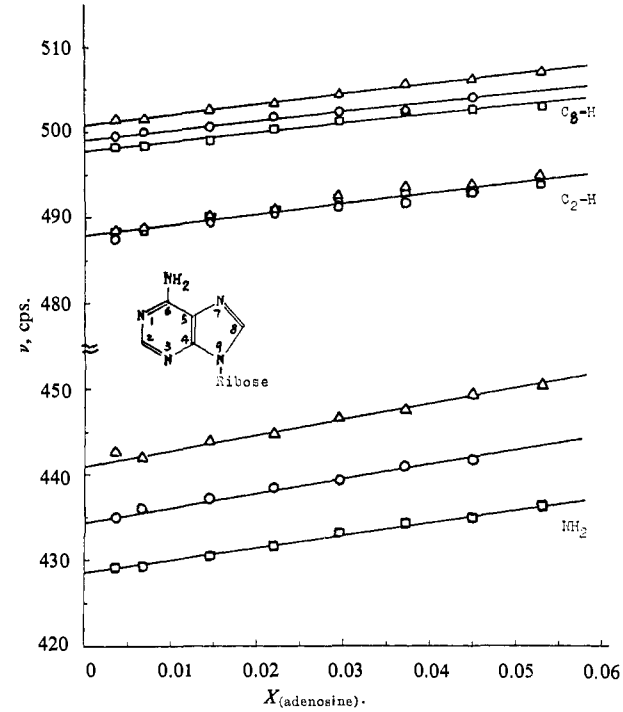


Figure 2. Pmr frequencies of adenosine protons as a function of mole fraction in dimethyl sulfoxide: △, 20.5°; ○, 36°; □, 54°.

The values of (MB) and ν_c can be calculated by iterative procedure based on successive approximations, using eq 1 and 4. The computations of K_c in eq 4 were performed using a CDC G20 computer.

Experimental Section

Materials. Adenosine, guanosine, cytidine, and uridine were obtained from Sigma Chemical Co. and used without further purification. DMSO was purified by vacuum distillation after drying over sodium hydroxide. Anhydrous $ZnCl_2$ was reagent grade.

Pmr Measurements. All spectra were obtained with a Varian A-60 nmr spectrometer. During the running of the spectra the temperature remained constant to within ±1°. Samples were prepared as described previously.[12] The frequencies were measured with respect to tetramethylsilane (TMS) as an internal standard and were calibrated by the usual side-band modulation technique to within ±0.2 cps.

Results and Discussion

(A) Self-Association of Nucleosides. The pmr spectra of adenosine, guanosine, cytidine, and uridine in DMSO and their peak assignments have been reported by several investigators.[5,7] The variation of the chemical shifts of the various protons in the purine or pyrimidine moiety of the nucleosides as a function of concentration in DMSO at various temperatures is shown in Figures 1–4. The aromatic CH protons do not manifest the pattern of upfield shifts which were shown by Ts'o, *et al.*,[3,13] to result from vertical stacking of the bases in aqueous medium, when the concentration is increased. Instead, the shifts vary with temperature and concentration in the manner expected for the formation of hydrogen-bonded complexes. Figure 4 shows that there is no apparent self-association of uridine in DMSO.

With the exception of C_2–H for adenosine in Figure 2 and C_5–H for cytidine in Figure 3, the intercepts of

(13) A. D. Brown, M. P. Schweizer, and P. O. P. Ts'o, *J. Am. Chem. Soc.*, 89, 3612 (1967).

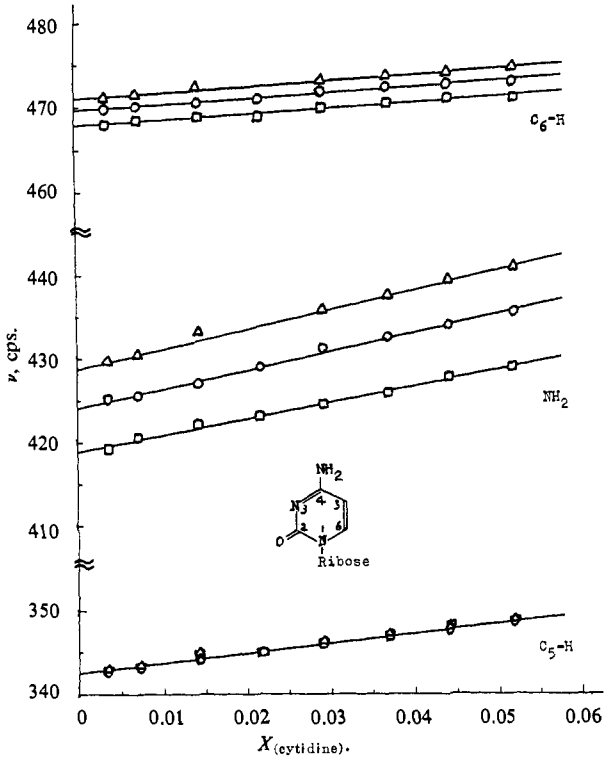


Figure 3. Pmr frequencies of cytidine protons as a function of mole fraction in dimethyl sulfoxide: △, 20.5°; ○, 35°; □, 54°.

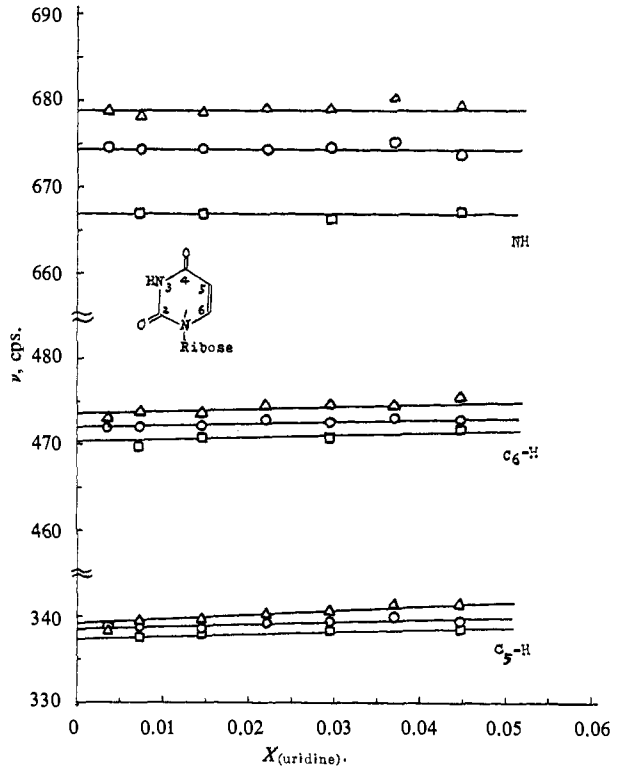


Figure 4. Pmr frequencies of uridine protons as a function of mole fraction in dimethyl sulfoxide: △, 20.5°; ○, 36°; □, 54°.

frequency *vs.* mole fraction of nucleoside decrease with increase in temperature. Part of this decrease must result from the decreased hydrogen bonding between monomeric nucleoside and the solvent DMSO at higher temperatures, since it is well known that hydrogen bonding causes a shift of the proton signal to lower field.[14]

(B) Binary Zn–Nucleoside Complexes. The effects of $ZnCl_2$ on frequencies of 0.1 *M* adenosine, guanosine, and cytidine in DMSO at 36° are shown in Figures 5–7. Formation constants of binary Zn–nucleoside complexes, K_c, were evaluated by the application of eq 4. Self-association of nucleoside at a total concentration of 0.1 *M* is considered negligible, since there was no change in K_c when B_0 for guanosine (eq 4) was varied from 0.05 to 0.15 *M*. The values of K_c for Zn complexes of nucleoside in dimethyl sulfoxide medium at 36° are 1.55 ± 0.05, 1.77 ± 0.04, and 7.49 ± 0.72 l./mole for nucleoside = A, G, and C, respectively. The proton frequencies which were used for the evaluation of K_c are those of 8H, 8H, and 6H for A, G, and C, respectively.

The large effects of $ZnCl_2$ on the frequencies of NH_2 and 8H in adenosine as shown in Figure 5 indicate that the Zn–A complex is a chelate ring involving NH_2 and 7N. The binding sites in the nucleoside are thus the same as those proposed by Harkins and Freiser[15] for adenine complex and by Frieden and Alles[16] for nucleotide complex. Since the intensity of the NH_2 resonance does not change with increasing concentration of $ZnCl_2$, the formation of the zinc chelate in DMSO does not result in the removal of a proton from the amino group. It is of interest to mention here that Harkins and Freiser[15] indicate that adenosine does not form metal complex and that Eichhorn, *et al.*,[6] conclude that 7N is the only binding site in adenosine toward copper (II).

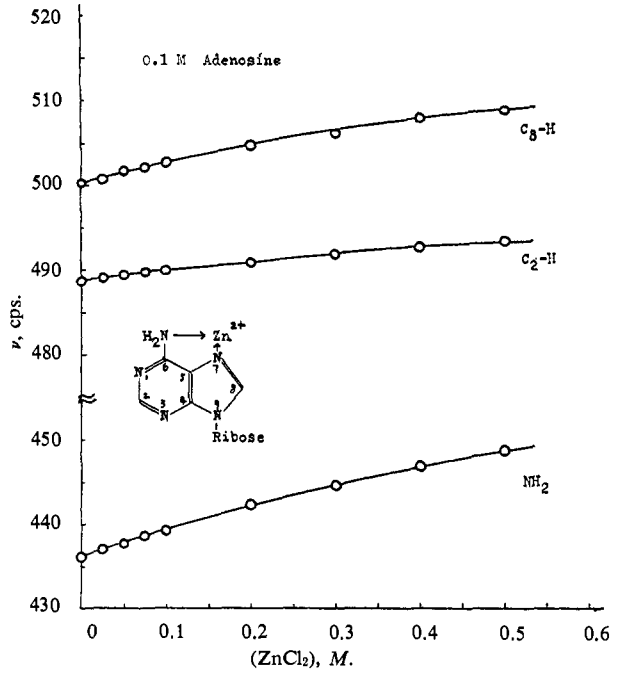


Figure 5. Pmr frequencies of adenosine protons in DMSO solutions containing 0.1 *M* adenosine and varying concentrations of $ZnCl_2$, 36°.

(14) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, p 400.

(15) T. R. Harkins and H. Frieser, *J. Am. Chem. Soc.*, **80**, 1132 (1958).

(16) E. Frieden and J. Alles, *J. Biol. Chem.*, **230**, 797 (1957).

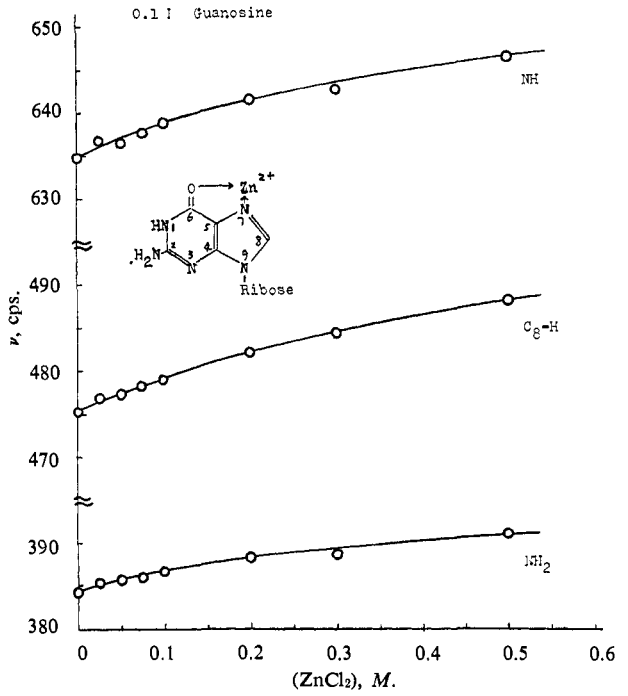


Figure 6. Pmr frequencies of guanosine protons in DMSO solutions containing 0.1 *M* guanosine and varying concentrations of $ZnCl_2$, 36°.

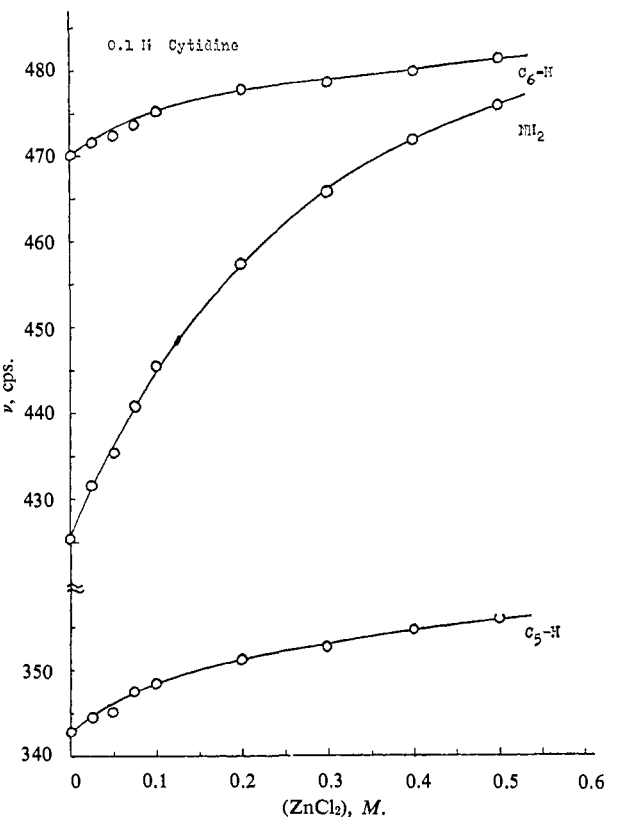
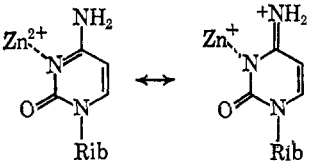


Figure 7. Pmr frequencies of cytidine protons in DMSO solutions containing 0.1 *M* cytidine and varying concentrations of $ZnCl_2$, 36°.

The large effects of $ZnCl_2$ on the frequencies of 8H and NH in guanosine as shown in Figure 6 indicate that zinc–G complex is a chelate ring involving 7N and the oxygen atom in guanosine. The NH proton signal becomes much broader on increasing $ZnCl_2$ concentration. In DMSO medium, K_c for Zn–G is 1.77. Although Albert[17] has reported that in aqueous medium for Zn–G log $K_1 = 4.6$, it must be mentioned that in his potentiometric titration studies the proton on 1N of guanosine is displaced by metal complexation, so that the ligand is the anion of guanosine. In our experiments the ligand is the uncharged guanosine molecule and the solvent is a strong hydrogen-bond acceptor as well as a powerful ligand toward zinc.

On adding $ZnCl_2$ to 0.1 *M* cytidine in DMSO (Figure 7), the 5H and 6H signals are shifted downfield to an equal extent. The extent of downfield shift of the amino protons and the broadening of the signal on adding $ZnCl_2$ are extraordinary, and the following structures may be written



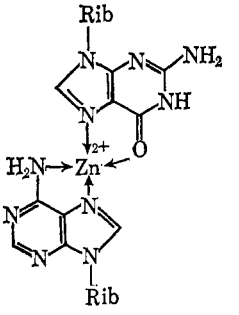
It is interesting to note that Eichhorn, *et al.*,[6] report that copper(II) binds to 3N of cytidine, and that Wang and Li[12] report that Zn binds to 3N of cytosine in DMSO. The value of K_c for Zn–C, 7.49 l./mole, is larger than for Zn–A and Zn–G, but smaller than for Zn–cytosine[12] ($K_c = 24.5$), possibly due to interference of ribose group with the approach of the metal ion.

(17) A. Albert, *Biochem. J.*, **54**, 646 (1953).

On adding $ZnCl_2$ (up to 0.5 *M*) to 0.1 *M* uridine solution in DMSO, the frequencies of 5H, 6H, and NH remain unchanged. This finding indicates that zinc does not form a complex with uridine in DMSO and is in line with the report of Eichhorn, *et al.*,[6] that copper(II) does not bind to thymidine.

(C) Possible Ternary Complexes of Zinc with Pairs of Nucleosides. Figure 8 gives pmr spectra of 0.1 *M* solutions of the nucleosides in the absence and presence of 0.1 *M* $ZnCl_2$ in DMSO. Conclusions about possible ternary complexes of $ZnCl_2$ with pairs of nucleosides are derived as follows.

A–G. On comparing Figure 8(1, 2, 5), the conclusion is reached that there is no appreciable interaction between A and G in DMSO. In an equimolar mixture of $ZnCl_2$, A, and G, both the A and G signals are in the same positions as those of A and G, each *separately* in the presence of an equivalent amount of $ZnCl_2$. The data demonstrate that in a mixture of $ZnCl_2$, A, and G in DMSO, a ternary complex is formed in which A is bonded to Zn, which in turn is bonded to G. It will be recalled that the formation constants of Zn–A and Zn–G



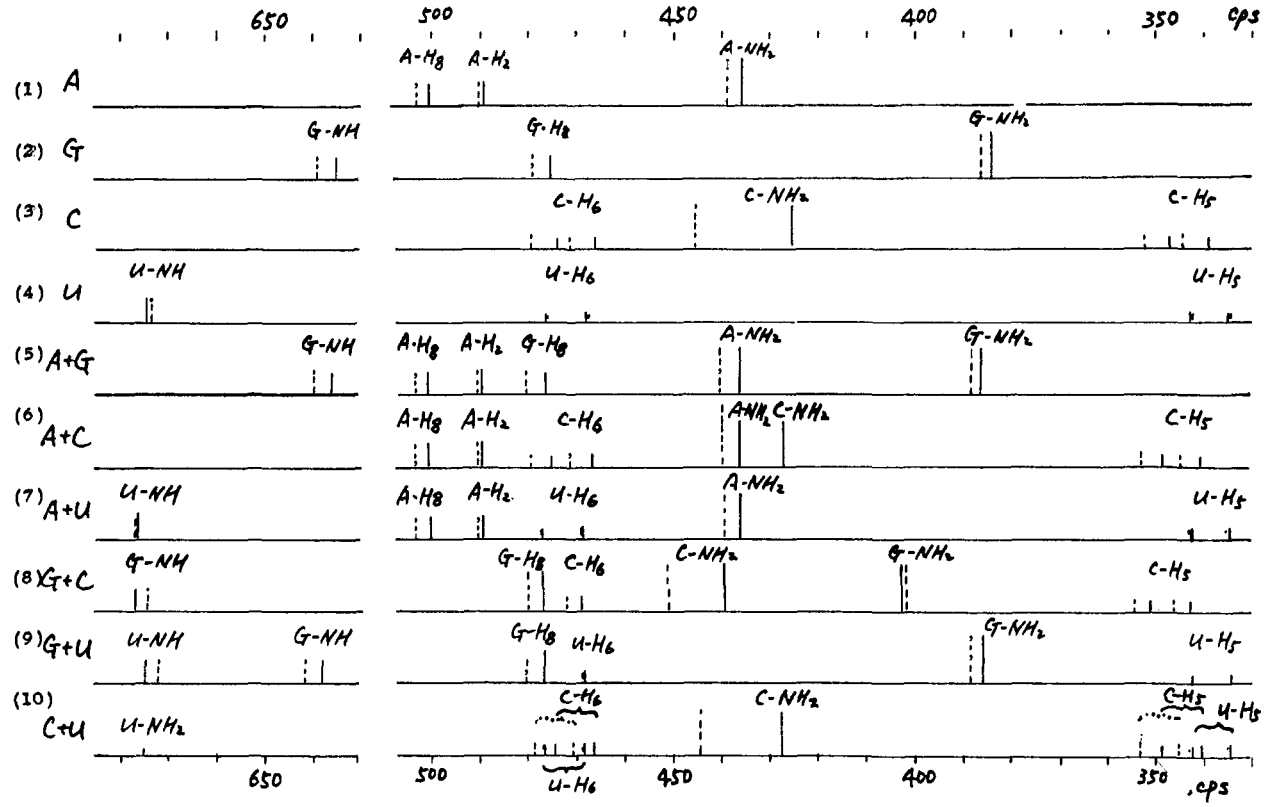
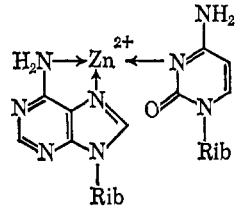


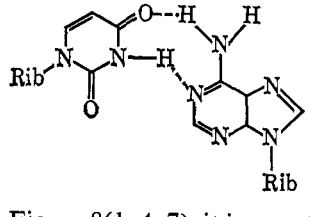
Figure 8. Pmr spectra of DMSO solutions of nucleosides in the absence and presence of $ZnCl_2$ (36°, TMS internal references): ——, nucleoside (0.1 *M*); ----, nucleoside (0.1 *M*) + $ZnCl_2$ (0.1 *M*).

complexes are about equal (K_c = 1.55 and 1.77, respectively). From what has already been said regarding the binding sites in A and G toward metal, a preferred structure for the ternary complex may be written

A–C. Interaction between A and C in DMSO is also negligible. On comparing Figure 8(1, 3, 6), the conclusion is again reached that in a mixture of $ZnCl_2$, A, and C in DMSO, a ternary complex is formed in which A is bonded to Zn, which in turn is bonded to C. A preferred structure for the ternary complex may be written

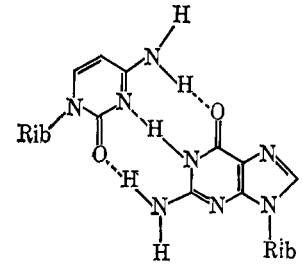


A–U. According to the Watson–Crick scheme,[18] this specific pair associates through hydrogen bonding in the following manner



On comparing Figure 8(1, 4, 7), it is seen that there is no interaction between A and U in DMSO. The same result was reported by Katz and Penman[2] who ascribe the lack of interaction to competition from DMSO. They demonstrate that in the relatively nonhydrogen-bonding solvent, chloroform, the adenine–uracil interaction is quite pronounced. In an equimolar solution of $ZnCl_2$, A, and U (Figure 8(7)), only the A signals are in the same positions as in Figure 8(1) in the presence of $ZnCl_2$. No interaction of Zn with U was observed, and hence the conclusion is reached that only the binary Zn–A complex is formed.

G–C. In an equimolar mixture of G and C, the large downfield shifts of both base signals are observed. The NH_2 and NH signals in G move downfield by 18.8 and 41.6 cps, respectively, while the NH_2 signal of C moves downfield by 14.9 cps. The result is in agreement with the finding of Katz and Penman[2] and of Shoup, *et al.*,[19] and indicates that the pairing utilizes a three-bond association.



The upfield shift of the guanosine NH and NH_2 and the downfield shift of cytidine protons in the G–C mixture on addition of $ZnCl_2$ (Figure 8(2, 3, 8)) suggest that

(18) J. D. Watson and F. H. C. Crick, *Nature*, **171**, 737 (1953).

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

the G–C complex is partially dissociated and that a binary Zn–C complex is formed. Additional experiments would have to be carried out before a full interpretation of the effect of $ZnCl_2$ can be given.

G–U and C–U. By considering Figures 8(2, 4, 9), and 8(3, 4, 10) and recalling that zinc does not interact with uridine, we propose that in equimolar mixtures Zn–G–U and Zn–C–U only binary complexes Zn–G and Zn–C, respectively, are formed.

Acknowledgment. The authors are deeply indebted to Dr. E. D. Becker for his helpful comments on the manuscript.

Electron Spin Resonance Studies of Substituted Triphenylmethyl Radicals[1a]

J. Sinclair and D. Kivelson[1b]

Contribution from the University of California, Los Angeles, Los Angeles, California 90024. *Received November 22, 1967*

Abstract: The esr spectra of some substituted triphenylmethyl radicals in dilute liquid solution have been examined and the isotropic hyperfine splittings and g values were determined. Line-width studies at different temperatures and in various solvents were made on halogen-substituted radicals, and experimental evidence was obtained which indicated that nuclear quadrupole relaxation was the dominant line-broadening mechanism. The effect of substituent groups on the unpaired spin distribution, as determined by proton and C^{13} splittings, was found to be very slight. The fluorine π-spin density does not seem to contribute much to the isotropic splitting a_F, and the data could be fitted to the equation $a_F = Q_{eff}\rho_c^{\pi}$ where $Q_{eff} = 57$ G and ρ_c^{π} is the spin density on the carbon adjacent to the fluorine. This value of Q can be understood on the basis of existing theory. Chlorine hyperfine splittings, about 0.1 of the magnitude of the corresponding proton splittings, were observed. The effect of substituents on the isotropic g value was examined and correlated with the expected dependence on spin–orbit coupling.

The electron spin resonance spectra of the triphenylmethyl (TPM) radical and some substituted triphenylmethyl radicals in liquid solution have been reported by several workers.[2–5] Isoptropic proton and C^{13} hyperfine splitting constants, determined from these spectra, have been used to estimate spin densities.

In this work several substituted triphenylmethyl radicals have been prepared and their esr spectra in dilute solutions have been obtained. Besides obtaining information on the effect of the substituent on the unpaired spin distribution, one is able to examine hyperfine splittings arising from the substituents (halogen atoms, methyl groups, etc.), to study the line widths and from these to obtain information about the relaxation mechanisms, and to study the effect of the substituents on the g values of the radicals.

Although the esr spectra of these radicals are very complicated because of the large number of magnetic nuclei interacting with the unpaired electron, these compounds are useful in a study such as this because many different substituted radicals may be easily prepared, the compounds are relatively stable and easy to handle, the lines are very narrow (~0.070 G) and, therefore, small effects in coupling constants and line widths can be observed.

Experimental Section

The radicals were prepared by the method of Gomberg.[6,7] The solvents were of reagent grade and used without further purification. The apparatus used for preparing and handling the samples is described elsewhere.[8] The concentrations of the solutions were decreased until the effects of spin–spin exchange were eliminated. The concentrations were probably in the range 3×10^{-3} to 1×10^{-4} M.

The spectra were taken with a Varian X-band spectrometer employing 100-kc modulation and a Fieldial control unit. Temperatures were regulated and measured with the Varian variable temperature apparatus. The magnetic field strengths were measured with a Harvey-Wells Model G-502 nmr probe; the radiofrequencies of the probe were measured with a Beckman Model 6146 electronic counter. The g values were determined by placing the unknown into one chamber of a dual cavity and a standard in the other. The two signals were detected separately and displayed simultaneously on a dual-channel recorder. The primary standard used in g value measurements was p-benzosemiquinone negative ion ($g = 2.00468 \pm 0.00002$).[9] Since this radical is relatively unstable, dilute solutions of diphenylpicrylhydrazyl (DPPH) in toluene were used as a secondary standard ($g = 2.00359 \pm 0.00005$).[10]

The splitting constants were determined by comparing computer simulated spectra with the observed spectra. The simulated spectra were calculated with trial values of the hyperfine splitting constants, Lorentzian line shapes, and a single mean line width ΔH. The splitting constants were determined to about 3%.

(1) (a) Supported in part by the National Science Foundation and the National Institutes of Health; (b) Alfred P. Sloan Fellow.
(2) (a) H. Jarrett and G. Sloan, *J. Chem. Phys.*, **22**, 178 (1954); (b) D. Chesnut and G. Sloan, *ibid.*, **33**, 637 (1960).
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(8) J. Sinclair, Ph.D. Thesis, University of Callfornia, Los Angeles, 1965.
(9) M. Blois, H. Brown, and J. Maling, "Free Radicals in Biological Systems," Academic Press Inc., New York, N. Y., 1961, p 117.
(10) Varian Technical Manual, V4502 EPR, pp 5–16.