Table II. ^{31}P Chemical Shift per ^{18}O as a Function of P-O Bond Type, N^a

source of ^{18}O	N	shift, ppm calcd[b]	shift, ppm obsd (av)
phosphoanhydride bridge (ADP, ATP)	1.00		0.0166[c]
phosphate anion	1.25	0.0208	0.0206
terminal-$P^{18}O_3$ anion (ADP, ATP)	1.33	0.0221	0.0220
ATP β-nonbridge ^{18}O	1.50	0.0249	0.0285

[a] $N = \sum(1a + 2b)/(a + b)$ where a = number of single bonds and b = number of double bonds. [b] Calculated from the value observed for $N = 1$. [c] Average of ^{31}P shifts due to the α-β bridge ^{18}O on α-^{31}P and β-^{31}P and to the β-γ bridge ^{18}O on β-^{31}P.

surprising, however, that even at 235 MHz where the β-γ bridge ^{18}O and nonbridge ^{18}O species on β-P of ATPβ,γ-$^{18}O_6$ are well resolved (see Figure 3), the spectrum of the γ-P($^{18}O_4$) gave no indication of any nonequivalence of the shifts due to the nonbridge ^{18}O as compared to the β-γ bridge ^{18}O. A simple five-line pattern corresponding to $^{18}O_0$, $^{18}O_1$, $^{18}O_2$, $^{18}O_3$, and $^{18}O_4$ was observed for each of the two regions of the γ-P doublet of the 63% ^{18}O sample at 235 MHz. The equivalence within experimental error of the chemical shift of the bridge and nonbridge oxygens on the γ-phosphate of ATP and the nonequivalence for the β-phosphate of ATP may be related to the axial symmetry in the chemical-shift tensors of the dianionic forms of mononucleotides and the axial asymmetry for the anionic form of diesters.[14] Since the chemical-shift tensor of the acid form of mononucleotides is no longer axially symmetric, it would be of interest to examine ATP $^{18}O_4$ in the acid form.

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Nucleoside Complexing. A Raman and ^{13}C NMR Spectroscopic Study of the Binding of Hard and Soft Metal Species

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Abstract: The influence of a wide variety of metal salts and complexes on the Raman and ^{13}C NMR spectra of three of the four common nucleosides (uridine, adenosine, and cytidine) in Me_2SO has been determined. Several related molecules also studied included deoxycytidine, 5-methyldeoxycytidine, 8-bromoadenosine, 6-dimethylaminopurine-9-riboside, and N^4,N^4-dimethylamino-1-methylcytosine. Inorganic species utilized included *cis*-$[Pt(Me_2SO)_2Cl_2]$, $HgCl_2$, $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Pb(NO_3)_2$, $Pb(ClO_4)_2$, $PbCl_2$, $Mg(NO_3)_2$, $Ca(NO_3)_2$, $Sr(NO_3)_2$, $Ba(NO_3)_2$, $BaCl_2$, $La(NO_3)_3$, $Pr(NO_3)_3$, $Lu(NO_3)_3$, $Lu(ClO_4)_3$, $Ga(NO_3)_3$, $LiClO_4$, NEt_4Cl, and NEt_4NO_3. The ^{13}C NMR shift dependencies on metal concentration were used to calculate formation constants for selected purine and pyrimidine derivatives. Constants obtained with $HgCl_2$ agreed well with literature values. However, our studies suggest that alkaline earth metal ions have a lower affinity for nucleosides than that suggested previously. Of the common nucleosides, cytidine has the greatest affinity for alkaline earth metal ions and these appear to bind to O-2. Raman and ^{13}C NMR studies with N^4,N^4-dimethylamino-1-methylcytosine are presented which support the O-2 binding mode. The only electrophile capable of interacting with N-3 of this pyrimidine is the H^+ ion. Conclusions drawn about binding sites to other nucleosides are in agreement with previous studies. A summary table specifying the importance of anion and cation binding to the four common nucleosides is included. This summary provides a rationale for all of the literature observations and should help to minimize the confusion currently surrounding the nature of the interactions of inorganic species with nucleosides in Me_2SO.

Introduction

Growing recognition of the importance of metal ion interactions with nucleic acids and nucleotides has stimulated an explosive growth in the number of studies devoted to understanding the chemistry of the complexes formed. This interest has led to at least seven recent reviews of this topic.[1-7]

The great promise of platinum(II) metalloantineoplastic

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agents[8,9] and the relatively high stability of complexes formed by Hg(II) and Cu(II) species[1,10] has resulted in considerable knowledge about the binding characteristics of these three metal systems. Comparative information on the binding of other metal species, in addition to having value in its own right, could provide further insight, say, into the reason for the effectiveness of certain platinum(II) antitumor agents, which are generally believed to function by binding to nucleic acids in the tumor cells.[9] Comparative information on binding may be of value in understanding the relationship between mutagenicity and carcinogenicity of some but not other metal species.[11] The origin of such effects is believed to be associated with binding of the metals to the nucleic acid template.

Another area of interest and one in which there is still considerable confusion involves the binding of biologically relevant alkali and alkaline earth metal ions to nucleic acid species. It is still unclear whether or not magnesium in MgATP is bound to the heterocyclic purine base.[1] If such an interaction exists, it must be relatively weak. It is difficult to observe metal binding to the heterocyclic base in nucleotide complexes, since phosphate-to-Mg bonding dominates the interaction. Complexes between alkaline earth metal species and nucleosides, which lack any phosphate groups, are expected to be weak, but the probability of observing an interaction with the heterocyclic base is increased.

In Me_2SO, the prospects of obtaining metal ion–nucleoside binding are enhanced because high concentrations of metal salt and nucleoside can be achieved. An additional advantage of this solvent is that amino and hydroxyl proton ^1H NMR resonances can be observed. There are several reports in the literature that salts of alkali and alkaline earth ions alter the ^1H and ^{13}C NMR spectra of nucleosides.[12-21] We demonstrated that the characteristic downfield shifts of ^1H NMR signals which accompany the addition of halide salts to the solutions of guanosine in Me_2SO can be wholly or partly the consequence of hydrogen-bonding interactions between the halide ion and the nucleoside.[19] This effect, which has now been generally accepted,[13,18] is an indirect consequence of the weak solvation of the anions by Me_2SO. We also demonstrated that a nucleoside can interact simultaneously with a metal center and an anion in this solvent.[19] In the case of guanosine, we suggested that alkaline earth cations do not bind to the base. (Our conclusions were later incorrectly generalized to encompass all metal ions and all nucleosides.[13]) The anion and cation can also form a complex which itself may interact with the nucleoside.[18] Finally, the ribose sugar can interact with both the cation and anion.[14,18]

Although NMR has been the only method of studying these interactions in Me_2SO, several nuclei have been examined (^1H, ^{13}C, ^{35}Cl, ^7Li), and both shift and relaxation measurements have been made.[12-21] These studies have also encompassed a variety of both paramagnetic and diamagnetic metal centers. Recently, we began to examine such interactions with Raman spectroscopy and Raman difference spectroscopy as well as with lanthanide shift reagents.[20] Our investigations have been aimed at clarifying the literature which contains confusing conclusions based on observations on the effects of salts of hydrogen-bonding anions. We have used instead, where possible, nitrate salts.

The application of Raman spectroscopy and Raman difference spectroscopy for the study of metal ion–nucleoside interactions in aqueous systems has been mainly developed by Tobias and his co-workers.[23] Raman spectroscopy has the advantage that environmental effects are relatively unimportant. A distinct change in the Raman spectrum of a nucleoside on addition of a metal salt is a clear indication of complex formation. In contrast, the addition of salts to solutions of ligands can indirectly cause changes in NMR parameters by alteration of the solvent properties, such as viscosity.[18] The molarity of Me_2SO is in the range of ~6 M and conceivably a 1 M concentration of salt (a concentration not infrequently employed in the literature) could coordinate all the solvent molecules.

In addition to examining the question of whether or not complexes are formed at all, we are also concerned with defining metal binding sites and gaining some insight into the relative stability of the complexes which are formed. As will be seen, methods which have been used for obtaining formation constants from NMR measurements[12,13,15,16,24,25] have frequently been incorrectly applied.

This paper describes our work on three of the common nucleosides (cytidine, adenosine, and uridine) and some relevant purine and pyrimidine derivatives. We will contrast the results, where possible, with work in progress on guanosine.

Experimental Section

Materials. The following were from Fisher: $La(NO_3)_3 \cdot 6H_2O$, $HgCl_2$, $Pb(NO_3)_2$, $Ba(NO_3)_2$, $Cd(NO_3)_2 \cdot 4H_2O$, and $Cr(NO_3)_3 \cdot 9H_2O$. The following were J. T. Baker chemicals: $PbCl_2$, $AgNO_3$, and $Mg(NO_3)_2 \cdot 6H_2O$. Alfa supplied $Pb(ClO_4)_2 \cdot 3H_2O$, $NaOCH_3$, $Lu(ClO_4)_3 \cdot 6H_2O$, and $Pr(NO_3)_3 \cdot 5H_2O$. Nucleosides and 2,4-dichloropyrimidine were from Sigma. The following were from Merck: $Zn(NO_3)_2 \cdot 3H_2O$, $Sr(NO_3)_2$, and $Ba(NO_3)_2$. The $Ga(NO_3)_3 \cdot 9H_2O$ was from K & K, the $LiClO_4$ from G. Frederick Smith, and the Me_2SO-d_6 (99.5 atom % D) from Aldrich. The $Lu(NO_3)_3 \cdot 5H_2O$ was from ROC/RIC and the tetraethylammonium salts were from Eastman.

NMR Studies. The ^{13}C NMR spectra were obtained on a Varian CFT-20 spectrometer operating at 20 MHz (1.8682 T) in the Fourier transform mode at a temperature of 32 °C and using an 8-mm probe. Me_2SO-d_6 containing Me_4Si as an internal standard was used as the solvent and provided the internal deuterium lock. Typically a total of 2000–3000 transients were acquired in 8K of memory using a pulse width of 10 μs (corresponding to a flip angle of 50–60°), a pulse delay of 2 s, and broad band proton decoupling. The 4000-Hz spectral width used results in an acquisition time of 1.023 s and in a digital resolution no better than 0.97 Hz, ~0.05 ppm. The chemical shifts reported are reproducible to ±0.04 ppm.[26] For high concentrations of lanthanide ions, as many as 10 000 transients were needed to achieve good signal to noise. The ^1H NMR spectra were recorded in the CW mode using either a JEOL MH 100 (32 °C) or a Varian HA 100 (28 °C) instrument. Solutions and references were as above.

Raman Studies. All Raman spectra were obtained on a Spex Industries Ramalog 5 spectrophotometer (Model 1401/14018 double grating monochromator, 1800 gratings/mm) containing an RCA C31034A photomultiplier for photon counting. The laser system consisted of a Spectra-Physics Model 165-8 argon ion laser, and the 5145-Å excitation was employed here. The spectrometer was externally driven by means of a programmed LAB-1180 system which contains a Nicolet 1180 data processor with 16K of memory, an ASR-33 Teletype, and a Dexter/2 monitor used for storage of required spectra. A Pace Electronics Variplotter Model 1100-D was used to obtain a hard copy of the display.

The samples (0.2 M) were prepared by dissolving in Me_2SO-d_6 the desired quantities of nucleic acid derivatives, metal nitrates, and anhydrous sodium perchlorate. The latter (0.1 M) has a strong band at 933 cm^{-1} which was used as an internal intensity standard and reference. Some of the solution (~50 μL) was introduced into capillary cells (1.5–2.0 × 90 mm) which were sealed and mounted horizontally in the cell compartment.

The intensity of the ~595-cm^{-1} peak of the Me_2SO-d_6 was maximized for each sample. All spectra were recorded using 400-mW laser power with a time constant of 0.1 s. The slit width was set at 244 μm, giving a resolution of ~2.25 cm^{-1}. The Raman spectra were recorded with a scan speed of 2 cm^{-1}/s for typical scans of 800 cm^{-1}. Thus, the eight scans for each sample required 65–70 min.

The displayed area of each spectrum was allocated 2048 storage points and was subjected to a nine-point least-squares smooth. The difference spectra were then obtained by performing the following series of subtractions:

$$[(\text{base} + \text{metal}) - (\text{base})] - [(\text{metal}) - (\text{solvent})]$$

Equilibrium Calculations. The analysis of metal ion–ligand equilibrium is greatly simplified by the assumption that a 1:1 complex ML is formed to the exclusion of all other complexes. Then the equilibrium constant K can be expressed as

$$K = \frac{ML}{(M_0 - ML)(L_0 - ML)}$$

where M_0 and L_0 are the initial concentrations of metal ion and ligand, respectively, and ML is the concentration of the complex.

The observation of only single resonances in the NMR spectra indicates rapid exchange between free and complexed ligand, and the chemical shift for the ligand, δ, is thus given[24] by

$$\delta = \frac{L}{L + ML}\delta_L + \frac{ML}{L + ML}\delta_{ML} \quad (1)$$

where δ_L and δ_{ML} are the chemical shifts of free and complexed ligand, respectively, and L is the concentration of free ligand. Defining the incremental chemical shift, $\Delta\delta$, as the difference between the chemical shift of free ligand and the observed shift, and the incremental limiting shift for total complex, Δ_{max}, as the difference between the shifts of free and complexed ligand, we can rewrite (1) as

$$\Delta\delta = \frac{ML}{L_0}\Delta_{max} \quad (2)$$

The equilibrium constant can now be given by

$$K = \frac{\Delta\delta}{(\Delta_{max} - \Delta\delta)\left(M_0 - \frac{\Delta\delta}{\Delta_{max}}L_0\right)} \quad (3)$$

and solving for $\Delta\delta$ we obtain

$$\Delta\delta = \frac{(M_0 + L_0 + K^{-1}) - \sqrt{(M_0 + L_0 + K^{-1})^2 - 4L_0M_0}}{2L_0}\Delta_{max} \quad (4)$$

This equation can be solved iteratively for the values of K and Δ_{max} that give the best agreement between the observed and calculated shifts, in the sense that they minimize the error square sum[12]

$$U(K, \Delta_{max}) = \sum_i (\Delta\delta^i_{obs} - \Delta\delta^i_{cal}) \quad (5)$$

The assumed stoichiometry was further verified[27] using a Scatchard equation of the form

$$\frac{\bar{\nu}}{L} = \frac{n}{K} - \frac{\bar{\nu}}{K} \quad (6)$$

where $\bar{\nu} = ML/M_0$ and n = number of ligands bound. In the 1:1 case, $\bar{\nu} = (L_0/M_0)\Delta\delta/\Delta_{max}$ and $\bar{\nu}/L_0 = \Delta\delta/(\Delta_{max} - \Delta\delta)M_0$ and a plot of $\bar{\nu}/L$ vs. $\bar{\nu}$ gave a straight line of y intercept approximately equal to the reciprocal of the slope, both of which are equal to K. These calculations were performed with locally written programs on a Dec-10 computer.

Preparations. 2,4-Dimethoxypyrimidine. The preparation of 2,4-dimethoxypyrimidine was carried out, with slight modifications, using the procedure of Hilbert and Johnson.[28] A solution of 30 g of 2,4-dichloropyrimidine dissolved in 150 mL of absolute CH_3OH was cautiously added to a solution of 25 g of sodium methoxide dissolved in 150 mL of absolute CH_3OH. The vigorous reaction was sufficient to raise the temperature of the solution to its boiling point. The reaction was completed after 10–15 min and the product was obtained in 80% yield as described.[28] The ^1H NMR spectrum of the product is as follows (ppm; intensities in parentheses): OCH_3, 3.96 (6); H-5, 6.55, J = 7 Hz (1); H-6, 8.35 (1).

1-Methyl-4-methoxy-2-oxypyrimidine. This product was prepared as described[28] in essentially quantitative yield, mp 150 °C. ^1H NMR: N(1)CH_3, 3.28 (3); OCH_3, 3.74 (3); H-5, 5.85, J = 7 Hz (1); H-6, 7.85 (1).

N^4,N^4-Dimethyl-1-methylcytosine. 1-Methyl-4-methoxy-2-oxypyrimidine (~2 g) was dissolved in 100 mL of a methanolic solution saturated with anhydrous dimethylamine in a bottle. The bottle was sealed tightly and set aside for 4.5 days at ~32 °C. The excess of solvent was then evaporated under diminished pressure, leaving behind a white solid. The crude product was dissolved in CH_3OH and the solution taken to dryness on a rotary evaporator. The process was repeated several times to remove all the unchanged dimethylamine. The product was collected in quantitative yield and air dried. ^1H NMR: N(4)$(CH_3)_2$, 3.04 (6); N(1)CH_3, 3.28 (3); H-5, 5.85, J = 7 Hz (1); H-6, 7.57 (1). Anal. Calcd for $C_7H_{11}N_3O$: C, 54.90; H, 7.19. Found: C, 55.27; H, 7.10.

N^4,N^4-Dimethyl-1-methylcytosinium Nitrate Salt. To an aqueous solution of 1 N HNO_3 (20 mL) was added 0.2 g of N^4,N^4-dimethyl-1-methylcytosine. The mixture was allowed to evaporate to dryness, leaving the white, crystalline N^4,N^4-dimethyl-1-methylcytosinium nitrate salt. The product was not recrystallized. Anal. Calcd for $C_7H_{12}N_4O_4$: C, 38.89; H, 5.55. Found: C, 38.76; H, 5.81.

N^4,N^4-Dimethyl-1-methylcytosinium Hydrochloride Salt. N^4,N^4-Dimethyl-1-methylcytosine (0.2 g) was added to an aqueous solution (20 mL) of 3 N HCl. The mixture was allowed to evaporate to dryness, leaving the crystalline N^4,N^4-dimethyl-1-methylcytosinium hydrochloride salt. The product was not recrystallized. Anal. Calcd for $C_7H_{12}N_3OCl$: C, 44.57; H, 6.37. Found: C, 44.91; H, 6.55.

***cis*-[Dichlorobis(dimethyl sulfoxide)platinum(II)].** This derivative was prepared as described in the literature[29] and was kindly provided by Mr. K. Wilkowski.

Results

The interactions of nucleosides with metal salts in Me_2SO have varying degrees of complexity. The Results section will be divided into four subsections. The first three subsections deal with each of the three nucleosides (Figure 1) and include in some cases derivatives of each nucleoside. The three sections will be ordered in terms of increasing complexity of binding, ending with cytidine, which has the most versatile binding modes. This section is followed by a fourth section of N^4,N^4-dimethyl-1-methylcytosine, a molecule which has proved to be useful in clarifying the conclusions we have drawn concerning the site of metal binding to the cytosine base of cytidine.

The metal species we have studied can be divided into three classes. First, and most controversial, are hard metal species such as the alkaline earth ions. We find (see subsection on cytidine) that all the alkaline earth ions have similar effects but that Ba^{2+} salts have the greatest effects. Therefore, we have generally employed $Ba(NO_3)_2$ in our studies. We have also found (see subsection on cytidine) that the effects of $Ba(NO_3)_2$ and $La(NO_3)_3$ on the ^{13}C NMR spectrum of cytidine are very similar and that the lanthanides are reasonable probes of the effect of alkaline earth ions in our system. Second, we have examined the effects of $HgCl_2$. There is general agreement[12,13,20,24,30] that $HgCl_2$ binds strongly to endocyclic ring nitrogens of nucleosides in Me_2SO solution. Our studies with this metal species were mainly aimed at establishing the degree of perturbation of ^{13}C NMR and Raman spectra expected from metal binding in Me_2SO. Finally, we examined $Zn(NO_3)_2$ and $Pb(NO_3)_2$. The Zn^{2+} ion has a known affinity for endocyclic nitrogen[1,2] but otherwise is "harder" than $HgCl_2$ and thus serves as a bridge to facilitate comparison between the hard and soft metal species we have studied.

Uridine. In Me_2SO, uridine has the simplest behavior of all the nucleosides examined in this study. The ribose portion of this molecule, as in all ribonucleosides, interacts with LiCl and it has been suggested that both Li^+ and Cl^- interact with the ribose hydroxyl group simultaneously.[18] Anion effects will be considered in the Discussion section.

Our ^{13}C NMR studies with uridine (see supplementary tables) clearly show that the hard metal species such as $Pb(NO_3)_2$ and $Ba(NO_3)_2$ do not cause appreciable shifts, with a maximum shift of 0.17 ppm in C-6 when $Ba(NO_3)_2$ (0.5 M) is added to uridine (0.2 M). In agreement with this finding, $La(NO_3)_3$ (0.5 M) causes ^{13}C NMR shifts of at most 0.2 ppm when added to uridine (0.2 M). These shifts are very likely only environmental effects since the identical experiment with the paramagnetic species $Pr(NO_3)_3$ (0.5 M) leads to a maximum shift of only ~0.25 ppm.

Similarly, Lippard and his co-workers[30] found that $HgCl_2$ (2.0 M) causes maximum shifts of ~0.3 ppm in uridine (0.5

Table I. Comparative Incremental Shifts of the ^{13}C NMR Resonances of Cytidine (0.2 M) on Addition of Various Salts[a]

salt	M	C-4	C-2	C-6	C-5	C-1′	C-4′	C-2′	C-3′	C-5′
HNO_3	0.2	6.49	8.02	−3.01	0.06	−0.25	−0.67	−0.15	0.56	0.69
$Ba(NO_3)_2$	0.7	0.56	−1.09	−0.10	−0.76	0.36	−0.24	−0.09	−0.08	0.02
$La(NO_3)_3$	0.5	0.74	−0.87	−0.07	−0.97	0.43	−0.33	0.03	−0.16	−0.09
$Lu(NO_3)_3$	0.3	1.05	0.71	−0.44	−0.55	0.19	−0.24	−0.02	0.00	0.05
$Lu(ClO_4)_3$	0.3	6.50	8.06	−2.91	0.04	−0.21	−0.57	−0.10	0.62	0.68
$Pr(NO_3)_3$	0.5	2.09	10.07	0.69	−1.43	1.10	−0.13	0.37	0.06	0.01
$Pb(NO_3)_2$	0.7	1.58	−1.16	−0.27	−1.80	0.43	−0.48	−0.04	−0.05	0.06
$Zn(NO_3)_2$	0.7	1.36	0.24	−0.74	−1.39	0.14	−0.59	0.03	−0.12	−0.03
$Cd(NO_3)_2$	0.7	1.32	−0.22	−0.85	−2.03	−0.25	−0.73	0.01	−0.13	0.03
$HgCl_2$	0.7	2.49	2.88	−0.96	−1.77	−0.72	−0.59	−0.01	0.31	0.42
$AgNO_3$	0.7	0.63	0.99	−0.69	−0.77	−0.35	−0.46	−0.08	0.00	0.24
$Pt(DMSO)_2Cl_2$	0.2	2.35	3.47	0.09	0.03	−0.77	−0.26	0.01	0.51	0.50

[a] In Me_2SO-d_6; negative shifts downfield.

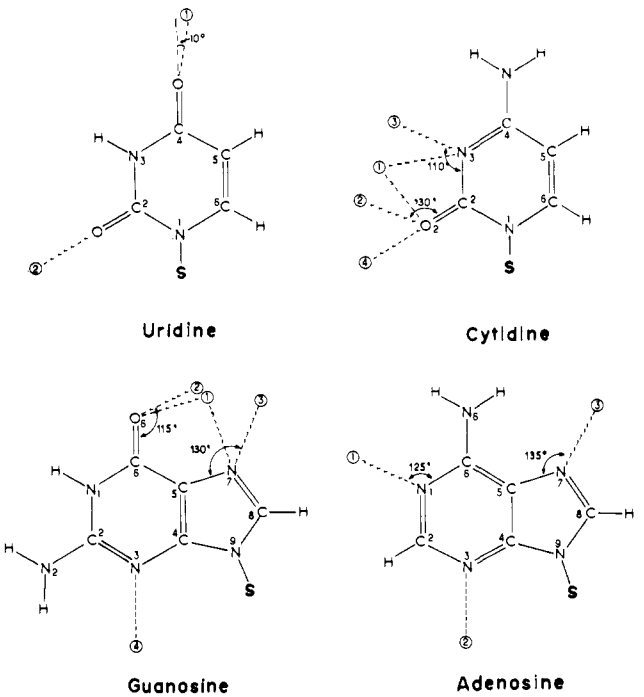


Figure 1. The four common nucleosides when S = ribose. Circled numbers indicate binding sites along the directions of highest electron density (adapted from ref 38).

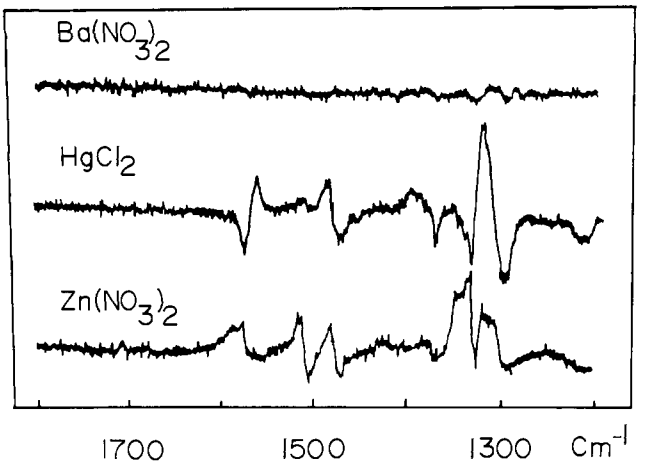


Figure 2. Raman difference spectra for adenosine (0.7 M) in Me_2SO-d_6 in the presence of the inorganic species (0.7 M) indicated.

M) and we have confirmed this finding. Also, $Zn(NO_3)_2$ (0.7 M) causes shifts of at most ~0.2 ppm. Complete ^{13}C NMR data will be found in the supplementary material.

In support of the conclusions drawn from the ^{13}C NMR studies, there are no detectable perturbations in the Raman spectrum of uridine (0.2 M) in the 900-1700-cm^{-1} region on addition of 0.7 M $HgCl_2$, $Zn(NO_3)_2$, or $Ba(NO_3)_2$. The Raman difference spectra obtained are essentially featureless except in spectral regions where strong solvent bands are present. The Raman bands in the 900-1800-cm^{-1} region for all the nucleosides do not arise from the ribose group but are attributable to the heterocyclic base.[31] Specific assignments of these bands to specific vibrational modes in the bases have been only tentatively made.[31]

Adenosine. This nucleoside is similar to uridine in not interacting with hard metal ions. For a 0.2 M solution of nucleoside, $Ba(NO_3)_2$ (0.7 M) causes a ^{13}C NMR shift of only 0.15 ppm (for C-8). Likewise, $La(NO_3)_3$ (0.5 M) causes a maximal shift of only 0.17 ppm (for C-1′) and $Pr(NO_3)_3$ (0.5 M) has its greatest influence on the resonances for C-8 and C-4′, which shift only 0.2 ppm.

The metal salts $HgCl_2$ and $Zn(NO_3)_2$ cause appreciable shifts in the ^{13}C NMR spectrum of this nucleoside. For a 0.2 M adenosine solution, $HgCl_2$ (0.7 M) causes a 1.20-ppm shift in the C-6 resonance (in good agreement with a previous study[30]), and $Zn(NO_3)_2$ (0.7 M) causes a 1.27-ppm shift in the C-5 resonance and a 1.03-ppm shift in the C-8 resonance. These changes in the ^{13}C NMR spectra are accompanied by large changes in the Raman spectrum in the 1300–1600-cm^{-1} region (Figure 2). The difference spectra for the $HgCl_2$ and the $Zn(NO_3)_2$ solutions are given here primarily for comparison with the difference spectrum for the $Ba(NO_3)_2$ solution. There is no controversy about the binding of $Zn(NO_3)_2$ and $HgCl_2$ to both N-1 and N-7. The Raman difference spectra are reminiscent of similar spectra taken for aqueous solutions where the major differences are in the same spectral region.[32] The dissimilar difference spectra for $HgCl_2$ and $Zn(NO_3)_2$ solutions may be a consequence of a different mix of N-1-, N-7-, and N-1- + N-7-bonded complexes. In support of this difference, the shift of C-2 moves upfield (0.36 ppm) on addition of $HgCl_2$ but downfield (0.47 ppm) on addition of $Zn(NO_3)_2$. The complex *cis*-$[Pt(DMSO)_2Cl_2]$ induces a large downfield shift of the C-2 resonance (1.55 ppm) when added in an equimolar amount (0.2 M).

The dependence of the ^{13}C NMR shifts on $HgCl_2$ concentration (supplementary material) leads to a formation constant of ~1 M^{-1}, Table I. This value agrees well with that obtained using ^1H NMR data.[12]

Cytidine. This nucleoside exhibits the most complex behavior of all the common nucleosides. A preliminary communication on our studies with cytidine has appeared.[20] Complete ^{13}C

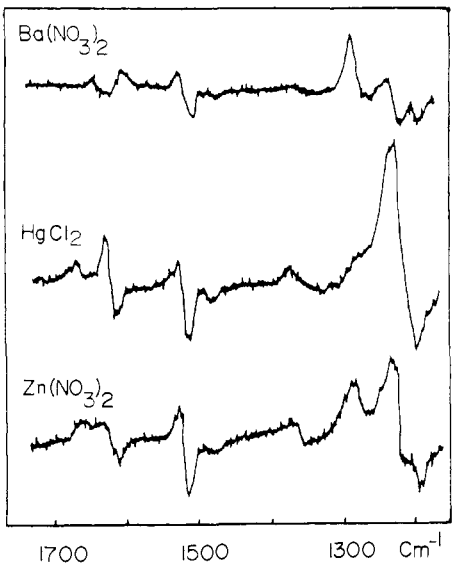


Figure 3. Raman difference spectra for cytidine (0.7 M) in Me_2SO-d_6 in the presence of the inorganic species (0.7 M) indicated.

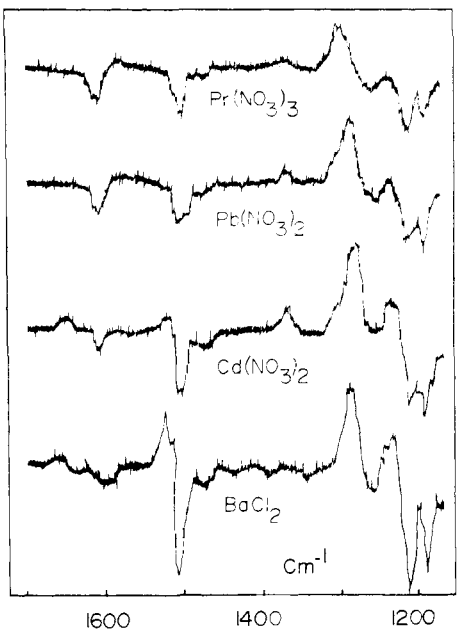


Figure 4. Raman difference spectra for cytidine (0.7 M) in Me_2SO-d_6 in the presence of several different inorganic species, all 0.7 M except $BaCl_2$, 0.3 M.

NMR spectral data on this nucleoside can be found in the supplementary material. We previously concluded that the site of attachment of metal species to this nucleoside depended on the nature of the metal ion: hard metal ions bind to O-2, soft metal species and H^+ bind to N-3, and zinc ion binds to both N-3 and O-2. In ^{13}C NMR, the characteristic change which signals the binding mode is the direction of shift of the C-2 resonance: upfield shifts signify strong N-3 binding and downfield shifts signify stronger interaction with O-2, Table I.

Cytidine is thus the only nucleoside for which there is clear evidence for the binding of alkaline earth metal ions. These metal ions bind to O-2. The cytosine base also interacts with Li^+ or LiCl, as evidenced by ^7Li line broadening.[18] However, the suggestion that this interaction involves N-3[18] is probably incorrect. The C-2 resonance of cytidine (0.2 M) is shifted downfield by ~0.5 ppm on addition of $LiClO_4$ (1.57 M).

We have briefly discussed Raman evidence for O-2 vs. N-3 binding to cytosine derivatives. Raman difference spectra for the addition of several salts to cytidine are given in Figures 3 and 4. There is relatively little effect of the metal species on the C=O, C=C, and C=N double bond frequencies[31] about 1500-1600 cm^{-1}, but considerably larger perturbations occur in the ~1200-1300-cm^{-1} region. The interpretation of these Raman spectral changes will be treated below. It should be noted, however, that several metal species induce Raman difference spectra similar to that produced by $Ba(NO_3)_2$ (Figure 4).

The changes in NMR chemical shifts which accompany the addition of labile metal species to solutions of ligands can be treated (as described in the Experimental Section and mentioned briefly in the previous subsections) to yield formation constants. We have investigated extensively the application of this approach to assess the formation constants when several metal species interact with cytidine, Table II. A comparison between the experimental points and the shifts predicted by theory is given in Figure 5.

The comparative results obtained for $Ba(NO_3)_2$ and $HgCl_2$ are considerably different from those reported by others.[12-14] In particular, our results suggest that the formation constant for $HgCl_2$ + cytidine is substantially larger than that for $Ba(NO_3)_2$ + cytidine. Both our Raman results (Figure 4) and our ^{13}C NMR data demonstrate that $BaCl_2$ and $Ba(NO_3)_2$ have similar effects. The literature conclusion that $BaCl_2$ and $HgCl_2$ have similar affinities for cytidine is different from our findings. We have therefore performed a competition experiment between $Ba(NO_3)_2$ and $HgCl_2$, and the data we obtained for three solutions of cytidine (0.2 M) each with (a) $Ba(NO_3)_2$ (0.7 M), (b) $HgCl_2$ (0.7 M), and (c) $Ba(NO_3)_2$ (0.7 M) and $HgCl_2$ (0.7 M) are given in Table III. As can be seen, the data for solutions (b) and (c) are similar. Since the C-2 resonance shifts in opposite directions with $Ba(NO_3)_2$ and $HgCl_2$ we would certainly have detected Ba complexation if these species had similar affinities for cytidine. For $BaCl_2$ (work in progress), we find that $K \sim 7.5$ M^{-1} as compared to values of 14-20 M^{-1} reported in the literature.[13,15]

Table II. Summary of Stability Constants[a]

ligand	M	salt	K, M^{-1}	K,[b] M^{-1}
cytidine[c]	0.10	$HgCl_2$	21 ± 2	
cytidine[c]	0.20	$HgCl_2$	22 ± 1	
cytidine	0.05	$HgCl_2$	22 ± 8	20 ± 2
cytidine	0.10	$HgCl_2$	23 ± 10	21 ± 6
cytidine	0.20	$HgCl_2$	18 ± 7	19 ± 4
deoxycytidine	0.20	$HgCl_2$	22 ± 14	19 ± 6
5-methyldeoxy-cytidine	0.20	$HgCl_2$	21 ± 12	18 ± 3
cytidine	0.10	$Ba(NO_3)_2$	2.2 ± 0.1	
cytidine	0.20	$Ba(NO_3)_2$	2.1 ± 0.2	
cytidine	0.20	$Zn(NO_3)_2$	2.8 ± 0.6	
cytidine	0.20	$Pb(NO_3)_2$	10 ± 2	
cytidine	0.20	$Pb(ClO_4)_2$	8 ± 2	
cytidine	0.20	$PbCl_2$	1.2 ± 0.1	
DMC	0.20	$HgCl_2$	1.3 ± 0.3	
DMC	0.20	$Pb(NO_3)_2$	2.7 ± 0.1	
1-methylcytosine	0.10	$Pb(NO_3)_2$	19 ± 1	
guanosine[37]	0.20	$HgCl_2$	1.7 ± 0.9	
1-methylguanosine[37]	0.20	$HgCl_2$	3.0 ± 0.6	
adenosine	0.20	$HgCl_2$	1.2 ± 0.3	

[a] In Me_2SO-d_6; determined using ^{13}C NMR except as noted. [b] Determined by excluding deviant resonances (C-4 and C-5 in cytidine). [c] ^1H NMR results.

N^4,N^4-Dimethyl-1-methylcytosine. Our studies with cytidine strongly suggested that O-2 and not N-3 is the principal binding site for most metal species. This finding is contradic-

Table III. Comparison of the Effects of $HgCl_2$ and $Ba(NO_3)_2$ on the ^{13}C NMR Spectrum of Cytidine (0.2 M)[a]

salt	C-2	C-4	C-5	C-6	C-1′	C-4′	C-2′	C-3′	C-5′
none	155.36	165.47	93.82	141.42	89.16	84.00	73.92	69.34	60.57
$HgCl_2$ (0.7 M)	152.41	162.95	95.47	142.38	89.88	84.58	73.92	69.02	60.13
$HgCl_2$ + $Ba(NO_3)_2$ (0.7 M) (0.7 M)	152.31	162.75	95.50	142.50	89.75	84.66	73.87	69.20	60.24
$Ba(NO_3)_2$ (0.7 M)	156.37	164.97	94.48	141.50	88.83	84.23	73.97	69.42	60.57

[a] In Me_2SO-d_6, in parts per million downfield from Me_4Si.

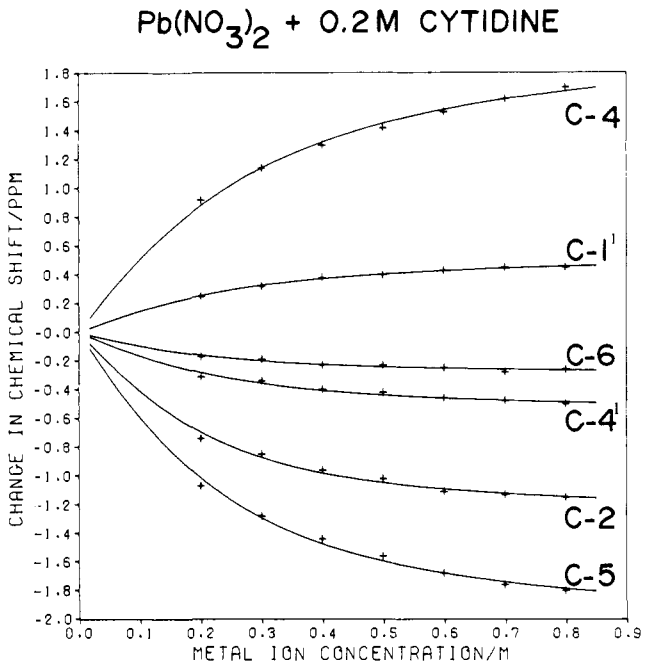
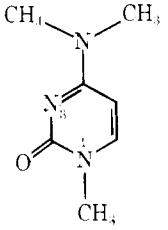


Figure 5. The chemical shift changes in cytidine (0.2 M) in Me_2SO-d_6 as a function of $Pb(NO_3)_2$ concentration. Computer-drawn plot and solid lines indicate theoretical fit based on data in the supplementary material.

tory to most solution studies in the literature[1] and furthermore is also contrary to most crystal structures on metal complexes of cytosine and its derivatives.[2,3,5] To verify our conclusions, we have examined the binding of metal species to N^4,N^4-dimethyl-1-methylcytosine (DMC). Molecular models of this



species indicate that the metal species are too large to bind to N-3 and that O-2 binding will be sterically favored. We also reasoned that the substitution of a dimethylamino group for an amino group would not significantly alter the electronic properties and binding in the six-membered heterocyclic ring. In agreement with this conclusion, we present in the supplementary material the shift values for the corresponding ring carbons in DMC and cytidine, which are comparable.

A characteristic phenomenon which we have used for cytidine in Me_2SO and others[33,34] have used for cytidine in aqueous solution is that the direction of ^{13}C chemical shifts for binding of electrophiles to a given ring site is the same regardless of the identity of the electrophile. Thus, $HgCl_2$ and

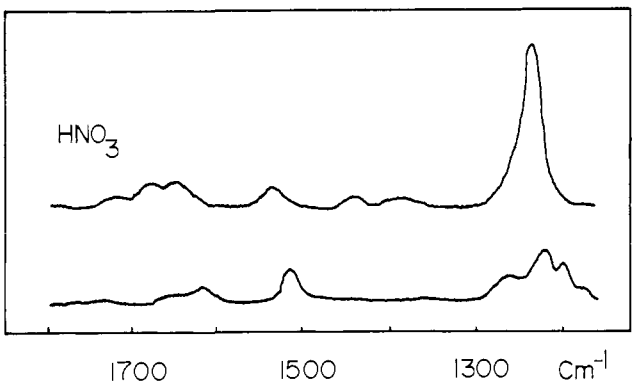


Figure 6. Raman spectra of cytidine and cytidine HNO_3, both 0.7 M in Me_2SO-d_6.

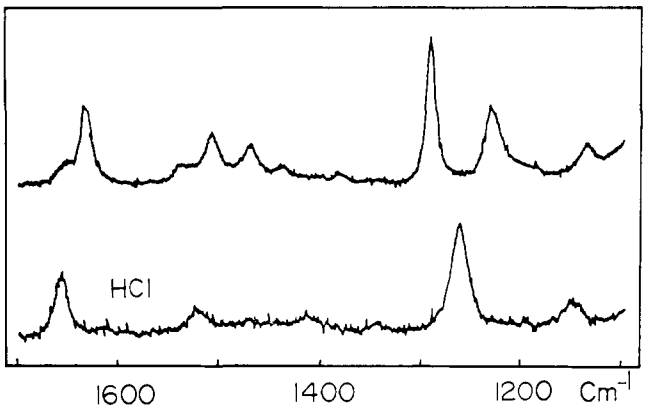


Figure 7. Raman spectra of N^4,N^4-dimethylamino-1-methylcytosine and its HCl salt, both 0.2 M in Me_2SO-d_6.

H^+ induce similar patterns of chemical-shift differences. These electrophiles also cause roughly similar patterns of change in the Raman spectrum of cytidine. The Raman spectrum of [cytidineH]NO_3 in Me_2SO-d_6 (Figure 6) is similar to that found when $HgCl_2$ is added to cytidine in this solvent. Our recent report[20] elucidating the ^{13}C NMR and Raman spectral changes attributable to O-2 binding of electrophiles represented the first examples of a deviation from this pattern.

The changes induced in the ^{13}C NMR spectrum of DMC by H^+ are quite different from those induced in DMC by all other electrophiles studied thus far (Table IV). In particular, the C-2 resonance is shifted upfield by H^+ as it is shifted by H^+ in cytidine. However, when $HgCl_2$ is added to DMC, the C-2 resonance is shifted downfield and the complex formed is relatively unstable (Table II). Recall that $HgCl_2$ shifts the C-2 resonance of cytidine upfield in Me_2SO. All metal salts examined also shift the C-2 resonance of DMC downfield (Table IV).

In the same manner, the Raman spectral changes which accompany the addition of a H^+ to DMC (Figure 7) are quite

Table IV. Comparative Incremental Shifts of the ^{13}C NMR Resonances of N^4,N^4-Dimethyl-1-methylcytosine (0.2 M) on Addition of Various Salts[a]

salt	M	C-2	C-4	C-5	C-6	CH_3	CH_3
HCl	0.2	6.36	6.76	−1.53	−1.97	0.15	2
HNO_3	0.2	6.36	6.89	−1.52	−1.87	0.15	2
$Ba(NO_3)_2$	0.7	−0.56	0.31	−0.61	−0.03	0.00	
$La(NO_3)_3$	0.7	−0.59	0.23	−0.99	−0.03	−0.13	
$Lu(NO_3)_3$	0.3	−0.48	0.16	−0.92	−0.09	−0.14	
$Pr(NO_3)_3$	0.7	−8.5	0.66	−1.39	−0.38		
$Pb(NO_3)_2$	0.7	−1.42	0.99	−1.55	−0.01	−0.03	−0.40
$Zn(NO_3)_2$	0.7	−1.13	0.47	−1.53	−0.14	−0.17	−0.83
$Cd(NO_3)_2$	0.7	−1.30	0.51	−1.61	−0.12	−0.25	−0.32
$HgCl_2$	0.7	−0.45	1.17	−1.34	−0.10	−0.19	−0.67
$AgNO_3$	0.7	−0.85	0.74	−1.83	−0.14	−0.47	−0.84
$Pt(DMSO)_2Cl_2$	0.2	0.06	0.11	0.03	0.02	−0.02	0.02

[a] In Me_2SO-d_6; negative shifts downfield.

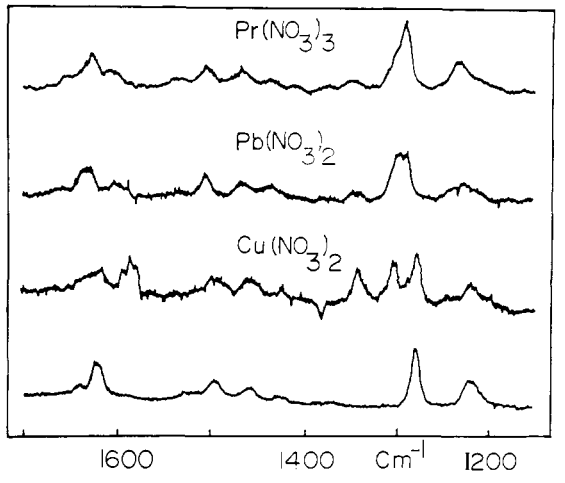


Figure 8. Raman spectrum of N^4,N^4-dimethylamino-1-methylcytosine (0.2 M) in Me_2SO-d_6 in the absence of metal salts and in the presence of the metal salts indicated (0.7 M).

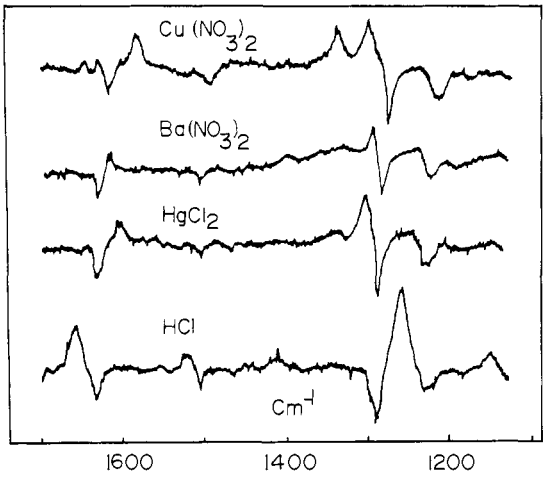


Figure 9. Comparison of Raman difference spectra for DMC (0.2 M) and DMC-HCl (0.2 M) with the difference spectra for DMC (0.2 M) in the presence of the salts indicated (0.7 M), all spectra for Me_2SO-d_6 solutions.

different from the changes which accompany the addition of bulkier electrophiles, namely, metal species (Figure 8). The Raman difference spectra are therefore characteristically different for H^+ and other electrophiles (Figure 9). The new bands at ~1450 cm^{-1} in the Raman spectrum of DMC may be due to $N\text{-}CH_3$ vibrational modes.[31]

In the pioneering work on the use of Raman spectroscopy to study metal-nucleoside interactions,[35] Lord and Thomas examined the mercuration and protonation of cytidine in H_2O. The shift to higher frequency of the total intensity in the double-bond region (1500-1800 cm^{-1}) and the corresponding shift to lower frequency of the intensity in the single-bond region (1200-1300 cm^{-1}) were interpreted as the "fixing" of the single and double bonds of the ring by protonation or mercuration. In the present study, the same direction of frequency shifts is observed in Me_2SO for mercuration and protonation of cytidine. However, the salts of hard metal ions caused the opposite pattern of shifts, particularly in the single-bond region. Protonation of DMC is accompanied by intensity shifts which are in the same direction as found for protonation of cytidine. However, with all metal salts investigated, the intensity shifts for DMC follow the pattern opposite to that found for protonation and mercuration of cytidine and protonation of DMC. These changes for DMC are quite pronounced (Figure 8) and follow the pattern observed with cytidine + salts of hard metal ions. Addition of an electrophile at O-2 apparently tends to "unfix" the double and single bonds of the cytosine ring.

Stability constants for DMC for two metal salts are presented in Table II. To allow a comparison to sterically less hindered systems, the interaction of $Pb(NO_3)_2$ with 1-methylcytosine was also examined.

Discussion

At present, the degree of interaction of the anions with metal ions in Me_2SO is generally not known. We are currently engaged in the study of this phenomenon as well as in a study of anion interactions with nucleosides. It was the aim of this study to avoid, where possible, the use of chloride salts since this anion is known to interact with nucleosides. Nevertheless, we shall refer to a few selected studies with chloride salts.[37] The use of $HgCl_2$ could not be avoided since $Hg(NO_3)_2$ (or $Hg(OAc)_2$) organomercurates some of the nucleosides. However, we feel that there is probably very little dissociation of Cl^- from $HgCl_2$. The site of interaction of Cl^- with cytidine is at the $NH_2/C(5)H$ site. We have found that Cl^- does not interact as strongly at this site with 5-methyldeoxycytidine.[37] The similarity in K for the $HgCl_2$ complex of these nucleosides (Table II) suggests that the interaction of Cl^- with the nucleosides is not a problem.

The order of stability and values of K reported previously for $HgCl_2$ using ^1H NMR and the least-squares procedure[12] are in excellent agreement with our results with ^{13}C NMR. The graphical estimation of K[24,25] appears to give values which are too high but the stability order, namely, cytidine > guanosine[37] > adenosine > uridine, is found in all cases. This same order is found in H_2O for the affinity of CH_3HgOH and $Hg(OH)_2$

toward available endocyclic nitrogens in cytidine, guanosine, and adenosine.[36]

The reported[15] $K \sim 3.5\ M^{-1}$ for $ZnCl_2$ + cytidine, using ^1H NMR, agrees well with our values of $K = 2.8\ M^{-1}$ for $Zn(NO_3)_2$ (Table II) and $K = 3.0\ M^{-1}$ for $ZnCl_2$.[37] The literature values[13,15] of K for $BaCl_2$ + cytidine of ~ 14-$20\ M^{-1}$ are higher than our value for $Ba(NO_3)_2$ ($\sim 2.2\ M^{-1}$) and $BaCl_2$ ($\sim 7.5\ M^{-1}$).[37] The higher value of K for $BaCl_2$ than for $Ba(NO_3)_2$ may result from anion binding to either the metal or the nucleoside; this problem is a subject of current investigation.

Our results with $PbCl_2$ (Table II) demonstrate some of the problems which may arise when chloride salts are employed. We find that $PbCl_2$ appears to form a much weaker complex with cytidine than does either $Pb(NO_3)_2$ or $Pb(ClO_4)_2$. (Both of these salts give similar K values.) The binding shifts, Δ_{max}, obtained with $PbCl_2$ are similar to those obtained with Pb salts of the oxy anions. This result suggests that most of the $PbCl_2$ does not dissociate. Accordingly, $PbCl_2$ does not produce the characteristically large downfield shifts we[19] and others[12,13,16,18] have reported with chloride salts.[37] Uncoordinated chloride ion would interact with N(1)H and NH_2 of guanosine.[17] A comparison of K values for cytidine and DMC is instructive. Since $HgCl_2$ is forced to bind to O-2 in DMC, it is not surprising that the formation constant for DMC is considerably lower than that for cytidine, where binding is at N-3. However, the K value for $Pb(NO_3)_2$ is also lower for DMC than for 1-methylcytosine and cytidine even though binding is at O-2 in all cases. We believe that the dimethylamino group probably causes some steric hindrance to binding of O-2 in DMC. Steric hindrance could force the metal to bind at an alternate site on the O-2, such as site 4 in Figure 1. Such a change in binding site could explain the reversal of the direction of the $Pr(NO_3)_3$ paramagnetic shift of C-2 in DMC as compared to the shift for other cytosine derivatives, Table V. We are currently exploring this matter further with other paramagnetic lanthanide ions.

For DMC, the order of stability is $Pb(NO_3)_2 > HgCl_2 > Pt(DMSO)_2Cl_2$ and the latter does not form a detectable complex. For cytidine, the stability order for various metal species appears to be $Pt(Me_2SO)_2Cl_2 > HgCl_2 > Pb(NO_3)_2 \sim Pb(ClO_4)_2 > BaCl_2 > Zn(NO_3)_2 \sim ZnCl_2 \geq Ba(NO_3)_2 \sim La(NO_3)_3$. For adenosine the order appears to be $Pt(Me_2SO)_2Cl_2 > HgCl_2 > Zn(NO_3)_2$. With adenosine, ^{13}C NMR shifts were inadequate for an assessment of K with hard metal species, and for uridine this was true for all metal species.

For salts of hard metal ions, the small ^{13}C NMR shifts for uridine and adenosine and the small residual Raman difference spectra for adenosine are caused by either solvent changes or by weak metal binding. The paramagnetic lanthanide salts should have produced much larger shifts if the metals were directly bound.

One potential cause of the minor spectral changes could be a slight amount of base protonation. In some cases, hydrated salts were used. Also, the metal ion could possibly bind to the sugar, leading to deprotonation of the hydroxyl group and protonation of a base site.

For adenosine, we observed similar effects for $La(NO_3)_3$ and the more acidic $Lu(NO_3)_3$. The species $Cr(H_2O)_6^{3+}$ did not have a significant effect on the ^{13}C NMR spectrum of adenosine. In contrast, $Cr(H_2O)_6^{3+}$ (along with $Lu(ClO_4)_3$ and $Ga(NO_3)_3$) had a significant influence on the spectrum of cytidine. In addition, the effects of $La(NO_3)_3$ and $Lu(NO_3)_3$ were considerably different. These changes in the cytidine spectrum were consistent with protonation at N-3 and suggest that cytidine is more basic in Me_2SO than the other nucleosides.

The ^{13}C NMR shifts of the sterically hindered nucleosides 6-dimethylaminopurine-9-riboside and 8-bromoadenosine (supplementary tables) were less sensitive to lanthanide salts. Such a result is consistent with either an environmental or a bonding origin for the small shifts in adenosine.

Table V. Comparative Incremental Shifts Obtained for Cytosine Derivatives (0.2 M in Me_2SO-d_6) with $Pr(NO_3)_3$ (0.3 M)[a]

	cytosine	1-methyl-cytosine	cytidine	N^4,N^4-dimethyl-1-methyl-cytosine
C-2	10.12	7.34	6.74	−3.40
C-4	2.01	1.86	1.56	0.35
C-5	−2.26	−1.34	−0.99	−0.69
C-6	0.18	0.37	0.58	−0.21

[a] Positive values upfield.

If the small spectral changes are reflecting bonding, then the patterns of stability toward hard metal species we have observed follow the trend cytidine ≫ guanosine[37] ≥ adenosine > uridine. The interaction of both hard and soft metal species with uridine is undetectable. The interactions with guanosine and adenosine are so weak as to be uncertain. Relaxation effects[18] with $^7Li^+$ also show that only cytidine definitely interacts with this cation.

In studies of the binding of alkaline earth chloride salts with nucleosides, the dependence of K's calculated from ^1H NMR data on the alkaline earth cation has been put forward as evidence for the interaction of the cation with all four nucleosides.[12-15] In this vein, the shift of the H-8 resonance of adenosine was used to evaluate the formation constant for the supposed complex formed by that nucleoside and alkaline earth metal ions.[12,14] We find that NEt_4Cl induces the same types of shifts in H-8.[37] However, NEt_4NO_3 does not cause any appreciable shifts in either the sugar or the base resonances of adenosine.[37]

At present there is no compelling ^1H NMR evidence for the binding of alkaline earth metal species to the base of any common nucleoside except cytidine, where nitrate and perchlorate salts induce significant shifts. There is currently a need to investigate the interaction of chloride salts of alkaline earth metal ions with nucleosides using ^{13}C NMR and Raman spectroscopy since M-ONO_2 species may exist. However, we find for lead that the nitrate salt has a much higher affinity for cytosine derivatives than does the chloride salt. In addition, the effect of $Pb(NO_3)_2$ and the nitrate salts of other hard metal species on the ^{13}C NMR spectrum of the nucleosides is quite comparable. It should be noted that $Pb(NO_3)_2$ is among the best diamagnetic metal salts in perturbing the ^{13}C NMR spectra of cytidine and DMC.

The results thus far with salts of hard metal ions fit in well with predictions made by the Pullmans,[38] who calculated the relative energies of metal binding by an SCF LCAO ab initio procedure. These calculations suggest that Na^+ would bind to the bases in the order guanosine ≃ cytidine ≫ uridine ≥ adenosine. According to these calculations, exocyclic oxygens are the important binding sites, in agreement with predictions which can be based on hard and soft acid and base theory.

This observed trend differs in detail from the theoretical calculations. However, we have demonstrated that other ligands in the coordination sphere of the metal center greatly influence the stability of the nucleoside complexes.[39] The theoretical computations assessed the interaction of a "naked" Na^+ ion with the bases. Our studies[39] have concentrated on the binding of metal species to endocyclic nitrogens. Attachment of a metal to the exocyclic groups poses different, less severe steric problems.

Table VI. Summary of Interactions of Cations and Anions with the Heterocyclic Base of Nucleosides in Me_2SO[a]

species	uridine	adenosine	guanosine	cytidine
soft metal salts	−	+	+	+
$Zn(NO_3)_2$	−	+	+	+
hard metal salts	−	−/+	−/+	+
$Pb(NO_3)_2$	−	−/+	−/+	+
Cl^- [b]	−	−	+	+
Li^+	−	−	−	+
NO_3^-	−	−	−	−

[a] No detectable interaction, −; detectable interaction, +; weak or no interaction, −/+. [b] Cl^- interacts with sugar hydroxyl groups.

Summary

We believe that virtually all of the NMR observations on metal ion-nucleoside interactions can be explained with only a few specified binding criteria. We have summarized these criteria in Table VI. The complex patterns reported in the literature are observed only when both the cation and the anion of the added salt can bind to the heterocyclic base. The summary view of these interactions in Table VI is consistent not only with all the NMR data but also with our Raman studies and (crudely) with theoretical predictions.

The present study demonstrates the utility and complementary nature of Raman and NMR spectroscopy. The contrasting behavior of DMC and cytidine represents an example in which both approaches point to the same conclusion. The results with DMC are unique in the sense that no other electrophile causes the same Raman spectral changes as does the H^+ ion. This finding contrasts with previous Raman spectroscopic studies on nucleic acid derivatives.[20,23,32-35] Nevertheless, an N(3),O(2) chelate cannot be ruled out.[20]

An interesting question relevant to the binding of antitumor agents to nucleic acids involves the possibility that atypical donor sites may be brought into the binding picture. This could happen if, in the polymer, a weak binding site is juxtaposed to one of the available coordination sites on Pt, which is held in place by strong endocyclic nitrogen bonds. Our study with *cis*-$[Pt(Me_2SO)_2Cl_2]$ and DMC is illuminating in that no detectable complex is formed.

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Supplementary Material Available: Effects of salts on ^1H and ^{13}C NMR spectra (23 pages). Ordering information is given on any current masthead page.

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