Aquation of the Anticancer Complex *trans*-$[RuCl_4(Him)_2]^-$ (Him = imidazole)†

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Aquation of the anticancer complex *trans*-$[H_2im][RuCl_4(Him)_2]$ **1** (Him = imidazole) has been studied in D_2O at a range of pH* (meter reading) values between 2.4 and 10 by observation of paramagnetically shifted ^1H NMR resonances. At 310 K, pH* 5.6, the half-life was 3.4 h and three products were detected, assigned as mono- and di-aqua species. The rate was similar at low pH*, but the reaction followed a different course at high pH*. Aquation was also studied by separation of the products on a reversed-phase column using HPLC, by conductivity measurements, and by EPR spectroscopy. Slow oxidation of the complex to Ru^{IV} appeared to take place in perchlorate, phosphate or acetate solutions. Cyclic voltammetric studies showed that reduction of **1** to Ru^{II} (E_m −0.14 V *versus* the normal hydrogen electrode, pH 7) was accompanied by the uptake of two protons with associated pK_a values of 6.26 and 3.87. Both aquation and the more favourable reduction of **1** at low pH may play a role in the biological activity of the complex.

The ruthenium(III) complex *trans*-$[H_2im][RuCl_4(Him)_2]$ **1** (Him = imidazole) is active against chemically induced colorectal tumours of the rat, reducing the tumour volume by up to 90%.[1] This and related complexes are therefore of clinical interest since such tumours are not responsive to $[PtCl_2(NH_3)_2]$ (cisplatin), and studies of preclinical pharmacology are currently in progress.[2] The *trans* geometry of **1** in the solid state has been established by X-ray crystallography.[3]

The mechanism of the biological activity of complex **1** is not understood. Ruthenium(III) complexes are usually considered to be kinetically inert and activation mechanisms involving *in vivo* reduction to Ru^{II} are often implicated in their biological activity.[4,5] Indeed the ruthenium(II) complexes *cis*- and *trans*-$[RuCl_2(dmso)_4]$ (dmso = dimethyl sulfoxide) possess good metastatic activity against murine tumours, and interact with DNA both *in vitro* and *in vivo*.[6,7] It has been reported that **1** binds to DNA and blocks its template-primer properties for DNA polymerase-catalysed DNA synthesis.[8] However, only aged (> 20 h) aqueous solutions of it react rapidly with DNA; fresh solutions in water or 2-morpholinoethanesulfonic acid buffer, pH 5.7, do not react. Aquation is known to be an important step in the activation of the anticancer drug cisplatin,[9] and we decided to investigate whether analogous reactions could occur for **1**.

In the present work we have studied the aquation of $[RuCl_4(Him)_2]^-$ **1** in aqueous solution by observation of paramagnetically shifted ^1H NMR resonances, and by EPR spectroscopy. Aquation was also detected by conductivity measurements and by reversed-phase HPLC. The redox chemistry of **1** was investigated by cyclic voltammetry and ^1H NMR spectra of the complex in blood plasma were also studied.

† *Non-SI unit employed:* G = 10^{-4} T.

Experimental

Materials and Methods.—The complex *trans*-$[H_2im][RuCl_4(Him)_2]$ was prepared as previously described.[1]

For EPR and cyclic voltammetry the buffers used were 50 mmol dm^{-3} sodium acetate (pH 4) or Good's buffers, *i.e.* N'-(2-hydroxyethyl)piperazine-N-ethanesulfonic acid (hepes) (pH 7) or 3-(cyclohexylamino)propanesulfonic acid (caps) (pH 11). Solutions also contained 100 mmol dm^{-3} $NaClO_4$. Fresh solutions containing $NaClO_4$ or NaCl gave identical results; long standing in $NaClO_4$ solutions leads to oxidation of Ru^{III} to Ru^{IV}. The EPR studies were also carried out in water in the presence of 100 mmol dm^{-3} $NaClO_4$.

Adjustments of pH* values (pH meter reading from D_2O solutions) for NMR experiments were made by addition of DNO_3 or NaOD to a D_2O solution of complex **1**, or by use of NaO_2CD_3–DNO_3 (pH* 4.5) or 50 mmol dm^{-3} phosphate–D_2O (pH* 7.3) buffers. Spectra of the buffered solutions were also acquired in the presence and absence of 200 mmol dm^{-3} $NaClO_4$.

Whole blood was obtained from a healthy volunteer and collected into glass tubes containing lithium heparin as anticoagulant. The plasma was separated from the red cells by centrifugation at 4 °C at 5000 revolutions min^{-1} for 3 min. It was either used immediately or transferred to plastic tubes, stored at 277 K, and used within 4 h.

Measurements of pH were made using a Corning pH meter.

NMR Spectroscopy.—Proton NMR spectra were recorded on JEOL GSX500 and GSX270 instruments, using 0.7 cm^3 of sample in 5 mm tubes. Typical pulsing conditions: 45° pulse, acquisition time 1.26 s, pulse delay 1.5 s, 32 K computer points, 250 transients, 298 or 310 K. Shifts were referenced to 1,4-dioxane, δ 3.765 and 3.762 at 298 and 310 K, with respect to sodium 3-trimethylsilyl[$^2H_4$]propionate. For quantitative work free induction decays were processed in absolute-intensity mode using exponential line broadenings of 20 Hz. Peak

heights were used to determine the relative amounts of products, since the peaks being compared all had similar linewidths. Rate constants were fitted to NMR data using the program KaleidaGraph.[10]

EPR Spectroscopy.—All EPR data were recorded on an X-band Bruker EPR 200 D spectrometer with the microwave source operated in levelled mode (nominal output: 0 dB = 200 mW). Microwave power levels (usually *ca.* 6 dB) were non-saturating for all spectra. The microwave frequency was determined continuously with a Systron Donner 1292A frequency counter. The d.c. magnetic field was calibrated with an AEG NMR fieldmeter, type GA-EPR 11/21-02. The field was modulated with a frequency of 100 kHz and an amplitude of 16 G. Sample cooling was with a laboratory-made helium-flow cryostat. The sample temperature (16–18 K) was calibrated with a dummy sample containing two 5 kΩ/0.1W Allen-Bradley carbon composition resistors just below and above the 1.5 cm measuring area of the TE_{102} cavity. The spectrometer was interfaced to a personal computer with software written in Asyst for data acquisition (as 1024 point spectra), correction of background signals, double integration procedures, and *g*-value determinations.

Cyclic Voltammetry.—Electrochemistry was carried out at 295 K on 20 μl of sample using the three-electrode microcell described previously.[11] Nitric acid-activated glassy carbon was used as the working electrode. The reference electrode was a saturated calomel electrode (0.246 V relative to the normal hydrogen electrode). All reported potentials have been recalculated with respect to the normal hydrogen electrode. The cyclic voltammetry was driven by a Wenking POS-73 potentiostat (Bank Electronic, Germany).

Conductivity.—Conductivity measurements were carried out using a digital conductometer 600 (Knick) with a platinated platinum electrode. The cell constant was calibrated to 12.88 mS cm^{-1} with a 0.1 mol dm^{-3} KCl solution in water (double distilled). Measurements were carried out at 298 K in a water-bath. Two solutions of complex **1** were studied: 0.81 and 0.9 mmol dm^{-3} dimethylformamide (dmf) (Merck) and water (double distilled) respectively. Solvent conductivities were 0.003–0.005 mS cm^{-1} for dmf and 0.001–0.003 mS cm^{-1} for water.

HPLC.—The HPLC investigations of complex **1** were carried out on a Nucleosil (300–7 μm) diol column (250 × 8 mm) at ambient temperature. The mobile phase used consisted of 70% MeCN (Merck) and 30% 5 mmol dm^{-3} KH_2PO_4 in doubly distilled water, pH 4.5. The flow rate was 3 cm^3 min^{-1} and UV detection was carried out at 210 nm. The complex was dissolved in water at 296 K and monitored over 10 h by removing 20 μl aliquots for injection onto the column.

Results and Discussion

NMR Data.—The ^1H NMR spectrum of a fresh solution of *trans*-$[H_2im][RuCl_4(Him)_2]$ **1** in D_2O at ambient temperature contained sharp resonances for the non-co-ordinated counter cation H_2im^+, and three broad paramagnetically shifted resonances for the protons of the co-ordinated imidazole ligands. The shifts are listed in Table 1. When **1** was dissolved in $(CD_3)_2SO$ two additional resonances were observed which disappeared on addition of D_2O. These can therefore be assigned to the NH protons of the H_2im^+ cation and co-ordinated Him, Table 1. The spectrum of a fresh D_2O solution at 310 K was similar to that at 298 K, although the paramagnetic shifts were smaller, as expected.

There are only a few previous reports of ^1H NMR spectra of ruthenium(III) complexes.[12–14] It can be seen from Table 2 that the ratios of the paramagnetic shifts for complex **1** agree well with ratios of $(3\cos^2\theta - 1)/r^3$, where θ is the dihedral angle between the principal symmetry axis and the Ru–H vector and *r* is the Ru–H distance (based on the crystal structure[3] of **1**). This suggests that the paramagnetic shifts are predominantly dipolar in origin.[15]

Proton NMR spectra of a yellow solution of complex **1** in D_2O at 310 K were recorded over 17 h. During this time the pH* dropped from 5.56 to 3.16, the solution became yellow-brown, and three new sets of peaks appeared in the high-field region of the spectrum. There was little change in the intensities of the resonances for the H_2im^+ counter ion. Spectra after 1, 6.8 and 16.6 h and a plot of the decrease in intensity of the resonance at δ −5.36 with time and increase in intensities of corresponding product resonances are shown in Fig. 1. Spectra were also recorded at 25 °C over nearly 48 h. Again three new sets of peaks appeared, though their relative intensities were less than those at 37 °C. Spectra of solutions of **1** left standing for 24 h at ambient temperature in the dark or in the light were similar, and variation of the complex concentration from 1 to 10 mmol dm^{-3} also did not affect the course of the reaction. On addition of $AgNO_3$ to an aged solution of **1** (14 d old),

Table 1 Proton NMR chemical shifts and linewidths ($\Delta\nu_{\frac{1}{2}}$/Hz) for complex **1** in D_2O (fresh solution, 8.2 mmol dm^{-3}, pH* 5.60, 298 K), hydrolysis products **2** and **3** (in solutions of **1** after 17 h at 37 °C, final pH* 3.2), and **1** in $(CD_3)_2SO$ (6.6 mmol dm^{-3}, 296 K)

δ($\Delta\nu_{\frac{1}{2}}$)					
1	**2**	**3a**	**3b**	**1**	Assignment
D_2O	D_2O	D_2O	D_2O	$(CD_3)_2SO$	
				14.20 (245)	$N^{1,3}HofH_2im^+$
8.62 (23)				9.04 (15)	C^2H of H_2im^+
7.44 (23)				7.68 (15)	$C^{4,5}HofH_2im^+$
				−2.82 (35)	N^3H of **1**
−5.87 (46)	−4.02	−2.31	−3.65	−7.83 (35)	C^4H of **1**
−15.83 (184)	−11.17	−6.19	−9.43	−17.50 (210)	C^2H of **1**
−21.25 (184)	−18.5	−15.13	−16.23	−19.00 (210)	C^5H of **1**

Table 2 Observed paramagnetic shifts [Δδ = δ (complex) − δ (free Him)] and calculated ratios of geometrical terms $[(3\cos^2\theta - 1)/r^3]$ for complex **1**

Proton	Δδ (D_2O)	Ratio	Δδ [$(CD_3)_2SO$]	Ratio	Geometrical ratio
C^4H	13.3	1.0:1	15.5	1.0:1	1:1
C^5H	28.7	2.2:1	26.7	1.7:1	1.65:1
C^2H	24.5	1.8:1	26.5	1.7:1	1.74:1
N^3H	(not seen)	—	17.02	1.1:1	1:1

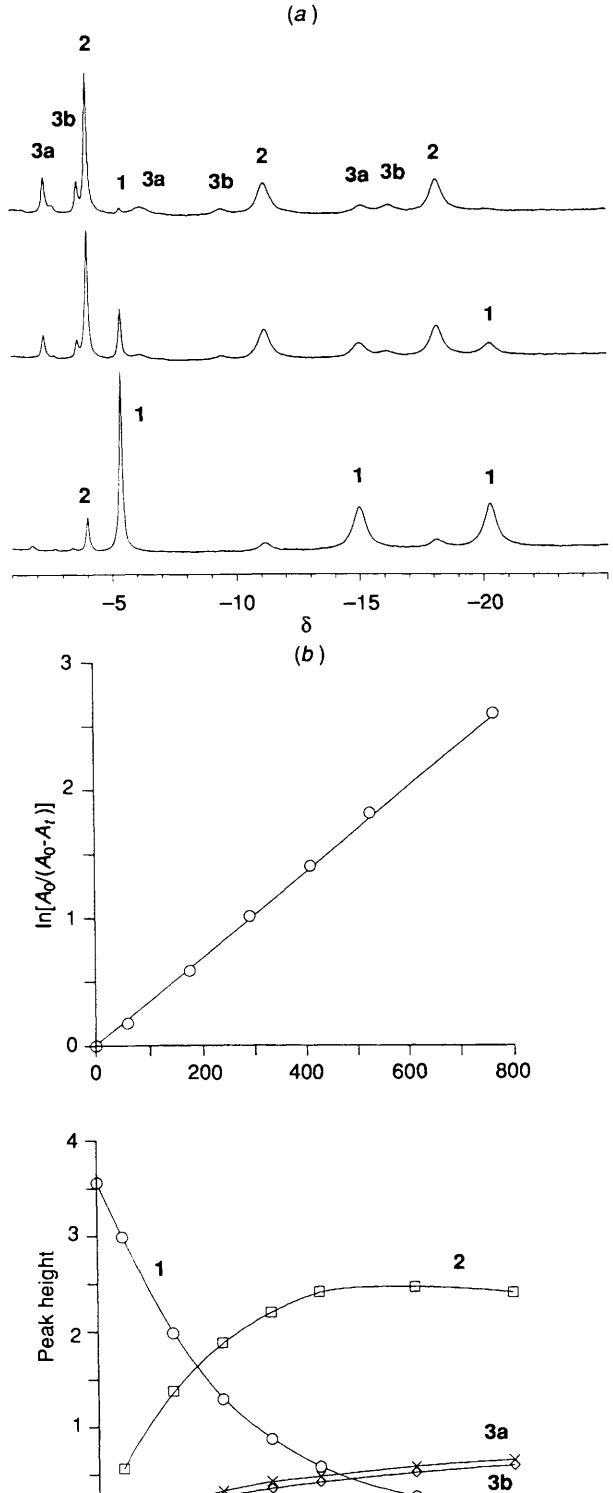
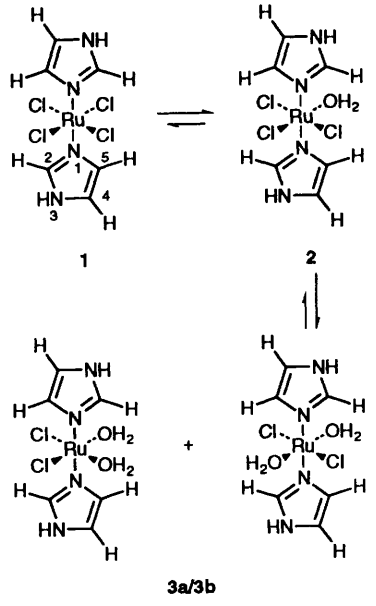


Fig. 1 (*a*) 270 MHz ^{1}H NMR spectra of *trans*-$[H_2im][RuCl_4(Him)_2]$ **1** in D_2O at 310 K after 1 (lower), 6.8 (middle) and 16.6 h (top). (*b*) Plot showing the variation in intensities of the sharpest paramagnetically shifted NMR peaks (assigned to C^4H) with time (lower), and first order rate plot for C^4H peak of **2**, where A_0 and A_t are peak intensities at times zero and *t* respectively (upper)

precipitation occurred suggesting that Cl^- ions had been released.

Appropriate plots showed that the disappearance of complex **1**, as monitored by the sharpest paramagnetically shifted



Scheme 1 Hydrolysis of complex **1** (charges on complexes omitted)

resonance C^4H, followed pseudo-first-order kinetics, with rate constants of 9.60×10^{-6} and 5.62×10^{-5} s^{-1} at 298 and 310 K, respectively, corresponding to half-lives of 20 and 3.4 h. The rate of decomposition of **1** in 0.15 mol dm^{-3} NaCl solution was slower, with a half-life of 4.6 h at 310 K, again accompanied by a drop in pH* (from 5.8 to 3.8). At low pH* (2.6, adjusted with DNO_3) the reaction proceeded at a similar rate (half-life 3.3 h, 310 K) to that in D_2O with no change in pH*.

These data are consistent with a stepwise aquation of complex **1** (Scheme 1). At 310 K, resonances due to **2** are clearly visible after less than 1 h, Fig. 1(*a*), and those due to **3a** and **3b** become apparent after *ca.* 3 h. After 10 h the amount of **2** begins to decline and the intensities of the resonances for **3a** and **3b** increase in parallel. Complex **2** is therefore likely to be a monoaqua complex and **3** a diaqua complex, perhaps as equal amounts of the *cis* and *trans* isomers (**3a** and **3b**). The greater *trans*-labilizing effect of chloride compared to oxygen in ruthenium(III) complexes might be expected to lead to a predominance of *cis*-diaqua species,[16] however this is not observed in the present case.

The drop in pH* which accompanies aquation could be due to deprotonation of the aqua species to form hydroxo complexes. Resonances for complexes **2** and **3** shifted little with the change in pH* during aquation, suggesting either that these aqua complexes have pK_a values outside this range or that the ^{1}H NMR shifts of the axial imidazole ligands are insensitive to such ionization. The latter is more probable since values of pK_a for H_2O co-ordinated to Ru^{III} have been reported[17] to lie within the range of pH* values used here, *e.g.* 2.6 for $[Ru(NO)(NO_3)_3(H_2O)_2]$, 4.1 for $[Ru(NH_3)_5(H_2O)]^{3+}$,[18] 4.7 for $[RuL(H_2O)]ClO_4$ {HL = [2-hydroxy-2-(2-pyridyl)ethyl]bis[2-(2-pyridyl)]-amine},[19] and 6 for the anionic complex $[Ru(NO)Cl_4(H_2O)]^-$.

In alkaline solution (pH* 10.1, adjusted with NaOD) the decomposition of complex **1** took a different course: there was a decrease in intensity of all the resonances due to **1** over 18.5 h at 25 °C, two new resonances appeared at δ 0.20 and 2.53, and the resonances of H_2im^+ counter ion shifted and decreased in intensity. The pH* dropped from 10.1 to 5.28 and the resultant solution was green.

For comparison with EPR data (see below), NMR studies were also carried out in NaO_2CD_3 (50 mmol dm^{-3}), in D_2O–phosphate buffers both in the presence and absence of $NaClO_4$ (200 mmol dm^{-3}), and in phosphate solutions at pH* 11.8. The spectra were different from those observed in D_2O alone. In the case of acetate the initially yellow solution became dark purple

and there was a general decrease in intensity of all the signals due to complex **1** with time, and a small pH* drop from 5.8 to 5.2 over 14 h. In addition, a number of new peaks were observed including two broad ones at δ 1.5 and 3.6, and one set of three with the same chemical shifts as those of complex **2** in D_2O. This species was present at highest concentration (*ca.* 28% of total) after 1.1 h and decreased in intensity thereafter. After 6 h a number of new peaks appeared in the imidazole region, δ 6.5–8.5. The presence of 200 mmol dm^{-3} perchlorate had no effect on the course of reaction in acetate buffer. In 50 mmol dm^{-3} phosphate (pH* 7.2) at 37 °C there was a general decrease in intensity of the resonances due to **1**, a colour change from yellow to purple, and a pH* drop from 7.2 to 6.6. No peaks due to the first aquation product **2** were present. Initially (after 11 min at 310 K) the only new peaks present were at δ 1.5 and 3.6 ($\Delta\nu_{\frac{1}{2}}$ *ca.* 270 and 189 Hz, respectively). After 13.2 h there were a number of new peaks present, δ −0.8, −0.5, 0.54 and 1.92, of comparable intensity to the signal at δ −5.36 of unreacted **1**. In the presence of both phosphate (50 mmol dm^{-3}, pH* 7.2) and perchlorate (200 mmol dm^{-3}) there was again a colour change from yellow to purple and a general decrease in intensity of the resonances due to **1**. However, there were also relatively intense new resonances present at δ 8.26, 6.09 and 6.07 and a fourth new peak after 1 h at δ 8.33 assignable to a diamagnetic Ru–Him complex (no new peaks were observed in the spectrum of Him alone in phosphate–ClO_4^-). These new peaks did not shift over the reaction time. In phosphate solution at high pH* (11.8) the colour changed from yellow to yellow-brown after 14.4 h. Decomposition of **1** in such a solution at 310 K was rapid, with no resonances for **1** being detectable after 1 h. These reactions clearly involve more than simple aquation and EPR studies (see below) suggested that the purple solutions may contain Ru^{IV}. It has previously been noted[17] that hydroxoruthenium(III) species are readily oxidized to Ru^{IV}.

Complex **1** was found to be soluble in blood plasma to at least 10 mmol dm^{-3} giving a yellow solution. Resonances for the intact complex were readily observable and a small upfield shift of the choline headgroup resonance of phospholipids at δ 3 suggested that a weak interaction of the negatively charged complex **1** with the positively charged NMe_3^+ phospholipid headgroups occurred. There was a general broadening of the other resonances especially in the glucose region due to the paramagnetic effects of Ru^{III}. The high chloride concentration in plasma (*ca.* 104 mmol dm^{-3}) would be expected to suppress aquation of **1**, but the time dependence of the spectrum was not monitored.

EPR Spectroscopy.—In the solid state complex **1** gave a single, very broad line with zero crossing at $g = 2.13$. No signal at all was observed from fresh solutions of up to 10 mmol dm^{-3} **1** in water alone. From 5 mmol dm^{-3} solutions in 50 mmol dm^{-3} buffer (sodium acetate pH 4, hepes pH 8 or caps pH 11) rather weak signals were found in the *g*-value range from 1.8 to 3.1, typical of d^5 ions such as Ru^{III}.

In the presence of 100 mmol dm^{-3} $NaClO_4$ the intensities of EPR peaks from buffered solutions of complex **1** increased by a factor of *ca.* 30. This is a commonly observed phenomenon in EPR spectra of frozen aqueous solutions of small transition-metal complexes, particularly those of Cu^{II}, and indicates that in the absence of perchlorate the compound forms (micro-) aggregates. Consequently, multiple spin coupling occurs and the EPR spectrum is broadened beyond detection. A fresh aqueous solution of **1** (4 mmol dm^{-3}, 100 mmol dm^{-3} $NaClO_4$) gave a spectrum with *g* values of 3.00, 2.34 and 1.2, and a similar spectrum was obtained in acetate buffer (100 mmol dm^{-3}, pH 4) with *g* values of 3.12, 2.44 and 1.2. The observed rapid increase in the inhomogeneous linewidth with decreasing *g* value is characteristic for EPR *g*-strain broadening in frozen solutions.[20] The average of these *g* values is close to the *g* value obtained for solid **1**. Although there are reports of ruthenium(III) EPR peaks being obtained at liquid-nitrogen temperatures, *e.g.* $[RuX(NH_3)_5]Cl_2$ (X = Cl, Br or I),[21] RuL_3 (L = 2-substituted quinolin-8-ol[22] or bidentate S donor ligand[23]), $[Ru(en)_2X_2]^+$ (en = ethane-1,2-diamine; X = Cl, Br, I or NCS)[24] or even at 27 °C *e.g.* $[NEt_4][RuCl_4(AsPh_3)_2]$,[25] in the present case reasonable resolution required lower temperatures (*ca.* 15 K).

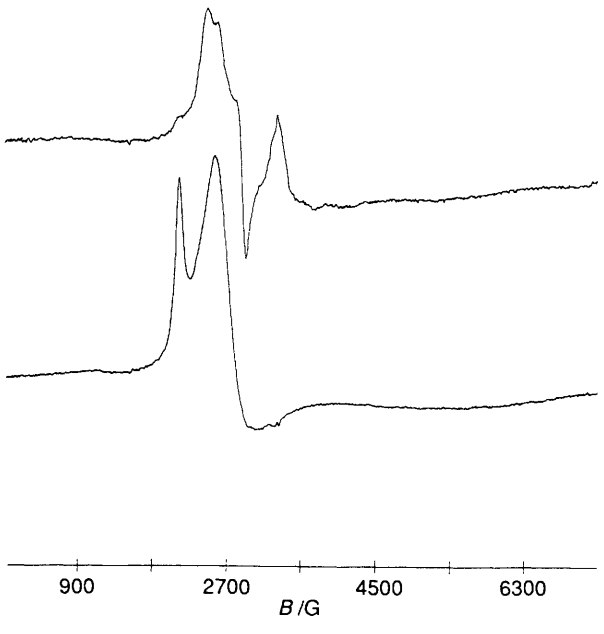


Fig. 2 The EPR spectra of 5 mmol dm^{-3} complex **1** at 15.5 K in hepes buffer at pH 7 after 0 (bottom) and 10 h (top) of aerobic incubation at 37 °C in the presence of $NaClO_4$ (100 mmol dm^{-3}). As well as peaks for hydrolysis products, the latter spectrum contains a peak at $g = 1.93$ due to a perchlorate-induced oxidation product (Ru^{IV}). The microwave frequency was 9.30 GHz

Aquation was studied by incubating an aqueous perchlorate solution at 310 K, removing aliquots at various times, freezing them in liquid nitrogen and recording their EPR spectra. Only minor spectral changes were observed after 1.5 h but by 6.5 h the original EPR peaks had been largely replaced by a different signal with *g* values of 2.64 and 2.36 and a third component which was too broad to measure, assignable to the aquation product **2**. The time-scale for aquation as measured by EPR is thus similar to that observed by NMR spectroscopy. Some typical EPR spectra are shown in Fig. 2. After 23 h this sample was dark purple and purple solutions such as this appeared to have peaks with *g* values of 1.93 and a broader signal at $g = ca.$ 1.4, probably due to Ru^{IV}. These EPR experiments were repeated under the same conditions except that $NaClO_4$ was added just before freezing the samples. The spectra were identical for the first few hours of aquation and only differed in the later stages since the colour changes only to brown and not to purple (*i.e.* no Ru^{IV} produced).

Cyclic Voltammetry.—A cyclic voltammogram of a fresh 0.2 mmol dm^{-3} solution of complex **1** in 10 mmol dm^{-3} hepes buffer, pH 7 is shown in Fig. 3. The same results were obtained with either 100 mmol dm^{-3} $NaClO_4$ or NaCl as supporting electrolyte. The Ru^{II}–Ru^{III} midpoint reduction potential, E_m, was pH dependent having a slope of $\Delta E = -118$ mV per pH unit around and above neutral pH (see Fig. 3). At lower pH the slope decreased to a value of $\Delta E \approx -59$ mV per pH unit around pH 4. At low pH (4–6) and low scan rate (10 mV s^{-1}) the peak-to-peak separation (ΔE_p) was 58–60 mV, close to the theoretical value of $58/n$ ($n = 1$) mV for a fully reversible system. Increasing the scan rate to 100 mV s^{-1} led to $\Delta E_p = 70$ mV. Above pH 6, ΔE_p increased to *ca.* 100 ± 10 mV at the highest pH values for a scan rate of 100 mV s^{-1}. Assuming a diffusion coefficient of 3.3×10^{-6} cm^2 s^{-1}, the heterogeneous

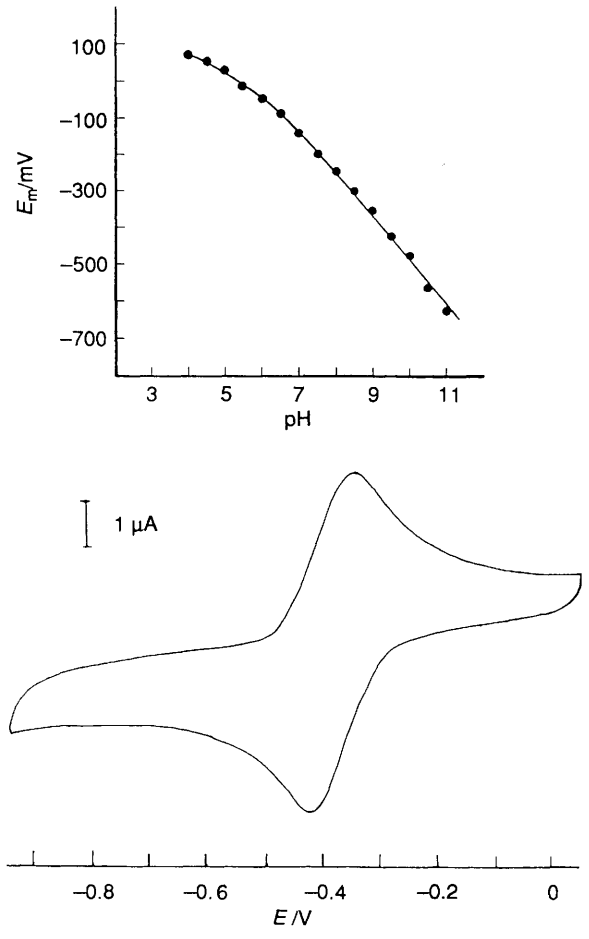


Fig. 3 Cyclic voltammogram (lower) of complex **1** at pH 7 and pH dependence (upper) of the reduction potential of **1**

rate constant is *ca.* 10^{-2} cm s^{-1} at pH < 7, and increases to *ca.* 10^{-3} cm s^{-1} at high pH. Complete stability of the reduced form on this time-scale can be deduced from the observed anodic to cathodic peak-current ratios of 1 ± 0.1 : 1 over the range pH 4–10, and therefore the mean of the cathodic and anodic potentials provides good estimates of the formal reduction potentials.

The observed dependence of E_m *vs.* pH suggests the existence of two pK_a values for the oxidized form of complex **1** in the pH range studied. Therefore, the data of Fig. 3 were fitted by equation (1).[26] An excellent fit was obtained for the parameters

$$E_m = E_m(\text{low pH}) + (RT/nF) \ln \{[H^+]^2/([H^+]^2 + K_{ox1}[H^+] + K_{ox1}K_{ox2})\} \quad (1)$$

E_m(low pH) = +92 ± 10 mV, pK_{ox1} = 3.87 ± 0.17, pK_{ox2} = 6.26 ± 0.21. At high pH (> *ca.* 7.3) the one-electron reduction is therefore coupled to the uptake of two protons. Such a process has been observed previously for the reduction of $[RuL(H_2O)]ClO_4$ {HL = [2-hydroxy-2-(2-pyridyl)ethyl]-bis[2-(2-pyridyl)]amine},[19] although in the present case it is not clear which groups are protonated. The only ionizable protons would appear to be the NH groups on the co-ordinated imidazoles but from ^{1}H NMR shifts there is no evidence that these ionize within this pH range. An interesting alternative is that d electrons on Ru^{II} are protonated. Such a mechanism has been suggested by Taube and co-workers[27] who proposed that the seven-co-ordinate intermediate $[Ru(NH_3)_6H]^{3+}$, which can be viewed as a ruthenium(IV) hydride species, occurs during the aquation of $[Ru(NH_3)_6]^{2+}$. A second protonation would effectively give a ruthenium(IV) dihydrogen complex.

Continuous cyclic voltammetry of solutions of complex **1** in the range pH 10–11 showed its conversion into another species having a 200 mV more positive E_m. The rate constant of the conversion at pH 11 was *ca.* 1 h^{-1}.

The calculated E_m value (−1.17 V) for the reduction of complex **1** using Lever's equation[28] differs considerably from that observed (+0.92 V), perhaps because the equation was formulated for neutral and positively charged complexes.

Conductivity.—The molar conductivities of fresh solutions of complex **1** in dmf and in water were determined to be 52 and 68 Ω^{-1} cm^2 mol^{-1}, respectively, at 298 K, *i.e.* typical of 1 : 1 electrolytes. The conductivity of the dmf solution remained constant with time, but that of the water solution (pH 4.2) increased to 143 Ω^{-1} cm^2 mol^{-1} after 22 h and to 260 Ω^{-1} cm^2 mol^{-1} after 2 d, consistent with aquation and the release of Cl^- ions on a similar time-scale to that observed by NMR spectroscopy.

HPLC.—The aquation products from an aqueous solution of complex **1** were also detected by HPLC. The reaction was monitored over 10 h at ambient temperature using a mobile phase of 70% MeCN–30% 5 mmol dm^{-3} KH_2PO_4 (pH 4.5). Initially only two peaks were observed, one at 15 min assignable to $[RuCl_4(Him)_2]^-$ **1**, and one at 3 min for the imidazolium cation H_2im^+. Based on peak area, a half-life for the decomposition of **1** of *ca.* 11.2 h (296 K) was calculated by graphical extrapolation.

Three new peaks were observed during the course of the reaction with retention times of 2.59, 3.23 and 3.45 min, similar to data we have briefly reported previously for complex **1** dissolved in physiological buffer.[29] A possible assignment of the peak at 3.45 min is the neutral complex $[RuCl_3(Him)_3]$, based on a comparison of its retention time and absorption spectrum with those of an independently prepared sample. After 24 h this was the major species present (*ca.* 60%), and the peak due to H_2im^+ had decreased to 5% of its original intensity. The peaks with retention times of 2.59 and 3.23 min are assigned to the aqua species $[RuCl_2(H_2O)_2(Him)_2]^+$ **3** and $[RuCl_3(H_2O)(Him)_2]$ **2**, respectively. The former peak also appears in the HPLC upon decomposition of $[RuCl_3(Him)_3]$. The UV spectra of both aqua species (λ_{max} 360 nm) are comparable to that of the isolated aqua complex, $[RuCl_3(H_2O)(mind)_2]$ (mind = 1-methylindazole).[30] The course of the aquation of **1** as monitored by HPLC appears to differ from that seen by NMR spectroscopy since ^{1}H NMR peaks for $[RuCl_3(Him)_3]$ (δ −20.0, −12.4, − 2.6, 1.4 and 2.0; 310 K, pH* 6.94) did not appear in spectra during aquation of **1**. This may be attributable to the different conditions necessary for the HPLC separation of the products compared to those in the NMR experiments, in particular the presence of high concentrations of acetonitrile, and the effect of the hydrophobic reversed-phase medium.

Conclusion

The data obtained show that the anticancer complex *trans*-$[H_2im][RuCl_4(Him)_2]$ **1** readily undergoes aquation in aqueous solution with a half-life of 3.4 h at 310 K (body temperature), a time-scale similar to that for aquation of the platinum drug cisplatin. For the latter complex the aqua adducts are much more reactive, *e.g.* towards DNA bases, than is the parent chloro complex, and there are indications that this is also the case for the ruthenium complex.[8] Three hydrolysed products of **1** were observed by ^{1}H NMR spectroscopy, assigned as the monoaqua adduct, formed initially, and isomeric diaqua adducts. The drop in pH* during aquation suggests that water ligands can dissociate resulting in the formation of hydroxo complexes, although the paramagnetically shifted imidazole ligand resonances seem to be insensitive to such ionization. Isolation of these aqua adducts and determination of their pK_a values by other techniques (*e.g.* potentiometry) would

be worthwhile. Attempts to control the pH during aquation with phosphate and acetate buffers led to side reactions including the production of ruthenium(IV) species. Aquation was also detectable by EPR spectroscopy, conductometric and HPLC studies. There was an apparent formation of $[RuCl_3(Him)_3]$ during the course of the HPLC separation, but this may be a consequence of the conditions employed. The reduction potential of **1** measured by cyclic voltammetry showed a curious pH dependence indicative of a one-electron reduction/two-proton uptake process, with pK_a values for the ruthenium(III) complex **1** of 3.87 and 6.26. It is not clear which groups on **1** become protonated during the reduction, although it is possible that the protons reside directly on the metal in the stable (electrochemical time-scale) ruthenium(II) product. The ease of reduction of **1** increases with decreasing pH, which has implications for its biological activity. Although extracellular compartments such as blood plasma have high pH values (7.4), there are compartments in cells which are quite acidic, including endosomes (pH 5.6) which handle transferrin, a possible transport agent for ruthenium complexes.[2,29] In these biological compartments reduction should be more favourable.

Acknowledgements

We thank the Cancer Research Campaign, Medical Research Council, SERC, Deutsche Krebshilfe (Dr. Mildred-Scheel-Stiftung für Krebsforschung), EC COST D1 (project D1-92-002) and HCM (ERBCHRXCT 920016) programmes, and University of London Intercollegiate Research Service for their support for this work.

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Received 25*th July* 1994; *Paper* 4/04547E