Metal Complexes of Alkylating Agents. IV. Complexes of Melphalan and Chlorambucil

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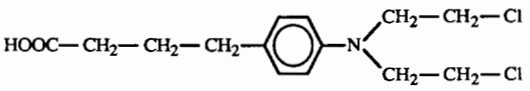
Abstract

Complexes of melphalan (mel) and chlorambucil (cam) that have been synthesized include $Cu(mel)_2(H_2O)_2$, Cu(sal-mel)(sal) where sal-mel is the Schiff base formed from salicylic acid and melphalan, $Pt(cyc)_2(mel)_2$ where cyc is cyclohexylamine, $(H_3C)_2Au(mel)$, and $Cu_2(cam)_4$.

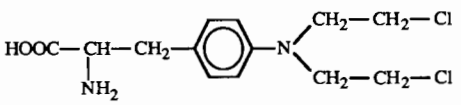
Introduction

Melphalan (mel), *p*-di(2-chloroethyl)-amino-L-phenylalanine or L-phenylalanine mustard (Fig. 1), is an alkylating agent used in the treatment of multiple myeloma and ovarian breast tumors [1, 2]. Chlorambucil (cam) is an aromatic mustard derivative that is used clinically to treat chronic lymphocytic leukemia and Hodgkin's disease [3].

Since metal complexes of phenylalanine and carboxylates are well known, the goal of this study was to synthesize and characterize metal complexes of melphalan and chlorambucil to determine what effect, if any, the 2-chloroethylamine functional group would have on the coordinating ability of the amino acid or carboxylate. We were particularly interested in complexes of platinum, copper, gold and rhodium since metal complexes of these metals have shown anticancer activity. The variety of copper carboxylate complexes [4, 5] and the anti-inflammatory activity of the copper complex with aspirin [6] were the basis of our interest in copper. We also decided to determine whether melphalan formed a Schiff base since a Schiff base of phenylalanine has been reported [7, 8].



Chlorambucil



Melphalan

Fig. 1. Structures of melphalan and chlorambucil.

Experimental

Materials

Melphalan and chlorambucil were used as obtained from Sigma Chemical Company. Salicylaldehyde and cyclohexylamine were used as obtained from Aldrich Chemical Company.

Preparation of $Cu(mel)_2(H_2O)_2$

$Cu(OH)_2$ was prepared according to a literature method [9]. Melphalan (0.30 g, 0.98 mmol) was added to the stirred suspension of $Cu(OH)_2$ (0.05 g, 0.52 mmol in 3.5 ml of water). After one hour, a blue–purple precipitate formed which was filtered with vacuum and washed with a large amount of hot water. Yield = 0.84 g (60.9%). Calc.: C, 44.26; H, 5.39; N, 7.94; Cl, 19.86. Found: C, 44.63; H, 5.24; N, 7.84; Cl, 20.11%.

Preparation of Cu(sal-mel)(H_2O)

Salicylaldehyde (1.06 ml, 9.96 mmol) and copper(II) acetate monohydrate (2.00 g, 10.0 mmol) in a mixture of 15 ml 1 N HCl and 15 ml ethanol was warmed to 50 °C. After 5 min of heating, melphalan (1.89 g, 6.20 mmol) in 1 ml 1 N HCl and 1 ml ethanol was added to the copper–salicylaldehyde solution. Sodium hydroxide (1 N) was added dropwise with continuous stirring, maintaining the temperature at 55 °C, until pH 5 was reached. After an additional 20 min of stirring at 55 °C, the solution was filtered and the dark green precipitate was washed with water, alcohol and ether. The precipitate was dried *in vacuo*. Yield = 1.30 g (26.7%). Calc. C, 49.18; H, 4.51; N, 5.74; Cl, 15.40. Found: C, 50.43; H, 4.46; N, 5.34; Cl, 15.65%.

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Preparation of Cu(sal-mel)(sal)$(H_2O)_{1/2}$

Cu(salicylaldehyde)$_2$ was prepared by a literature method [8]. Melphalan (0.40 g, 1.31 mmol) was added to a solution of Cu(sal)$_2$ (0.20 g, 0.65 mmol) in 25 ml water. The heterogeneous mixture was stirred at 50 °C for 1 h. The dark green precipitate was filtered via suction, washed with hot water and dried *in vacuo*. Yield = 0.24 g (67.9%). Calc.: C, 50.74; H, 4.41; N, 5.15; Cl, 12.65. Found: C, 50.95; H, 4.37; N, 5.34; Cl, 12.87%.

Preparation of Pt(cyc)$_2$(mel)$_2$

A solution of K_2PtCl_4 (1.24 g, 3.00 mmol) in 12 ml of water was added to a solution of KI (7.80 g, 47.0 mmol) in 12 ml of water. The resulting solution was stirred for 5 min at 40 °C. Cyclohexane (1.5 ml, 14.0 mmol) was then added to the KI–K_2PtCl_4 solution and the mixture was stirred for 10 min at 40 °C. The bright yellow product, Pt(cyc)$_2$I$_2$, was collected by vacuum filtration, washed with hot water, cold ethanol, ether and dried. Yield = 1.77 g (91.2%). Silver nitrate (0.22 g, 1.29 mmol) in 10 ml of water was placed in a flask wrapped in aluminum foil. This solution was stirred at 50 °C for 5 min. To the $AgNO_3$ solution at 50 °C was added Pt(cyc)$_2$I$_2$ (0.43 g, 0.06 mmol). The mixture was stirred at 55 °C for 4 h, cooled in an ice bath at 0 °C for 30 min, and AgI was removed by filtration. During the filtration process, the filtrate was diluted by half with water. To the filtrate was added, with stirring, melphalan (0.40 g, 1.31 mmol) in 1.0 ml of 1 N KOH at room temperature. The resulting white precipitate was immediately filtered, washed with a copious amount of hot water and dried in a dessicator. Yield = 0.19 g (77.1%). Calc.: C, 44.84; H, 5.90; N, 8.26; Cl, 13.77. Found: C, 44.23; H, 5.88; N, 8.48; Cl, 13.68%.

Preparation of Au(CH$_3$)$_2$(mel)(H$_2$O)

Pyridinotrichlorogold (1.0 g, 2.6 mmol) (Aesar) was dissolved in 8 ml of freshly-distilled, warm pyridine. This solution was then cooled in an ice bath, yielding a suspension of dipyridinodichlorogold(III) chloride, and then 3 M CH_3MgI (2.6 ml, 7.83 mmol) (Aldrich) in diethyl ether was added dropwise over a 15 min period. The addition of the Grignard reagent changed the orange suspension to a black–brown suspension which was stirred for 10 min and then water (5.5 ml) was slowly added, followed by petroleum ether (8 ml) and concentrated HCl (10.6 ml). The solution was filtered and the aqueous layer was extracted with ten 50 ml portions of petroleum ether. The combined organic layers were extracted with three 20 ml portions of water, then treated dropwise with ethylenediamine (1 ml) with appearance of a white precipitate (dimethylgold(III) ethylenediamine iodide). This compound was extracted with water and, upon acidification with 6 M HCl, a white solid formed which was collected and dried *in vacuo*. This compound was identified as $[(CH_3)_2AuI]_2$. To a stirred solution of this (151 mg, 0.213 mmol) in 6 ml of petroleum ether was added a solution of $AgNO_3$ (76 mg, 0.45 mmol) in 6.3 ml of water. The petroleum ether was removed *in vacuo* and the AgI was removed by filtration. A solution of melphalan (130 mg, 0.43 mmol) in 1 ml of 1 N KOH was added to the filtrate. A white precipitate formed which was collected, washed with ether and dried *in vacuo*. Yield = 123 mg (53.9%). Calc.: C, 32.79; H, 4.55; N, 5.10; Cl, 12.93. Found: C, 32.81; H, 4.16; N, 5.37; Cl, 12.88%.

Preparation of Cu$_2$(cam)$_4$

The sodium salt of chlorambucil was prepared by dissolving chlorambucil (200 mg, 0.658 mmol) in 6.6 ml of 0.1 N NaOH. This solution was added to a solution of $CuCl_2 \cdot 2H_2O$ (56 mg, 0.330 mmol) in 1 mmol of water and a green precipitate appeared immediately. Water (1 ml) was added, and the suspension was stirred for 30 min before collecting the dark green solid, which was washed with water and dried *in vacuo* (42.1% yield). Calc.: C, 50.19; H, 5.38; N, 4.18; Cl, 21.21. Found: C, 50.30; H, 5.53; N, 4.11; Cl, 20.86%.

Preparation of Rh$_2$(asp)$_4$·xH$_2$O

To a stirred solution of acetylsalicylic acid (0.68 g, 3.8 mmol) in 20 ml of 50% aqueous ethanol was added solid $RhCl_3 \cdot 3H_2O$ (0.50 g, 1.9 mmol) (Alfa). The resulting brick-red solution was stirred under nitrogen at 70–80 °C for 1 h before filtering to yield a dark green solid which was washed with water, ethanol, and ether and dried *in vacuo*. Two different aquated products have been identified. Calc. for $[Rh(asp)_2(H_2O)]_2$: C, 45.10; H, 3.34. Found: C, 45.13; H, 3.21%. Calc. for $[Rh(asp)_2(H_2O)]_2 \cdot 2H_2O$: C, 43.46; H, 3.62. Found: C, 43.29; H, 3.19%.

Preparation of Rh$_2$(3,5-diisopropylsalicylate)$_4$·xH$_2$O

To a stirred solution of 3,5-diisopropylsalicylate (dips) (0.85 g, 3.8 mmol) in 20 ml of 50% aqueous ethanol was added solid $RhCl_3 \cdot 3H_2O$ (0.50 g, 1.9 mmol) and the resulting solution was stirred for 1.5 h at 75 °C in a stoppered flask. A blue–green solid precipitated out and was collected by filtration, washed with water, and dried *in vacuo*. As with the aspirin complex, two different aquated products have been identified. Calc. for $[Rh(dips)_2(H_2O)]_2 \cdot 2H_2O$: C, 53.70; H, 6.54. Found: C, 53.93; H, 6.44%. Calc. for $[Rh(dips)_2(H_2O)]_2 \cdot (H_2O)(C_2H_5OH)$: C, 54.45; H, 6.72. Found: C, 54.82; H, 6.67%.

Elemental Analysis

Carbon, hydrogen, nitrogen and halogen analyses were performed by Desert Anal, Tucson, AZ.

Infrared Spectra

Spectra were recorded on either a Perkin-Elmer 727 or 1430 infrared spectrometer and samples were prepared as potassium bromide disks, Nujol mulls, or run neat.

Nuclear Magnetic Resonance Measurements

^1H and ^{13}C NMR spectra were recorded on a Jeol FX-90 Fourier Transform NMR Spectrometer.

Electron Paramagnetic Resonance Measurements

The EPR spectra were obtained at room temperature and liquid nitrogen temperature on a Varian E-3 EPR Spectrometer.

Magnetic Susceptibility Measurements

The Faraday method was used to measure the magnetic susceptibility of Cu(II) complexes. The measurements were made at 295 K with mercury(II) tetrathiocyanatocobaltate(II) as the reference.

Results and Discussion

Melphalan Complexes

The complexes of melphalan were found to have properties similar to those of analogous phenylalanine derivatives as described by other authors. Spectroscopic data support the structures shown in Figs. 2, 3 and 4. For most metal–amino acid complexes a strong absorption occurs around 1640–1600 cm^{-1} which corresponds to the asymmetric stretching frequency of the carboxyl group [8]. This characteristic range of absorption bands indicate that the amino acids are acting as bidentate ligands. Strong to medium bands do exist in this region for $Cu(mel)_2(H_2O)_2$, Cu(sal-mel)(H_2O), and Cu(Sal-mel)(sal)$(H_2O)_{1/2}$ but not for $Pt(cyc)_2(mel)_2$. The coordination of the carboxyl group of the Schiff base complexes can be verified by a band at approximately 1720 cm^{-1}. Presence of this CO stretching band is characteristic of uncoordinated carboxyl groups while absence of the band appears to indicate that the carboxyl group is coordinating to the metal ion [7]. This band was not observed for either of the two Schiff base complexes which implies that the carboxyl group is involved in coordination.

The effective magnetic moment values at 295 K for $Cu(mel)_2(H_2O)_2$, Cu(sal-mel)(H_2O) and Cu(sal-mel)(sal)$(H_2O)_{1/2}$ are 1.96, 1.87 and 1.87 BM respectively. These values are similar to the value of 2.03 BM obtained for $Cu(L\text{-phe})_2(H_2O)_2$ and the published value of 1.87 BM for the Schiff base copper(II) complex of L-phenylalanine [10].

The EPR spectra of $Cu(mel)_2(H_2O)_2$ and $Cu(L\text{-phe})_2(H_2O)_2$ at both 298 and 77 K are characteristic of spectra for elongated tetragonal Cu(II) complexes. The g values calculated from the spectral data are: $g_\perp = 2.06$ and $g_\parallel = 2.19$ for $Cu(L\text{-phe})_2(H_2O)_2$ and $g_\perp = 2.06$ and $g_\parallel = 2.21$ for $Cu(mel)_2(H_2O)_2$. The EPR spectrum of Cu(sal-mel)(H_2O) is a single broad peak (165 gauss from minimum to maximum) at both 295 and 77 K. The calculated g_{ave} is 2.13 at 298 K and 2.12 at 77 K. The broad peak indicates a fast relaxation time because of interaction between adjacent Cu(II) ions.

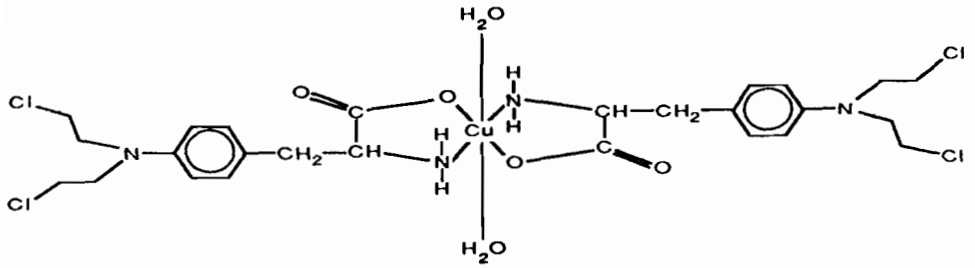
The visible spectral data are also indicative of six-coordinate Cu(II) with a single broad peak centered between 615 and 675 nm. The absorption band for $Cu(mel)_2(H_2O)_2$ has a maximum at 615 nm with a molar absorptivity value of 73 while Cu(sal-mel)(H_2O) has a maximum at 675 nm with a molar absorptivity of 22.

The gold complex of melphalan has been further characterized with NMR spectroscopy. The ^1H NMR spectrum is of limited use due to the sparingly soluble nature of the complex. In DMSO-D_6 we find a broad region of poor resolution at δ 4.06–2.34 which integrates for fifteen protons. This is assigned to the thirteen non-aromatic protons in the ligand melphalan plus two from the water which is present. The gold–methyl protons lie at δ 0.72 and δ 0.55 with respect to TMS. These methyl groups are anisochronous due to the symmetry present in the complex. This anisochronous behavior is also found in other dimethylgold(III) complex spectra [11–13]. The $^{13}C\{^1H\}$ NMR spectrum also shows this anisochronous behavior which has been observed in other dimethylgold complexes [14], since we observe gold–methyl carbon peaks at 17.13 and 14.69. We assign a peak at δ 180.8 to the carbonyl carbon and the aromatic assignments are as follows: δ 145.0 (C–N), 130.2 (*m*-C to mustard group), 125.2 ($C-CH_2$), 112.1 (*o*-C to mustard group). The C_α and C_β peaks for the mustard group are at δ 52.2 and 40.9, respectively. Peaks at 57.4 and 54.8 can be assigned to the aliphatic methylene and methine carbon atoms, respectively. These data are in agreement with the proposed structure shown in Fig. 4.

All of the spectroscopic evidence supports the bonding of the ligands to metal ions as monodentate and bidentate ligands by coordinating to the metal ion through the carboxylate group in the monodentate case and through the carboxylate and amine groups in the bidentate case. In the $Pt(cyc)_2(mel)_2$ compound, melphalan is a monodentate ligand with coordination to the platinum through the carboxylate group.

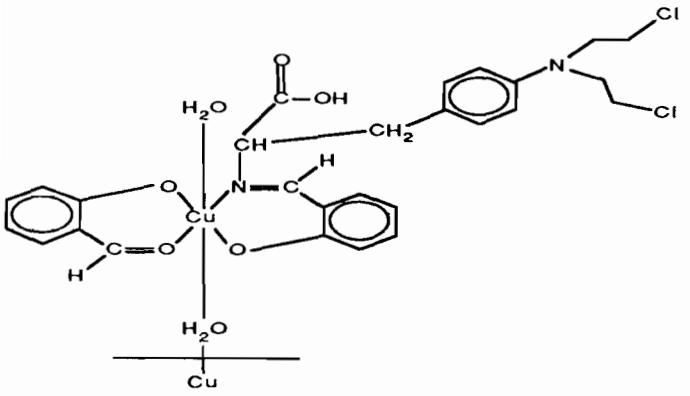
Complex of Chlorambucil with Cu(II)

Magnetic susceptibility, EPR and IR data support the assignment of the dimeric structure shown in Fig. 5. Copper(II) carboxylates are generally dimeric with subnormal magnetic moments [15]. The Cu(II) complex with chlorambucil is represented as a dimer in Fig. 5 on the basis of magnetic susceptibility mea-



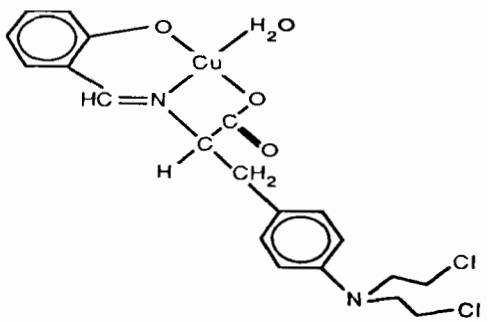
$Cu(mel)_2(H_2O)_2$

(a)



$Cu(sal\text{-}mel)(sal)(H_2O)_{1/2}$

(b)

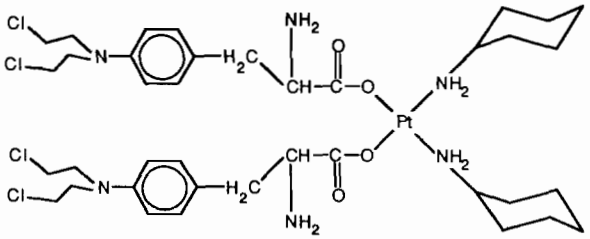


$Cu(sal\text{-}mel)(H_2O)$

(c)

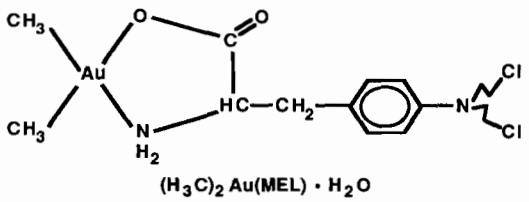
Fig. 2. Proposed structures for copper(II) complexes of melphalan.

surements at 295 K which give a calculated effective magnetic moment of 1.17–1.26 BM for different preparations of the complex.



$Pt(cyc)_2(mel)_2$

Fig. 3. Proposed structure for Pt(II) complex of melphalan.

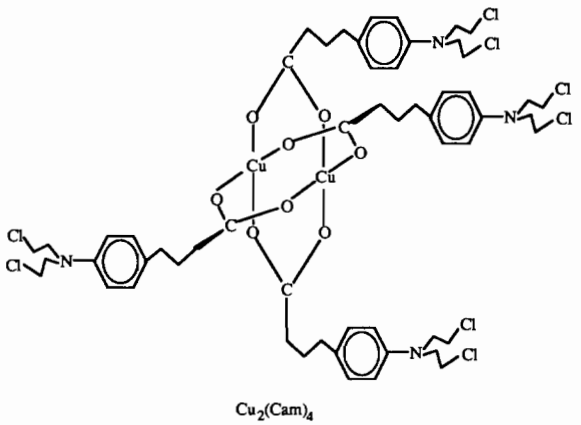


$(H_3C)_2Au(MEL) \cdot H_2O$

Fig. 4. Proposed structure for $(CH_3)_2Au(mel)\cdot H_2O$.

The antisymmetric and symmetric carboxylate vibrational modes of chlorambucil are assigned at 1708 and 1442 cm^{-1}, respectively. Upon coordination the 1708 band shifts to 1579 cm^{-1} while the 1442 cm^{-1} band does not appear to shift. Normally the asymmetric band shifts to lower frequencies and the symmetric band shifts to higher frequencies. For example, copper(II) acetate monohydrate has antisymmetric and symmetric carboxylate bands at 1605 and 1425 cm^{-1}, respectively, whereas the values for acetic acid are 1725 and 1408 cm^{-1}, respectively. The lack of shift of the symmetric band in the chlorambucil complex is puzzling although the amount of shift in symmetric bands of other Cu(II) carboxylates is very small compared to the magnitude of the antisymmetric band shift.

The EPR spectrum of $Cu_2(cam)_4$ at 77 K is shown in Fig. 6. The compound gives no EPR spectrum at room temperature. The lack of an EPR spectrum at room temperature can be attributed to rapid electron exchange between Cu(II) ions. Since pure complexes are being used, the lack of a spectrum at room temperature is not surprising. However, cooling the sample to 77 K gives an EPR spectrum that includes hyperfine splitting derived from the interaction of the



$Cu_2(Cam)_4$

Fig. 5. Proposed structure for Cu(II) complex of chlorambucil.

$3d^9$ electron with ^{63}Cu. This type of spectrum has been reported for both anhydrous Cu(II) carboxylate complexes and Cu(II) carboxylate complexes with axial ligands other than water [15]. Since the structure of $Cu_2(asp)_4$ is the anhydrous dimer [16], we obtained the powder EPR spectrum of $Cu_2(asp)_4$ at 77 K for comparison with the spectrum of $Cu_2(cam)_4$. The values for $g_\perp$, $g_\parallel$ and $A_\parallel$ are similar. Values for $Cu_2(cam)_4$ are $g_\perp = 2.07$, $g_\parallel = 2.35$, $A_\parallel = 145 \times 10^{-4}$ cm^{-1}. Values for $Cu_2(asp)_4$ are $g_\perp = 2.04$, $g_\parallel = 2.28$, $A_\parallel = 150 \times 10^{-4}$ cm^{-1}. However, the $Cu_2(cam)_4$ spectrum also shows hyperfine coupling in the $g_\perp$ region. This could indicate a lower symmetry for the $Cu_2(cam)_4$ dimer or resolution of $A_\perp$ hyperfine coupling peaks. The $A_\perp$ value of 15×10^{-4} cm^{-1} is typical of values reported from single crystal studies of doped Cu(II) complexes. The similarities between the EPR spectra of $Cu_2(asp)_4$ and $Cu_2(cam)_4$ provide support for the structure shown in Fig. 5.

Complexes of Rhodium with Carboxylates

The rhodium(II) carboxylates have been studied extensively, both for their unique structural properties and for their potential as catalysts and antitumor agents [17, 18]. The rhodium dimers have a structure similar to the copper carboxylates. We were not successful in the synthesis of a rhodium complex with chlorambucil. However, we were able to isolate two different adducts of both dirhodium(II) tetraaspirinate and dirhodium(II) tetradiisopropylsalicylate.

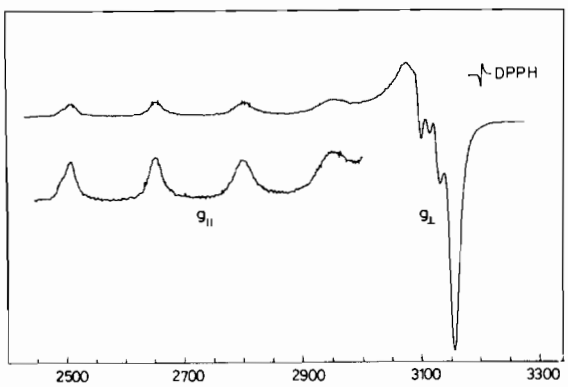
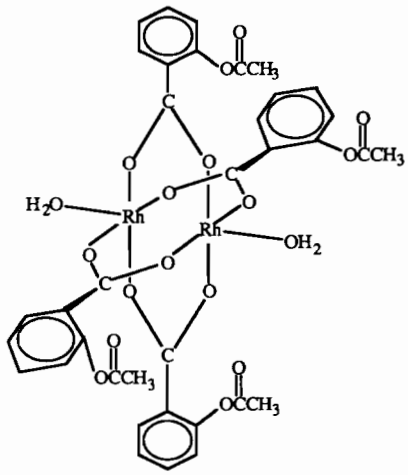


Fig. 6. EPR spectrum of $Cu_2(cam)_4$ at 77 K.



$Rh_2(Asp)_4 \cdot 2H_2O$

Fig. 7. Proposed structure for $Rh_2(asp)_4$.

The IR spectra of the rhodium complexes provide evidence for the dimeric structure shown in Fig. 7. The antisymmetric and symmetric stretching bands for the carboxyl C=O in aspirin are found at 1600 and 1364 cm^{-1}. In $Rh_2(asp)_4$ the antisymmetric band shifts to lower wavenumbers and splits into two bands (1590 and 1561 cm^{-1}). The symmetric band shifts to higher wavenumbers and also splits into two bands (1400 and 1380 cm^{-1}). These changes are characteristic of rhodium carboxylate dimers [17]. In $Rh_2(dips)_4$ complexes the antisymmetric band shifts from 1657 cm^{-1} in the uncomplexed acid to 1558 cm^{-1} in the complex and the symmetric band shifts from 1520 cm^{-1} in the acid to 1540 cm^{-1} in the complex. These IR results agree well with those observed in dimeric rhodium complexes of other salicylates [19]. The X-ray structure of dirhodium(II) salicylate has been determined by Cotton *et al.* in which an ethanol and a water molecule are coordinated axially with a molecule of each also in the crystal lattice [20].

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