Kinetic studies of substitution on 
\[(\text{H}_2\text{O})(\text{tap})_2\text{RuORu}(\text{tap})_2(\text{H}_2\text{O})\]²⁺ ion by 
DL-penicillamine at physiological pH

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The reaction of DL-penicillamine with the title complex has been studied spectrophotometrically in aqueous solution as a function of concentrations of the substrate complex and ligand, pH and temperature. The reactivity of different donor centers (i.e., (N), (N=O) and (O=O)) have been compared. At pH 7.4 the reaction is a two-step process. Unlike other systems studied with the same substrate, the second step in the present case is ligand independent, ascribed to the lower difference between the two steps \((k_1 = 1.78 \times 10^{-3} \text{ s}^{-1} \text{ and } k_2 = 0.67 \times 10^{-4} \text{ s}^{-1}\) respectively at 50°C) in comparison to the other systems studied earlier \((k_1 = 10^{-3} \text{ s}^{-1} \text{ and } k_2 = 10^{-2} \text{ s}^{-1}\). An associative interchange mechanism is proposed for both the steps. The activation parameters \((\Delta H^* = 19.2 \pm 1 \text{ kJ mol}^{-1}, \Delta S^* = -239 \pm 3 \text{ JK}^{-1} \text{ mol}^{-1}; \Delta H^* = 65.6 \pm 5.8 \text{ kJ mol}^{-1} \text{ and } \Delta S^* = -123 \pm 17 \text{ JK}^{-1} \text{ mol}^{-1})\) support the proposition. The activation parameters have been compared with other systems studied earlier.

Nucleophilic substitution reactions of ruthenium(II/III) complexes are well studied. Literature shows that no single mechanism can explain these reactions and the reaction path depends on the nature of the complexes as well as that of the incoming ligands. Substitution reactions of ruthenium(II/III) complexes are important due to their wide applications as DNA binders, antitumour, anti-HIV, antiamebic, anticancer, antileukemic, antimitastatic, antifungal and immuno-suppressive agents. Ruthenium complexes can also be used in wastewater treatment.

The aim of the present work is to study the complex formation of ruthenium(II) with some bioactive molecules and to carry out kinetic studies of substitution reaction of DL-penicillamine on the complex cation, \(\text{[(H}_2\text{O})(\text{tap})_2\text{RuORu}\text{(tap)}_2\text{(H}_2\text{O})\]²⁺\) near the physiological pH.

In continuation of our earlier works related to the reactivity of the complex ion \(\text{[(H}_2\text{O})(\text{tap})_2\text{RuO}\text{-Ru}(\text{tap})_2\text{(H}_2\text{O})\]²⁺\) \(\text{[tap} = 2-(m\text{-tolylazo})\text{pyridine, an unsymmetrical bidentate N,N-chelator with azoimine (-N=N-C=N-) function]}\) we report herein the interaction of DL-penicillamine (β,β-dimethyl-cysteine), a degradation product of β lactum antibiotic, penicillin with the title complex. The present study has been carried out in aqueous solution and at pH 7.4, under which conditions most of the ruthenium(II) complexes are oxidised to ruthenium(III); however, in the present case, the +2 state of the metal ion in \(\text{I}\) is quite stable due to the presence of an excellent π-acceptor ligand, tolylazopyridine (tap).

**Experimental**

The compounds, \(\text{cis\text{-diaqua\text{-bis\{-2\-(m\text{-tolylazo)\text{-pyridine}\}\text{ruthenium(II) diperchlorate, monohydrate, cis\{-Ru(tap)}_2\text{(H}_2\text{O})_2\text{\{ClO}_4\text{_2\text{H}_2\text{O and the reacting complex ion }\text{[(H}_2\text{O})(\text{tap})_2\text{RuORu(tap)}_2\text{(H}_2\text{O})\]²⁺ (I)}\)}\} were prepared following the methods described earlier\(^1\). The product, \(\text{[(tap)]}_2\text{Ru(µ-O)(µ-DL-pen)}\text{Ru(tap)}_2\]²⁺, \(\text{(2)}\) of the reaction between complex \(\text{I}\) and DL-penicillamine (DL-pen) was prepared by mixing the reactants in 1:10 ratio and thermostated at 50°C for 72 h. The spectrum of \(\text{2}\) shows good complexation between DL-pen and \(\text{I}\). Repeated attempts to isolate the solid product failed, but the composition in solution was determined by Job’s method of continuous variation. The metal:DL-pen ratio was found to be 2:1. The pH of the solution was adjusted by adding NaOH/HClO₄ and the measurements were made with a Systronics digital pH meter with an accuracy of ±0.01 unit. Doubly distilled water was used to prepare all the kinetic solutions. All chemicals used were of AR grade. The reactions were carried out at constant ionic strength \((0.1 \text{ M NaClO}_4)\).

Kinetic measurements were carried out on a Shimadzu UV 2101PC spectrophotometer attached to a thermoelectric cell temperature controller (model TB 85 thermo bath, accuracy ±0.1°C). The conventional mixing technique was followed and pseudo-first order conditions were employed throughout. The progress of the reaction was followed by measuring the decrease in absorbance at 600 nm, where the spectral difference between the substrate and the product complex was maximum. Plots of \(\text{ln(A}_t\text{-A}_\infty)\) against time where \(\text{A}_t\) and \(\text{A}_\infty\) are the absorbances at time \(t\) and at infinite time are non-
linear until the later stages of reaction suggesting a consecutive type of mechanism (Fig. 1). The method of Wyeh and Hamm, as described earlier\(^\text{13}\), was adopted to calculate the rate constants for two consecutive steps. The rate data represented as an average of duplicate runs are reproducible within ± 4%.

**Results and discussion**

The \(pK_1\), \(pK_2\) and \(pK_3\) values\(^\text{17}\) of DL-penicillamine are 1.90, 7.88 and 10.58 respectively at 25°C, which correspond to the following three dissociation equilibria:

\[
\begin{align*}
\text{HSCMe}_2\text{CH(NH}_3^+\text{)}\text{CO}_2\text{H} & \rightleftharpoons \text{HSCMe}_2\text{CH(NH}_3^+\text{)}\text{CO}_2^- + H^+ \quad (LH^-) \\
\text{HSCMe}_2\text{CH(NH}_3^+\text{)}\text{CO}_2^- & \rightleftharpoons \text{SCMe}_2\text{CH(NH}_3^+\text{)}\text{CO}_2^- + H^+ \quad (L^-) \\
\text{SCMe}_2\text{CH(NH}_3^+\text{)}\text{CO}_2^- & \rightleftharpoons \text{SCMe}_2\text{CH(NH}_2\text{)}\text{CO}_2^- + H^+ \quad (L_2^-)
\end{align*}
\]

\(pK_1 = 1.90\) …(1)  
\(pK_2 = 7.88\) …(2)  
\(pK_3 = 10.58\) …(3)

Thus at \(pH\) 7.4 the zwitterionic form of DL-penicillamine (\(LH_2\)) is the major species (~ 75%). The anionic species (\(L^-\)) due to its better electron availability will also be a competitor for the interaction. As far as outersphere association is concerned, the second species, \(L^-\), will be a better species for the outersphere association.

The first acid dissociation equilibrium of the complex, \([\text{Ru(tap)}_2(\text{H}_2\text{O})_2]^{2+}\), at 25°C is 6.6 (ref. 13) and is represented as:

\[
[\text{Ru(tap)}_2(\text{H}_2\text{O})_2]^{2+} \rightleftharpoons [\text{Ru(tap)}_2(\text{H}_2\text{O})(\text{OH})]^+ + H^+ \quad (L_2^-)
\]

At \(pH\) 7.4 the complex ion exists in dimeric oxo-bridged form, \([[\text{H}_2\text{O}(\text{tap})_2\text{RuORu(tap)}_2(\text{H}_2\text{O})]]^+\) (ref. 13).

\[
\text{[(tap)}_2\text{Ru} \overset{\text{O}}{\longrightarrow} \text{Ru(tap)}_2]^{2+} \quad (1)
\]

At \(pH\) 7.4, the mononuclear species exists as the hydroxoqua form. Two such species assemble to form the dinuclear oxo-bridged diaqua complex due to thermodynamic force arising mainly from \(\pi\)-bonding\(^\text{18}\) (O\(^2-\) donor, Ru\(^{II}\) acceptor) which is favourable for the 4d ion, Ru\(^{III}\). Such strong covalency reduces the acidity of the coordinated water.

The oxo-bridge formation is solely dependent on the \(pH\). Electrochemical studies show that there is a \(pH\)-potential domain where the \(\mu\)-oxo structures stay intact. Variable temperature study does not show any effect on oxo-bridge formation, which is in agreement with the fact that oxo-bridge formation is solely \(pH\)-dependent\(^\text{19}\).

Job’s method of complexation indicates a 2:1 metal-ligand ratio in the product complex, which is possible only when a bridged product is formed with the ligand. At constant temperature, \(pH\) (at 7.4) and fixed concentration of 1, the \(\ln(A_t - A_{\infty})\) versus time \((t)\) plot for different ligand concentrations indicates a two-step process. The first step is dependent on ligand concentration and with increasing ligand concentration a limiting rate is reached. However, unlike the other systems studied the second step here is independent of ligand concentration.

The rate constant for such a process can be evaluated by assuming the following scheme:

\[
\begin{align*}
(1) & \rightarrow B \rightarrow (2), \\
\text{where } B & = [[\text{H}_2\text{O}(\text{tap})_2\text{RuORu(tap)}_2(\text{DL-pen})]]^+.
\end{align*}
\]
Step (1) \( \rightarrow B \)

The rate constant \( k_{1\text{obs}} \) for \( (1) \rightarrow B \) was evaluated by the method of Wyeh and Hamm as described in an earlier paper. A similar procedure was applied for each ligand concentration in the range \( 1.0 \times 10^{-3} \text{ mol dm}^{-3} \) to \( 5.0 \times 10^{-3} \text{ mol dm}^{-3} \) at constant complex \( (1) \) concentration of \( 1.0 \times 10^{-4} \text{ mol dm}^{-3} \) and \( pH \) 7.4 in the \( 50-65^\circ C \) temperature range and at constant ionic strength (0.1 mol dm\(^{-3}\) NaClO\(_4\)). The rate increases with increase in [ligand] and reaches a limiting value (Fig. 2), which is probably due to the completion of the outersphere association complex formation. Since the metal ion reacts with immediate environment, further change in [Ligand] beyond the saturation point will not affect the reaction rate and a gradual approach towards limiting rate is observed. The outersphere association complex is probably stabilised through hydrogen bonding\(^{10,21}\) between coordinated water and the approaching ligand. At this stage, the interchange of the ligands from the outer sphere to the inner sphere takes place, i.e., the ligand attacks one of the ruthenium(II) center and the coordinated water moves out.

Based on the above findings, Scheme 1 is proposed for the \( (1) \rightarrow B \) step.

\[
\text{Scheme 1}
\]

Based on Scheme 1, a rate expression can be derived for the \( (1) \rightarrow B \) step (Eq. 5).

\[
d[B]/dt = k_1 K_E [(H_2O)(tap)_2RuORu(tap)_2(H_2O)^{2+}][LH^-]/(1 + K_E[LH^-])
\]  

or,

\[
d[B]/dt = k_{1\text{obs}} [(H_2O)(tap)_2RuORu(tap)_2(H_2O)^{2+}]_T
\]  

where \( T \) stands for total concentration of Ru(II).

Thus,

\[
k_{1\text{obs}} = k_1 K_E [LH^-]/(1 + K_E[LH^-])
\]  

The equation can be represented as follows:

\[
1/k_{1\text{obs}} = 1/k_1 + 1/k_1 K_E [LH^-]
\]  

The plot of \( 1/k_{1\text{obs}} \) versus \( 1/[LH^-] \) is linear (Fig. 3) with an intercept of \( 1/k_1 \) and slope \( 1/k_1 K_E \) at all temperatures studied. The \( k_1 \) and \( K_E \) values obtained from the intercept and from slope to intercept ratios are as follows: \( 10^3 k_1 \) values are 1.78, 2.00, 2.22 and 2.50 s\(^{-1}\); while \( K_E \) values are 140, 156, 195 and 236 dm\(^3\) mol\(^{-1}\) s\(^{-1}\) at 50, 55, 60 and 65\(^\circ\) C respectively.
Step B → (2)

The rate constants calculated from latter linear portion of the graphs [ln (A − A∞) versus time plots] show that this step is not dependent on [Ligand]. The coordinated DL-penicillamine in any of the ruthenium(II) center in the first step now attacks the second ruthenium(II) center. The intermediate here is also probably stabilized through hydrogen bonding between coordinated water and the approaching DL-penicillamine. The 10^4k2 (which is the k2(obs) also) values for the B → (2) step are 0.67, 0.86, 1.37 and 2.00 s\(^{-1}\) at 50, 55, 60 and 65°C respectively.

Based on the experimental findings, a two-step associative interchange mechanism is proposed for the substitution process. The dependence of the second step on the ligand concentration is not observed here due to the fact that the difference in rate between the two steps is very small. The outersphere association complex is formed in a very fast step and the interchange of outersphere to innersphere complex occurs in a very rapid step. But we shall not consider the general idea that since cyclisation is taking place, the second step should be independent of ligand concentration. It should be kept in mind that here two metal centers are involved. The coordinated DL-penicillamine in any of the ruthenium(II) centers in the first step now attacks the second ruthenium(II) center like a metalloligand and two distinct ligand dependent steps should be observed. This has been observed in our earlier studies\(^{12-15}\) (k1~10\(^{-3}\) s\(^{-1}\) and k2~10\(^{-5}\) s\(^{-1}\)). However, for the present system (k1~10\(^{-3}\) s\(^{-1}\) but k2~10\(^{-4}\) s\(^{-1}\)), we could not observe the dependence of the rate on the concentration of the ligand in the second step.

Effect of temperature on the reaction rate

The activation parameters for the steps (1) → B and B → (2) were evaluated from the linear Eyring plots. The activation enthalpies and entropies are:

\[ \Delta H^\ddagger_1 = 19.2 ± 1 \text{ kJ mol}^{-1}, \quad \Delta S^\ddagger_1 = -239 ± 3 \text{ J K}^{-1} \text{ mol}^{-1}, \]
\[ \Delta H^\ddagger_2 = 65.6 ± 5.8 \text{ kJ mol}^{-1}, \quad \Delta S^\ddagger_2 = -123 ± 17 \text{ J K}^{-1} \text{ mol}^{-1}. \]

Low \( \Delta H^\ddagger \) values support ligand participation in the transition state for both the steps. Positive energy required for the bond breaking process is partly compensated from the negative energy obtained from bond formation in the transition state and hence a low value of \( \Delta H^\ddagger \) is observed. Large negative \( \Delta S^\ddagger \) value on the other hand suggests a more compact transition state than the starting complex which also corroborates the assumption of a ligand participating transition state.

Mechanism

The bonding mode of DL-penicillamine is not fully understood, as it was not possible to isolate the solid product. In the studied reaction condition i.e. at pH 7.4, DL-penicillamine exists in the deprotonated form. Initially, an S¯ attack on one of the two ruthenium(II) centers is assumed. This step is ligand dependent and with increasing ligand concentration, a limiting rate is reached. This may be due to the formation of outersphere association complex. The outersphere association complex here is possibly stabilized through hydrogen bonding. The spontaneous formation of outersphere association complex is also supported by a negative \( \Delta G^\circ \) value calculated from the temperature dependence of the \( K_E \) values. The coordinated DL-penicillamine in any of the ruthenium(II) centers now attacks the second ruthenium(II) center like a metalloligand and we
obtain a S-atom bridged product. The second step here is independent on the concentration of the ligand. This has been ascribed to the fact that as the difference in rate between the two steps is not appreciable, we could not observe the ligand dependence in the second step. However, this step should be similar to first step as has been observed in many earlier studies. If the cyclisation occurs in the same metal, generally we observe (in case of consecutive reactions) that the second step is independent of ligand concentration. It is to be noted here that the second step is not a normal cyclisation step like in chelation in a single central atom. Here two metal centers are available and after attachment of the ligand to one of the metal center the environment of the two centers will no longer remain same. For this complex, to behave as a bridging ligand, as indicated from the Job’s method, monoatom sulphur bridge is with the best prospects22.

Based on the above observations, mechanism shown in Scheme 2 is proposed.

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References
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