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KINETICS OF SUBSTITUTION OF PYRAZINE MONO-N-OXIDE AND ISONIAZID ON AQUOPENTAMMINERUTHENIUM(II)

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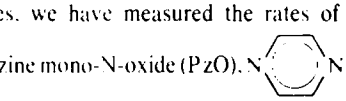
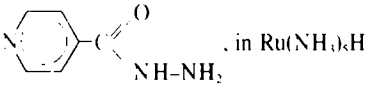
Abstract—The substitution of pyrazine mono-N-oxide in aquopentaammineruthenium(II) presents the following kinetic parameters: $k(25°C) = 3.0 \times 10^2\ M^{-1}\ s^{-1}$, $\Delta H^{\ddagger} = 16.4$ kcal/mole^{-1}, $\Delta S^{\ddagger} = -10$ cal K^{-1} mole^{-1}. Up to 20% of the oxide is involved in parallel redox reaction. Isoniazid substitutes yielding a complex bound through the pyridinic nitrogen, by a path involving the neutral molecule, $k(25°C) = 9.2 \times 10^{-1}\ M^{-1}\ s^{-1}$, $\Delta H^{\ddagger} = 16.6$ kcal/mole^{-1}, $\Delta S^{\ddagger} = -7$ cal K^{-1} mole^{-1}, and by an acid path, $k(25°C) = 5 \times 10^{-1}\ M^{-1}\ s^{-1}$. The two possible (D and Id) mechanisms for substitution in $Ru(NH_3)_5H_2O^{2+}$ are discussed.

INTRODUCTION

Allen and Ford[1] and later Shepherd and Taube[2] have studied the mechanism of substitution of various ammines in $Ru(NH_3)_5H_2O^{2+}$, finding that a dissociative mechanism (Id or D) is operative, with small changes in rates on changing the ligand. In some cases a path was found which was pH-dependent, and it was suggested that this path implied the interaction of a proton with the πd electrons of Ru(II). These data, together with the kinetic results for the acid catalyzed aquation, allowed the evaluation of some stability constants, giving an idea of the relevance of π backbonding to the stability of complexes of aromatic ammines. For the case of isonicotineamide, a lower limit of $K_{stab} = 10^8\ M^{-1}$ was established at 25°C[2].

In the course of our studies of the reactions of coordinated ammines, we have measured the rates of substitution of pyrazine mono-N-oxide (PzO),  , which provides a good comparison with pyrazine (Pz), serving as a check on the lack of discrimination of $Ru(NH_3)_5H_2O^{2+}$ towards different ligands. In addition, this study was aimed at an understanding of the redox interaction (or lack of) between PzO and Ru(II), as previous work[3] had indicated that the complex could be obtained and was stable, whilst PzO was easily reduced by V(II) and Cr(II), in a reaction where coordination between the metallic center and the oxygen atom of PzO is involved.

On the other hand, we also present here kinetic data on the substitution of isonicotinic acid hydrazide (isoniazid, INAH),  , in $Ru(NH_3)_5H_2O^{2+}$, which was undertaken because of our interest in the coordination chemistry of this powerful tuberculostatic agent. Previous reports[4] on complexes of INAH showed chelation through the hydrazide moiety, and there are no kinetic data on its complexation reactions, other than the results on the redox decomposition of the copper(II) and manganese(III) complexes[5] and indirect evidence from pH-metric titrations[4(d)]. By comparison with the data on isonicotineamide, an estimate of the stability constant of $Ru(NH_3)_5INAH^{2+}$ is obtained.

EXPERIMENTAL

Reagents. $Ru(NH_3)_5H_2O^{2+}$ was prepared in solution either by the standard procedure[6] of dissolving $[Ru(NH_3)_5Cl]Cl_2$ in a solution of silver trifluoroacetate (in the molar ratio 1:2) or, in the case of more dilute solutions, by simply dissolving the slightly soluble solid chloride. This solution was deaereated and reduced with freshly prepared amalgamated zinc under an atmosphere of argon. To ensure protection against oxidation by oxygen, high purity argon (La Oxígena), previously scrubbed through two flasks containing Cr(II) was employed.

$[Ru(NH_3)_5Cl]Cl_2$ was prepared from recrystallized $Ru(NH_3)_6Cl_3$ and recrystallized from hot 0.1 M HCl.

Pyrazine mono-N-oxide was prepared from recrystallized pyrazine (Fluka) following essentially the procedure of Klein and Berkowitz[7], by oxidation with 30% H_2O_2 in glacial CH_3COOH at 75–80°C. The product was recrystallized twice, from $HCCl_3$ and from benzene; purity was checked by IR, UV, m.p. and elementary analysis.

INAH was a gift from G. Ramón Laboratories (Buenos Aires) and was purified following standard techniques. Iodometric titration[8] showed the purified substance to be not less than 99% INAH.

All other reagents were analytical grade and were used as provided.

Kinetic experiments. Doubly distilled water (once from alkaline $KMnO_4$) was employed. Deaereated solutions of $Ru(NH_3)_5H_2O^{2+}$ and ligand were mixed under argon in a Zwickel flask[9], which allowed transfer to the cell of a Beckman DK-2A spectrophotometer. Syringe transfer of $Ru(NH_3)_5H_2O^{2+}$ was found to be inadequate, as extensive oxidation took place. The course of the reaction was followed at 527 nm (maximum for the PzO complex)[3] or at 476 nm (maximum for the INAH complex). Ionic strength was kept constant at 0.1 M by the addition of KCl. Experiments were performed in the pH range 1.5–5.0 and at temperatures ranging from 15 to 35°C. All runs were carried out in a large excess of ligand, under pseudo first order conditions.

RESULTS AND DISCUSSION

Pyrazine mono-N-oxide as a ligand

A large bathochromic shift accompanies the replacement of Pz by PzO in $Ru(NH_3)_5L^{2+}$ [3], which is similar to the shift attending protonation of pyrazine (λ_{max} for $Ru(NH_3)_5PzH^{3+}$ is 529 nm)[10]. This shift implies that the π interaction between Ru(II) and the aromatic moiety is increased substantially upon oxidation of the terminal nitrogen of Pz. A kinetic study substantiates this conclusion[3]: the reducibility of the

N–O moiety is arrested upon coordination in spite of the lower frequency of the N–O vibration, indicating a loss in stabilization energy as the prevailing factor. This increased interaction however should not be reflected in the rates of formation; in fact, the dipole moment of PzO which is 1.66 D[11] would in any case favour the interaction of Ru(II) with the oxygen end of PzO when the reactants approach.

The results of the kinetic measurements are shown in Table 1. Good second order rate constants are obtained during the first 40% of reaction. At higher conversions, some deviations from linearity were evident, and noticeable differences between expected and experimental values of A_∞ were found. This discrepancy, amounting usually to 20%, was taken into account, correcting accordingly the rate constants: values tabulated in the last column of Table 1 are the corrected rate constants. The extent of the divergence did not correlate noticeably with either reactants concentrations, or with pH. It showed however a slight increase with increasing temperature. The ratios $(A^{\infty}_{exper}/A^{\infty}_{calcd})$, which we take as a measure of the deviation from the simple stoichiometry, are shown in Table 2, for several typical experiments. Occasionally, experiments with a much lower yield in Ru(II) were encountered, but these were disregarded as arising from air leaks into the solution. In order to eliminate the possibility that a similar and systematic air leak was responsible for the divergence, stoichiometric experiments were conducted complexing $Ru(NH_3)_5H_2O^{2+}$ generated *in situ*, in the presence of amalgamated zinc. Similar differences (15–20%) were found with the absorbance values calculated using the reported extinction coefficient[3] obtained by dissolving weighted amounts of the recrystallized solid. This value was checked and confirmed in the course of the present work. On the other hand, no discrepancies were found in experiments performed with pyrazine as the incoming ligand. The yield of Ru(II)–Pz according to published values for the extinction coefficient[2] was in no case lower than 95%.

Table 1. Rate constants for the reaction $Ru(NH_3)_5H_2O^{2+} + PzO$ at I = 0.1 M (KCl)

[Ru(II)] × 10⁴/M	[PzO] × 10³/M	pH	t/°C	k × 10²/M⁻¹ sec⁻¹
1.65	12.9	4.5	20.0	2.03
1.64	26.0	4.5	20.0	1.96
1.31	14.5	4.5	20.0	1.94
2.25	100.0	4.8	20.0	1.77
2.25	26.0	4.5	20.0	1.90
0.62	6.25	4.5	20.0	2.23
0.62	26.0	4.5	20.0	1.96
1.65	14.5	4.5	15.0	1.16
1.65	26.0	4.5	15.0	1.17
1.68	50.0	4.5	15.0	1.06
1.65	100.2	4.5	15.0	0.98
0.64	26.0	4.5	15.0	1.13
0.60	26.0	4.5	25.0	2.80
1.20	26.0	4.5	25.0	2.92
1.65	26.0	4.5	25.0	2.84
2.20	26.2	4.5	25.0	3.20
1.60	12.9	4.5	25.0	3.15
1.65	50.4	4.5	25.0	2.90
1.60	100.3	4.5	25.0	3.15
1.65	26.0	4.5	30.1	5.06
1.60	50.0	4.5	30.1	5.23
1.60	100.2	4.5	30.1	4.80
1.60	26.0	4.5	35.1	7.30
1.60	50.0	4.5	35.1	7.82
1.68	100.2	4.5	35.1	7.83
1.65	26.0	5.0	25.0	2.86
1.65	26.0	4.2	25.0	2.65
1.68	26.0	3.9	25.0	2.90
1.65	26.0	3.2	25.0	2.73
1.65	26.1	2.5	25.0	3.18
1.65	26.1	2.0	25.0	3.00
1.65	26.1	1.5	25.0	3.20

Table 2. The stoichiometry of the reaction $Ru(NH_3)_5H_2O^{2+} + PzO$†

[Ru(II)] × 10⁴/M	[PzO] × 10³/M	pH	t/°C	% Yield Ru(II)–PzO‡
1.65	26.0	5.0	25.0	88
1.65	26.0	4.5	25.0	82
1.65	26.0	4.2	25.0	75
1.68	26.0	3.9	25.0	79
1.65	26.0	3.2	25.0	89
1.65	26.1	2.5	25.0	85
1.65	26.1	2.0	25.0	72
1.65	26.1	1.5	25.0	80
1.60	12.9	4.5	25.0	81
1.60	100.3	4.5	25.0	83
1.64	26.0	4.5	20.0	91
1.65	26.0	4.5	15.0	92
1.65	26.0	4.5	30.1	80
1.60	26.0	4.5	35.1	73

†Only typical experiments shown.

‡Calculated as $(A^{\infty}_{exper}/A^{\infty}_{calcd}) \times 100$; A^{∞}_{calcd} was obtained using $\epsilon = 1.4 \times 10^4\ M^{-1}\ cm^{-1}$.

The measured rate constant, once corrected, corresponds to the complexation reaction through nitrogen, and is in excellent agreement with previous data for other heterocycles[2]. In Table 3, the rate constants and activation parameters for various incoming ligands are shown. It can be seen that all activation enthalpies are the same within the experimental error, and the differences in rates are to be traced to an entropic factor arising from differing steric situations. Thus, the results for PzO and Pz at 25°C are in excellent (although somewhat fortuitous, in view of the compensation of differences in $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$) agreement; the ratio k_{Pz}/k_{PzO} is 1.87 at 25°C and seems to reflect simply the availability of two positions of coordination in Pz as compared to one for PzO.

Substitution in $Ru(NH_3)_5H_2O^{2+}$ has been view from the perspective of a D mechanism[1, 2]:

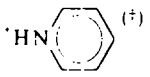
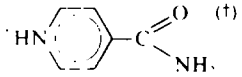
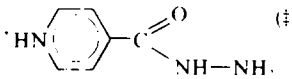
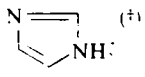
$$Ru(NH_3)_5H_2O^{2+} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Ru(NH_3)_5^{2+} + H_2O \qquad (1)$$

$$Ru(NH_3)_5^{2+} + L \xrightarrow{k_2} Ru(NH_3)_5L^{2+} \qquad (2)$$

$k_{-1}[H_2O]$ being much larger than $k_2[L]$ for all cases studied so far. Consequently, the second order experimental rate constants have been interpreted as (k_1k_2/k_{-1}). The rate constants k_2 and k_{-1} are assigned similar activation enthalpies, thus accounting for the constancy of the experimental value, which corresponds to k_1.

All differences in rates being attributed only to steric factors in k_2, there is no *a priori* reason to disregard oxygen coordination during the formation of the PzO complex. If a truly dissociative D mechanism is accepted as suggested by Allen and Ford[1] the most reasonable interpretation of the data involves the simultaneous formation of both isomers, followed by a rapid aquation,

Table 3. Rate parameters for the substitution of various ligands in $Ru(NH_3)_5H_2O^{2+}$

Ligand	$k_{25°} \times 10^2$/M^{-1} sec^{-1}	$\Delta H^{\ddagger}$/kcal mole^{-1}	$\Delta S^{\ddagger}$/cal K^{-1} mole^{-1}
Pyridine†	9.3	16.9	−6.6
β-picoline†	9.1	16.9	−6.7
α-picoline†	0.3	—	—
Pyrazine†	5.6	17.5	−5.7
3-methylpyrazine‡	5.0	—	—
Pyrazine oxide‡	3.0	16.4	−10
Isonicotinamide†	10.5	—	—
Isoniazid‡	9.2	16.6	−7.7
	0.31	16.9	13
	0.36	—	—
	0.5	—	—
	0.27	—	—
$OOC-CH_2CN$‡	120	15	−8
NCS⁻ †	40	—	—

†Data from Ref. [2].
‡This work.

isomerization and/or redox decomposition of the oxygen bound complex. This is not unreasonable, as aquation of Ru(II)–O bonds is fast: for neutral carboxylic acids, values around $k = 20\ sec^{-1}$ have been reported[12, 13], and even negatively charged carboxylates aquate with rate constants higher than $1\ sec^{-1}$[12, 13]. Water itself is thought to exchange with $k = 2.7\ sec^{-1}$[2], and these values are several orders of magnitude higher than the pseudo first order rate constants measured in our work. The question remains as to how safe it is to compare a possible Ru–O bond in the PzO case with the Ru–O in a carboxylate complex, in view of the very different donor and acceptor properties of oxygen in the two cases, although the rapid aquation is surely reasonable in any case.

If a Ru(II)–O bond is formed at all, it could be expected that oxidation of the Ru(II) by transfer of the oxygen atom could result, with release of pyrazine. PzO is in fact reduced to Pz by other reductant ions, such as V(II), upon coordination through oxygen[3]. Oxidation of the Ru(II) is probably responsible for the discrepancies between the theoretical and actual yields of the Ru(II)–PzO complex, but whether this occurs via oxygen bonding is an open question. In view of the short time of residence of oxygen bound ligands, we may infer that the reaction is fast, with rate constant of the same order of magnitude as the aquation (taking into account the yield of redox products and assuming similar rates of formation of oxygen and nitrogen bound complexes). It should be pointed out that in the comparable reaction of $Ru(NH_3)_5H_2O^{2+}$ with N_2O[14] no evidence was reported for oxygen coordination: on the other hand, a redox interaction occurs in the reaction with some pyridinic derivatives, but then only at high acidities[2].

It should be mentioned however that even though some evidence has been presented in favour of a D mechanism, an Id mechanism is much more likely to be operative. It has been pointed[1] out that the rather small changes in the rates of substitution when the charge of the ligand is varied are better interpreted as resulting from changes in the diffusional rate (k_2) rather than as a result of varying degrees of outer sphere interactions. The changes in fact are not so very small: X ions replace water 100 times faster than neutral ligands, and these in turn show rate constants 10 times larger than monopositive ions: see Table 3. These factors are larger than those found in the substitution reactions of $Fe(CN)_5H_2O^{3-}$ which are supposed to be more dissociative[15]. This fact, together with the results for methylpyrazinium ion[16] and 2,6-lutidine[2], where outer sphere association has been diagnosed, and the rather large rates for acetonitrile[2] indicate that outer sphere association may be widespread in these systems[17]. For methylpyrazinium ion (Mpz^+) the following scheme has been proposed[16]:

$$\begin{array}{ccc} & & M\ldots L \xrightarrow{k_3} (NH_3)_5RuMpz^{3+} + H_2O \\ & \overset{k_1}{\rightleftharpoons} & \uparrow\downarrow \\ (NH_3)_5RuOH_2^{2+} + Mpz^+ & & \downarrow\uparrow \\ & \underset{k_{-3}}{\rightleftharpoons} & \\ & & X \xrightarrow{k_4} \text{Redox decomposition} \end{array} \qquad (3)$$

where the equilibrium between M...L and X is pH dependent, higher acidities favouring the formation of X. In fact, all our data are consistent with Scheme (3), with the difference that the M...L⇄X interconversion would not be effected by pH, and we have much lower values of K_1 and K_2. Under this scheme, $k_{exper} = k_3K_1$, and the constant activation enthalpy for a whole series of amines is interpreted by assuming that ΔH_1 is not very sensitive

to the nature of L, or that it is very small. K_1 values would then be governed by the basicity and long range π interactions[16], and some kind of compensation could be operative here (as it is in the stability constants of iron(II) prussides with aromatic amines[15]) rendering K_1 values rather insensitive to the nature of the ligand.

In the case of PzO, no acid-catalyzed path is detected, and this is in agreement with its low basicity (pK_a = 0.05)[18].

Isoniazid as a ligand

Some years ago much attention was devoted to the complexes of isoniazid in connection with the role that they might play in the inhibition of *Mycobacterium t.* growth. All the complexes reported, resulting from the interaction of isoniazid with several aquo ions were chelates in which coordination was achieved through the hydrazide function, with release of protons[4]. As expected, in the presence of pentammineruthenium(II), coordination is achieved through pyridinic nitrogen; the same is true for low spin pentacyanoferrate(II)[19]. The charge-transfer band of the ruthenium–isoniazid complex resembles closely the band of the isonicotinamide complex[10] (λ_{max} are 476 and 479 nm, and log ϵ are 3.96 and 4.20 respectively); the similarity also shows up in the rates of substitution in pentacyanoferrate(II)[19].

Isonicotinamide substitutes in pentammineruthenium(II) by a direct and by an acid-catalyzed path[2]. The rate data for isoniazid can also be fitted into a rate law of the form $v = k_0[INAH] + k_H[INAH_2^+]$, as shown in Table 4. The precision of the data was not good, as experimental difficulties, resulting probably from the oxidation of the complex were great, especially at lower pH values. The acid path has been interpreted in terms of a pre-equilibrium involving H^+ and t_{2g} electrons on the Ru(II)[2], i.e. actual reactants would be $(NH_3)_5Ru(OH_2)H^{3+}$ and the free base. It is striking however that the less basic amines, e.g. Pz or PzO, which could be present as free bases at pH values where protonation on Ru(II) is kinetically important, do not show such a path. Probably the basicity of the amine is an important ingredient in "bridging" H^+ and Ru(II). Anyway, as expected, INAH and isonicotinamide show similar acid path constants, and this fact, together with the similarity of the visible spectrum, indicate that the stability constant for the INAH complex should be similar to the value reported for isonicotinamide (K_{stab} higher than $10^8\ M^{-1}$)[2], and this is among the highest stability constants reported for the interaction of INAH with divalent ions: see Table 5.

Table 4. Rate constants for the substitution of isoniazid on $Ru(NH_3)_5H_2O^{2+}$†

t/°C	pH	$k \times 10^2/M^{-1}\ sec^{-1}$
25.0	5.0	8.7 (9.2)‡
25.0	4.5	8.5
25.0	4.0	6.0
25.0	3.5	2.0
25.0	2.8	1.4 (0.5)§
20.0	5.0	4.9 (5.2)‡
15.0	5.0	3.2 (3.4)‡
10.0	5.0	1.7 (1.8)‡

†Rate constant k in the third column calculated as k_{exp}/c^0, where c^0 stands for total concentration of INAH.

‡Figures within parentheses are k_0 in the equation rate = $k_0[INAH] + k_H[INAH_2^+]$, and were calculated from the experimental value at the highest pH, and corrected using pK_a = 3.8.

§Figure within parentheses is k_H in the above equation, calculated from the experimental value at the lowest pH (2.8); pH values below 2.8 were not studied to avoid complications from the second protonation of INAH ($pK_a \cong 2$).

Table 5. Stability constants for 1:1 complexes of INAH

Metallic center	pK	Reference
Cu^{2+}	10.0; 8.0	[4(d), 4(a)]
Zn^{2+}	7.2; 5.4	[4(d), 4(a)]
Ni^{2+}	5.6; 5.5	[4(d), 4(a)]
Co^{2+}	4.6; 4.8	[4(d), 4(a)]
Cd^{2+}	3.4	[4(d)]
Mn^{2+}	4.1	[4(d)]
$Fe(CN)_5^{3-}$	4.6	[19]
$Ru(NH_3)_5^{2+}$	8	This work

All the data therefore point to a close similarity between INAH and isonicotinamide as potential competitors for attachment to $Ru(NH_3)_5^{2+}$, or $Fe(CN)_5^{3-}$ [19], showing similar high affinities; this aspect might be of interest when discussing the interactions of isoniazid and related substituted pyridines with metal ions in biological systems.

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