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# Synthesis of S-2-Aminoethyl-L-Cysteine and S-2-Aminoethyl-D,L-Penicillamine Complexes with Pt(II) and Pd(II). Interpretation of IR and $^1$H NMR Spectra and Conformational Implications

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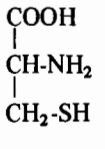
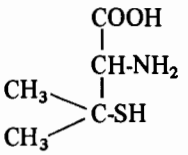
*The Pt(II) and Pd(II) complexes of S-2-aminoethyl-L-cysteine and S-2-aminoethyl-D,L-penicillamine hydrochloride salts have been prepared. The structure of the complexes was studied by elemental analysis, infrared and $^1$H NMR spectra. It was concluded that the amino acid derivatives behave as bidentate ligands, being bound to the metals via the sulphur atom and the amino group near the carboxylic group. There are also indications of a possible equilibrium between the uncoordinated carboxylic group and the electrostatic form of $COO^- \cdots M$ (M = Pt or Pd). The metal complexes are being tested for antitumour activity.*

## Introduction

The discovery by Rosenberg *et al.* [1] that *cis*-dichlorodiammineplatinum(II) had strong antitumour activity prompted other researchers to prepare analogues, using mainly the transition metals Pt(II) and Pd(II) containing different ligands, producing groups with varying biological activity [2].

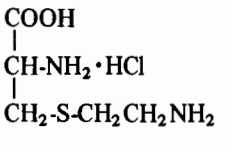
The coordination chemistry of L-cysteine and its derivative, D-penicillamine, has been studied extensively with nontransition

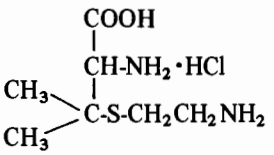




and transition metals and reviewed up to 1979 by Gergely and Sóvágó [3]. A number of authors have reported the formation of complexes of Pd(II) with L-cysteine and D-penicillamine [4], of Pt(II) and Pd(II) complexes with L-cysteine [5] and Pt(II) and Pd(II) complexes with S-methyl-L-cysteine [6], and have studied the coordination sites and their ring conformation by spectroscopic and X-ray techniques.

We report here the synthesis of Pt(II) and Pd(II) complexes of S-2-aminoethyl-L-cysteine hydrochloric salt (S-2-Am-L-Cys·HCl) and S-2-aminoethyl-D,L-penicillamine hydrochloric salt (S-2-Am-D,L-Pen·HCl)





and the interpretation of their structure by infrared and $^1$H NMR spectroscopy and elemental analysis. Our final aim is to study, in cooperation with the National Cancer Institute (USA), the antitumour activity of the complexes in L-1210 lymphoid leukaemia test system in mice and to relate this activity with the structural features of the complexes.

In our attempts to synthesize these particular complexes we considered two important facts. First, that amino acids and their derivatives have been used as transfer agents of biological alkylating compounds. Since platinum complexes behave like alkylating compounds we hope that amino acids will increase the diffusibility of the metal complexes and consequently their biological activity inside the cell. Second, the discovery in 1958 by Connors and Ross [7] that S-2-chloroethyl cysteine ($HOOC\text{-}CH(NH_2)CH_2\text{-}S\text{-}CH_2CH_2Cl$) was the simplest amino acid derivative with biological activity. Although a monofunctional compound not likely to be a tumour growth inhibitor, this compound showed outstanding mutagenic activity in *Drosophila*.

TABLE I. Elemental Analysis of Pt(II) and Pd(II) Complexes.

| Compound | Molecular formula (hydrochloric salts) | M.P. °C | Analyses % Calcd./Found | | |
|---|---|---|---|---|---|
| | | | C | H | N |
| (S-2-Am-L-Cys·HCl)$PtCl_2$ | $C_5H_{13}N_2O_2SPtCl_3$ | 258–60 | 13.7<br>13.4 | 2.8<br>2.6 | 6.0<br>5.7 |
| (S-2-Am-L-Cys·HCl)$PdCl_2$ | $C_5H_{13}N_2O_2SPdCl_3$ | 130–32 | 15.8<br>16.0 | 3.4<br>3.7 | 7.4<br>7.1 |
| (S-2-Am-DL-Pen·HCl)$PtCl_2$ | $C_7H_{17}N_2O_2SPtCl_3$ | 305–07 | 16.9<br>17.3 | 3.4<br>3.2 | 5.6<br>5.3 |
| (S-2-Am-DL-Pen·HCl)$PdCl_2$ | $C_7H_{17}N_2O_2SPdCl_3$ | 135–37 | 20.7<br>19.8 | 4.2<br>3.8 | 6.9<br>6.5 |

TABLE II. Proton Chemical Shifts of S-2-Aminoethyl-L-Cysteine and S-2-Aminoethyl-D,L-Penicillamine and their Complexes with Pt(II) and Pd(II) in ppm.

| Compound | δC–H | $\delta CH_2$ | $\delta NH_3^+$ | $\delta NH_3$ | $\delta CH_3$ |
|---|---|---|---|---|---|
| S-2-Am-L-Cys·HCl[a] | 3.95 | 3.4–2.75 | 4.7 | | |
| (S-2-Am-L-Cys·HCl)$PtCl_2$ [a] | 3.3 | 3.0–2.75 | 4.4 | | |
| (S-2-Am-L-Cys·HCl)$PdCl_2$ [b] | 3.3 | 3.2–2.7 | 4.85 | 8.0 | |
| S-2-Am-DL-Pen·HCl[a] | 3.7 | 3.35–2.8 | 4.75 | | 1.5–1.4 |
| (S-2-Am-DL-Pen·HCl)$PtCl_2$ [a] | 3.3 | 3.0–2.7 | 4.6 | | 1.6–1.5 |
| (S-2-Am-DL-Pen·HCl)$PdCl_2$ [b] | 3.3 | 2.85–2.7 | 4.7 | 8.2 | 1.6–1.4 |

[a] Compound was dissolved in $D_2O$. [b] Compound was dissolved in DMSO.

## Results and Discussion

Elemental analyses of the complexes for C, H and N (Table I) strongly support the ratio 1:1, amino acid derivative:metal, when compared to theoretical calculations.

Of the various vibrational modes in the infrared and far infrared spectra we looked at the stretching vibrations of the $NH_2$ group, the C–S group and the OH and C=O of the carboxylic group in the molecule of the complexes which are the most likely to change due to complexation. Also we looked at the stretching vibrations of the metal–S and metal–Cl bonds (metal = Pt or Pd) which are formed in the complexes.

In the $^1H$ NMR spectra we looked at the proton chemical shifts for the C–H unit of the coordination site metal–N–C–H and the -$CH_2$- groups around the sulphur atom.

### *S-2-Aminoethyl-L-Cysteine Hydrochloride Salt Complexes*

The IR spectra of the free ligand showed absorption bands at 3360sh and in the region 3100–2900s$_b$ $cm^{-1}$, assigned to the OH of the carboxylic group, the $NH_3^+$, the $NH_2$, the CH stretching vibrations and the result of their intermolecular hydrogen bondings.

In the Pt(II) complex we observe two strong bands, at 3460 and in the region 3200–3080 $cm^{-1}$. In the Pd(II) complex the bands appear at 3220sh and in the region 3100–2900s $cm^{-1}$. The IR data in this region cannot give us any indication of which of the amino groups, if any, is coordinated to the metals. A clearer information can be obtained from the $^1H$ NMR data.

The C–H group signal for the free ligand appears at 3.95 ppm whereas in the Pt(II) and Pd(II) complexes the signal appears at 3.3 ppm for both. The upfield shifts for both complexes suggest that the $NH_2$ group near the carboxylic group is the one which bounds to the metals.

The absorption bands at 675w, 665m and 610s $cm^{-1}$ in the IR spectra of the free ligand can be assigned to the C–S group stretching vibration. In the Pt(II) complex there is only one strong band at 668 $cm^{-1}$ and in the Pd(II) complex the absorption bands appear at 672s, 662vw and 605w $cm^{-1}$. The shifts in the absorption bands and the changes in their

intensities suggest complexation of the sulphur atom to the metals. Additional information on the previous suggestion comes from the far infrared with the stretching vibration mode for the metal–S bond in both complexes at *ca.* 400w $cm^{-1}$ [8].

The free ligand exhibits a medium absorption band at 1662 $cm^{-1}$ for the asymmetric stretching vibration of the carboxylic group. In the Pt(II) and Pd(II) complexes we observe absorption bands at 1625vs and 1740w and at 1640vs, 1650vs and 1730vs respectively. The absorption bands for the carboxylic group in the region 1620–1660 $cm^{-1}$ depend on the medium in which the IR was taken and their presence in the complexes can be interpreted as an indication for the $COO^{-}\cdots M$, possibly in equilibrium with an uncoordinated carboxylic group, as the new absorption bands in the region 1740–1730 $cm^{-1}$ in the complexes suggest. According to A. Alain *et al.* [6] the asymmetric stretching vibration for the $COO^{-}\cdots M$ unit should occur at about 1640 $cm^{-1}$.

The stretching vibration band for the metal–Cl bond in the Pt(II) and Pd(II) complexes appears at 335w and 328w $cm^{-1}$ respectively.

The $^1H$ NMR spectra of the free ligand for the $-CH_2-$ groups around the sulphur atom showed a multiplet in the region 3.4–2.75 ppm. In the Pt(II) complex the bands appear in the region 3.0–2.75 ppm and in the Pd(II) complex in the region 3.2–2.7 ppm. The slight upfield shift that we observe in the region of the $-CH_2-$ can be taken as an indication that two of the $-CH_2-$ groups are affected by the complexation of the sulphur atom to the metals.

*S-2-Aminoethyl-D,L-Penicillamine Hydrochloride Salt Complexes*

An analysis of the infrared and $^1H$ NMR data is described below for the free ligand and its two complexes.

The IR of the free ligand exhibits absorption bands at 3400w and in the region $3060–2940s_b$ $cm^{-1}$, assigned to the OH of the carboxylic group, the $NH_3^+$, the $NH_2$ and the CH groups stretching vibrations and their intermolecular bondings. In the Pt(II) complex we observe absorption bands in the region $3480–3320sh_b$, in the region 3180–3120s and at 2960s $cm^{-1}$. In the Pd(II) complex the absorption bands appear at 3500 $m_b$, $3100s_b$ and 2900w $cm^{-1}$.

The determination of which of the amino groups is the complexation site in the complexes was resolved by using the $^1H$ NMR data. In the free ligand the band for the C–H group appears at 3.7 ppm, whereas in both the Pt(II) and Pd(II) complexes the band appears at 3.3 ppm. The upfield chemical shift suggests that the $NH_2$ group near the carboxylic group is the one which binds to the metals.

The stretching vibration mode for the C–S group in the free ligand can be assigned to two bands at 680m and 605s $cm^{-1}$. In the Pt(II) and Pd(II) complexes there appears to be only one medium band at 670 and 665 $cm^{-1}$ respectively. The changes of absorption frequencies in this part of the IR spectrum suggest complexation of the sulphur atom to the metals. Also, as an additional information, we observe in both complexes a weak band at 395 $cm^{-1}$ which can be assigned to the metal–S bond.

The IR spectra of the free ligand show a medium absorption band at 1662 $cm^{-1}$ for the asymmetric stretching vibration of the carboxylic group. In the case of the Pt(II) and Pd(II) complexes the absorption bands appear at 1650s, 1660m and 1733s, and at 1650s, 1720–1725vs respectively. Again the presence of absorption bands at around 1650–1660 can be interpreted as an indication for the unit $COO^{-}\cdots M$, possibly in equilibrium with an uncoordinated carboxylic group, as the new absorption band in the complexes at 1730–1720 $cm^{-1}$ suggests.

The stretching vibration mode for the metal–Cl bond in both complexes is observed at 315, medium for the Pt(II) and as a shoulder for the Pd(II).

The $^1H$ NMR spectra of the free ligand for the $-CH_2-$ groups show a multiplet in the region 3.35–2.8 ppm. In the Pt(II) complex the bands appear in the region 3.0–2.7 ppm and in the Pd(II) complex in the region 2.85–2.7 ppm. Here again we have a slight chemical shift which can be taken as an indication that one of the $-CH_2-$ groups is affected by the complexation of the sulphur atom to the metals.

From the above discussion of the infrared and $^1H$ NMR data and the elemental analysis we conclude that both ligands behave as bidentates, being bound to the metals via the sulphur atom and the amino group near the carboxylic group. Also, there are indications of an equilibrium between the electrostatic attraction $COO^{-}\cdots M$ and the uncoordinated carboxylic group.

## Experimental

*Preparation of S-2-Aminoethyl-L-Cysteine and S-2-Aminoethyl-D,L-Penicillamine Hydrochloride Salts [9]*

L-cysteine hydrochloride (0.1 mol, 15.7 g) was dissolved in 70 ml of water and the solution was cooled in an ice bath. Ethylenimine (0.115 mol, 5.7 ml) was added with stirring, along with few drops of phenolpthalein. More ethylenimine (approximately 1 ml) was added to give a slight pink colour and the stirring continued for 30–60 minutes. The end of the reaction can be tested by the nitroprusside test for the SH group. The reaction mixture was evaporated to one-half of the original volume under reduced pressure and an equal volume of ethanol was added. After storing the mixture at 0 °C overnight, the solid precipitate was collected and recrystallized from water–ethanol. Yield 70%; m.p. 195–197 °C.

*Anal.* Calcd for $C_5H_{13}N_2O_2SCl$ (200.7): C, 29.92; H, 6.52; N, 13.96. Found: C, 30.05; H, 6.18; N, 13.38.

The same method was used for the preparation of S-2-Am-DL-Pen·HCl. The recrystallization was performed with a solution of ethanol–acetone and addition of ether to form the precipitate. After storing at 0 °C overnight, the precipitate was washed with acetone and dried in a vacuum desiccator. Yield 65%; m.p. 166–168 °C.

*Anal.* Calcd for $C_7H_{17}N_2O_2SCl$ (228.45): C, 36.79; H, 7.44; N, 12.25. Found: C, 34.24; H, 7.40; N, 11.86.

*Preparation of Pt(II) and Pd(II) Complexes*

The complexes were prepared with the following general method in a ratio of 1:1 (free ligand: metal).

The free ligand (0.001 mol) was added with stirring to a solution of $PtCl_2$ (or $PdCl_2$) in 2 N HCl (approximately 30 ml), which contained 0.001 mol of the metal. The mixture was stirred with a magnetic stirrer at room temperature and the solution was then titrated with 1 N NaOH solution. The addition of the NaOH solution was followed by parallel monitoring of the pH of the mixture. In the case of Pt(II) complexes the pH remained in the range 5.0–6.5 and in the case of Pd(II) complexes in the range 2.5–3.0 until the end of the reaction.

After one hour stirring the reaction mixture was allowed to rest for the complex to be deposited and then evaporated at 40 °C under reduced pressure. The precipitate was filtered and washed with ether or acetone. Complexes were recrystallized from DMF by addition of ether or acetone and finally dried in a vacuum desiccator over silica gel.

Some of the complexes are highly hygroscopic, so care should be taken to use dried solvents. Yields for complexes were in the range 70–85%. Purity of the complexes was tested by thin layer chromatography.

Melting points were taken on a Büchi Mel-temp apparatus and are uncorrected.

*Infrared Absorption Spectra*

Infrared spectra were obtained by means of a Perkin-Elmer Infrared Spectrophotometer Model 283 B (4000–200 $cm^{-1}$) from samples prepared in accordance with the KBr disk technique. Absorption bands are expressed in $cm^{-1}$.

*$^1H$ NMR Spectra*

$^1H$ NMR spectra were obtained by dissolving the free ligands and their complexes in $D_2O$ or DMSO. Spectra were recorded with a Varian EM 360 proton NMR spectrometer (60 MHz) at room temperature. Chemical shifts were measured with respect to internal TMS in DMSO or deuterated 3-(trimethylsilyl)-1-propanesulphonic acid sodium salt hydrate (or DSS) in $D_2O$ and are expressed in ppm.

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