Binding of a Tetraammineruthenium Complex to Imidazole and Purine Derivatives under Equilibrium Conditions

Gilbert M. Brown, James E. Sutton, and Henry Taube*

Contribution from the Department of Chemistry, Stanford University, Stanford, California 94305. Received April 30, 1977

Abstract: Using a kinetic method, the equilibrium constants for the reactions $trans$-$Ru(NH_3)_4(SO_3)H_2O$ + L = $trans$-$Ru(NH_3)_4SO_3L$ + H_2O have been measured as $\geq 46 \times 10^3$, 11.8×10^3, 1.4×10^3, 1.1×10^3, 0.76×10^3, and 8 ± 7 for L = 1-methylimidazole, imidazole, 1.9-dimethylguanine, 1-histidine, 1-methylguanosine, and adenosine, respectively (25 °C, μ = 0.1, pH 8.63 except for 1-histidine where it was 8.11). The systems are quite labile, and $t_{1/2}$ for approach to equilibrium in a typical case (1-methylguanosine at 4.1×10^{-3} M) is 3.3 s. The equilibrium can be frozen by oxidizing Ru(II) to Ru(III) using H_2O_2 at a pH of 3.8 or lower. In this operation, the sulfito complex is converted to the sulfato; $trans$-$Ru(NH_3)_4(SO_4)L^+$ can in turn readily be converted to $trans$-$[Ru(NH_3)_4(I)L]^{2+}$ by using Ru(II) as a catalyst for the replacement of SO_4^{2-} by I^-. Titration of $trans$-$[Ru(NH_3)_4(SO_2)(H_2O)]^{2+}$ with base using slow sweep cyclic voltammetry to follow the potential of the Ru(III)·S(IV)–Ru(II)·S(IV) couple shows that S(IV) is bound to Ru(III) as SO_3^{2-} over the whole pH range covered (0.3–7.6) and confirms, within the limits of experimental error, the published values[7] for the two acid dissociation constants of S(IV) bound to Ru(II) (pK_a = 1.90 and 5.05 in present work). $E_{1/2}$ for the couple $[trans$-$Ru(NH_3)_4(SO_3)H_2O]^{+/0}$ is 0.3 V vs. NHE (μ = 1.00). A direct measure of the rate of hydration of the SO_2 complex shows that coordination of SO_2 to Ru(II) decreases the rate of hydration by a factor of approximately 10^5.

Pentaammineruthenium has been shown to form stable complexes with a variety of nitrogen heterocycles in both the Ru(II) and Ru(III) oxidation states.[1] Complexes of pentaammineruthenium with imidazole,[2,3,4] guanine,[5] and xanthine[6] derivatives have been described recently. These complexes are substitution inert, and have made possible some detailed studies of the effects of metal ion coordination on the properties of the ligands.

Pentaammineaquoruthenium(II) shows a high equilibrium selectivity for unsaturated nitrogen sites in reactions involving substitution of coordinated H_2O, but because the reactions are slow, the product distribution for most of the pentaammineruthenium(II) complexes of nitrogen heterocycles thus far studied appear to be kinetically controlled. Some substitution reactions of $trans$-tetraammineaquo(sulfur dioxide)ruthenium(II) have been studied by Isied.[7] The coordinated sulfur(IV) takes the forms SO_2, HSO_3^-, and SO_3^{2-}, and in the latter state exerts a strong trans labilizing effect in Ru(II). This effect of sulfur(IV) makes possible the substitution of coordinated H_2O in $trans$-$Ru(NH_3)_4(SO_3)(H_2O)$ by various ligands as an equilibrium process. Isied[8] has also demonstrated that complexes of the type $trans$-$[Ru^{II}(NH_3)_4(SO_2)(L)]^{2+}$, where L is a derivative of pyridine or a related aromatic heterocycle, can be oxidized in strong acid to the corresponding substitution inert $trans$-sulfatotetraamminepyridineruthenium(III) complexes with hydrogen peroxide.

We find that the binding of the imidazole and purine derivatives shown below to $trans$-$[Ru(NH_3)_4(SO_3)(H_2O)]$ can be carried out as an equilibrium process and that the rapidly established equilibrium can be frozen by oxidizing the trans-labilized sulfur(IV) ruthenium(II) complex to a substitution inert sulfato ruthenium(III) complex.

Experimental Section

Materials. House-line distilled water was purified by redistillation from alkaline permanganate. Imidazole (MCB or Aldrich) was purified by recrystallization first from CH_2Cl_2/pentane and then from benzene. Isonicotinamide (Aldrich) was purified by recrystallization from hot water. Pyrazine was purified by sublimation. 1-Methylimidazole (Aldrich) was redistilled, collecting the fraction which boiled at 196 °C. 1-Histidine (MCB), adenosine (Aldrich), and 1,9-dimethylguanine (Fluka) were used without further purification. 1-Methylguanosine was prepared by the method of Broom et al.[9]
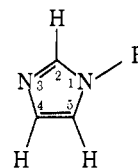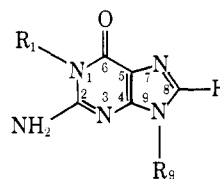

R = H (imidazole)
R = CH₃ (1-methylimidazole)
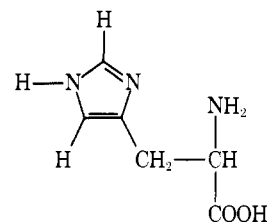
histidine

R_1 = R_9 = CH₃ (1,9-dimethylguanine)
R_1 = CH₃; R_9 = ribose (1-methylguanosine)
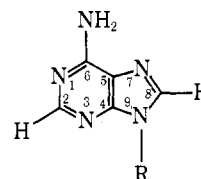
R = ribose (adenosine)

Tris(hydroxymethyl)aminomethane (Sigma 7–9 grade) was recrystallized from 95% ethanol. 2,6-Lutidine (MCB) was purified by distillation, collecting the 143–144 °C fraction. Hexamethylenetetraammine (Baker) was used as received. 1,4-Diazobicyclo[2.2.2]octane hydrotrifluoroacetate was prepared by the general method outlined by Quagliano et al.[10] Diazobicyclo[2.2.2]octane (Dabco) (10 g) was dissolved in 60 mL of acetone, warmed to 50 °C, and added

to a 50 °C solution of 7 mL of trifluoroacetic acid in 20 mL of acetone. The resulting solution was allowed to stand at 50 °C for 1 h, and the precipitate was collected by filtration and purified by reprecipitation from ethanol/ether. Because Dabconium trifluoroacetate is hygroscopic, it was stored in a vacuum desiccator over Drierite.

Standard solutions of HCl, NaOH, and Na$_2$S$_2$O$_3$ were prepared by dilution of Titrisol (E. Merck) ampules. Solutions of trifluoroacetic acid were prepared by diluting either trifluoroacetic anhydride (MCB) or the pure acid (MCB) with water and were standardized by titration with 0.100 N NaOH to a phenolphthalein end point. Solutions of trifluoromethanesulfonic acid were generously provided by Michael Willis; the acid had been purified by low-pressure distillation. Standard solutions of Cr(VI) were prepared by dissolving a weighed amount of primary standard potassium dichromate (Mallinckrodt) in water. Solutions of H$_2$O$_2$ were prepared by dilution of commercially available solutions. Hydrogen peroxide solutions were standardized by iodometric methods, using Mo(VI) as a catalyst for the reaction of H$_2$O$_2$ with I$^-$. A standard solution of Fe(III) in 0.4 M CF$_3$SO$_3$H was generously provided by Heinz Krentzien. All other chemicals were of reagent or spectroscopic quality and were used without further purification.

Preparation of Ruthenium Complexes. *trans*-[Ru(NH$_3$)$_4$(SO$_2$)-(Cl)]Cl was prepared from [Ru(NH$_3$)$_5$Cl]Cl$_2$[11] following the literature procedure.[12] Microanalyses were performed by the Stanford University Microanalytical Laboratory.

trans-Tetraammineaquo(sulfur dioxide)ruthenium(II) Tetrafluoroborate. One gram of freshly prepared *trans*-bis(bisulfito)tetraammineruthenium(II),[12] an intermediate in the preparation of *trans*-[Ru(NH$_3$)$_4$(SO$_2$)Cl]Cl, was dissolved at 50 °C in a minimum volume of 1 M HBF$_4$. The solution was filtered while still warm and an equal volume of 48% HBF$_4$ was added. This solution was placed in the freezer compartment of a refrigerator overnight, whereupon the product crystallized as very fine, light brown needles. It was collected by filtration, washed with ethanol and ether, and air dried. Additional product was obtained as a red-brown powder by adding ethanol to the filtrate. The combined yield was in excess of 60%. Anal. Calcd for [Ru(NH$_3$)$_4$(SO$_2$)(H$_2$O)](BF$_4$)$_2$: Ru, 23.8; N, 13.2; H, 3.32. Found: Ru, 24.2; N, 13.5; H, 3.42.

trans-Tetraammineimidazole(sulfur dioxide)ruthenium(II) Tetrafluoroborate. A solution containing 250 mg of imidazole (4.1 mmol) in 5–6 mL of water was degassed for 0.5 h with a stream of argon. To this solution 100 mg of [Ru(NH$_3$)$_4$(SO$_2$)Cl]Cl (0.33 mmol) was added, and the mixture was allowed to react under argon for 10 min. The solution was rapidly filtered in air and immediately acidified with 6 mL of 48% HBF$_4$. The resulting brown solution was put in a stoppered flask and cooled to −5 °C for 3 h. The product (red-brown) which crystallized was collected by filtering, washed with ethanol and ether, air dried, and further dried in a vacuum desiccator overnight, yield 71 mg (44%). Anal. Calcd for [Ru(NH$_3$)$_4$(SO$_2$)(imidazole)]-(BF$_4$)$_2$: C, 7.59; H, 3.40; N, 17.7. Found: C, 7.41; H, 3.36; N, 17.5. It should be noted that this preparation was irreproducible. If the desired product did not crystallize before the aquation of imidazole became significant, the solid obtained would be contaminated with [Ru(NH$_3$)$_4$(SO$_2$)(H$_2$O)](BF$_4$)$_2$ (light-brown crystals). We were able to prepare pure salts of neither (Ru(NH$_3$)$_4$(SO$_2$)(1-methylimidazole)]$^{2+}$ nor [Ru(NH$_3$)$_4$(SO$_2$)(histidine)]$^{2+}$ presumably because of the complication just described.

trans-Tetraammineimidazole(sulfato)ruthenium(III) Chloride. *trans*-[Ru(NH$_3$)$_4$SO$_2$Cl]Cl (200 mg) was dissolved in 3 mL of argon-degassed water and a tenfold excess of imidazole was added. HCl (6 M, 2 mL) was added turning the solution brown, and then 5 mL of 30% H$_2$O$_2$ was immediately added, resulting in a yellow solution. An excess of acetone was added and the solution allowed to stand in the freezer for 2 days. The product was collected by filtration, washed with acetone, and air dried, yield 200 mg (80%). The same product could be isolated by adding the H$_2$O$_2$ before the HCl, the solution now first turning pink, then yellow. Anal. Calcd for [Ru(NH$_3$)$_4$(SO$_4$)(imidazole)]Cl: C, 9.77; H, 4.37; N, 22.79. Found: C, 9.96; H, 4.50; N, 22.59.

trans-Tetraammineimidazoleiodoruthenium(III) Iodide. *trans*-[Ru(NH$_3$)$_4$SO$_4$(imidazole)]Cl was dissolved in a small amount of water and the resulting solution was saturated with KI. A small piece of zinc amalgam was introduced into the solution until the color became blue, whereupon it was removed. A blue crystalline product slowly formed which was collected by filtration, washed with ethanol, and then dried in a vacuum desiccator overnight. Anal. Calcd for

[Ru(NH$_3$)$_4$(I)(imidazole)]I$_2$: C, 5.83; H, 2.61; N, 13.60. Found: C, 6.03; H, 2.83; N, 13.57.

trans-Tetraammine(1,9-dimethylguanine)iodoruthenium(III) Iodide. *trans*-[Ru(NH$_3$)$_4$SO$_4$(1,9-dimethylguanine)]Cl was prepared using the same procedure as for [Ru(NH$_3$)$_4$SO$_4$(imidazole)]Cl but substituting 1,9-dimethylguanine for imidazole. The crude product was isolated and 50 mg of this solid was dissolved in 2 mL of water. The procedure for preparing [Ru(NH$_3$)$_4$I(imidazole)]I$_2$ was then followed, yield 6 mg (~10%). Anal. Calcd for [Ru(NH$_3$)$_4$I(1,9-dimethylguanine)]I$_2$: C, 11.53; H, 3.09; N, 16.57. Found: C, 11.53; H, 2.90; N, 17.29.

Rate Measurements. The rates of substitution were measured under pseudo-first-order conditions (ligand in excess) in buffered solution with the ionic strength adjusted to 0.10 M using NaCl. Slower reactions were followed by monitoring the change in absorbance on Cary Model 14 or Model 15 spectrophotometers. The temperature was controlled to within ±0.2 °C in the spectrophotometer cell compartments. Solutions of ligand and buffer were degassed and thermostated at 25.0 °C in a cell fitted with a rubber septum cap. The reactions were initiated by syringing in a small amount (0.25–0.50 mL) of a degassed solution of Ru(NH$_3$)$_4$(SO$_3$)(H$_2$O).

The rate of aquation of *trans*-Ru(NH$_3$)$_4$(SO$_3$)(imidazole) was measured by trapping the aquo complex as Ru(NH$_3$)$_4$(SO$_3$)(isonicotinamide). A buffered solution containing isonicotinamide (M = 0.10 M) in a 5-cm path cell was degassed and thermostated at 25.0 °C in the Cary Model 14 sample compartment. A weighed amount of [Ru(NH$_3$)$_4$(SO$_2$)(imidazole)](BF$_4$)$_2$ was rapidly dissolved in degassed 0.1 M NaCl and a small aliquot was immediately added by syringe to the cell. The rate of appearance of the product at 415 nm was shown to be independent of the concentration of isonicotinamide.

The rates of faster reactions were measured using a stopped-flow apparatus, which consisted of a thermostated Aminco-Morrow flow system adapted to fit on a Beckman DU spectrometer. Changes in transmittance at a fixed wavelength were measured using an EMI Model 6256B photomultiplier tube powered by a Fluke Model 412A high-voltage power supply. The photomultiplier tube cathode was connected to the inverting input of a Philbrick Model 1024 operational amplifier. Zero suppression and dark current adjustment were included in an adder configuration along with damping circuitry. Transmittance data were obtained from photographs of a storage oscilloscope trace (Tektronix Model D11 oscilloscope). The measuring system of this stopped-flow apparatus has a band-pass in excess of 10 kHz. The conversion of transmittance to absorbance was done by standard procedures, and the data were fitted to the integrated first-order rate expression

$$\log \frac{\Delta A_t}{\Delta A_0} = -\frac{k_{obsd}t}{2.303}$$

where ΔA_t is $(A_t - A_\infty)$ and ΔA_0 is $(A_0 - A_\infty)$. Solutions were degassed with argon and loaded into the stopped-flow instrument with all-glass syringes. The mixing chamber and observation port region of the flow instrument were maintained anaerobic with a stream of argon.

Electrochemical Measurements. Electrochemical measurements were made either on an instrument constructed locally by G. M. Tom using standard operational amplifier circuitry or, later, on a Princeton Applied Research Model 173 potentiostat and Model 175 universal programmer system. Formal potentials were measured by cyclic voltammetry using a carbon paste electrode. We gratefully acknowledge the gift of carbon paste from Dr. R. M. Wightman and Professor R. N. Adams of the University of Kansas. Potentials were measured against a saturated calomel electrode (SCE) at 25 °C and are uncorrected for junction potentials. Potentials were corrected to the normal hydrogen electrode scale by adding 0.244 V to the observed value. The voltammetric measurements were made in a standard H cell, with the test solution compartment separated from the reference electrode compartment by a fine porosity glass frit.

Solutions of *trans*-Ru(NH$_3$)$_4$(SO$_3$)(L), where L is any one of the ligands studied, were generated by adding solid [Ru(NH$_3$)$_4$(SO$_2$)-(Cl)]Cl or [Ru(NH$_3$)$_4$(SO$_2$)(H$_2$O)](BF$_4$)$_2$ to a buffered, degassed solution of the ligand with μ = 0.10 M. Ligand was in all cases at a concentration high enough to convert the aquo to the ligand complex virtually completely at equilibrium (ruthenium at the 1–2 mM concentration range).

The pH dependence of the redox couple Ru^{III}(NH$_3$)$_4$(SO$_3$)(H$_2$O)$^+$

$+ e^- \rightarrow Ru(NH_3)_4(S^{IV})(H_2O)^{n+}$ was measured at 25 °C with the ionic strength held constant at 1.0 M ($NaCF_3COO$). In these experiments, a special cell was used, designed to hold both a combination pH electrode and the electrodes for cyclic voltammetry. Because acetate, citrate, and phosphate form complexes with Ru(II), buffers based on these anions could not be used and the weakly coordinating buffers diazobicyclo[2.2.2]octane, hexamethylenetetraammine, 2,6-lutidine, and tris(hydroxymethyl)aminomethane were used for pH control.

Other Measurements. Electronic spectra were recorded on Cary Model 14, Cary Model 15, or Beckman Acta MVII spectrophotometers. Measurements of pH were made on a Brinkman Model 101 digital pH meter using a Metrohm glass microelectrode. ^1H NMR spectra were recorded on a Varian Model T-60 spectrometer.

Stoichiometry. The extent of oxidation of *trans*-[$Ru^{II}(NH_3)_4$-(S^{IV})(L)]$^{n+}$, where L is imidazole or 1,9-dimethylguanine, by H_2O_2 was determined by cyclic voltammetry. The sulfur(IV) complex of ruthenium(II) and the product of oxidation, [$Ru^{III}(NH_3)_4(SO_4)$-(L)]$^+$, have well-separated voltammetric waves ($\Delta E_{1/2} \geq 0.34$ V) and the concentration of Ru(II) was determined from the peak current at constant scan rate.

The stoichiometry of the reaction of [$Ru(NH_3)_4(SO_2)(H_2O)$]$^{2+}$ with Cr(VI) was measured by spectrophotometric titration. The equivalence point was determined from Job plots. Titrations were carried out in a flow-through vessel described by Clarke.[13]

Results

Site of Bonding. The ^1H NMR spectrum of a solution of *trans*-$Ru(NH_3)_4(SO_3)$(imidazole) in D_2O showed distinct resonances for the C_2, C_4, and C_5 protons at 7.18, 7.37, and 7.95 ppm vs. DSS. The appearance of three resonances is consistent with coordination of Ru(II) at the N_3 site of imidazole and rules out bonding at C_2 to form an imidazolium complex. The histidine complex also has Ru(II) bound at the unsaturated ring nitrogen site as shown by NMR. The resonances of ring protons (C_2 and C_5 positions at 7.95 and 7.10 ppm) and the change in the $-CH_2-$ resonances upon coordination by Ru(II) (≤ 0.15 ppm) is consistent with this interpretation. The binding site of *trans*-$Ru(NH_3)_4(SO_3)(H_2O)$ on the guanine derivatives is expected to be N_7.[5] The N_1 and N_9 sites are blocked in both 1,9-dimethylguanine and 1-methylguanosine and the N_3 site is expected to be sterically hindered by the substituent at the N_9 position. The site of binding in adenosine is suggested by Clarke's work as N_1.[13,14] The bonding of [$Ru^{II}(NH_3)_5(H_2O)$]$^{2+}$ to adenine sites in DNA occurs readily only after the DNA is denatured.[14] In normal DNA the N_1 site is involved in hydrogen bonding and is unavailable for coordination.

Kinetics of Substitution. The rate of approach to equilibrium for the reaction of the sulfur(IV) ruthenium(II) complex with the various ligands was studied as a function of free ligand concentration. The observed pseudo-first-order rate constants are listed in Table I. These rate measurements apply to the reaction of *trans*-$Ru(NH_3)_4(SO_3)(H_2O)$ with the entering ligands; in the pH range used in this study (6.93–8.63), the HSO_3^- and SO_2 forms make a negligible contribution to the observed rate.[7]

For the reaction

$$Ru(NH_3)_4(SO_3)(H_2O) + L$$

$$\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Ru(NH_3)_4(SO_3)(L) + H_2O \quad (1)$$
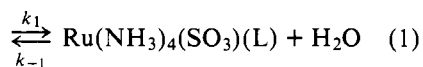
where L is any one of the imidazole and purine derivatives, the forward and reverse rates of reaction 1 are competitive, and plots of k_{obsd} vs. the concentration of L are linear with a positive intercept. The observed pseudo-first-order rate constant for the approach to equilibrium in reaction 1 is given by the expression

$$k_{obsd} = k_1[L] + k_{-1}$$

Table I. Pseudo-First-Order Rate Constants for the Approach to Equilibrium in the Reaction of $Ru(NH_3)_4(SO_3)(H_2O)$ with Imidazole and Guanine Derivatives

Ligand	[L], M^a	k_{obsd}, s^{-1} ^{b}	No. of determinations
Imidazole	4.91×10^{-4}	$2.63 \pm 0.13 \times 10^{-2}$	(3)
	9.80×10^{-4}	$4.64 \pm 0.16 \times 10^{-2}$	(3)
	2.36×10^{-3}	$10.7 \pm 0.1 \times 10^{-2}$	(3)
	0.62	30.3 ± 0.8^c	(3)
	9.56×10^{-4}	3.89 ± 0.30 $\times 10^{-2}$ ^{d}	(2)
		3.66 ± 0.20 $\times 10^{-3}$ ^{e,f}	(4)
		4.03×10^{-3} ^{e,g}	(1)
		4.45×10^{-3} ^{e,h}	(1)
1,9-Dimethyl-guanine	0.48×10^{-3}	0.71×10^{-1}	(1)
	0.81×10^{-3}	0.85×10^{-1}	(1)
	1.00×10^{-3}	$1.03 \pm 0.02 \times 10^{-1}$	(3)
	1.35×10^{-3}	1.12×10^{-1}	(1)
	2.01×10^{-3}	$1.47 \pm 0.07 \times 10^{-1}$	(3)
	3.00×10^{-3}	$2.30 \pm 0.04 \times 10^{-1}$	(3)
	4.02×10^{-3}	$2.68 \pm 0.03 \times 10^{-1}$	(3)
1-Methylgua-nosine	1.03×10^{-3}	$1.33 \pm 0.01 \times 10^{-1}$	(3)
	2.05×10^{-3}	$1.96 \pm 0.02 \times 10^{-1}$	(3)
	4.10×10^{-3}	$3.10 \pm 0.05 \times 10^{-1}$	(3)
1-Methylimid-azole	1.00×10^{-3}	$4.28 \pm 0.06 \times 10^{-2}$	(3)
	2.86×10^{-3}	$1.34 \pm 0.05 \times 10^{-1}$	(3)
	4.78×10^{-3}	$2.15 \pm 0.06 \times 10^{-1}$	(3)
Adenosine	0.98×10^{-2}	$1.38 \pm 0.06 \times 10^{-1}$	(3)
	1.96×10^{-2}	$1.48 \pm 0.11 \times 10^{-1}$	(3)
Histidine	5.87×10^{-3}	2.95×10^{-2}	(2)
	3.84×10^{-3}	2.16×10^{-2}	(2)
	2.10×10^{-3}	1.28×10^{-2}	(2)

^{a} Concentration of ligand is the concentration of unprotonated species at the pH of the experiment, 8.63 unless otherwise noted. ^{b} 25.0 $\pm$ 0.2 °C with ionic strength maintained at 0.10 M using NaCl. ^{c} pH 7.9 $\pm$ 0.1 imidazole–imidazolium buffer. ^{d} pH 6.93. ^{e} Rate of aquation of $Ru(NH_3)_4(SO_3)$(imidazole) as determined in presence of isonicotinamide. ^{f} pH 8.63. ^{g} pH 6.93. ^{h} pH 5.86.

Values of k_1 and k_{-1} were calculated from the slope and intercept of plots of k_{obsd} vs. [L]. No evidence for rate saturation at high concentration of entering ligand was observed even with imidazole at 0.62 M.

For the entering ligands imidazole and 1-methylimidazole, the value of the intercept was small in relation to the value of k_{obsd}, necessitating a direct measure of the rate of loss of the ligand. For imidazole, this was done by adding solid samples of *trans*-[$Ru(NH_3)_4(SO_2)$(imidazole)](BF_4)$_2$ to buffered solutions; the loss of imidazole was made irreversible by trapping the product aquo complex using isonicotinamide. We were unable to prepare salts of the 1-methylimidazole derivative, and therefore were unable to measure k_{-1} for this ligand with any precision.

The values of k_1 and k_{-1} defined by eq 1 are included in Table II along with Isied's data for the substitution of pyrazine and isonicotinamide. All the rates of substitution (k_1) are within a factor of about 4, provided that the reaction site on the ligand is not sterically hindered. The binding site on adenosine is likely to be N_1, which is sterically hindered. The slow rate of substitution of histidine can be interpreted as evidence that conversion of the ligand to the tautomeric form[15] shown on the right in eq 2 occurs before reaction with the Ru(II). If the form shown on the right of eq 2 reacts at the rate of the other imidazole species shown in Table II, we can infer that the equilibrium quotient governing reaction 2 is about 0.1.

The values of k_1 and k_{-1} in Table II were used to calculate

Table II. Rates and Equilibrium Constants for the Reaction of Nitrogen Heterocycles with $Ru(NH_3)_4(SO_3)(OH_2)$ at 25 °C

L	k_1, M^{-1} s^{-1}	k_{-1}, s^{-1}	K_{II},[a] M^{-1}
1-Methylimidazole[b]	45.8	$<1 \times 10^{-3}$	$\geq 46 \times 10^3$
Imidazole[b]	43.3	$3.66 \pm 0.20 \times 10^{-3}$	11.8×10^3
1,9-Dimethylguanine[b]	57.8	4.2×10^{-2}	1.38×10^3
1-Histidine[c]	4.42	$3.9 \pm 0.8 \times 10^{-3}$	1.1×10^3
1-Methylguanosine[b]	57.4	7.6×10^{-2}	0.76×10^3
Adenosine[b]	1.0 ± 0.5	0.13 ± 0.03	8 ± 7
Isonicotinamide[d]	24	6.4×10^{-3}	3.8×10^3
Pyrazine[d]	13.5	4.7×10^{-3}	2.9×10^3
Imidazole[c]	37	4.0×10^{-3}	

[a] Equilibrium constant for the reaction of L with Ru^{II}-$(NH_3)_4(SO_3)(H_2O)$. [b] pH 8.63, Tris–HCl buffer, $\mu = 0.1$ M. [c] pH 8.11, Tris–HCl buffer, $\mu = 0.1$ M. [d] pH 8.35, 0.1 M $NaHCO_3$; ref 7. [e] pH 6.93, phosphate buffer, $\mu = 0.1$ M.

$$(2)$$
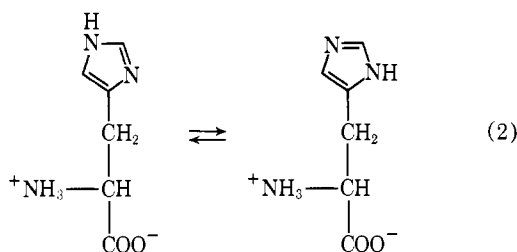
the affinity of the various ligands for the sulfur(IV) ruthenium(II) center.

One-Electron Oxidation of $[Ru(NH_3)_4(S^{IV}(OH_2)]^{n+}$. A spectrophotometric titration of *trans*-$[Ru(NH_3)_4(SO_2)-(H_2O)]^{2+}$ with Cr(VI) in $[H^+] = 0.1$–1.0 M media shows a net 1e$^-$ oxidation of the complex as a distinct stage. From Job's plots, the ratio of moles of Ru(II) complex to equivalents of Cr(VI) is 0.98 ± 0.03 (3 Ru(II):1 Cr(VI)). The Ru(II) complex can also be oxidized by Fe(III), but in this case a Job's plot was curved in the region of the equivalence point (CF_3SO_3H medium). When HCl was used to keep the solution acidic, a Job's plot indicated 1:1 stoichiometry. (Later it will be demonstrated that Cl$^-$ is coordinated to the Ru(III)–S(IV) complex.)

Cyclic voltammetry also shows that the sulfur(IV) complex of tetraammineaquoruthenium(II) undergoes a 1e$^-$ oxidation to form the ruthenium(III) complex. The separation of the anodic and cathodic peak potentials, the ratio of the cathodic to anodic peak currents, and the relative magnitude of the current are consistent with a reversible one-electron oxidation process. The half-wave potentials calculated from cyclic voltammetry are pH dependent, as shown in Figure 1a. The pH dependence of the $E_{1/2}$ values demonstrates that the sulfur(IV) complex of Ru(III) takes the form $[Ru^{III}(NH_3)_4(SO_3)-(H_2O)]^+$ throughout the range pH 0.3–8.7. The reactions corresponding to the three separate pH regions are

$$Ru(NH_3)_4(SO_3)(H_2O)^+ + e^- \rightarrow Ru(NH_3)_4(SO_3)(H_2O)$$

$$Ru(NH_3)_4(SO_3)(H_2O)^+ + e^- + H^+ \rightarrow Ru(NH_3)_4(HSO_3)(H_2O)^+$$

$$Ru(NH_3)_4(SO_3)(H_2O)^+ + e^- + 2H^+ \rightarrow Ru(NH_3)_4(SO_2)(H_2O)^{2+} + H_2O$$
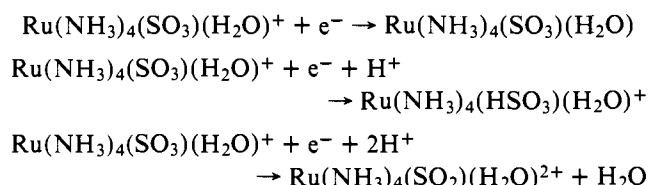
and respectively show slopes of 0, 60, and 120 mV/pH unit. The breaks in the $E_{1/2}$ vs. pH plot are the pK_a values for the successive deprotonations of S(IV) bound to Ru(II).

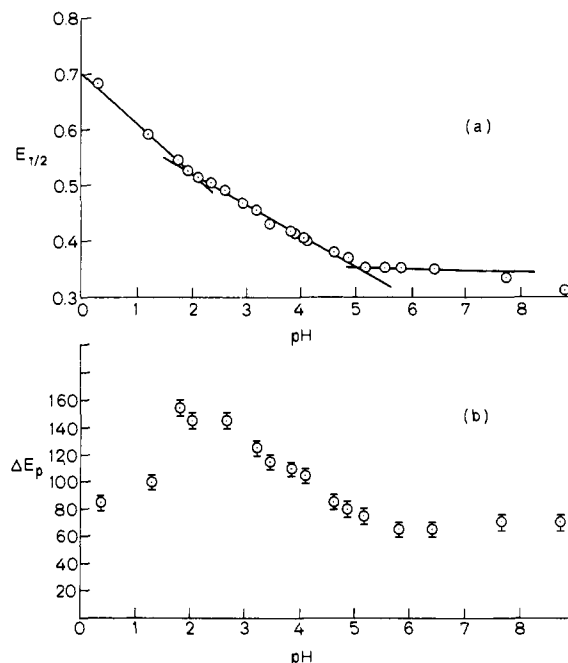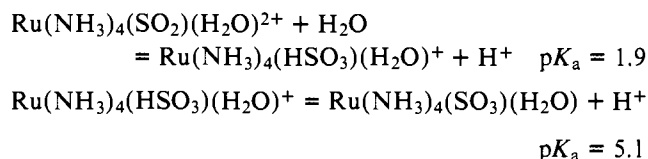**Figure 1.** (a) $E_{1/2}$ for the *trans*-$[Ru^{III/II}(NH_3)_4(S^{IV})H_2O]$ couple vs. pH (in volts vs. NHE; $\mu = 1.0$, 25 °C). (b) Peak to peak separation ($\Delta E_p = E_{pa} - E_{pc}$, mV) in the cyclic voltammograms of *trans*-$[Ru(NH_3)_4-(S^{IV}H_2O]$ at 0.1 V/s scan rate as a function of pH; $\mu = 1.0$ M, 25 °C.

The pK_a values found in this work

$$Ru(NH_3)_4(SO_2)(H_2O)^{2+} + H_2O$$
$$= Ru(NH_3)_4(HSO_3)(H_2O)^+ + H^+ \quad pK_a = 1.9$$

$$Ru(NH_3)_4(HSO_3)(H_2O)^+ = Ru(NH_3)_4(SO_3)(H_2O) + H^+$$
$$pK_a = 5.1$$

are in good agreement with those reported by Isied[7] (2.15 and 5.05) when allowance is made for the differences in ionic strength of the solvent medium.

The separation of the anodic and cathodic peak potentials vs. pH at a scan rate of 0.10 V/s is shown in Figure 1b. At pH 0 and pH >5.5 the peak separation is that expected for a reversible one-electron oxidation. The peak separation shows its maximum value at the first pK_a of the coordinated S(IV) deprotonation. The large peak separation is related to the slow rate of hydration of the $Ru^{II}(SO_2)$ species and the slow rate of protonation and dehydration of the $Ru^{II}(HSO_3^-)$ species.

$$RuSO_2^{2+} + H_2O \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} RuHSO_3^+ + H^+ \quad (3)$$
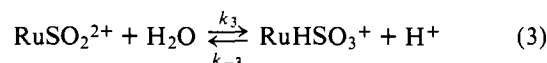
In an experiment to determine the rate of hydration, a solution of $Ru(NH_3)_4(SO_2)(H_2O)^{2+}$ at pH 1.2 was rapidly mixed with a buffer in the stopped-flow spectrometer to bring the final pH to 3.0. The observed first-order specific-rate for approach to equilibrium was 3.6×10^1 s^{-1} at 25.0 °C. Using the known equilibrium constant for reaction 3 ($pK_a = 2.15$ at $\mu = 0.2$ M), k_3 and k_{-3} are calculated to be 3.2×10^1 s^{-1} and 4.5×10^3 M^{-1} s^{-1}, respectively. The rate constant for hydration of uncoordinated SO_2 is 3.4×10^6 s^{-1}.[16] This retardation in the rate of hydration by five orders of magnitude can be attributed to stabilization of S(IV) in the SO_2 form by back-bonding; the presence of "extra" electron density in the S(IV) orbitals retards the attack by the nucleophile H_2O.

There is no evidence for rapid loss of S(IV) from the Ru(III) complex. In an experiment designed to test this possibility, a solution of $Ru(NH_3)_4(SO_2)(H_2O)^{2+}$ at pH 0 was oxidized

with 1 equiv of Cr(VI). Cyclic voltammetry was used to monitor the stability of the Ru(III) complex and no change in $E_{1/2}$ for the reduction of Ru(III) to Ru(II) or in the current was observed for 2 h after oxidation by Cr(VI). This result is in contrast with the results of Elson et al.[17] for oxidation of the similar complex $Ru(NH_3)_5(SO_2)^{2+}$. Sulfur(IV) is lost from the pentaammineruthenium(III) complex with a rate constant of 2.4×10^{-4} s^{-1}.

Properties of *trans*-[Ru(NH₃)₄(SO₃)(H₂O)]⁺. During the determination of the pH dependence of the half-wave potentials of the tetraammineaquosulfur(IV) ruthenium(II) ruthenium(III) couple, the identity of the buffering substance was observed to have large effects on the observed voltammetric behavior. Potentially coordinating anions such as phosphate, citrate, acetate, and chloride caused significant shifts in the half-wave potential and in some cases the voltammograms were electrochemically irreversible. These effects are caused by coordination of these anions to $[Ru(NH_3)_4(SO_3)]^+$. The reaction of Cl⁻ with $[Ru(NH_3)_4(SO_3)(H_2O)]^+$ was studied in some detail.

Solutions of *trans*-[Ru(NH₃)₄(SO₃)(H₂O)]⁺, generated by Cr(VI) oxidation of the Ru(II) complex in weakly coordinating media (CF_3SO_3H or CF_3COOH), have a weak absorption band at 320–330 nm ($\epsilon \le 500$ M^{-1} cm^{-1}) in addition to absorption due to $Cr(H_2O)_6^{3+}$ (280 nm). In the presence of Cl⁻, a new absorption band appears at 325 nm. The calculated extinction coefficient is 3.3×10^3 M^{-1} cm^{-1} based on the measured affinity of Cl⁻ for the $[Ru(NH_3)_4(SO_3)(H_2O)]^+$ ion. This band is tentatively assigned to $p_{Cl^-} \rightarrow d_{Ru}$ LMCT. There is also a weak band at 530 nm (ϵ 80 M^{-1} cm^{-1}), which causes more concentrated solutions of $[Ru(NH_3)_4(SO_3)(H_2O)]^+$ to appear red. The position of the band at 530 nm is not sensitive to the presence of chloride ion.

The rate of approach to equilibrium for the reaction

$$Ru(NH_3)_4(SO_3)(H_2O)^+ + Cl^-$$
$$\underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} Ru(NH_3)_4(SO_3)(Cl) + H_2O \quad (4)$$
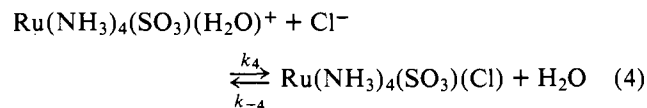
was measured under pseudo-first-order conditions. The observed pseudo-first-order rate constants are summarized in Table III. A plot of k_{obsd} vs. the concentration of Cl⁻ was linear indicating a rate law of the form

$$k_{obsd} = k_4[Cl^-] + k_{-4}$$

The values of k_4 and k_{-4} at 25.0 °C are respectively 24.3 M^{-1} s^{-1} and 8.1 s^{-1}. There was no evidence for rate saturation at the highest concentration of Cl⁻ used, 0.50 M. For comparison, the rate constant for aquation of Cl⁻ in $[Ru(NH_3)_5Cl]^{2+}$ is 7×10^{-7} s^{-1} at 25 °C.[18]

The affinity of Cl⁻ for the sulfitoruthenium(III) center was determined from the ratio of the rate constants in eq 4. The value at 25.0 °C is 3.0 M^{-1}, as compared to the affinity of Cl⁻ for $[Ru(NH_3)_5(H_2O)]^{3+}$, 1.1×10^2 M^{-1},[19a] 95 M^{-1}.[19b] A decrease in the affinity of Cl⁻ for the tetraammineaquosulfitoruthenium(III) complex compared to pentaammineaquoruthenium(III) is expected considering the differences in charge.

Solutions of the sulfitoruthenium(III) complex are stable for periods of time in excess of 1 h in acidic media (pH 0–2). However, in neutral solution the complex decomposes. At pH 7, a solution of $Ru^{III}(NH_3)_4(SO_3)(H_2O)$ was added to an imidazole buffer. Under these conditions it is assumed that imidazole substituted for the coordinated H_2O. Cyclic voltammetry was used to monitor the stability of this solution. A new peak appeared with time in the voltammograms at -0.12 V vs. NHE in addition to that at 0.33 V vs. NHE attributable to $[Ru(NH_3)_4(SO_3)(imidazole)]^{+/0}$ couple. No further changes were observed after approximately 0.75 h, and the

Table III. Pseudo-First-Order Rate Constants for the Approach to Equilibrium in the Reaction of Cl⁻ with $[Ru^{III}(NH_3)_4(SO_3)-(H_2O)]^+$

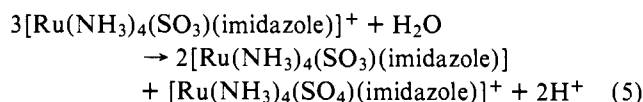| [Cl⁻], M	k_{obsd}, s^{-1} ^{a}	[Cl⁻], M	k_{obsd}, s^{-1} ^{a}
0.050	9.8 ± 0.8	0.200	13.3 ± 0.4
0.070	10.3 ± 0.3	0.300	15.1 ± 0.4
0.100	10.9 ± 0.2	0.500	20.7 ± 1.6

^{a} 25.0 ± 0.2 °C and ionic strength 1.0 M ($NaCF_3COO$) at [H⁺] = 0.10 M (HCF_3COO). Error limits are the average deviations from three or more determinations.

Table IV. Half-Wave Potentials and Stabilities of Nitrogen Heterocyclic Complexes

L	$E_{1/2}$, V ^{a}	K_{III}, M^{-1} ^{b}	K_{II}/K_{III}
Pyrazine	$\sim$0.51	$\sim$1.2	$\sim$2.5 × 10³
Isonicotinamide	0.464	8.6	4.3 × 10²
1-Methylguanosine	0.351	150	5.15
1,9-Dimethylguanine	0.341	400	3.48
Imidazole	0.337	4.3 × 10³	2.75
1-Histidine	0.336	430	2.65
H₂O	0.309	1	1

^{a} Potentials are vs. NHE at 25 °C ($\mu = 0.1$ M). $[Ru(NH_3)_4(SO_3)(L)]^+ + e \rightarrow Ru(NH_3)_4(SO_3)(L)$ error is estimated to be ± 0.010 V. ^{b} Calculated equilibrium constant for the reaction of L with $[Ru^{III}(NH_3)_4(SO_3)(H_2O)]^+$.

final ratio of the cathodic peak currents for the two redox processes was approximately 2:1. This result suggests the decomposition reaction is a disproportionation of the sulfitoruthenium(III) species, as shown in the equation

$$3[Ru(NH_3)_4(SO_3)(imidazole)]^+ + H_2O$$
$$\rightarrow 2[Ru(NH_3)_4(SO_3)(imidazole)]$$
$$+ [Ru(NH_3)_4(SO_4)(imidazole)]^+ + 2H^+ \quad (5)$$

However, when H_2O is the position trans to SO_3^{2-}, the decomposition of the Ru(III) complex yields ill-defined products, at least one of which is highly colored.

Formal Potentials of the [Ru(NH₃)₄(SO₃)(L)]⁺/⁰ Couples. The Ru(II)/Ru(III) redox couples were electrochemically reversible, and with the majority of the ligands employed in this study, the values of $E_{1/2}$ were determined by cyclic voltammetry. The results are summarized in Table IV.

For the complex $Ru(NH_3)_4(SO_3)(pyrazine)$, the cyclic voltammogram was irreversible at slow scan rates (0.1 V/s) but became reversible at faster scan rates (2.5 V/s) as judged from the ratio of the cathodic to anodic peak currents. The irreversibility at slow scan rates is due to loss of pyrazine from the Ru(III) complex. From the cyclic voltammetry data at 0.1 V/s, the rate constant for loss of pyrazine from the Ru(III) complex can be estimated to be 0.3 s^{-1}.

The affinities of the various ligands for the sulfitoruthenium(III) center were calculated from the formula

$$E'_{aquo} = E'_L - 0.059 \log K_{II}/K_{III}$$

where E'_{aquo} and E'_L are respectively the formal potentials of the aquo and ligand Ru(II)/Ru(III) couples and K_{II} and K_{III} are the affinities of the ligand for Ru(II) and Ru(III), respectively.

Oxidation of Tetraamminesulfur(IV) Ruthenium(II) Complexes by H₂O₂. Excess H_2O_2 in $\sim$1 M acid is known to oxidize aromatic heterocyclic derivatives of tetraammine(sulfur dioxide)ruthenium(II) quantitatively to the corresponding O-bound sulfatoruthenium(III) complex.[8] Evidence to be cited shows that this reaction involves attack of H_2O_2 on coordinated SO_2, followed by or coincident with oxidation of the ruthenium center.

Table V. Absorption Spectra for *trans*-[Ru(NH$_3$)$_4$(L)I]$^{2+}$

| L = 1,9-DMG		L = imidazole	
λ, nm	ϵ, M^{-1} cm^{-1}	λ, nm	ϵ, M^{-1} cm^{-1}
235	1.78×10^4	281	2.31×10^3
275 (sh)		377	4.52×10^2
320 (sh)	1.4×10^3	562	2.33×10^3
375 (sh)	4.4×10^2		
575	2.88×10^3		

In an imidazole–imidazolium buffer at pH 7, *trans*-[Ru(NH$_3$)$_4$(SO$_3$)(imidazole)] is not rapidly oxidized by hydrogen peroxide. A large excess of H$_2$O$_2$ does oxidize Ru(II) to Ru(III), but there is no evidence for oxidation of S(IV) to S(VI) at short times (5–10 min). At this pH, coordinated S(IV) is predominantly in the SO$_3^{2-}$ form. There is evidence for production of a sulfatoruthenium(III) complex on a time scale long enough to be accounted for as a product of disproportionation of the sulfitoruthenium(III) complex, but it should be noted that our evidence does not rule out a slow oxidation of coordinated S(IV) to S(VI) by H$_2$O$_2$.

At a pH of 3–4, H$_2$O$_2$ oxidizes the tetraammine(sulfur(IV)) ruthenium(II) complex of 1,9-dimethylguanine in the time of mixing. With an excess of H$_2$O$_2$ ([complex] = 1×10^{-3} M, [H$_2$O$_2$] = 5×10^{-3} M), the sulfur(IV) ruthenium(II) complex is quantitatively converted to the corresponding sulfatoruthenium(III) complex. In this pH range, the Ru(II)·S(IV) complex is predominantly in the HSO$_3^-$ form.

These results show that it is an acid form of the sulfur(IV) ruthenium(II) complex which is most reactive with H$_2$O$_2$. Ruthenium(II) complexes such as [Ru(NH$_3$)$_6$]$^{2+}$ and [Ru(NH$_3$)$_5$(py)]$^{2+}$ do not react rapidly with H$_2$O$_2$.[20] In both cases the rate of disappearance of Ru(II) shows a zero-order dependence on the concentration of Ru(II), indicating that the reaction proceeds by a catalyzed path involving impurities. Furthermore, coordinated S(IV) is known to be rapidly converted to the SO$_3^{2-}$ form upon oxidation of Ru(II) to Ru(III). The reaction of hydrogen peroxide with the sulfur(IV) ruthenium(II) complex must then occur by attack on coordinated SO$_2$, likely giving an intermediate similar to that shown for the reaction of H$_2$O$_2$ with uncomplexed SO$_2$.[21] Oxidation of Ru(II) to Ru(III) would occur in subsequent steps.
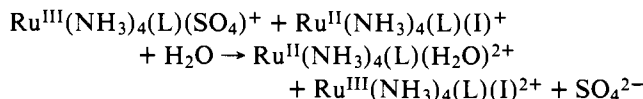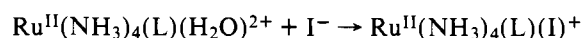
$$SO_2 + H_2O_2 \longrightarrow HOO-S\begin{smallmatrix} \nearrow OH \\ \searrow O \end{smallmatrix} \longrightarrow H_2SO_4$$
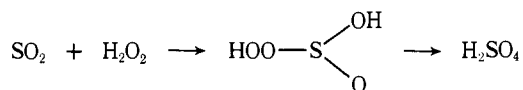
Catalyzed Substitution of Coordinated SO$_4^{2-}$ by I$^-$. Endicott noted that the presence of a small amount of [Ru(NH$_3$)$_5$(H$_2$O)]$^{2+}$ caused the equilibrium

$$Ru(NH_3)_5(H_2O)^{3+} + Cl^- \rightleftharpoons Ru(NH_3)_5Cl^{2+} + H_2O$$
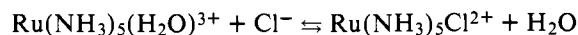
to be established very rapidly.[19a] This rapid equilibration occurs because of rapid substitution of Cl$^-$ at the Ru(II) center and rapid electron transfer between the species [Ru^{III}(NH$_3$)$_5$(H$_2$O)]$^{3+}$ and [Ru^{II}(NH$_3$)$_5$Cl]$^+$. Similarly, coordinated SO$_4^{2-}$ is rapidly substituted by I$^-$ in the complexes *trans*-[Ru(NH$_3$)$_4$)(SO$_4$)(L)]$^+$, where L is imidazole or 1,9-dimethylguanine, when small amounts of [Ru^{II}(NH$_3$)$_4$-L(H$_2$O)]$^{2+}$ are introduced. Cyclic voltammetry shows that coordinated SO$_4^{2-}$ is lost very rapidly when *trans*-[Ru(NH$_3$)$_4$(SO$_4$)(imidazole)]$^+$ is reduced at the electrode. Peaks due to the [Ru(NH$_3$)$_4$(imidazole)(H$_2$O)]$^{3+/2+}$ couple appear after the first scan, and the current due to the aquo couple increases with each succeeding scan. The measured rate constant[8] for loss of SO$_4^{2-}$ from *trans*-[Ru(NH$_3$)$_4$(SO$_4$)-(isonicotinamide)]$^+$ is ~0.3 s^{-1}; from *trans*-[Ru(NH$_3$)$_4$(SO$_4$)-2,9-dimethylguanine] it exceeds 1 s^{-1}.

Catalytic amounts of [Ru^{II}(NH$_3$)$_4$(L)(H$_2$O)]$^{2+}$ can be conveniently introduced in I$^-$ solutions of [Ru^{III}(NH$_3$)$_4$(L)-(SO$_4$)]$^+$ by partial reduction with amalgamated zinc. The substitution reaction involves the steps

$$Ru^{II}(NH_3)_4(L)(H_2O)^{2+} + I^- \rightarrow Ru^{II}(NH_3)_4(L)(I)^+$$

$$Ru^{III}(NH_3)_4(L)(SO_4)^+ + Ru^{II}(NH_3)_4(L)(I)^+$$
$$+ H_2O \rightarrow Ru^{II}(NH_3)_4(L)(H_2O)^{2+}$$
$$+ Ru^{III}(NH_3)_4(L)(I)^{2+} + SO_4^{2-}$$

The net reaction is substitution of coordinated SO$_4^{2-}$ by I$^-$ with Ru(NH$_3$)$_4$(L)(H$_2$O)$^{2+}$ as a catalyst.

Spectrophotometric measurements on the iodo complexes are summarized in Table V. The band at 562 nm for the imidazole species can be identified as LMCT (p$_I \rightarrow$ d$_{Ru^{III}}$) on the basis that pentaammineimidazoleruthenium(III) shows no absorption in this region.[3] For the latter ion, the longest wavelength band is at 430 nm. Coordination of I$^-$ shifts it to 377 nm, consonant with the idea that this band is also LMCT (in this case imidazole to Ru(III)). Pentaammine(1,9-dimethylguanine)ruthenium(III)[5] shows absorption at 579 nm but with an extinction coefficient of only 0.57×10^3. This absorption is presumably buried in the band with a maximum at 575 nm (ϵ 2.9×10^3) for the iodotetraammine complex.

Discussion

For ligands where there is no ambiguity in the binding site, the rates of substitution at the sulfitotetraammineruthenium(II) center are remarkably similar. The rate constants (k_1) fall in the range 13.5–57.4 M^{-1} s^{-1} (the data for adenosine and histidine excepted), the narrow range being consistent with a process in which bond breaking is more important than bond formation.

The substitution by imidazole was studied in more detail than for the other ligands. At pH 6.93 and 5.86, the aquation of imidazole is not assisted significantly by acid. Results could not be obtained at a lower pH without complication from conversion of coordinated SO$_3^{2-}$ to HSO$_3^-$. Paths involving both substitution of imidazolium ion and acid-catalyzed aquation of imidazole have been noted for the pentaammineruthenium(II) case.[3]

At a high concentration of entering imidazole, no evidence for rate saturation was noted. Despite this fact, the similarity in rate constants for a variety of entering ligands shows that the rate of loss of coordinated water is to some extent rate limiting. If a dissociative mechanism is operative, the absence of significant rate saturation at even the highest ligand concentrations shows that the rate constant for loss of coordinated H$_2$O must be greater than 30 s^{-1}. Shepherd has estimated the rate constant for loss of H$_2$O from Ru(NH$_3$)$_5$(H$_2$O)$^{2+}$ to be ~3 s^{-1}.[22] The labilization by SO$_3^{2-}$ of the trans position seems to be even greater in the ruthenium(III) complex. The rate constant for loss of Cl$^-$ from *trans*-[Ru^{III}(NH$_3$)$_4$(SO$_3$)(Cl)] when compared to acid hydrolysis of [Ru^{III}(NH$_3$)$_5$Cl]$^{2+}$ shows an enhancement by more than seven orders of magnitude. Part of this enhancement is no doubt due to the difference in charge of the two complexes, but there is nonetheless a significant intrinsic rate enhancement. The trans labilizing effect has been observed in complexes of Co^{III}(NH$_3$)$_5$(SO$_3$) and Co^{III}-(en)$_2$(SO$_3$). The reaction of Cl$^-$ with these species has not been reported, but the rate of loss of OH$^-$ has been studied with the Co(III) systems. The rate constants at 25 °C for loss of OH$^-$ from *trans*-Co(NH$_3$)$_4$(SO$_3$)(OH)[24a] and *trans*-Co(en)$_2$-(SO$_3$)(OH)[24b] are respectively 7 and ≥ 3 s^{-1}. The rate constant for loss of H$_2$O from *trans*-[Co(en)$_2$(SO$_3$)(H$_2$O)]$^+$ is 13 s^{-1}.[24b] It seems likely that the rate of loss of chloride is bracketed by those recorded for OH$^-$ and H$_2$O. It appears that the lability of trans complexes of Ru^{III}(NH$_3$)$_4$(SO$_3$) is com-

parable with the analogous sulfitotetraamminecobalt(III) complexes.

The affinity of the series of nitrogen heterocycles for *trans*-$Ru^{II}(NH_3)_4(SO_3)(H_2O)$ is listed in Table II. The affinity of the imidazole and guanine derivatives for this sulfur-(IV) complex of ruthenium(II) roughly parallels that expected from a consideration of the pK_a of the ligand binding site. The imidazole derivatives are stronger bases than the N_7 site of guanine and this is reflected in the equilibrium constants. The correlation between pK_a and log K_{II} is expected to be poor since the binding of Ru(II) to these ligands is subject to steric effects not found in the protonation equilibrium.
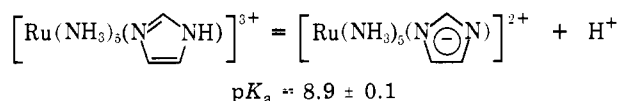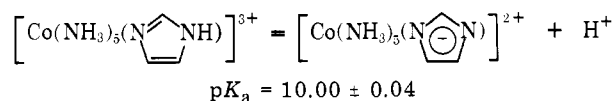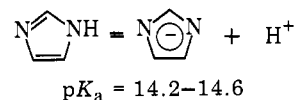
The N_1 site of adenosine is more basic than the N_7 site. Clarke has suggested that $Ru(NH_3)_5{}^{2+}$ binds at N_1 rather than the less hindered N_7 site.[14] If in fact N_1 is the binding site, the disparity between the affinities of the adenosine and guanine bases in DNA is expected to be even greater than that recorded for the free bases. In native biological materials such as DNA, the N_1 site of adenosine is involved in hydrogen bonding to other bases and is unavailable for metal ion coordination.

The ratio, K_{II}/K_{III}, of the affinity of a ligand for the tetraamminesulfitoruthenium center in the Ru(II) and Ru(III) oxidation states is a useful quantity. For pentaammineruthenium complexes this ratio has been shown to be sensitive to the back-bonding capabilities of the ligands.[25] A ligand such as N_2 makes a stable complex with $Ru^{II}(NH_3)_5$ by virtue of a $d_\pi \rightarrow \pi^*$ back-bonding but has little capacity for σ bonding and consequently makes a very weak complex with Ru(III). The ratio K_{II}/K_{III} for N_2 binding to pentaammineruthenium is 8×10^{16}.[25] Hydroxyl ion and hydrosulfide ion are on the other extreme, making more stable complexes with Ru(III) than with Ru(II) and the K_{II}/K_{III} ratios are 1×10^{-9} and 6×10^{-8}, respectively.[25] Where comparisons can be made, the ratio K_{II}/K_{III} shows the same trend for the $Ru(NH_3)_4$-$(SO_3)^{+/0}$ center as found for $Ru(NH_3)_5{}^{3+/2+}$. For pyridine, imidazole, and Cl^-, the affinity ratios for pentaammineruthenium are respectively 4×10^3, 1.5, and 4×10^{-1}.[25] The values of K_{II}/K_{III} measured for isonicotinamide, imidazole, and Cl^- in this study are respectively 4.3×10^2, 2.8, and ≤ 1. Some bonding principles established for complexes of $Ru^{II}(NH_3)_5$ are thus applicable to trans complexes of $Ru^{II}(NH_3)_4(SO_3)$.

For the ligands isonicotinamide and pyrazine, the affinity for $[Ru^{III}(NH_3)_4(SO_3)(H_2O)]^+$ is much smaller than that observed for the corresponding Ru(II) complex. The relatively strong binding of these weak bases (but rather strong π acids) to Ru(II) is attributable to back-bonding. The imidazole and guanine ligands are stronger bases and make relatively strong bonds to both Ru(II) and Ru(III). The smaller ratio of K_{II}/K_{III} measured for the imidazole and guanine derivatives indicates the binding of these ligands to *trans*-$[Ru^{II}(NH_3)_4$-$(SO_3)(H_2O)]$ is primarily an acid-base reaction with a much smaller contribution from back-bonding.

There is clear evidence that $Ru^{III}(NH_3)_5$ is incapable of engaging in $d_\pi \rightarrow \pi^*$ back-bonding to the degree with which it occurs in $Ru^{II}(NH_3)_5$. The explanation for this effect is the small radial extension of the d orbitals of Ru(III) compared to Ru(II).[1] Ligands such as imidazole and guanine have low-energy π orbitals which may engage in multiple bonding with the partially filled d_π orbitals of Ru(III). Multiple bonding of a $\pi \rightarrow d_\pi$ nature has been postulated to occur in imidazole complexes of Fe(III) porphyrins.[26] It would be appropriate to examine the available data to ascertain whether or not there is evidence for $\pi \rightarrow d_\pi$ multiple bonding in imidazole complexes of Ru(III). A comparison of the pK_a values for deprotonation of imidazole,[27] $Co(NH_3)_5(imidazole)^{3+}$,[28] and $Ru(NH_3)_5$-$(imidazole)^{3+}$[3] suggests that some transfer of charge from imidazole to Ru(III) does occur. Co(III) has a completely filled

d_π subset and can only affect the pK_a of coordinated imidazole by σ polarization. The affinity of imidazole and guanine ligands for Ru(III) show that in general stronger bases make stronger

$$N \overset{\frown}{\underset{\smile}{}} NH \;\rightleftharpoons\; N \overset{\frown}{\underset{\smile}{(-)}} N \;+\; H^+$$

$$pK_a = 14.2\text{–}14.6$$

$$\left[Co(NH_3)_5(N\overset{\frown}{\underset{\smile}{}}NH) \right]^{3+} \;\rightleftharpoons\; \left[Co(NH_3)_5(N\overset{\frown}{\underset{\smile}{}}N) \right]^{2+} \;+\; H^+$$

$$pK_a = 10.00 \pm 0.04$$

$$\left[Ru(NH_3)_5(N\overset{\frown}{\underset{\smile}{}}NH) \right]^{3+} \;\rightleftharpoons\; \left[Ru(NH_3)_5(N\overset{\frown}{\underset{\smile}{}}N) \right]^{2+} \;+\; H^+$$

$$pK_a = 8.9 \pm 0.1$$

bonds to Ru(III). These values are dependent on both the σ and π bonding effects which are difficult to separate. There is some evidence for imidazole functioning as a π donor to Ru(III) but it is difficult to assess the magnitude of the effect.

The oxidation of nitrogen heterocyclic complexes of tetraammine(sulfur dioxide)ruthenium(II) by hydrogen peroxide deserves further comment. Hydrogen peroxide reacts with the SO_2 form of coordinated S(IV) rather than with HSO_3^- or $SO_3{}^{2-}$. This is not a surprising result in the context of H_2O_2 acting as a nucleophile. The SO_2 form of S(IV) has available unfilled p orbitals into which H_2O_2 can donate a pair of electrons. In analogy with the mechanism proposed on the basis of oxygen tracer studies[21] for H_2O_2 reacting with SO_2 in acidic solution, we postulate that an intermediate with the structure

$$
\begin{array}{c}
H \\
O \\
HOOS^{(IV)}Ru^{(II)} \\
O
\end{array}
$$
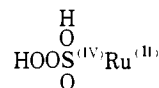
is formed. The detailed mechanism of the reaction, however, remains obscure. The mechanism must account for the fact that H_2O_2 does not ordinarily oxidize Ru(II) ammines at all rapidly (though OOH^- activated by coordination to S(IV) may do so), for the fact that excess H_2O_2 must be used, and for the fact that while conversion to a ruthenium(III) sulfato complex is virtually complete under the conditions described, when the oxidation of *trans*-$Ru^{II}(NH_3)_4(SO_2)pyrazine^{2+}$ takes place at high Cl^- concentration, a substantial amount of *trans*-$Ru^{III}(NH_3)_4(Cl)(pyrazine)^{2+}$ [29] is formed. Collapse of $Ru^{II}S(O)(OH)OOH$ to $Ru^{II}[SO_4{}^{2-}]$ would create a labile coordination position and may be one step in the reaction.

The question of the potential use of this ruthenium ammine complex as a specific labeling reagent for the bases in DNA will be considered by making comparisons with other substitution inert metal ions. The only metal ions of this kind for which a reasonable amount of information has been accumulated are low-spin Co(III) and Pt(II). With regard to the bases themselves, we will restrict the discussion to double-stranded DNA in which the only coordination positions to be considered are the N_7 positions of guanine and adenine. The purine N_1 positions are involved in hydrogen bonding as are the N_3 pyrimidine sites. These aspects of metal ion binding to DNA have been discussed in a recent review by Marzilli.[30] Co(III) ammine complexes appear to bind too weakly to be particularly useful as labeling agents.[30,31] The complex $Co(acac)_2(NO_2)_2{}^-$ shows good selectivity for adenine sites in reactions involving substitution of coordinated NO_2^-.[32] The basis for selectivity in this complex is thought to be a favorable hydrogen bonding interaction between oxygen on coordinated acetylacetonate and the exocyclic amine group (C_6) on adenine. No such hydrogen bonding arrangements exist for guanine binding to a $Co(acac)_2(NO_2)$ center. The interaction of Pt(II) complexes with DNA and its constituent bases has been much studied. Substitution reactions of Pt(II) complexes are normally slow,

and Pt(II) does form robust complexes with both adenine and guanine sites. Conclusions which may be drawn from a review of the literature[30] would suggest that Pt(II) complexes suffer the same problems as noted for $Ru(NH_3)_5(H_2O)^{2+}$ in the introduction. This problem is that product distributions are kinetically controlled rather than thermodynamically controlled.

The usefulness of most labile metal centers for specific labeling is limited, because compositions respond so rapidly to changes in conditions which often are a concomitant of doing the experiments of locating the metal ions. An advantage of the system we have described is that the high discrimination of the equilibrium labeling can be exploited and the system can then be fixed in this composition by oxidizing $[Ru(NH_3)_4$-$SO_2H_2O]^{2+}$ to the sulfatoruthenium(III) complex. In principle, specificity can be further improved by substituting NH_3 in the cis positions by other groups.

Conclusions

The rate data in Table II demonstrate that equilibrium is established very rapidly in the reaction of *trans*-$Ru(NH_3)_4$-$(SO_3)(H_2O)$ with nitrogen heterocyclic ligands. With 1,9-dimethylguanine at 1×10^{-3} M, the pseudo-first-order rate constant is 0.1 s^{-1}. This equilibrium can be "frozen" by oxidation of S(IV) to S(VI) using H_2O_2 as the oxidant at pH $\leq$ 4. The position of the rapidly established equilibrium is sensitive to the steric and electronic properties of the entering ligand. These facts make this ruthenium ammine system an attractive candidate for labeling biological materials. The strong (>50) discrimination between guanine and adenine binding sites should make it possible to label the guanine sites in DNA specifically, the equilibrium then being frozen by oxidation of S(IV) to S(VI) and Ru(II) to Ru(III). Furthermore, the coordinated SO_4^{2-} can be selectively replaced by a heavy atom as I$^-$ in a Ru(II)-catalyzed substitution process.

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Reactions of Coordinated Molecules. 12.
Preparation of *cis*-(OC)₄Re[CH₃C(O)][CH₃CN(C₆H₅)(H)]: the Ketamine Tautomer of a Metallo-β-ketoimine Molecule

C. M. Lukehart* and Jane V. Zeile

Contribution from the Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235. Received June 22, 1977

Abstract: The rhenium metalloacetylacetone complex, *cis*-(OC)₄Re[C(CH₃)O⋯H⋯OC(CH₃)], reacts with two primary aromatic amines, H₂NR (where R is phenyl or *p*-tolyl), affording complexes of the type *cis*-(OC)₄Re[CH₃C(O)][CH₃CN(R)-(H)]. The crystal and molecular structure of the *N*-phenyl complex was determined commercially on an Enraf-Nonius CAD4 diffractometer by using Mo Kα radiation: *Pbca*, $a = 6.838$ (2) Å; $b = 18.807$ (5) Å; $c = 23.729$ (5) Å; $\alpha = \beta = \gamma = 90°$; $Z = 8$; $d_{calcd} = 2.004$ g/cm³; $R_1 = 0.033$; $R_2 = 0.040$. The molecular geometry and the spectroscopic data and, especially, the chemical reactivity of the complex are consistent with the chemical formulation of the complex as a metallo-β-ketoimine molecule. The electronic structure is interpreted as a zwitterionic metal complex having a multiple C–N bond. The geometrical isomerization about this bond was followed using ¹H NMR.

In a previous communication, we reported the preparation of the first example of a "metalloacetylacetone" molecule, **1**.[1] This complex was characterized as the metallo analogue of the symmetrical, enol tautomer of acetylacetone where the methine group is replaced formally by the Re(CO)₄ moiety. Several other examples of these "metallo-β-diketone" molecules having various substituents and metallo moieties were prepared recently.[2] We are currently investigating the simi-