Electrochemistry, Synthesis, and Spectra of Pentaammineruthenium(III) Complexes of Cytidine, Adenosine, and Related Ligands

Michael J. Clarke

Contribution from the Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02167. *Received August 16, 1977*

Abstract: The synthesis of pentaammineruthenium(III) complexes of cytidine, adenosine, and tubercidin by redox catalysis is reported. The spectroscopic and chemical properties of these complexes favor coordination site assignments as the exocyclic nitrogens of cytidine, adenosine, and tubercidin, but do not preclude binding to the adjacent pyrimidine ring nitrogens for the corresponding Ru(II) complexes. An N-7 coordinated complex of guanine is also reported. These complexes exhibit strong ligand to metal charge transfer bands in the visible and near-ultraviolet. The cytidine and adenosine complexes are remarkably acidic (having pK_a values of 3.15 and 3.64, respectively) with displacement of a proton from the exocyclic amine. The acidity of these complexes is attributed to the close juxtaposition of the Ru(III) to the ionizable proton and to intramolecular hydrogen bonding between a coordinated ammine and an adjacent nitrogen following proton loss. Values of pK_a for the Ru(II) complexes have also been measured. Cyclic voltammetry studies indicate that the cytidine and adenosine complexes dissociate or isomerize following reduction to the Ru(II) state. Implications of the chemistry of these complexes regarding their formation and behavior in biochemical systems are discussed.

Introduction

The effect of transition metals on nucleotides, nucleic acids, and their constituent bases has implications relating to (1) heavy metal toxicity,[1,2] (2) the efficacy of transition metal antitumor[3,4] agents, and (3) the use of metals as labels in structure studies by x-ray or electron microscopy techniques.[5-7] Chemical mechanisms bearing on the toxicity of heavy metals have been the focus of considerable investigation in recent years. In particular, the discovery by Rosenberg[8] that platinum ammines are effective as anticancer drugs has provoked the search for similar antitumor agents based on platinum and other transition metals. Many of these drugs appear to interfere with the replication of DNA by binding directly to the cellular DNA.[9] Since they are not selective for tumor cells, the platinum drugs in use at the present time show a number of undesirable side effects. It is hoped that by using other metal ions and by paying careful attention to their chemical properties, more selective and less toxic agents can be developed. Ruthenium ammine complexes are particularly attractive in this regard since (1) a reasonable amount of chemical information concerning them already exists,[10,11] (2) their redox properties and reaction rates can be varied by chemical modification of the ligands,[12] and (3) several ruthenium complexes have been shown to inhibit cellular DNA synthesis in vitro at a level similar to that of *cis*-$Cl_2(NH_3)_2Pt^{II}$ [13,14] and have also shown antitumor activity in animal studies.[13]

Ruthenium(II) ammine complexes generally undergo substitution reactions more rapidly than the corresponding Ru(III) ions[10,11,15] and the reduction potentials of many Ru(III) ammine complexes are such that they may be reduced by a variety of reductants found in biological systems.[13,18] On the other hand, many Ru(II) ammine complexes can be oxidized fairly rapidly by oxygen.[16] It should be possible to take advantage of these properties to design Ru(III) ammine or amine complexes which would be activated by cellular reduction. Owing to the differences in oxygen content often present between normal and solid tumor cells,[17] the Ru(II)/Ru(III) ratio should be higher in solid tumors than in the surrounding tissue. A higher rate of metal binding to DNA is therefore expected in solid tumor cells, thereby providing a possible means for selective tumor toxicity.

Compounds containing the pentaammineruthenium group are presently under investigation and have been shown to inhibit cellular DNA synthesis.[13a] Since these complexes do not have cis leaving groups, it is unlikely that they act entirely analogously to the platinum agents. Nevertheless, it is probable that they function by binding to cellular DNA and provide a convenient system for study.

In order to test the approach of cellular activation of the metal complex by reduction and to understand the binding of this class of compounds to nucleic acids we have been studying the interactions of $(NH_3)_5Ru^{II \text{ and } III}$ with DNA. The $(NH_3)_5Ru(OH_2)^{2+}$ ion appears to bind rapidly to DNA and subsequent aerial oxidation of these solutions yields colored products.[18,19] The present investigation was undertaken in an effort to understand (1) the interactions of the pentaammineruthenium group with adenine and cytosine ligands and (2) the origin of the bands in the visible spectra of $[(NH_3)_5Ru^{III}]_n$-DNA in order to determine if they could be used to assess the type and degree of metal binding to DNA. Moreover, the systematic study of a firmly bound metal ion on the physical and chemical properties of the constituent bases of nucleic acids will provide basic information necessary not only to understand the mechanism of metal-containing drugs, but also in designing base-specific labels to aid in the sequencing of nucleic acids by electron microscopy.

Experimental Section

Chemicals and Reagents. Chloropentaammineruthenium(III) chloride was prepared by the method of Vogt, Katz, and Wiberly[20] and recrystallized from 0.1 m hcl. Cytidine, adenine, and adenosine (Aldrich), deoxyguanosine (ICN), 1-methylcytidine (Sigma), and tubercidin (Calbiochem) were used without further purification. Standard acid, base, electrolyte solutions, and ion exchange resins were prepared according to previously reported methods.[21]

Equipment. Spectra were recorded on a Cary Model 14 spectrophotometer. Electrochemical measurements were made on a cyclic voltammetry apparatus constructed in this laboratory based on Analog Devices 514K operational amplifiers for the potentiostat, current, and voltage follower circuits. Platinum button or disk electrodes (Beckman) or a hanging drop mercury electrode (Brinkmann) were used as the indicator electrode against a standard calomel reference electrode. The $(NH_3)_6Ru^{III-II}$ couple gave an E_f of 49 mV (corrected vs. SHE) on this system. Measurements of pH were made with a Metrohm combination glass electrode. Atomic absorption measurements were made on a Perkin-Elmer Model 300 atomic absorption spectrophotometer, using a Perkin-Elmer Ru-Pt combination hollow cathode lamp.

Synthesis of Compounds. The cytidine complex was prepared by (method I) allowing an argon-purged solution of chloropentaammi-

0002-7863/78/1500-5068$01.00/0

neruthenium(III) trifluoroacetate (0.7 mmol) to react with a twofold excess of the ligand in a 0.01 M phosphate buffer solution at pH 7 over zinc amalgam. After 40 min the zinc was removed and oxygen was bubbled through the solution for at least 1 h. The solution was then chromatographed on a 6 × 1.5 cm Biorex-70 ion exchange column eluted with 0.1 and 0.2 M ammonium acetate. The orange band eluted with 0.2 M ammonium acetate was collected, rotary evaporated, and redissolved several times to remove all of the ammonium acetate. The red solid was then dissolved in a minimum amount of water and a saturated solution of $NaClO_4 \cdot 2H_2O$ in ethanol was added until precipitation began. Concentrated solutions that were allowed to stand for days at 4 °C produced an undesired brick-red precipitate. A red-orange microcrystalline material appeared on cooling after several hours. The precipitate was collected, washed with ethanol and ether, and stored in a vacuum desiccator. Anal. Calcd for $[(Cyd^-)(NH_3)_5Ru](ClO_4)_2 \cdot 3H_2O$: H, 4.91; C, 15.86; N, 16.44; Cl, 10.40; Ru, 14.82. Found: H, 4.38; C, 15.58; N, 16.41; Cl, 10.04; Ru, 14.45.

Method II. The cytidine complex was also observed to form in good yield under similar reaction conditions but with no reductant present and only 5% of the starting material in the Ru(II) form. The Ru(II) was prepared in a separate vessel and then syringed into the flask containing the chloropentaammineruthenium(III) and the cytidine.

Method III. The cytidine complex was also observed to form over a period of days in solutions containing only cytidine and chloropentaammineruthenium(III) at pH 7.

The 1-methylcytosine complex was prepared by similar methods except that a saturated solution of ammonium hexafluorophosphate was added dropwise to induce crystallization. Anal. Calcd for $[(1MeCyt^-)(NH_3)_5Ru](PF_6)_2$: H, 3.68; C, 9.99; N, 18.63. Found: H, 3.57; C, 10.02; N, 18.55.

The adenosine complex was prepared by analogy to method I, except that the solid obtained by evaporation of the ammonium acetate eluent was dissolved in a minimum of water and 1 M HBr was added dropwise until the solution turned from blue to red (pH 3). The solution was rotary evaporated again and the solid was taken up in alcohol, collected by filtration, and then stored in a desiccator. This procedure often resulted in the formation of a highly charged purple material which could be removed by subsequent repurification on an ion-exchange column. This material seemed to form when concentrated solutions of the adenosine or adenine complexes were allowed to stand or were in the process of rotary evaporation. Anal. Calcd for $[(Ado)(NH_3)_5Ru]Br_3 \cdot H_2O$: H, 4.25; C, 16.88; N, 19.69; Br, 33.71. Found: H, 3.86; C, 16.93, N, 19.95; Br, 34.6. The adenosine complex was observed to form by method II in comparable yield. The complex was also observed to form by method III over a period of days.

The adenine complex was prepared by acid hydrolysis of the adenosine or deoxyadenosine complexes. This was usually accomplished in the course of an additional ion exchange chromatography step of the original reactant solution after aerial oxidation. The solution was placed on a 3-4-cm AG-50 ion exchange column and eluted with 1-4 M HCl. The major portion of the material separated as a red band which was collected and rotary evaporated to dryness. Acidification was only attempted on solutions that had been thoroughly oxidized and allowed to stand overnight. Solutions that were not so treated often rapidly decomposed on the addition of acid. Varying amounts of a dark blue-purple material usually adhered to the top of the column and were not eluted. The red solid was redissolved and chromatographed a second time on the Biorex 70 column. Two blue-purple bands were observed to elute with 0.6-0.8 M ammonium acetate. The first of these contained the adenosine complex and the second the adenine. The bromide salt of the adenine complex was obtained in a manner similar to that described for the adenosine complex. Anal. Calcd for $[(Ade)(NH_3)_5Ru]Br_3 \cdot 2H_2O$: H, 4.05; C, 10.06; N, 23.46; Br, 40.15; Ru, 16.9. Found: H, 3.67; C, 10.06; N, 23.57; Br, 40.21; Ru, 16.8.

Since concentrated solutions of the adenine and adenosine complexes often formed a dark species of apparent high charge, spectral measurements were usually made by preparing the compounds in dilute solution. This was normally done by method I except that after the ion exchange step on the carboxylate column the required fractions were put on a second short (3-4 cm) carboxylate ion exchange column and water was passed through the column to elute the ammonium acetate. The desired complex was then eluted with 0.05 M HCl, which was neutralized with a LiOH solution immediately after elution from the column. The complexes themselves serve as convenient indicators

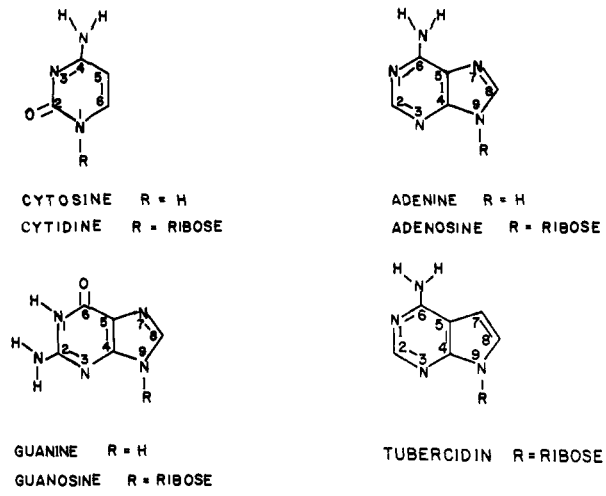


Figure 1.

of this titration. The neutralized solution was then rotary evaporated to one-half the original volume and stored in the dark at 4 °C. Solutions prepared in this manner were usually about 10^{-4} M in the complex and were normally stable for weeks.

Complexes with tubercidin, 1-methyladenosine, and 3-methylcytidine were prepared by mixing a 20% excess of ligand with a 0.1 mM solution of chloropentaammineruthenium(III) trifluoroacetate in air over a few small pieces of zinc amalgam for at least 1 h. Purification was effected by chromatographic separation on a Biorex-70 column eluted with ammonium formate. Reactant solutions containing 1-methyladenosine yielded numerous multicolored products. Red (λ_m 536 nm) and orange (λ_m 498 nm) bands which eluted with 0.8-1.0 M buffer contained the bulk of the product. A second chromatographic separation was required to resolve these two bands. This second chromatographic step revealed that solutions containing the red complex decomposed on standing overnight to yield several colored products. Therefore, spectra were taken on samples in solution immediately after chromatographic separation.

The guanine complex was prepared by acid hydrolysis of the deoxyguanosine complex made by previously reported methods.[21,22] The hydrolysis procedure involved adjusting the oxidized reactant solution to be approximately 1 M in HCl. This solution was allowed to sit at 45 °C for 1 h and then was placed on a 4-cm AG-50 column and eluted with 1-5 M HCl. The purple solution eluted from the column was rotary evaporated to dryness and then placed on a 6-cm Biorex 70 column. Usually a single blue band eluted from this column with 0.8-1.0 M ammonium acetate. This fraction was evaporated to eliminate the ammonium acetate and the blue solid taken up in a minimum of 1 M HCl. Alcohol was added to induce the formation of a gelatinous precipitate which was sucked dry on a sintered glass filtration funnel and washed with copious amounts of ethanol before storing in a desiccator. Anal. Calcd for $[(Gua)(NH_3)_5Ru]Cl_3 \cdot 2H_2O$: H, 5.04; C, 12.52; N, 29.20; Cl, 22.17; Ru, 21.0. Found: H, 4.97; C, 12.85, N, 29.17; Cl, 21.90; Ru, 20.6.

Determination of Ru by Atomic Absorption Spectroscopy. Determinations of ruthenium in solution were made by a modification of the method of Rowston and Ottaway[23] using hexaammineruthenium(III) chloride as a standard diluted with the same media as the sample. A solution of Cu(II) and Cd(II) sulfates was used to suppress interfering substances and to enhance the absorption signal of the ruthenium. This was prepared by dissolving 0.393 mol of $CuSO_4 \cdot 5H_2O$ and 0.216 mol of $CdSO_4 \cdot 8H_2O$ in 1 l of 1 M HCl. Solutions prepared for atomic absorption determinations contained 5% (v/v) of the Cu/Cd solution. The ruthenium line at 349.9 nm was employed using a 0.2-nm slit setting and a lean air-acetylene flame. The sensitivity of this method is approximately 3 μM and calibration curves were usually linear in the range 3-300 μM.

Physical Measurements. Molar absorptivities were usually determined at $\mu = 0.1$ in LiCl media. Spectrophotometric determinations of pK_a values were performed on the Ru(III) complexes according to previously reported techniques.[22] Values for the Ru(II) complexes were determined from plots of E_f vs. pH for the complex. Formal reduction potentials were determined by cyclic voltammetry to be at a

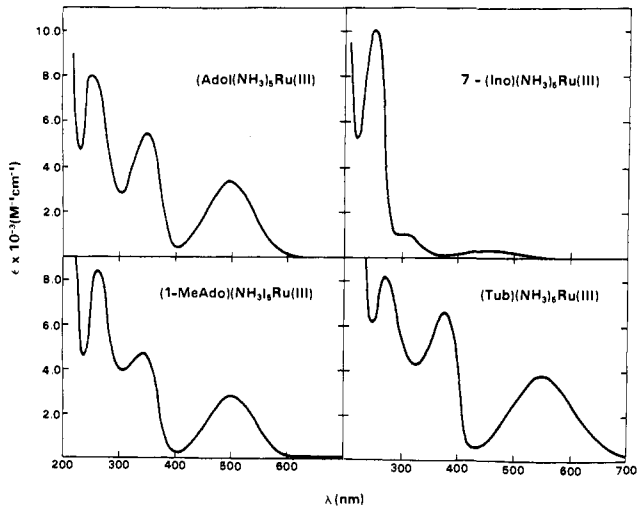


Figure 2. Neutral ligand spectra of adenosine, tubercidin, inosine, and orange 1-methyladenosine complexes.

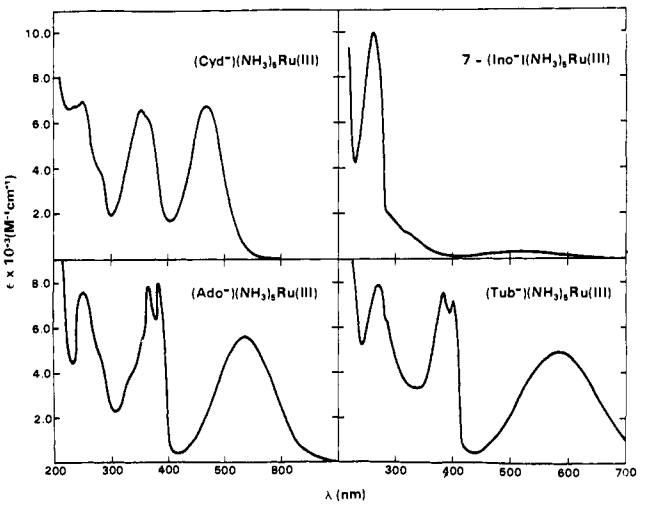


Figure 4. Deprotonated-ligand spectra of adenosine, tubercidin, cytidine, and inosine.

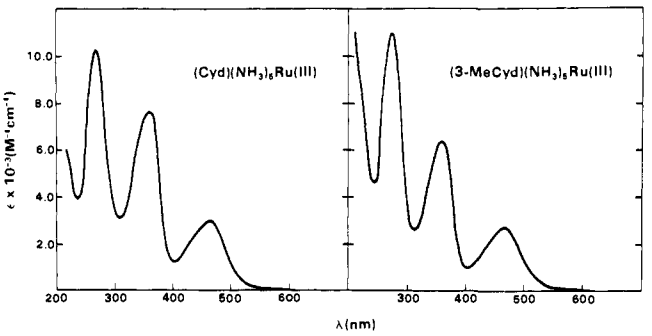


Figure 3. Neutral-ligand spectra of cytidine and 3-methylcytidine complexes.

point midway between the anodic and cathodic peaks. Cyclic voltammetry measurements were made in buffers of $\mu = 0.1$ with ruthenium concentrations in the millimolar range.[21,30] Unless otherwise noted, scan rates were 125 mV/s. The electrode areas were 0.02 cm^2 for the HMDE, 0.88 cm^2 for the platinum disk, and 0.16 cm^2 for the platinum button. Magnetic susceptibility measurements were made at room temperature on a Cahn Faraday magnetic susceptibility balance using $Hg[Co(CNS)_4]$ as a standard.

Results

Synthesis. Despite being formed in argon-purged solutions over zinc amalgam, the color of the Ru(III) cytidine and adenosine complexes persisted throughout the preparation of these complexes by method I. The characteristic color of the Ru(III) species disappeared only when the pH of these solutions was lowered below pH 3. Freshly oxidized solutions of the adenosine and cytidine complexes prepared by method I often decomposed rapidly on addition of 1 M HCl. Owing to their instability and extreme sensitivity to air oxidation, efforts to isolate Ru(II) cytidine and adenosine complexes were abandoned. The Ru(III) complexes of cytidine and adenosine appeared to form in good yield when only 5% of the ruthenium starting materials existed as Ru(II). These complexes were also observed to form over extended periods of time with only Ru(III) present. The synthesis of the guanine complex is similar to that reported previously for a series of guanine derivatives.[21]

When 1-methyladenosine was allowed to react with chloropentaammineruthenium(III) via redox catalysis (method II) followed by chromatographic separation, numerous products were evident. Separation on an AG-50 column eluted with 1 M HCl yielded a red band as the major product. Attempts to isolate a solid from this band yielded crystals of *cis*-$[Cl_2(NH_3)_4Ru]Cl$. Ion exchange chromatographic separation of the reactant mixture on a carboxylate column at neutral pH resulted in the isolation of two major products, which behaved as 3+ ions, and several minor products of lower charge. The major products were resolved only with difficulty into red and orange components. The red complex decomposed on standing to yield a blue complex which behaved as a 2+ ion on the ion exchange column at pH 7.

The behavior of the cytidine, adenosine, tubercidin, and guanine complexes on a carboxylate ion-exchange column eluted with a pH 7 buffer indicated the ions to be near 2+ in charge. The adenine and guanine complexes were less easily eluted than the cytidine, probably owing to increased interactions of their larger, planar aromatic structures with the resin. Aside from this, compounds of higher pK_a were retained longer on the column. Purification of concentrated solutions of adenine and adenosine complexes invariably yielded highly charged species which eluted only with molar concentrations of eluant. Magnetic moments for all compounds were determined to be in the range of 2.1–2.3 μ_B.

Spectra. Some pertinent UV-visible spectra of these complexes are shown in Figures 2–4. A common factor in the spectra of all the compounds is the presence of visible and near-UV bands which cannot be attributed to the ligand alone. With increasing pH these bands usually disappeared with more intense absorptions appearing at lower energy. The spectra of the 3-methylcytidine and 1-methyladenosine complexes did not vary in the 0–10 pH range. The blue decomposition product of the red 1-methyladenosine complex exhibited the two closely spaced peaks in the near-ultraviolet region, which are characteristic of an anionic adenine ligand, and a broad absorption around 600 nm, which can also be attributed to a lower energy ligand to metal charge transfer transition (LMCT) resulting from ligand deprotonation.

Electrochemistry. Formal reduction potentials for the complexes as determined by cyclic voltammetry are listed in Table I. The observed separation between the anodic and cathodic peaks for the cytidine complex was normally about 70 mV over the entire pH range with i_a approximating i_c. However, when the potential was held at −0.3 V (vs. SCE) for intervals greater than 1 min in solutions saturated with isonicotinamide at pH 4.7, a new peak was observed to grow in centered at 137 mV positive of SCE. This new couple was not apparent in buffers at the same pH which did not contain isonicotinamide. The reduction potential was observed to be pH

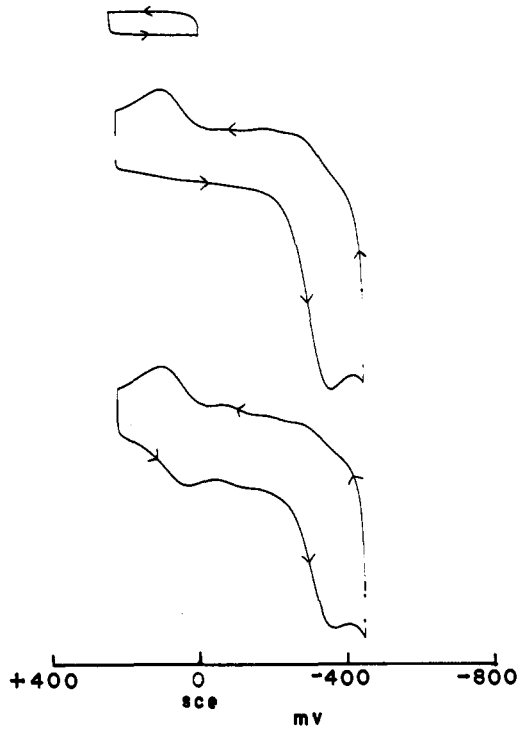


Figure 5. Cyclic voltammetry scans of $(Ade)(NH_3)_5Ru^{III}$ at 125 mV/s on platinum disk electrode in pH 4.4 buffer, $\mu = 0.1$: (A) initial scan, (B) initial scan to reduce complex, (C) subsequent scan over entire region of interest.

Table I. Formal Reduction Potentials of $(NH_3)_5Ru^{III}$-L Complexes at $\mu = 0.1$

ligand	probable[f] binding site	E_f vs. NHE, V	media
[Cyd]	N-4	0.09	0.01 M $HClO_4$
			0.09 M $LiClO_4$
3-MeCyd	N-4	0.09	0.1 M LiCl
$[Cyd^-]^c$	N-4	−0.30	0.1 M LiOH
[Ado]	N-6	−0.04	0.1 M $HClO_4$
$[Ado^-]^c$	N-6	−0.41	0.01 M LiOH
			0.09 M $LiClO_4$
[Ade]	N-6	−0.05	0.1 M $LiClO_4$, pH 4
[Gua]	N-7	0.15	0.01 M $HClO_4$
			0.09 M $LiClO_4$
$[Gua^-]^d$	N-7	−0.07	glycine/LiOH, pH 9
$[Gua^{2-}]^{d,e}$	N-7	−0.20	0.1 M LiOH
$[Guo]^a$	N-7	0.19	acetate/acetic acid/LiCl, pH 5.9
$[Guo^-]^{a,e}$	N-7	0.11	glycine/LiOH, pH 9.5
pyrimidine[b]	N-1	0.43	0.1 M HCl

[a] Taken from ref 21. [b] Taken from ref 19. [c] Deprotonated at exocyclic amine. [d] Deprotonated at N-9. [e] Deprotonated at N-1. [f] N-4 and N-6 are exocyclic nitrogens.

dependent in the pH range between the pK_a values of the Ru(II) and Ru(III) complexes.

The reduction potential of the adenine complex decreased with increasing pH over the pH range 1–12 and had a value of −0.17 V (vs. NHE) at pH 7.4. Cyclic voltammetry on a HMDE showed a single cathodic peak and two smaller anodic peaks, one in a position about 75 mV positive of the cathodic peak and the second at a slightly more positive potential. The first anodic peak increased with increasing pH and scan rates, while the second appeared to decrease. At pH 11.7 and a scan rate of 1250 mV/s the couple appeared to approach reversibility. Additional anodic peaks were evident when a platinum disk electrode was used at pH 4.4 and scans made over more positive potentials (Figure 5). The initial scan on this system showed a significantly larger third anodic peak which appeared at a potential 90 mV positive of the SCE. Subsequent scans exhibited a second cathodic peak at +20 mV. Subsequent scans also often showed an additional couple seen as being centered at −110 mV in Figure 5. The cyclic voltammetry behavior of the adenosine complex was analogous to that of the adenine complex except that the couple approached reversibility at lower pH and appeared to be essentially reversible at pH 11.7. The reduction potential of the adenosine complex was pH dependent in the pH range 4–11.

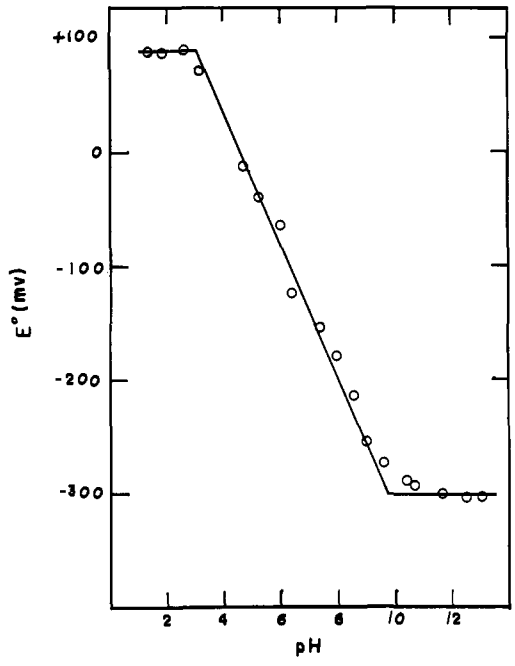


Figure 6. E_f vs. pH plot of $(Cyt)(NH_3)_5Ru^{III}$.

Table II. pK_a for $(NH_3)_5RuL$ Complexes at $\mu = 0.1$

ligand	probable[f] binding sites	probable deprotonation site	Ru(III)[a]	Ru(II)[b]
[Cyd]	N-4	N-3, N-4	3.15	9.7
[lMeCyt]	N-4	N-3, N-4	3.31	
[Ado]	N-6	N-1, N-6	3.64	11.3
[Ade]	N-6	N-1, N-6	4.54	
$[Ade^-]^d$	N-6	N-7, N-9	9.88	
[Tub]	N-6	N-1, N-6	4.0	
[Gua]	N-7	N-9	5.23	8.9
$[Gua^-]^e$	N-7	N-1	9.54	11.8
$[Guo]^c$	N-7	N-1	7.36	9.0

[a] Determined spectrophotometrically. [b] Determined from electrochemical data. [c] Taken from ref 21. [d] Deprotonated at exocyclic amine. [e] Deprotonated at N-9. [f] N-4 and N-6 are exocyclic nitrogens.

The reduction potential of the guanine complex was pH dependent in the pH range 5–12. The couple appeared to be reversible over this range, exhibiting single anodic and cathodic peaks of equal area and usually separated by 60–80 mV.

pK_a Values. Measured pK_a values are summarized in Table II. Isosbestic points were present in the spectrophotometric determinations of the pK_a values for the Ru(III) complexes. Values for the Ru(II) complexes were determined from plots of the formal reduction potential vs. pH as shown in Figure 6.

The sloping lines in these plots were constrained to have a slope of 59 mV per pH unit and to begin from a pH value equal to the spectrophotometrically determined pK_a of the Ru(III) complex. The 3-methylcytidine complex and red and orange complexes of 1-methyladenosine did not exhibit protonation equilibria in the pH range 0–10.

Discussion

Coordination Sites. Since the guanine complex was prepared from the corresponding deoxyguanosine complex, binding at N-7 is expected. Reasons for this have been discussed elsewhere.[21,22] In addition the molecular structure of the (Hyp)-$(NH_3)_5Ru^{III}$ complex, prepared analogously from deoxyinosine, exhibits metal coordination at N-7.[24]

The N-3 atom on cytidine is the most electron rich and the most basic toward protonation,[2] and is the preferred coordination site for Pt(II) ammines[25] and methylmercury[26] at low pH. This site is somewhat sterically hindered by the exocyclic ammine and the carbonyl groups; however, an ammine–metal ion should be able to hydrogen bond with the adjacent oxygen atom, thereby stabilizing the molecule.[7] Direct metal binding to the exocyclic oxygen has been shown in the case of Ag(I),[27] but is unlikely here as will be shown in the discussion of the spectra of these complexes. Monodentate coordination to the exocyclic amine has been indicated with methylmercury at high pH where this site is deprotonated.[26]

Of the three ring nitrogens available on adenosine, N-3 is sterically hindered by the ribose to the attack of a bulky metal ion,[7,21] leaving only the N-1 and N-7 sites. In cases where favorable steric and hydrogen bonding interactions between a coordinated ligand and the exocyclic amine occur, N-7 coordination of adenosine has been established.[29,30] However, when these favorable interactions are absent, binding at N-1 might be expected since this site is the most basic toward protonation and the least sterically hindered by the exocyclic amine. Methylmercury(II) coordinates adenosine at the N-1 position[26] and platinum ammines at both N-1 and N-7 in neutral solution. At high pH methylmercury(II) may also bind adenosine at the exocyclic nitrogen.[26]

The present case involves deprotonation of the ligand upon the redox-catalyzed addition of Ru(III). Neutral cytidine and adenosine are not particularly good ligands[29] and the driving force for complexation appears to be provided by the negative charge residing on these ligands resulting from proton loss. In the case of adenosine, deprotonation of the exocyclic amine should increase the electron density on the pyrimidine ring nitrogen to a markedly greater extent than on the more distant N-7 and so favor coordination at N-1 over N-7. A similar situation has been noted for 3-(7MeHyp)$(NH_3)_5Ru^{III}$ [22] where coordination at N-3 is stable only at pH values where the pyrimidine ring is deprotonated. Proton loss from the exocyclic amine would also favor coordination at the exocyclic nitrogen. Studies with the linkage isomers of (Hyp)$(NH_3)_5Ru^{III}$ indicate that the metal may move between adjacent coordination sites as a function of the protonation state of the hypoxanthine ligand.[22] Therefore, while N-1 should be the preferred binding site when the metal initially adds as Ru(II), it may move to the adjacent exocyclic nitrogen on oxidation to Ru(III) and deprotonation of the ligand.

The behavior of the pentaammineruthenium ion with DNA is also indicative of coordination on the exocyclic or adjacent pyrimidine ring nitrogens. When this ion is allowed to bind to helical DNA, little binding to adenine is observed; however, when allowed to bind to denatured DNA followed by the extraction of the Ru-base residues, spectra identical with those reported here for the adenine complex are observed.[18] Thus when the exocyclic-N and N-1 sites, which are involved in hydrogen bonding on the interior of helical DNA, become available on separating the strands, metal labeling of adenine occurs to a much greater degree. The N-7 purine sites are on the exterior of helical DNA and coordination to N-7 guanine sites is observed in both normal and single-stranded DNA.

The tubercidin complex cannot be coordinated at the 7 position since this site is occupied by a carbon atom. Similarly, 1-methylcytidine and 1-methyladenosine should not coordinate on the pyrimidine ring nitrogen since this is blocked by a methyl group. The presence of two products (orange and red) of tripositive charge with 1-methyladenosine may be attributed to the occurrence of both N-7 and exocyclic nitrogen coordination or, since *cis*-$[Cl_2(NH_3)_4Ru]Cl$ was observed to form on standing in 1 M HCl, to the formation of either linkage isomer and a N-7, exocyclic-N chelate.

Spectra. The absorption bands in the range 200–290 nm are attributed to π–π^* transitions on the ligands. The absorptions in the near-UV and visible portions of the spectrum are due to the presence of the metal. The intensity of these bands and the occurrence of similar bands at lower energy on deprotonation of the ligand both indicate that these absorptions are due to ligand to metal charge transfer transitions (LMCT).

The LMCT bands in the spectrum of the tubercidin are shifted slightly to lower energy relative to those of the adenosine complex, as would be expected in substituting the less electronegative carbon for nitrogen. Analogous bands are observed at higher energy on removal of the electron-donating imidazole ring and addition of the electronegative carbonyl as in the cytidine complex. The LMCT spectra of the neutral ligand adenosine, cytidine, and tubercidin complexes are quite similar when these effects are taken into account.

Strikingly similar absorption patterns can be observed in the spectra of the neutral-ligand cytidine, 1-methylcytidine, adenosine, tubercidin, and 1-methyladenosine complexes (Figures 2 and 3) and in the deprotonated cytidine, adenosine, and tubercidin complexes (Figure 4). These spectral similarities indicate a common binding site, i.e., the exocyclic nitrogen. Moreover, these spectra can be contrasted with those of the corresponding inosine (Figures 2 and 4), guanosine,[21] and xanthine[31] complexes, which have been shown to be coordinated at N-7. Previous studies have shown that the intensity of the LMCT absorptions in complexes of this type is a function of the coordination site,[22] so that the large differences in the molar absorptivities of the isoelectronic inosine and adenosine complexes suggest that they do not share a common binding site.

Reduction Potentials. The reduction potentials of the neutral ligand adenine, adenosine, and cytidine complexes are less than might be expected for a pentaammineruthenium group coordinated to a pyrimidine or imidazole ring[12,21,22,31] but are in the range expected for pentaammineruthenium(III) complexed by amines.[12] This must in some way be attributed to the presence of the exocyclic amine and is most easily accounted for by assuming binding to this site. Moreover, the reduction potentials for the 3-methylcytidine complex, which cannot involve N-3 coordination, and the neutral-ligand cytidine complex are nearly identical.

Assuming pyrimidine ring coordination, an amine group in a position ortho to the coordination site would be expected to increase the available electron density at this site and thereby stabilize Ru(III) relative to Ru(II). The juxtaposition of an electron-donating group should decrease any metal to ligand back-donation which would also lower the reduction potential somewhat relative to the analogous pyrimidine complex. However, it is doubtful that this would account for the relatively large differences in reduction potential between the complexes reported here and the analogous pyrimidine species (see Table I). The single exception is the new couple evident at 0.30 V (vs. NHE) in the cyclic voltammetry studies of the adenine and adenosine complexes (Figure 5).

The relative reduction potentials of the adenine and cytidine

complexes can be accounted for by the lack of the electron-withdrawing carbonyl group and the presence of an electron-donating imidazole ring in going from cytidine to adenine. The pH behavior of the reduction potentials is as expected for a single proton equilibrium in the case of the adenosine and cytidine complexes, and two consecutive proton equilibria in the case of the guanine complex.

Stability of Complexes. Unlike previously reported pentaammineruthenium complexes with pyrimidine ligands, no appreciable back-bonding appears to be present in the case of the cytidine and adenosine derivatives and the Ru(II) oxidation state is therefore not stabilized. The magnetic susceptibility values of the complexes reported here indicate the metal to exist as Ru(III). The low reduction potentials of the complexes also indicate that Ru(III) is the air-stable form. The reduction potentials of the cytidine and adenosine complexes are in keeping with the observation that only very small amounts of oxygen are required to maintain the Ru(III) state during their synthesis.

The ion exchange behavior of these complexes and the elemental analysis of the cytidine complexes indicate that, with the exception of the 1-methyladenosine and 3-methylcytidine complexes, they exist as 2+ ions at neutral pH. Their ion exchange behavior in acid and the elemental analysis of the compounds which were precipitated from acidic solutions indicate that these complexes exist as 3+ ions in the pH range 1–3. Since a coordinated ammine proton would not be expected to be extracted below pH 12[32] these observations strongly indicate deprotonation of the heterocyclic ligand at neutral pH.

The cytidine, adenosine, and guanosine complexes were observed to form slowly over a period of days when the starting material was present only as Ru(III). However, the cytidine and adenosine complexes formed rapidly and in good yield with only a small amount of Ru(II) present. Formation of these species by redox catalysis is therefore suggested:

$$Ru^{II} + LH \rightleftarrows Ru^{II}LH \qquad L = \text{nucleoside}$$

$$Ru^{III} + Ru^{II}LH \rightleftarrows Ru^{III}L^- + Ru^{II} + H^+$$

where the Ru(III) is present as the chloro, phosphato, or hydroxy pentaammineruthenium ions and the Ru(II) ion exists predominantly as the corresponding aquo species. This type of catalysis has been employed in the synthesis of a number of Ru(III) acido complexes[33] and is expected here on the basis of the relative reduction potentials of the stated complexes under the reaction conditions employed. Formation of the guanosine complex by redox catalysis is not expected.

The instability of the cytidine and adenine complexes in acidic solutions in the presence of even small quantities of Ru(II) suggest that these complexes can also be decomposed by redox catalysis. The scheme suggested for this dissociation is merely the reverse of that given for the catalytic formation of these complexes at neutral pH. In strongly acidic solutions this scheme would involve the protonated cationic ligand species. The relative reduction potentials of the species involved at low pH are consistent with these schemes.

The cyclic voltammetry investigation of the adenine and adenosine complexes reveals that the reduced forms of these complexes are unstable over a broad pH range. The first anodic peak evident in the cyclic voltammetry scans of these complexes on the HMDE increased with increasing scan rates and is attributed to the oxidation of the respective Ru(II) complex. The pH behavior of this peak indicates that the decomposition of these complexes increases with hydronium ion concentration. The second anodic peak is attributed to the oxidation of the most likely decomposition product, $(H_2O)(NH_3)_5Ru^{II}$. The third anodic peak, which became apparent when the Pt-disk electrode was employed (Figure 5), remains unidentified, but, if exocyclic-N coordination of Ru(III) is assumed, may be due to the formation of the N-1 bound Ru(II) complex. The reduction potential for this species occurs in the range which might be expected for coordination to a pyrimidine ring with electron-donor substituents, and $(NH_3)_5Ru^{II}$ would be expected to bind preferentially to the N-1 site.

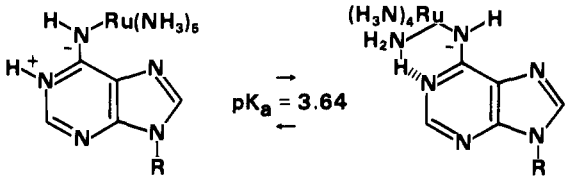
While the cyclic voltammetry scans of the cytidine complex appear reversible over a broad pH range, it must be noted that loss of the cytidine upon reduction to give the $(H_2O)(NH_3)_5Ru^{II}$ ion would be masked since the redox potentials of these two complexes are similar over a broad pH range and so would yield coincident anodic and cathodic peaks. The appearance of a new couple after brief electrolytic reduction in the presence of isonicotinamide indicates decomposition of the complex. The new couple appears in the range expected for the isonicotinamidepentaammineruthenium complex, and it is likely that the complex forms via the aquo species as an intermediate. The higher reduction potential of the cytidine complex relative to the adenosine indicates that the Ru(II) cytidine species is more stable relative to the Ru(III) ion than for the corresponding adenosine ions. It is therefore reasonable to expect that the Ru(II) cytidine complex would dissociate from the exo-N site somewhat more slowly than the adenosine complex.

The instability of the reduced cytidine and adenosine complexes relative to the corresponding guanine,[21] inosine,[22] and pyrimidine complexes appears to be caused by the presence of the exocyclic amine adjacent to the ring nitrogens. Assuming either exocyclic-N or adjacent pyrimidine nitrogen binding and protonation at the other site, space-filling models show that the juxtaposed proton is in an excellent position to add onto an octahedral face of the metal center and so form a structure similar to that proposed as an intermediate in the acid-catalyzed hydrolysis of $(NH_3)_6Ru^{2+}$.[34] In the case of an exocyclic-N bound adenosine complex, this could also occur via N-7. These facile means of protonating the metal center may account for the rapid dissociation or isomerization of these complexes at low pH.

The decomposition of the red 1-methyladenosine complex, which probably involves N-7 metal coordination, to yield species which apparently contain an anionic adenine ligand can be attributed to the loss of the ribose. Loss of this group from purine nucleosides is known to be acid catalyzed, with the most effective protonation site for catalysis being N-7.[36] In the case of the N-7 bound $Guo(NH_3)_5Ru^{III}$ complex, the observed rate of proton-catalyzed hydrolysis is decreased relative to that of the free ligand, since the metal blocks the preferred protonation site and makes it more difficult for the proton to add to the purine ligand.[21] However, the metal itself may function as an effective general acid catalyst at this site and so promote ribose loss exclusive of purine protonation. Owing to dissociation of the metal at a rate greater than that of ribose loss in the case of the guanosine complex at neutral pH, this effect has not been quantitated.[21] However, it appears that in the present case metal-catalyzed loss of the ribose occurs more rapidly than metal–purine dissociation.

pK_a Values. The pK_a for the loss of the first proton from the guanine complex is 4.4 orders of magnitude more acidic than that for free guanine and agrees well with the previously reported value for the similar 1-methylguanine complex.[21] The pK_a for the loss of the second proton from this complex is 3 orders of magnitude greater than that for the free ligand and is similar to that reported for the analogous deprotonation in $(Hyp^-)(NH_3)_5Ru^{III}$.[22] The increase in the acidity of purines bound via N-7 to Ru(III) is caused by electrostatic effects and is therefore dependent upon the proximity of the ionizable proton to the metal center.[31] The pK_a values for the loss of the first and second protons from the Ru(II) guanine complex are 0.7 orders of magnitude more acidic than the corresponding

Scheme I



values for the free ligand and compare favorably with those of analogous Ru(II) complexes previously reported.[21]

The pK_a values for the cytidine and adenosine complexes are at least nine orders of magnitude more acidic than those reported for the free ligands.[2,35] The change in the spectra of these complexes on deprotonation indicates that the heterocyclic ring is strongly affected. Since it is unlikely that a proton bound to a carbon is lost, displacement of a proton from the exocyclic nitrogen is most probable. The enormous increase in the acidity of these ligands is most easily accounted for by assuming exocyclic-N coordination. The similarity of the spectra of the pyrimidine ring methylated complexes with those of the corresponding cytidine and adenosine species suggests that the proton resides on the adjacent pyrimidine ring nitrogen. Deprotonation occurring from this site (N-1 of adenosine, N-3 of cytidine) would provide a favorable steric arrangement for a strong hydrogen-bonding interaction in which a hydrogen would be shared between a coordinated ammine and the adjacent pyrimidine ring nitrogen. Alternatively, space-filling models assuming N-1 or N-7 coordination on adenosine or N-3 coordination on cytidine indicate that an exocyclic amine proton is located directly on one of the octahedral faces of the metal ion. Following deprotonation, a similar hydrogen-bonding situation can occur between a coordinated ammine and the resulting exocyclic amide, so that the acidity of these complexes is compatible with both exocyclic-N and pyrimidine ring binding. The adenine complex exhibits an additional proton equilibrium involving the loss of a proton from the imidazole ring.

The Ru(II) adenine, adenosine, and cytidine complexes are much less acidic than the Ru(III) species. This is primarily due to the lower charge on the metal ion.

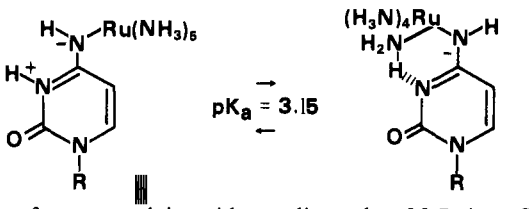
Conclusion

The physical properties of the Ru(III) complexes presented here are most consistent with coordination to the exocyclic nitrogen of cytidine and adenosine and least consistent with N-7 binding on adenosine, but do not rule out coordination on the pyrimidine ring for the Ru(II) species. Schemes I and II summarize the probable forms of the $Cyd(NH_3)_5Ru^{III}$ and $Ado(NH_3)_5Ru^{III}$ complexes. Hydrogen bonding between a coordinated ammine hydrogen and the N-7 of adenosine is possible in both the neutral ligand and deprotonated ligand forms, but space-filling models indicate N-1 hydrogen bonding to provide a more favorable steric arrangement in the deprotonated form. The higher reduction potentials of the cytidine relative to the adenosine complexes are consistent with some small degree of back-bonding which would indicate that resonance forms containing an exocyclic imine are more important with cytidine as the ligand.

The redox-catalyzed formation of the cytidine and adenosine complexes may involve initial attack of $(H_2O)(NH_3)_5Ru^{II}$ on a ring nitrogen followed by migration of the metal to the exocyclic nitrogen upon oxidation and deprotonation of the ligand. The linkage isomerization indicated by the cyclic voltammetric study of the adenosine complex is consistent with this. While the 1-methyladenosine and 3-methylcytidine complexes could not form by this mechanism, preparations with these ligands gave smaller yields than with the others reported here.

The metal-assisted nucleoside hydrolysis suggested for the red 1-methyladenosine complex could provide a mode for purine loss from a nucleic acid coordinated at N-7 sites. Such metal-assisted depurination occurring intracellularly could result in toxicity or mutagenesis.

Scheme II



The compounds reported here all exhibit strong absorptions in the visible region which allows them to be used as convenient probes in biochemical systems. Spectral bands which can be attributed to the adenine, cytidine, and guanine complexes have been observed with complexes of $(NH_3)_5Ru$–DNA.[18] The extent of specific base labeling can now be determined by standard photometric methods. The reduction potentials reported here indicate that the metal would likely reside in the lower oxidation state when coordinated to deoxyguanosine in an anoxic reducing environment provided by a biological system. The adenosine, cytidine, and guanine complexes are expected to exist as Ru(III) ions at neutral pH unless strong reducing agents are present. In the presence of strong reductants the cytidine and adenosine complexes would dissociate or isomerize.

Coordination to the adenine and cytidine sites of DNA in a biological system would appear possible by a form of redox catalysis requiring that only a portion of the metal ion exist in the Ru(II) form. However, it must be noted that base complexation by this method would compete with coordination by a variety of anions.[33] Monodentate coordination of cytidine and adenosine is therefore not expected to predominate in a biological system.

Since nucleoside coordination to Ru(III) was also observed in the absence of Ru(II), base binding under completely aerobic conditions may be expected to occur but at a much slower rate than in an environment where significant amounts of Ru(II) are allowed to form.

Acknowledgment. We wish to thank Professor Henry Taube (Stanford University) for helpful discussions at the outset of this work and Dr. Roy Magnuson (Boston University) for useful insights. Mr. Jerome Chaklos provided excellent assistance in the construction of the electrochemical apparatus and in performing the cyclic voltammetric measurements. Mr. Edward Schwarz ably performed many of the atomic absorption measurements. Support for this work was provided by the National Institutes of Health under Grants IROI-CA-18522 (Boston College) and GM 13638 (Stanford University). We are grateful to the Department of Chemistry at Wheaton College for the use of their Faraday balance.

Supplementary Material Available: Figures of the cyclic voltammetry scans for the cytidine and adenosine complexes and graphs of E_f vs. pH for the guanine and adenosine complexes. Tabulation of molar absorptivities and peak maxima (6 pages). Ordering information is given on any current masthead page.

References and Notes

(1) G. L. Eichhorn, "Inorganic Biochemistry", Vol. II, G. L. Eichhorn, Ed., Elsevier, Amsterdam, 1973, Chapters 33 and 34.
(2) R. M. Izatt, J. J. Christensen, and J. H. Rytting, *Chem. Rev.*, **71**, 439 (1971).
(3) T. A. Connors and J. J. Roberts, Ed., "Platinum Coordination Compounds in Cancer Chemotherapy", Springer-Verlag, West Berlin, 1974.
(4) M. J. Cleare, *Coord. Chem. Rev.*, **12**, 349 (1971).
(5) L. Marzilli, *Prog. Inorg. Chem.*, **23**, 255 (1977).
(6) D. Hodgsen, *Prog. Inorg. Chem.*, **23**, 211 (1977).
(7) L. Marzilli and T. J. Kistenmacher, *Acc. Chem. Res.*, **10**, 146 (1977).
(8) B. Rosenberg, *Naturwissenschaften*, **60**, 399 (1973).
(9) J. M. Pascoe and J. J. Roberts, *Biochem. Pharmacol.*, **23**, 207 (1974).
(10) H. Taube, *Surv. Prog. Chem.*, **6**, 1 (1973).

(11) P. C. Ford, *Coord. Chem. Rev.*, **5**, 75 (1971).
(12) (a) H. S. Lim, D. J. Barclay, and F. Anson, *Inorg. Chem.*, **11**, 1460 (1972); (b) J. A. Marchant, T. Matsubara, and P. C. Ford, *ibid.*, **16**, 2160 (1977).
(13) (a) A. D. Kelman, M. J. Clarke, S. D. Edmonds, and H. J. Persie, "Proceedings of the Third International Symposium on Platinum Coordination Complexes in Cancer Chemotherapy", Wadley Institute, Dallas, Texas, 1977, p 274; (b) M. J. Clarke, S. D. Pell, and A. D. Kelman, unpublished results from the National Cancer Institute's Screening Program for potential anticancer drugs.
(14) C. Monti-Bragadin, L. Ramani, L. Samer, G. Mestroni, and G. Zasinovich, *Antimicrob. Ag. Chemother.*, **7**, 825 (1975).
(15) J. A. Broomhead, F. Basolo, and R. G. Pearson, *Inorg. Chem.*, **3**, 826 (1964).
(16) J. R. Pladziewicz, T. J. Meyer, J. A. Broomhead, and H. Taube, *Inorg. Chem.*, **12**, 639 (1973).
(17) (a) C. T. Gregg in "Growth, Nutrition and Metabolism of Cells in Culture", Vol. 1, G. H. Rothblat and V. J. Cristofalo, Ed., Academic Press, New York, N.Y., 1972; (b) P. Vaupel, *Microvasc. Res.*, **13**, 394 (1977).
(18) M. J. Clarke, M. Buchbinder, and A. D. Kelman, *Inorg. Chim. Acta*, **27**, L86 (1978).
(19) M. J. Clarke, Ph.D. Thesis, Stanford University, 1974.
(20) L. H. Vogt, J. L. Katz, and S. E. Wiberly, *Inorg. Chem.*, **4**, 1158 (1965).
(21) M. J. Clarke and H. Taube, *J. Am. Chem. Soc.*, **96**, 5413 (1974).
(22) M. J. Clarke, *Inorg. Chem.*, **16**, 738 (1977).
(23) W. B. Rowston and J. M. Ottaway, *Anal. Lett.*, **3**, 411 (1970).
(24) K. Eriks, T. Hayden, S. Edmonds, and M. J. Clarke, to be submitted for publication.
(25) S. Mansy, G. Y. H. Chu, R. Duncan, and R. S. Tobias, *J. Am. Chem. Soc.*, **100**, 607 (1978).
(26) (a) R. B. Simpson, *J. Am. Chem. Soc.*, **86**, 2059 (1964); (b) S. Mansy, J. P. Frick, and R. S. Tobias, *Biochim. Biophys. Acta*, **378**, 319 (1975).
(27) L. G. Marzilli, T. J. Kistenmacher, and M. Rossi, *J. Am. Chem. Soc.*, **99**, 2797 (1977).
(28) S. Mansy, B. Rosenberg, and A. J. Thomson, *J. Am. Chem. Soc.*, **95**, 1633 (1973).
(29) T. Sorrell, L. A. Epps, T. J. Kistenmacher, and L. G. Marzilli, *J. Am. Chem. Soc.*, **99**, 2173 (1977).
(30) A. D. Collins, P. DeMeester, D. Goodgame, and A. C. Skapski, *Biochim. Biophys. Acta*, **402**, 1 (1975).
(31) M. J. Clarke and H. Taube, *J. Am. Chem. Soc.*, **97**, 1397 (1975).
(32) G. Navon, D. Waysbort, and M. Evenor, *Inorg. Chem.*, **14**, 514 (1975).
(33) J. Stritar and H. Taube, *Inorg. Chem.*, **8**, 2281 (1969).
(34) P. C. Ford, J. R. Kuempel, and H. Taube, *Inorg. Chem.*, 7, 1976 (1968).
(35) B. McConnell, *Biochemistry*, **13**, 4516 (1974).
(36) J. A. Zoltewicz, D. F. Clark, T. W. Sharpless, and G. Grahe, *J. Am. Chem. Soc.*, **92**, 1741 (1970).

The Influence of Axial Ligands on Metalloporphyrin Visible Absorption Spectra. Complexes of Tetraphenylporphinatozinc

Mario Nappa and Joan S. Valentine*

Contribution from the Department of Chemistry, Rutgers, the State University of New Jersey, New Brunswick, New Jersey 08903. *Received December 19, 1977*

Abstract: Electronic absorption spectra for a number of Zn(TPP)L and $Zn(TPP)X^-$ (TPP = tetraphenylporphinato) complexes in benzene or toluene are reported as well as spectra of ZnTPP in several noncoordinating solvents. Two principal effects are seen upon addition of axial ligands to ZnTPP: a red shift of the entire spectrum relative to that of ZnTPP and an increase in $\epsilon_\alpha/\epsilon_\beta$ that is correlated with the magnitude of the red shift. The magnitude of the red shift is shown to depend on the charge and polarizability of the axial ligand and not on the strength of the Zn-ligand bond. Equilibrium constants for complexation of the halides to ZnTPP increase in the order I < Br < Cl < F but the opposite order of red shifts is observed. IR spectra of $Zn(TPP)(NCS)^-$ show it to be N bound. Complexes of ZnTPP with crown ethers and imidazolate are observed. The spectra of the superoxide and imidazolate complexes are similar and fall between those of the hydroxide and cyanide complexes. The spectrum of the *n*-butylthiolate complex resembles that of carboxycytochrome P-450 in that the Soret band is abnormally red shifted and a new "hyper" band appears on the short-wavelength side of the Soret band.

The dependence of the electronic spectra of metalloporphyrins on the nature of their axial ligands makes heme a natural reporter of its immediate environment in hemoproteins. Thus considerable effort has been expended in cataloguing and interpreting the spectra of iron and other metalloporphyrins and the effects of varying axial ligation.[1-3] A particularly interesting example is the observation of the unusually long wavelength Soret band of the carbonyl complex of cytochrome P-450,[4,5] the successful synthesis of carboxy ferrous porphyrin thiolate complexes that reproduce the essential features of its spectrum,[6-9] and the theoretical analysis of this spectrum as an example of a "hyper" spectrum.[10]

Understanding the effects of axial ligands on the electronic spectra of iron porphyrins as opposed to other metalloporphyrins is the ultimate goal in this field because of its biological relevance. Unfortunately such interpretation has not been straightforward because of complications introduced by partially filled d orbitals and ambiguities in coordination numbers, spin states, and oxidation states. In the course of our recent studies of complexes of zinc porphyrins, we observed substantial spectral shifts of the electronic absorption spectrum as a function of the nature of axial ligands. Although such effects have been observed previously for a limited range of ligands,[11-17] we have extended the range of ligands and studied these spectra in some depth for three reasons. First, zinc porphyrin complexes provide a simpler system than those of iron in which to study a wide range of different ligands. This is because the metal is unambiguously in the 2+ oxidation state; the four-coordinate zinc porphyrin will accept one and only one axial ligand to form five-coordinate complexes;[12,13,18] and, since the electronic configuration is d^{10}, there are no empty d orbitals involved in the bonding. Secondly, we have made a number of complexes of zinc porphyrins with the relatively uncommon ligands superoxide,[19] imidazolate, crown ethers, and *n*-butylthiolate. Interpretation of the visible absorption spectra of these complexes allows us to compare some of the properties of these ligands with those of several more thoroughly studied ligands. Finally, examination of the visible spectra of other metalloporphyrins with different axial ligands has shown that the effects we see in the relatively straightforward zinc system can be seen in more complicated systems as well.

Experimental Section

Materials. Tetraphenylporphinatozinc (ZnTPP) was synthesized and purified from the chlorin impurity by literature methods.[20] It was recrystallized from toluene and dried in vacuo at 100 °C for 2 days. (Mesoporphinato IX dimethyl ester)zinc (Zn(MesoIXDME)) was

0002-7863/78/1500-5075$01.00/0