Ag(2). This type of asymmetry in which the shortest Ag–C distance is 2.47 ± 0.02 Å is usually observed in Ag(I)–aromatic complexes while the longer Ag–C distance may be as much as 2.7 Å regardless of stoichiometry, anion, or packing considerations.

The anthracene molecule is planar well within experimental error, and the bond distances and angles are not too dissimilar from that of the free molecule.[33,34] The largest distortion from that of the free molecule is the elongation of the C(2)–C(3) distance from 1.418 Å in free anthracene (measured at 290°K)[34] to 1.487 (12) Å in this complex. Since this is of the order of seven–eight standard deviations, it appears that the distortion is real. While the explanation for this ring distortion is not readily apparent, it should be noted that the distortion is of the same type and in the same direction as the distortion in bis(cyclohexylbenzene)(silver perchlorate).[5] In both cases the C–C bonds adjacent to the carbon of the "longer" Ag–C interaction have been elongated. In the anthracene case, both C(2) and C(3) are the carbons associated with a "long" Ag–C interaction, and the C(2)–C(3) bond shows a much greater distortion. It is unfortunate that comparison with benzene(silver perchlorate)[3] is impractical because of the disorder in the silver positions and the magnitude of the errors in the C–C bonds in both the *m*-xylene[6] and the *o*-xylene[7] structures preclude an analysis of ring distortion. Similar difficulties exist in most of the other known structures. It is obvious that an adequate explanation of the reason for ring distortion in Ag–aromatic complexes will have to wait until more evidence is available in terms of structures of silver complexes with polysubstituted aromatics.

(33) D. W. J. Cruickshank and R. Sparks, *Proc. Roy. Soc., Ser. A*, **258**, 270 (1960).
(34) R. Mason, *Acta Crystallogr.*, 17, 547 (1964)

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Supplementary Material Available. A listing of structure factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-74-5407.

Pentaammineruthenium–Guanine Complexes

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Abstract: The synthesis of several pentaammineruthenium(II and III)–guanine complexes is reported, in which the metal is believed to be bound to N_7. The Ru(III) compounds exhibit a broad low energy guanine-to-metal charge-transfer absorption, while the Ru(II) complexes show a metal-to-ligand charge transfer in the ultraviolet. The effect of Ru(II and III) on the acidity of the protons at N_1 and N_9 is investigated. Electrochemical potentials are reported for the complexes over a broad pH range. At low pH the Ru(III) nucleoside complexes undergo acid-catalyzed hydrolysis of the sugar–purine bond at a much slower rate than do the corresponding free nucleosides.

The interaction of metal ions with nucleotides has been the subject of considerable investigation for the past several years.[1] Areas of interest include: the effect of metal ions on the stability of nucleic acids,[2,3] synthesis of heavy-atom derivatives as aids in determining the structure of RNA by X-ray crystallography,[4] the participation of metal ions in the biological function of nucleic acids,[5] the use of heavy metal derivatives of nucleosides as cytological stains,[6] and the sequencing of nucleic acids by electron microscopy with the aid of a metal ion complex binding selectively to sites along the polynucleotide chain.[7,8]

Ruthenium is a sufficiently heavy atom to be of use in studies of structure by X-ray and electron microscopy. The aquopentaammineruthenium(II) ion shows a unique selectivity for heterocyclic nitrogen bases. This property has made it possible for us to synthesize a number of purine complexes with a ruthenium ammine species bound to the N-7 site (Figure 1). These complexes are substitution inert for ruthenium in both the 2+ and 3+ oxidation states. This feature simplifies the results and prepares the way for a systematic study of the effect of both a di- and tripositive metal ion on the purine moiety.

Experimental Section

Chemicals and Reagents. Chloropentaammineruthenium(III) chloride was prepared by refluxing hexaammineruthenium(III) chloride, obtained from Matthey Bishop, Inc., in 6 *M* HCl for 4 hr followed by crystallization from 0.1 *M* HCl.[9] The compounds 1-methylguanosine and 2′-deoxy-1-methylguanosine were prepared by methylating the corresponding ribosides (Aldrich Chemical Co.)

(1) R. M. Izatt, J. J. Christensen, and J. H. Rytting, *Chem. Rev.*, 71, 439 (1971).
(2) G. L. Eichhorn, *et al.*, *Advan. Chem. Ser.*, No. 100, 135 (1971).
(3) G. L. Eichhorn, E. Tarien, and J. Butzow, *Biochemistry*, 10, 2014 (1971).
(4) S. H. Kim, G. J. Quigley, F. L. Suddath, A. McPherson, D. Sneden, J. J. Kim, J. Weinzierl, and A. Rich, *Science*, 179, 285 (1973).
(5) W. Szer and S. Ochoa, *J. Mol. Biol.*, 8, 823 (1964).
(6) R. J. Barrnett, *J. Roy. Microsc. Soc.*, 83, 143 (1964).
(7) M. Beer and I. Moudrianakis, *Proc. Nat. Acad. Sci. U. S.*, 48, 409 (1962).
(8) D. Gibson, M. Beer, and R. Barrnett, *Biochemistry*, 10, 3699 (1971).
(9) L. H. Vogt, J. L. Katz, and S. E. Wiberly, *Inorg. Chem.*, 4, 1158 (1965).

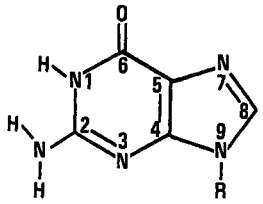

Figure 1. Guanine ring structure. Guanine, R = H; guanosine, R = ribose.

with methyl iodide according to the method of Broom, *et al.*[10] 1,9-Dimethylguanine was obtained from Fluka AG, Buchs, Switzerland, and was used without further purification. Hydrochloric acid solutions were standardized against standard NaOH or made up directly with Tritrisol (Brinkmann Instruments, Inc.). Lithium chloride solutions were prepared by dissolving lithium carbonate with hydrochloric acid, adjusting to neutral pH, and standardizing the solution by potentiometric titration with standard silver nitrate. Microanalyses were performed by the Stanford Microanalytical Laboratory, Stanford, Calif.

Equipment. All spectra were taken on a Cary 15 spectrophotometer. Measurements of pH were made with a Metrohm combination glass electrode on a Beckman Expandomatic pH meter standardized with Beckman certified buffers. Electrochemical potentials were measured on a cyclic voltammetry apparatus constructed in this laboratory by Mr. Glenn Tom using a platinum button as the indicator electrode and a standard calomel reference electrode.

Ion Exchange. The ion exchange resins, AG-50 W X-2 and Bio-Rex 70, 200–400 mesh, were purchased from Biorad Laboratories. The AG-50 resin was purified according to a standard method used in this laboratory.[11] The Bio-Rex 70 resin was converted to the ammonium form by first slurrying the resin three times with a one-bed volume of 1 *M* HCl followed by a water rinse. The resin was again slurried three times with a one-bed volume of 1 *M* ammonium acetate, filtered, and washed several times with water.

Synthesis of Complexes. (1-Methylguanosine)pentaammineruthenium(III) chloride was prepared by allowing an argon purged solution of chloropentaammineruthenium(III) trifluoroacetate at pH 3–4 to react with a stoichiometric amount of 1-methylguanosine over zinc amalgam for 30 min. After removing the zinc from the solution, the mixture was oxidized by bubbling air through for 1 hr. Ion exchange was then carried out on a 10-cm AG-50 column. On elution, several bands developed. A small red band eluted with 1–2 *M* HCl is believed to be a tetraammine nucleoside complex. A second band eluted with 2–3 *M* HCl was shown to be comprised of (1-methylguanosine)pentaammineruthenium(III). It overlapped somewhat with a smaller final blue band believed to be a triammineruthenium species containing two guanosine ligands. Bands one and three were not characterized. The eluate solutions were roto-evaporated to dryness, the residue was then redissolved in a minimum of water, and the solution was filtered. Finally ethanol was added to induce crystallization, and crystals were collected after cooling in a refrigerator. These were washed with ethanol and stored in a vacuum desiccator. *Anal.* Calcd for $[m^1Guo(NH_3)_5Ru]Cl_3 \cdot 2H_2O$: C, 21.1; N, 22.4; H, 5.47; Cl, 17.0. Found: C, 20.9; N, 22.5; H, 5.13; Cl, 16.7.

(1,9-Dimethylguanine)pentaammineruthenium(III) chloride was prepared by the above method. *Anal.* Calcd for $[m_2^{1,9}Gua(NH_3)_5Ru]Cl_3 \cdot 2H_2O$: C, 17.2; N, 28.6; H, 5.35; Cl, 21.7. Found: C, 16.9; N, 28.7; H, 5.32; Cl, 21.5.

(Guanosine 5′-monophosphate)pentaammineruthenium(III) chloride was prepared in the same way. Slow crystallization, resulting in needle-like crystals, was induced by ethanol diffusion into an aqueous solution of the complex. *Anal.* Calcd for $[GMP(NH_3)_5Ru]Cl_2 \cdot H_2O$: C, 18.9; N, 22.0; H, 4.74; Cl, 11.1; P, 4.86; Ru, 15.8. Found: C 19.0; N, 21.7; H, 4.66; Cl, 11.2; P, 4.66; Ru, 15.7.

The same method was followed for the preparation of (guanosine)pentaammineruthenium(III) chloride except that the reactant solution was adjusted to pH 2 to promote dissolution of the ligand. The compound was brought out of solution as a gel by addition of ethanol and then sucked as dry as possible on a fritted disk with repeated ethanol washings. The compound was dried overnight in a vacuum desiccator. *Anal.* Calcd for $[Guo(NH_3)_5Ru]Cl_3 \cdot 2H_2O$: C, 19.6; N, 22.9; H, 5.27; Cl, 17.4. Found: C, 19.8; N, 22.9; H, 4.70; Cl, 17.4.

For the preparation of (2′-deoxy-1-methylguanosine)pentaammineruthenium(III) chloride, the pH of the solution was adjusted to 3–4. The oxidized solution was ion exchanged on a 6-cm Bio-Rex 70 column using ammonium acetate as the eluent. Numerous bands were evident and are believed to be due to species resulting from loss of sugar, loss of ammonia, and the addition of more than one purine ligand.

A small amount of (1-methylguanine)pentaammineruthenium(III) eluted as a dipositive ion in 0.4 *M* ammonium acetate while the desired deoxyguanosine complex eluted in 1 *M* ammonium acetate. The eluate solution was roto-evaporated to a viscous liquid. It was redissolved in 1 *M* lithium chloride and ethanol was added to induce crystallization. *Anal.* Calcd for $[m^1dGuo(NH_3)_5Ru]Cl_3 \cdot 2H_2O$: C, 21.7; N, 23.0; H, 5.62; Cl, 17.4; Ru, 16.6. Found: C, 21.4; N, 23.1; H, 5.27; Cl, 17.4; Ru, 16.5.

Compounds containing the (1-methylguanine)pentaammineruthenium(III) ion were isolated from solutions used in studying the kinetics of hydrolysis of the methylated nucleoside complexes. Compounds were also isolated from solutions of the (1-methylguanosine)pentaammineruthenium(III) complex in 2 *M* HCl heated at 56° for 2 days and from 1 *M* HCl solutions of the 2′-deoxy-1-methylguanosine complex heated at 56° for 2 hr. The solutions were purified by ion exchange on a 10-cm AG-50 column. A red band eluting with 1–2 *M* HCl was usually present in these solutions. A particularly large amount of this red complex determined to be chloro(1-methylguanosine)tetraammineruthenium(III) was isolated from a hydrolysate mixture using a solution prepared by the method usually employed for synthesizing the 1-methylguanosine complex—but not purified by ion exchange. *Anal.* Calcd for $[m^1Guo(NH_3)_4RuCl]Cl_2 \cdot 2H_2O$: C, 21.7; N, 20.7; H, 5.13; Cl, 17.5; Ru, 16.6. Found: C, 21.3; N, 21.0; H, 4.95; Cl, 17.6; Ru, 16.6. Ionic chloride calcd, 11.6; found, 11.6.

The partially protonated 1-methylguanine complex was eluted by 3–4 *M* HCl. The fraction containing this complex was then roto-evaporated to dryness. It was redissolved in a solution of $NaBF_4$ and ethanol was added to induce crystallization. *Anal.* Calcd for $[m^1Gua(NH_3)_5Ru](BF_4)_3 \cdot {}^1/_2H_2O$: C, 11.6; N, 22.5; H, 3.73. Found: C, 11.4; N, 22.5; H, 3.53.

Determination of Acid Association Constants. The values of pK_a were determined spectrophotometrically for the ruthenium(III) species using the relation

$$pK_a = pH + \log \frac{A' - A}{A - A''}$$

where A' is the absorbance of the protonated species, A'' is the absorbance of the deprotonated species, and A is the absorbance of a mixture of both species at a given pH. All absorbance readings were taken on solutions of the same concentration of the complex in 0.1 *M* LiCl. The pH was varied by adding a fraction of a drop of 0.1 *M* HCl or 0.1 *M* LiOH and the pH was determined before and after the absorbance reading. At least six values of the pK_a were determined in this fashion.

Values of pK_a for the ruthenium(II) species were determined using the relation

$$pK_a(II) = \Delta E/59 + pK_a(III)$$

In this equation $pK_a(II)$ refers to the loss of a proton from the Ru(II) species, $pK_a(III)$ is the spectrophotometrically determined pK_a value for loss of a proton from the Ru(III) species; ΔE is the difference (in millivolts) between the potential of the Ru(III)–Ru(II) couple when both complexes are in the protonated forms and when they are both in the deprotonated forms. Electrochemical values were determined by cyclic voltammetry at a sweep rate of 100–200 mV/sec in buffer solutions adjusted to an ionic strength of 0.1 with a LiCl stock solution. The media used were hydrochloric acid (pH 1–2), glycine–hydrochloric acid (pH 2–3.5), sodium acetate–acetic acid (pH 3.8–5.5), dibasic–monobasic sodium phosphate (pH 6–8), tris(hydroxymethyl)aminoethane (Sigma Chemical Co.)–hydrochloric acid (pH 7.5–8.5), glycine–lithium hydroxide (pH 9–10.5), and lithium hydroxide (pH 11–13).[12]

Kinetic Measurements. All solutions were adjusted to an ionic

(10) A. D. Broom, L. B. Townsend, J. J. Jones, and R. K. Robins, *Biochemistry*, 3, 494 (1964).

(11) R. G. Gaunder, Ph.D. Thesis, Stanford University, 1969.

(12) G. Gomori, "Methods in Enzymology," Vol. I, S. P. Colowick and W. O. Kaplan, Ed., Academic Press, New York, N. Y., 1955, p 138.

strength of 1.0 with LiCl and were equilibrated overnight at 56.0° in a thermostated water bath. Reactant solutions sampled over extended periods were sealed in glass ampoules; otherwise the solutions were kept in tightly stoppered glass vessels. Solutions of the free ligands were sampled by taking aliquots and diluting them with an appropriate NaOH solution to give a pH of approximately 12. The decrease in absorbances at 258 nm for 1-methylguanosine and at 256 nm for 2′-deoxymethylguanosine were measured. The slope of log $(A - A_\infty)$ *vs.* time as determined by a linear least-squares treatment using a 90% confidence interval yielded the pseudo-first-order rate constant k_{obsd}[4]. Usually at least 20 points were taken and the plots were linear over at least 3.5 half-lives.

The hydrolysis of the (2′-deoxy-1-methylguanosine)pentaammineruthenium(III) complex was followed by diluting an aliquot of the reactant solution with an appropriate phosphate buffer so that the resulting solution had a pH of 7.8–8.0 and immediately recording the spectrum. The first-order specific rate was then determined by observing the decrease in absorbance at 380 nm and determining the slope of log $(A_\infty - A)$ *vs.* time. In the case of the (1-methylguanosine)pentaammineruthenium(III) ion, absorbance readings were made at 380, 570, and 660 nm on both the reactant solution and aliquots diluted with an appropriate buffer to pH 7.8–8.0. The necessary simultaneous equations were then solved to give the concentrations of starting material and the hydrolyzed product (1-methylguanine)pentaammineruthenium(III). The observed pseudo-first-order rate constants for loss of sugar from the complex were determined by a nonlinear least-squares computer fit to the equation derived in the Appendix. Whenever possible k_{obsd}[1] and k_{obsd}[3] were determined directly by observing the decrease in absorbance in the reactant solution at 573 nm. When this was not feasible the value was given by the computer least-squares fit. Due to the loss of ammonia from these complexes a gradual shift in the visible band toward higher energy became evident after approximately 10–12 days, and no points were taken at $t > 2$ weeks.

Results

Spectra. The spectrophotometric properties of the various ruthenium–guanine complexes are summarized in Table I. The spectrum of (1-methylguanine)pentaammineruthenium(III) shown in Figure 2 is typical of the various complexes in their neutral and protonated ligand forms. It must be noted that because deprotonation is affected by methyl substitution, the spectra do vary considerably at pH >5. Characteristic of the spectra for the neutral ligands is a broad absorption band (width at half-height $\sim 5 \times 10^3$ cm^{-1}) in the visible range having an extinction coefficient (M^{-1} cm^{-1}) of several hundred. When the charge on ruthenium(III) is decreased by substituting a chloride for an ammonia or when the guanine ligand is protonated, this band shifts to higher energy. In complexes which are able to lose a proton from the guanine ligand, the band moves to lower energy as the concentration of acid is decreased. At pH 8 the (1-methylguanine)pentaammineruthenium(III) ion exhibits this band at lower energy with approximately a threefold increase in intensity. In the spectrum of (guanosine)pentaammineruthenium(III) at pH 10, this band is shifted toward lower energy by the same amount (Table II) but with no increase in oscillator strength.

A second higher energy band is evident in the spectra of the Ru(III) complexes listed in Table I. This band occurs at approximately 320 nm for all except the chloro(1-methylguanosine)pentaammineruthenium(III) where it appears at 335 nm. When the 1,9-dimethylguanine complex is protonated by placing it in 9 *M* HCl, the band at 329 nm disappears and a band forms at 302 nm. In the spectrum of the 1-methylguanine complex at pH 8, this band shifts to lower energy (*cf.* Table I) and increases in intensity. Other complexes show no significant change in either the intensity or energy of this band at higher pH.

The high energy bands in the uv portion of the spec-

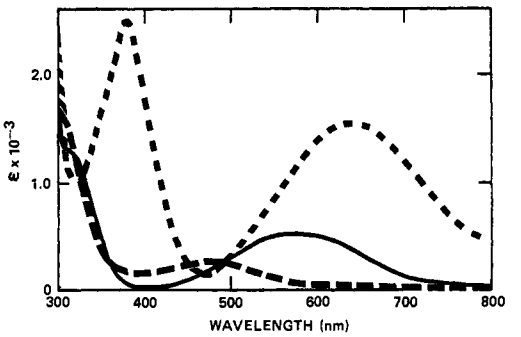

Figure 2. Visible spectra of $(m^1Gua)(NH_3)_5Ru^{III}$: solid line pH 2.5, 0.1 *M* LiCl (neutral ligand); short dashed line pH 7.9, 0.1 *M* LiCl (deprotonated ligand); long dashed line 9 *M* HCl (protonated ligand).

Table I. Spectra of Ruthenium–Guanine Complexes in 0.1 *M* LiCl[a,b]

	Ru(III)		Ru(II)	
Ligand	λ_{max}	$\epsilon_{max} \times 10^{-3}$ (M^{-1} cm^{-1})	λ_{max}	$\epsilon_{max} \times 10^{-3}$ (M^{-1} cm^{-1})
1,9-Dimethylguanine[c]	249	11.3	254	13.0
	274 (i)	7.8	262 (i)	12.0
	329	1.46	364	2.0
	579	0.566		
2′-Deoxy-1-methyl-	256	12.1	254	15.0
guanosine[c]	275 (i)	9.0	363	2.2
	320 (s)	1.3		
	580	0.487		
1-Methylguanosine[c]	257	11.9	255	14.0
	280 (i)	8.2	360	1.9
	319	1.18		
	575	0.466		
1-Methylguanosine[c,d]	252	13.2	254	15.0
	275 (i)	9.4	350	2.3
	335	2.07		
	536	0.247		
Guanosine[c]	252	13.2	252	13.0
(pH 4.0)	316	1.26	365	2.1
	567	0.441		
Guanosine[e]	216	22.7	259	12.0
(pH 10.7)	257	9.22	350	2.2
	273	9.27		
	316	1.3		
	624	0.433		
1-Methylguanine[e]	247	11.6		
(pH 2.5)	273	7.76	248	13.1
	315 (s)	1.24	361	2.0
	576	0.531		
1-Methylguanine[e]	237 (i)	9.1	251	10.5
(pH 7.9 for Ru(III),	279	6.96	268	10.8
pH 11.2 for Ru(II))	378	2.47	350 (s)	1.9
	639	1.56		
Guanosine 5′-mono-	251	12.6	251	15.6
phosphate[c]	321	1.26	370	2.4
(pH 4.0)	560	0.420		
Guanosine 5′-mono-	215	22.4		
phosphate[e]	257	8.3		
(pH 10.4)	274	8.8		
	318	1.25		
	615	0.391		

[a] Pentaammineruthenium except where superscript (*d*) appears. [b] Key: (i) represents an inflection point and (s) a shoulder. [c] Neutral guanine ring. [d] The inorganic radicals here are chlorotetraammineruthenium(III) and tetraammineaquoruthenium(II). [e] Guanine ligand is deprotonated.

Table II. Ligand-to-Metal Charge Transfer Band Energies, $\mu = 0.1$

IIa Visible Band Energies[a]

Ligand	pH	$10^{-4}\nu_m$, cm^{-1}	$10^{-4}\Delta\nu_m$, cm^{-1}	Width at half-height ($cm^{-1} \times 10^{-4}$)	Oscillator strength ($\times 10^2$)
1-Methylguanine	2.5	1.74	0.17	0.52	1.27
1-Methylguanine	7.9	1.56		0.47	3.50
Guanosine	4.0	1.76	0.16	0.52	1.05
Guanosine	10.0	1.60		0.58	1.09

IIb Near-Ultraviolet Band Energies[b]

Ligand	pH	$10^{-4}\nu_m'$, cm^{-1}	$10^{-4}\Delta\nu_m'$, cm^{-1}	$10^4(\nu_m' - \nu_m)$, cm^{-1}
1-Methylguanine	2.5	3.17	0.53	1.43
1-Methylguanine	7.9	2.64		1.08
Guanosine	4.0	3.16	0	1.40
Guanosine	10.0	3.16		1.56

[a] Attributed to a $\pi \rightarrow t_{2g}$ charge transfer. [b] Attributed to a $\pi' \rightarrow t_{2g}$ charge transfer where π' is an orbital of lower energy than π.

trum are similar in position and intensity to those of the free ligand. For example, neutral 1-methylguanine has absorption maxima (extinction coefficient) at 249 nm (10.2×10^3) and 273 nm (8.1×10^3) and the monoanion absorbs at 277 nm (8.1×10^3). These values are quite similar to those listed in Table I for the (1-methylguanine)pentaammineruthenium(III) ion and are attributed to $\pi \rightarrow \pi^*$ transitions on the ligand.[13] At high pH the guanosine and guanosine 5′-monophosphate complexes have an additional peak at 215 nm that does not exist as a distinct absorption in the free ligands.

The ruthenium(II) guanine complexes exhibit a broad band around 360 nm in addition to the ligand peaks in the uv. This band appears to shift to higher energy (350 nm) at pH 11 for the (guanosine)pentaammineruthenium(II) complex.

Electrochemistry. Formal potentials for the various ruthenium(III–II) guanine couples are summarized in Table III. Potential measurements were made by

Table III. Formal Reduction Potentials of Ruthenium(III–II)–Guanine Complexes, $\mu = 0.1$

Ligand	E_f (mV) *vs.* nhe	Supporting electrolyte
Chloro-1-methylguanosine	−160	0.01 *M* HCl, 0.09 *M* LiCl
1-Methylguanine	152	0.01 *M* HCl, 0.09 *M* LiCl
1-Methylguanine	−73	Glycine–LiOH buffer pH 10.5
1,9-Dimethylguanine	158	0.01 *M* HCl, 0.09 *M* LiCl
2′-Deoxy-1-methylguanosine	181	0.01 *M* HCl, 0.09 LiCl
Guanosine	190	Acetate–acetic acid buffer pH 5.9
Guanosine	110	Glycine–LiOH buffer pH 9.5
1-Methylguanosine	198	0.01 *M* HCl, 0.09 *M* LiCl

cyclic voltammetry and the formal potentials were taken to be at a point half the distance between the anodic and cathodic peaks. Except for the inner sphere chloride complex, the peak separations were independent of sweep rate and were somewhat larger than the theoretical value of 58 mV, but, since the hexaammine couple, used as a standard, gave a peak separation in the same range, we have no reason to believe that the couples are not reversible. The chloro(1-methylguanosine)pentaammineruthenium(III) ion appeared to decompose upon reduction, presumably by loss of a chloride.

The positive potentials between 150–200 mV indicate some degree of stabilization of the ruthenium(II) entity by the purine ligand relative to the potential for the hexaammineruthenium(II–III) couple (51 mV).[14] The negative potential of the chloro(1-methylguanosine)-pentaammineruthenium(II–III) couple indicates stabilization of the ruthenium(III) entity by the negative ion in the inner sphere. This potential can be compared to that for the chloropentaammineruthenium-(II–III) couple of −42 mV.[14]

pK_a values as determined by spectrophotometry and cyclic voltammetry are summarized in Table IV. There appeared to be some decomposition of the

Table IV. pK_a Measurements at 25°

Ligand	Probable deprotonation site	Ru(III)	Ru(II)
1-Methylguanine	N_9	5.44 ± 0.08[a,b,f]	9.2 ± 0.1[b,f]
1-MethylguanineH$^+$	C_2–NH_2, N_3	-0.38 ± 0.01[a,g]	
Guanosine	N_1	7.36 ± 0.05[a,b,f]	8.7 ± 0.2[b,f]
GuanosineH$^+$	C_2–NH_2, N_3	~ -1.3[a,g]	
Free Ligands			
1-Methylguanine	N_7, N_9	10.5[c]	
1-MethylguanineH$^+$	N_7	3.1[c]	
GuanineH$_2^{2+}$	C_2–NH_2, N_3	−1.3[d]	
Guanosine	N_1	9.5[c]	
GuanosineH$^+$	N_7	1.9[e]	
GuanosineH$_2^{2+}$	C_2–NH_2, N_3	−2.43[d]	
1-MethylguanosineH$^+$	C_2–NH_2, N_7	2.2[e]	

[a] Measured spectrophotometrically. [b] Measured by cyclic voltammetry. [c] Reference 13. [d] Reference 15. [e] Reference 1. [f] $\mu = 0.1$. [g] $\mu = 1.0$.

(guanosine)pentaammineruthenium(III) ion at pH >9 as evidenced by a time-dependent increase in absorption in the 700–800 nm range and a concomitant loss of the isosbestic point at 596 nm in the determination of pK_a for the species. Addition of a pentaammineruthenium(III) ion appears to have a profound effect on the acidity of the purine moiety. Adding pentaammineruthenium(III) enhances the acidity of neutral 1-methylguanine by approximately five orders of magnitude, of guanosine by two orders of magnitude, and of the monoprotonated cationic guanine species by more than three orders of magnitude.

The effects of the pentaammineruthenium(II) ion on the acidity of the neutral guanine are not nearly so pronounced. Addition of it to 1-methylguanine increases the ionization constant by a factor of 18 while its effect on guanosine is less than 10.

Kinetics. The kinetics for the loss of the sugar moiety from the (1-methylguanosine)pentaammineruthenium(III) complex is complicated by the dissocia-

(13) J. H. Lister, "Purines, The Chemistry of Heterocyclic Compounds," A. Weissberger and E. C. Taylor, Ed., Wiley-Interscience, New York, N. Y., 1971, p 493.

(14) H. S. Lim, D. J. Barclay, and F. Anson, *Inorg. Chem.*, 11, 1460 (1972).

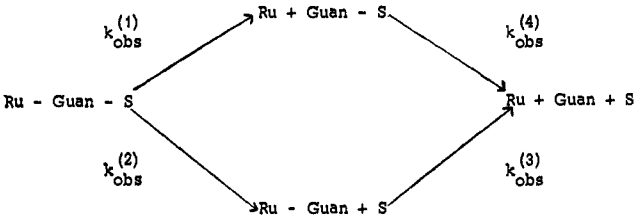

Figure 3. Reaction scheme for Ru(III) nucleosides.

tion of the purine from the ruthenium (see Figures 3 and 4). The expression for this dissociation is given by

$$\text{rate} = k_{obsd}[S] \quad (1)$$

$$k_{obsd}^{(1,3)} = k_0 + [H^+]k_1 \quad (2)$$

where $k_0 = 6.3 \pm 0.6 \times 10^{-7}$ sec^{-1} and $k_1 = 9.6 \pm 0.6 \times 10^{-7}$ M^{-1} sec^{-1} for the 1-methylguanosine complex and $k_0 = 3.8 \times 0.5 \times 10^{-7}$ sec^{-1} and $k_1 = 1.3 \pm 0.2 \times 10^{-6}$ M^{-1} sec^{-1} for the 1-methylguanine complex. Since the rates corresponding to the $k_{obsd}^{(1)}$ and $k_{obsd}^{(3)}$ terms are much slower than the rate of hydrolysis of the sugar–purine bond in the 2′-deoxy-1-methylguanosine complex, these terms can be neglected at low pH and the data treated as simple first-order kinetics.

The hydrolysis of free purine nucleosides is known to be hydronium ion catalyzed with rates given by eq 3,[15,16] where $k = 1.8 \pm 0.2 \times 10^{-4}$ M^{-1} sec^{-1} for 1-

$$k_{obsd}^{(2,4)} = [H^+]k \quad (3)$$

methylguanosine and $k = 0.12 \pm 0.01$ M^{-1} sec^{-1} for 2′-deoxy-1-methylguanosine. These hydrolysis rates are decreased by a factor of 30 when the pentaammineruthenium(III) ion is present at N_7. The rates $k_{obsd}^{(2)}$ are of the form of eq 3 and $k = 5.0 \pm 0.5 \times 10^{-6}$ M^{-1} sec^{-1} for the (1-methylguanosine)pentaammineruthenium(III) complex and $k = 4.0 \pm 0.2 \times 10^{-3}$ M^{-1} sec^{-1} for the (2′-deoxy-1-methylguanosine)pentaammineruthenium(III) ion (Table V).

Discussion

Structure. The similarity of the spectra of the Ru(III) complexes of the neutral ligands, a similarity that embraces both energies and extinction coefficients, makes it likely that there is a common binding site for the metal in all of the complexes. If this proposition is accepted, N_1 and N_9 are excluded as binding sites because those positions in some of the complexes are preempted by carbon-containing radicals. In studies with xanthine derivatives,[16] it has been found that substitution of CH_3 at N_3 prevents the binding of Ru to N_9, and thus substitution at N_9 is expected to prevent Ru binding at N_3. The spectra referred to above, it should be noted, are rather similar to those obtained with 1,3,-9-trimethylxanthine as ligand,[16] and ruthenium can coordinate to this only at N_7. On the basis of the arguments advanced we conclude that the binding site for ruthenium for the series is at N_7.

It should be noted that on the basis of other experience[17] with oxygen derivatives as ligands, the bonding of pentaammineruthenium(II) to phosphate is expected to be both labile and unstable. This does not preclude the formation of ruthenium–phosphate bonds. Such combinations could arise when Ru(II) is used as a catalyst for the formation of Ru(III)–phosphate complex—but phosphate complexes are not expected to have spectra of the kind which are observed here (and are not in question for the present work).

Space-filling models also indicate that the C_6=O group should interact with ammonia ligands cis to the purine. Marzilli[18] has already noted a hydrogen bonding interaction between an ammine nitrogen bound on a cobalt(III) atom on N_7 and the C_6=O group. A steric interaction between the C_6=O and the pentaammineruthenium groups could be responsible for the labilization of an ammonia and subsequent substitution of a chloride in the 1-methylguanosine complex.

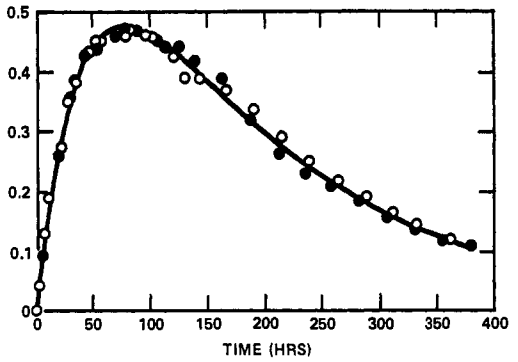

Figure 4. $[m^1Gua(NH_3)_5Ru^{III}]/[m^1Guo(NH_3)_5Ru^{III}]_0$ *vs.* time in 1.0 *M* HCl at 56°. Solid line calculated from least-squares computer fit to equation derived in the Appendix.

Table V. Observed Rate Constants, $\mu = 1.0$, $T = 56°$

$m^1Guo(NH_3)_5Ru^{III}$			
[H$^+$]	$10^6k_{obsd}^{(1)}$, sec^{-1}	$10^6k_{obsd}^{(2)}$, sec^{-1}	$10^6k_{obsd}^{(3)}$, sec^{-1}
1.00	1.57	4.94	1.66
1.00	1.61	4.92	1.68
0.40	1.04	2.18	1.02
0.10	0.672	0.467	0.486
0.01	0.677	0.06 (est)	0.353

1-Methylguanosine	
[H$^+$]	$10^4k_{obsd}^{(4)}$, M^{-1} sec^{-1}
1.00	1.76
1.00	1.65
0.21	0.398

$(m^1dGuo)(NH_3)_5Ru^{III}$	
[H$^+$]	$10^4k_{obsd}^{(2)}$, M^{-1} sec^{-1}
0.204	7.83
0.051	2.00
0.010	0.424

2′-Deoxy-1-methylguanosine	
[H$^+$]	$10^4k_{obsd}^{(4)}$, M^{-1} sec^{-1}
0.01	12.1
3.1×10^{-3} [a]	3.59

[a] Formate buffer.

(15) (a) J. A. Zoltewicz, P. F. Clark, T. W. Sharpless, and G. Grahe, *J. Amer. Chem. Soc.*, **92**, 1741 (1970); (b) N. K. Kochetkov and E. I. Budovskii, "Organic Chemistry of Nucleic Acids," Plenum Press, London, 1971, Chapter 8.

(16) M. J. Clarke and H. Taube—work to be submitted for publication.

(17) J. Stritar and H. Taube, *Inorg. Chem.*, **8**, 2281 (1969).

(18) L. G. Marzilli, T. J. Kistenmacher, and C. H. Chang, *J. Amer. Chem. Soc.*, **95**, 7507 (1973).

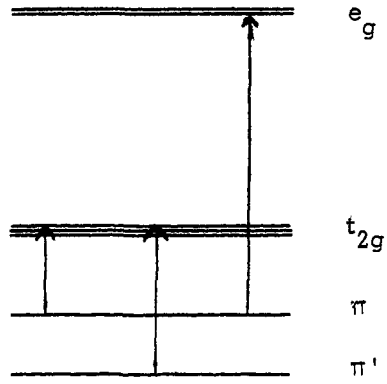

Figure 5. Energy level diagram.

Substitution of a methyl group for a proton at N_1 on guanine enhances the solubility of the ligand and tends to prevent gel formation of the ruthenium–guanine complexes when they are precipitated from solution.[19] Remarkably the guanosine 5′-monophosphate complex, which is not alkylated at N_1, formed crystals on slow precipitation. Concentrated solutions of 1,9-alkylated guanine complexes readily formed crystals on cooling.

pK_a Values. Addition of the pentaammineruthenium(III) ion at N_7 of guanine has a marked effect on the acidity of the neutral and monoprotonated guanine species. The most acidic site on guanine itself is thought to be N_1,[1] but the effect of a ruthenium(III) ion at N_7 is to cause the N_9 proton to ionize more easily by several orders of magnitude. This is undoubtedly due to the effect of a tripositive ion situated close to the N_9 site. The effect on the more distant N_1 site is much less pronounced but the ionization constant is still approximately 100 times more acidic. The ionization constants for loss of a proton from the monoprotonated cationic guanine species are also affected by addition of a Ru(III) to the purine. The ruthenium atom blocks N_7, the most basic site on the guanine ring, and forces protonation at the N_3 or exocyclic amine sites. This coupled with the inductive effect of the tripositive ion increases the acidity of these species by over three orders of magnitude.

The effect of the pentaammineruthenium(II) ion on the acidity of the guanine is diminished on two counts. First, because of its lower charge, it has a smaller inductive effect, and, secondly, it tends to back-donate electron density into the π system of the purine ring.

The effect of the pentaammineruthenium(III) ion on the hydrogen bonding and ring-stacking properties of guanosine is pertinent to the general effect of metal ions on the stability of polynucleotides as well as to the potential usefulness of ruthenium as a base-specific label. Bonding of ruthenium(III) ammine at N_7 can affect the base pairing of guanosine in two ways: first, the cis-aquo or ammine ligands would be expected to hydrogen bond to the adjacent carbonyl oxygen and weaken its ability to bond with cytosine;[18] secondly, the metal ion at N_7 increases the acidity of the N_1 proton causing it to be partially ionized at neutral pH. The pK of cytosine for accepting a proton at N_3 is approximately 4[1] and it is by no means certain that the cytosine would accept the N_1 proton from guanosine to form an internal cation–anion pair. A metal ion bound to N_7 would also polarize the π-electron cloud of guanosine which might be expected to affect its tendency toward ring stacking with other aromatic systems. In all the effects mentioned, ruthenium(II) would differ significantly from ruthenium(III).

Spectra. The broad absorption band exhibited by the ruthenium(III) complexes in the visible portion of the spectrum (~570 nm) is believed to be a charge transfer from a π orbital in the guanine to a t_{2g} level on the ruthenium.[20] Supporting this conclusion is the shift in absorption maximum of this band when a negative ion is also bound to the metal center. This decreases its effective charge, thus shifting the absorption to shorter wavelengths, as in the spectrum of the chloro(1-methylguanosine)pentaammineruthenium(III) ion. In harmony with this interpretation, the band is observed to shift to higher energy when a positive charge is placed on the purine by protonation and to lower energy when a negative charge is left on the purine by deprotonation (Figure 2).

The band at 335 nm (ϵ 2.07 × 10^3) in the spectrum of the chloro(1-methylguanosine)pentaammineruthenium(III) ion is thought to be a charge transfer from the chloride to the ruthenium(III) analogous to the charge transfer band at 327 nm (ϵ 1.90 × 10^3) for the chloropentaammineruthenium(III) ion.[21] The other complexes exhibit a partially resolved band or shoulder around 320 nm which can be attributed to a purine-to-ruthenium charge transfer. Evidence for this is its shift to higher energy on protonation of the guanine and shift to lower energy and increase in intensity on deprotonation of the N_9 site in the case of the 1-methylguanine complex.

This second higher energy ligand-to-metal charge transfer band (ν_m') could be either a $\pi \rightarrow e_g$ or a $\pi' \rightarrow t_{2g}$ transition where π' represents a ligand orbital of lower energy than π (Figure 5). If there are no back-bonding interactions, the ligand field strength of a "pyridine" ligand is approximately that of ammonia so that the local symmetry around the Ru(III) can be considered to be octahedral.[22] The Δ value for the $t_{2g} \rightarrow e_g$ orbital splitting for hexaammineruthenium(II) has been calculated as 2.71 × 10^4 cm^{-1} [23] and that for ruthenium(III) is expected to be even greater. Since the separation between the higher and lower energy (ν_m) charge transfer bands (Table II, $\nu_m' - \nu_m$) is only 1.4 × 10^4 cm^{-1}, it seems unlikely that ν_m' is a $\pi \rightarrow e_g$ transition. The more likely interpretation is that this transition is from a lower lying π level on the purine. This would account for the difference in the spectra when either the N_1 or N_9 protons are removed from the purine ring since the various molecular orbitals on the purine would be perturbed in different ways by loss of a proton at nonequivalent sites. If both charge transfers were from the same π level on the ligand, the expectation would be that they would shift the same amount on de-

(19) J. F. Chanlot and W. Guschlbauer in "The Purines—Theory and Experiment," The Jerusalem Symposium on Quantum and Biochemistry, IV, the Israel Academy of Sciences and Humanities, Jerusalem, 1972, p 205.

(20) A. B. P. Lever, "Inorganic Electronic Spectroscopy," Elsevier, New York, N. Y., 1968, pp 124, 224–248.

(21) J. N. Armor, Ph.D. Thesis, Stanford University, 1970.

(22) C. K. Jorgensen, "Absorption Spectra and Chemical Bonding In Complexes," Pergamon Press, London, 1962, p 109.

(23) H. H. Schmidtke and D. Garthoff, *Helv. Chim. Acta*, **49**, 2039 (1966).

protonation, *i.e.*, $\Delta\nu_m'$ would equal $\Delta\nu_m$ for the same ligand.

The band located around 360 nm in the spectra of the ruthenium(II) guanine complexes is considered to be a metal-to-ligand charge transfer from a filled metal t_{2g} orbital to an empty π^* orbital on the purine. Evidence for this is its shift to higher energy (350 nm) on deprotonation of the guanosinepentaammineruthenium(II) complex. Since the N_7 site to which the ruthenium is bound can be considered to be a "pyridine" nitrogen, the charge transfer becomes analogous to those observed by Ford and Gaunder for a series of ruthenium(II)–pyridine complexes and assigned as $t_{2g} \rightarrow \pi^*$ transitions.[24] Other investigators have noted the correlation between metal-to-ligand back-donation, reduction potential of the complex, reducibility of the ligand, and energy of this sort of charge transfer transition[14,22,25] In general, the more available the ligand π^* orbitals are toward accepting an electron from Ru(II), the more Ru(II) is stabilized relative to Ru(III) and the lower the energy of the charge transfer bands. The reduction potential of the complexes reported here compared to those for the hexaammine couple and for the pyridine–pentaammine couple indicate that neutral guanine ligands are capable of accepting electron back donation from Ru(II) but not to the extent that pyridine itself does.

Kinetics

The kinetics of the hydrolysis of the 1-methylguanosinepentaammineruthenium(III) complex indicate that the Ru–N_7 bond cleaves by both an acid-independent and an acid-dependent pathway (Figures 3 and 4). The acid-independent pathway is analogous to the loss of ammonia from hexaammineruthenium(III),[21] which loses ammines at a rate independent of the acid concentration at room temperature. That the acid-independent rate for dissociation is smaller for the 1-methylguanine complex than for 1-methylguanosine is due to the fact that 1-methylguanine is a slightly better base than 1-methylguanosine as evidenced by their pK_a values in Table IV.

The pathway for the hydrolysis of the sugar–purine bond in the ruthenium–nucleoside complexes involves protonation of the nucleoside as does the hydrolysis of the free nucleosides.[15a] Zoltewicz has proposed a scheme for the acid catalyzed hydrolysis of purine nucleosides involving initial protonation at N_7 or diprotonation at N_7 and N_3 (C_2–NH_2). This step is followed by the rate determining cleavage of the N_9–C bond. The presence of a ruthenium atom at N_7 precludes protonation at this site and forces protonation at N_3 or the exocyclic amine. Since the hydrolysis rates for the free ligands are considerably greater than those for the corresponding ruthenium complexes, it is apparent that involving a proton in this way is less effective than protonation at the N_7 site.

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Appendix

The expression for the rate of change of $[m^1Guo(NH_3)_5Ru^{III}]$ is

$$\frac{d[Ru\text{-}G\text{-}S]}{dt} = k_{obsd}^{(1)}[Ru\text{-}G\text{-}S] - k_{obsd}^{(2)}[Ru\text{-}G\text{-}S]$$

$$[Ru\text{-}G\text{-}S] = [Ru\text{-}G\text{-}S]_0 e^{-(k_{obsd}^{(1)} + k_{obsd}^{(2)})t}$$

For $[m^1Guo(NH_3)_5Ru^{III}]$ the expression is given by

$$\frac{d[Ru\text{-}G]}{dt} = k_{obsd}^{(2)}[Ru\text{-}G\text{-}S] - k_{obsd}^{(3)}[Ru\text{-}G]$$

$$\frac{d[Ru\text{-}G]}{dt} + k_{obsd}^{(3)}[Ru\text{-}G] = k_{obsd}^{(2)}[Ru\text{-}G\text{-}S]_0 e^{-(k_{obsd}^{(1)} + k_{obsd}^{(2)})}$$

This first-order linear differential equation has the solution[26,27]

$$\frac{[Ru\text{-}G]}{[Ru\text{-}G\text{-}S]_0} = \frac{k_{obsd}^{(2)}}{k_{obsd}^{(3)} - k_{obsd}^{(1)} - k_{obsd}^{(2)}}[e^{-(k_{obsd}^{(1)} + k_{obsd}^{(2)})t} - e^{k_{obsd}^{(3)}t}]$$

(24) P. Ford, D. F. P. Rudd, R. Gaunder, and H. Taube, *J. Amer. Chem. Soc.*, **90**, 1187 (1968).

(25) A. M. Zwickel and C. Creutz, *Inorg. Chem.*, **10**, 2395 (1971).

(26) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," Wiley, New York, N. Y., 1965, p 166.

(27) NOTE ADDED IN PROOF. Values of pK_a for the pentaammineruthenium(II)–guanosine and 1-methylguanine complexes have been determined spectrophotometrically and are 9.0 ± 0.2 and 8.9 ± 0.1, respectively.