Synthesis and Characterization of Potential Anticancer Platinum(II) and Palladium(II) Complexes. Infrared, Nuclear Magnetic Resonance Studies, Structure and Binding Site Determination

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Interactions of Pd(II) and Pt(II) with (4-amino-2-methyl-5-pyrimidinyl-methylthio) acetic acid (AMM PTA) in aqueous medium (pH = 3.5 and pH = 11.9) resulted in the formation of new complexes which have been characterized by elemental analysis, IR, ^1H, and ^{13}C NMR spectroscopic techniques. Platinum (II) is bound to two ligands through sulphur and oxygen donor atoms to form square planar complexes which in H_2O/D_2O mixture (3:2) is a mixture of cis and trans isomers as evident from NMR studies. Palladium(II), on the other hand, seems to bind weakly to the sulfur donor atom. In both Pd(II) and Pt(II) complexes, the amino group seems to be in the form $\overset{+}{N}H--\bar{C}l$ and $\overset{+}{N}H--\bar{O}H$ at low and high pH, respectively. A complete assignment of ^1H and ^{13}C NMR spectra resonances is presented for both the ligand and the complexes.

Introduction

Without any dint of doubt, the road to finding solution to cancer problems has been very rough and unpredictable. Realistically, there have been some successes, but these few successes have been clouded with numerous failures. As a consequence, to a lay man and the cancer patients, the battle against cancer is a lost battle, but to researchers, it is a challenging battle which must not only be fought but won; it is a battle whose end is not only imminent but at hand.

There are few basic requirements which must be met by potential anticancer complexes, namely (1) solubility in the common solvent, water (2) little or no side effect and (3) quickness in acting. Although, numerous potential anticancer complexes have been reported, only few of them look promising. Even the few that look promising are either too toxic or not reasonably soluble [1–6].

The reason for undertaking this work is twofold, namely, (1) possible use of these complexes to cure cancer and (2) for the interesting chemistry surrounding this particular ligand and its complexes. The ligand, AMMPTA is unique since it contains a wide variety of coordination sites, namely, the heterocyclic nitrogen, the amine group, the carboxyl group and the sulfur donor atom. The comparison of the donor properties in this ligand may be more straightforward than in thiamine where the pyrimidine ring is conjugated with the thiazolium ring [7–11].

Recently, [12], a group of researchers claimed that both the heterocyclic nitrogen and the amine group coordinated to the metal. Even more recently [13] Kozlowski *et al.* in their studies of Pd(II) and Pt(II) complexes with sulfur containing amino acids suggested that two donor groups (S, NH_2 or COO) might be involved in the coordination. They further claimed that differences do exist between the complex species created in solution due to various conformations and coordination sites. Furthermore, sulfur atom was found to be the most effective donor for both Pd(II) and Pt(II) at any pH, adding that the nitrogen donor seemed to be more reactive than the carboxyl donor.

In order to find out whether these findings are unique to their systems or these findings could be extended to similar systems, we have undertaken studies on the interactions of Pd(II) and Pt(II) with AMMPTA. Meanwhile, the biological activities of these complexes are under investigation.

Experimental

Potassium tetrachloroplatinate(II) and potassium tetrachloropalladate(II) were purchased from Aldrich Chemical Company and used without further purification. The ligand (4-amino-2-methyl-5-pyrimidinyl-methylthio)acetic acid (AMMPTA) was prepared according to the literature method [14].

A. Preparation of $Pt(AMMPTA)_2(OH)_2 \cdot H_2O$

Aqueous solutions of K_2PtCl_4 and AMMPTA were mixed in 1:2.5 mole ratio and the reactions mixture (pH = 11.9) was magnetically stirred at room temperature for 24 hours. The solution first turned light

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TABLE I. ^{13}C NMR Chemical Shifts of AMMPTA and Its Pt(II) and Pd(II) Metal Complexes.

Carbon	COO^-	C2′	C4′	C6′	C5′	CS	SC	2′-CH_3
AMMPTA	178.20	167.00	162 50	154.96	111 45	36.78	29 87	24 54
$Pt(AMMPTA)_2(OH)_2 \cdot H_2O$ pH = 11.9, white, mp = 252 °C	172.81	168 02	162.90	154.81	107.96	40 51	32 91	24 49
$Pt(AMMPTA)_2Cl_2 \cdot H_2O$ pH = 3 5, orange, mp = 250 °C	172 80	168 14	163.07	155 09	107.92	40.49	32.90	24.60
$Pd(AMMPTA)_2Cl_4 \cdot H_2O$ pH = 3.5, orange, mp = 248 °C	177 65	166.34	162.85	152 97	111 70	37 17	30 08	24.12
$Pd(AMMPTA)_2$ Complex[a] pH = 11.9, orange, mp = 211 °C	177 73	167 99	162.52	154 82	111 49	38.96	32 38	24.68

[a]Elemental analysis was not performed

yellow and as it evaporated down, precipitate formed. This precipitate was washed with ice cold distilled water, acetone and ether. The semi-white precipitate was dried at room temperature for a couple of days. Further drying in the oven (120 °C) for about six hours did not change the color of this compound. *Anal.* Calcd. for $Pt(AMMPTA)_2(OH)_2 \cdot H_2O$, $PtC_{16}N_6H_{26}O_7$ (Mw = 673.09 pH = 11.90): C, 28.53; H, 3.86; Cl, 0.00%. Found: C, 29.15; H, 3.86; Cl, 0.11%. *Anal.* Calcd. for $Pt(AMMPTA)_2Cl_2$, $PtC_{16}N_6H_{24}O_4Cl_2$ (Mw = 691.99, pH = 3.5): C, 27.75; H, 3.18; Cl, 10.25%. Found: C, 27.92; H, 3.49; Cl, 11.05%.

B. Preparation of $Pd(AMMPTA)_2Cl_4 \cdot H_2O$

Anal. Calcd. for $PdC_{16}N_6H_{24}O_5Cl_4$ (Mw = 692.20, pH = 3.5): C, 27.75; H, 3.47; Pd, 15.37%. Found: C, 28.99; H, 3.40; Pd, 14.60%. At high pH (11.9), mixture of aqueous solutions of K_2PdCl_4 and AMM PTA turned yellow almost immediately, but no precipitate formed not even when the reaction mixture almost completely evaporated. Precipitate was forced out of solution, employing solvent mixture 1:1 volume ratio methanol/ether. The solid compound isolated in this way was found to be extremely water soluble. Elemental analyses were performed by Galbraith Laboratories, Inc. of Knoxville, Tennessee and Atlantic Microlab, Inc. of Atlanta, Georgia.

Instrumentation

Carbon-13 and ^1H NMR spectra were recorded on Nicolet 200 MHz instrument, employing mixture of H_2O/D_2O (3:2) as solvent and dioxane as internal reference standard. All ^1H and ^{13}C NMR chemical shifts are expressed in parts per million (ppm) downfield from dioxane. Infrared spectra were recorded on Perkin-Elmer 621 Grating Infrared Spectrophotometer. The melting points of these complexes were determined in capillaries, employing Mel-Temp device and were uncorrected.

Results and Discussion

Carbon-13 NMR spectra

The ^{13}C NMR assignments of the ligand and the complexes have been made for the first time, based on the known ^{13}C NMR spectra of thiamine hydrochloride [7–9] and off-resonance spectra. Table I shows the chemical shifts of the ligand and the complexes, while Figs. 1, 2 and 3 show the spectra.

The ligand has resonances at 167.00, 162.50, 154.96 and 111.45 ppm, which can be assigned to the four carbons of the pyrimidine ring, C2′, C4′, C6′ and C5′ respectively. In the off-resonance spectrum, C6′ appears as doublet as expected. The resonance at 178.15 ppm is definitely due to the carbonyl carbon, COO^-. Other resonances appear at 36.78, 29.87 and 24.54 ppm which are ascribed to ′CS–, –SC– and 2′-CH_3, respectively.

In the complexes, $Pt(AMMPTA)_2(OH)_2$ and $Pt(AMMPTA)_2Cl_2$, the COO^- and C5′ resonances moved upfield by 5.34 and 3.59 ppm, respectively. While –CS– and –SC– shifted downfield by 3.73 and 3.04 ppm, respectively. Other resonances remain practically unaffected. The significant chemical shifts experienced by COO^- (5.34 ppm), –C5′ (3.373 ppm) and –SC (3.04 ppm) carbons are indicative of complexation through carbonyl carbon and sulfur donor atom. These chemical shifts (5.34–3.04 ppm) are smaller than that observed for C6′ of Pt(II) and Pd(II) complexes reported earlier [9, 15–17] where N1′ position of the pyrimidine ring is the coordination site but comparable to some of those observed by other investigators [18] in similar systems.

In the complex, $Pd(AMMPTA)_2Cl_4 \cdot H_2O$, –SC– and –CS– resonances shifted downfield by 2.51 and 2.18 ppm, respectively. All other resonances either remain practically unaffected or shifted by less than 1.00 ppm. The fact that the carbons adjacent to the sulfur donor atom are the most shifted (3.18–2.51 ppm) confirms our assumption that Pd(II) is weakly bound to the sulfur donor atom.

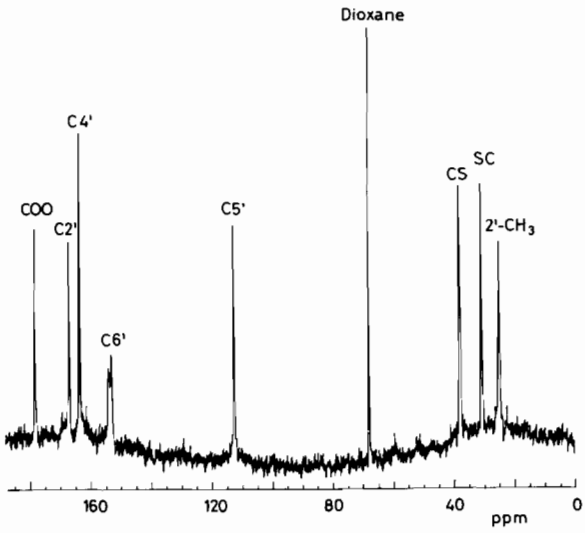

Fig. 1. ^{13}C NMR spectrum of AMMPTA in H_2O/D_2O with 2 drops of 1.0 *M* NaOH.

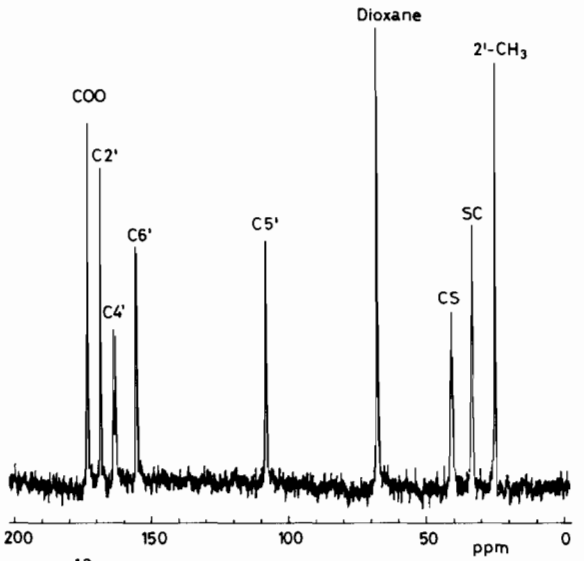

Fig. 2. ^{13}C NMR spectrum of $Pt(AMMPTA)_2(OH)_2 \cdot H_2O$ in H_2O/D_2O with 2 drops of 1.0 *M* NaOH.

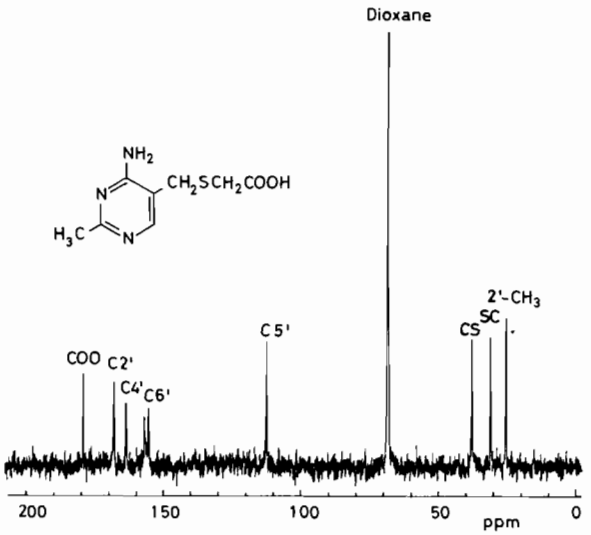

Fig. 3. ^{13}C N.M.R. spectrum of $Pd(AMMPTA)_2Cl_4 \cdot H_2O$ in H_2O/D_2O with 2 drops of 1.0 *M* NaOH.

Proton NMR Spectra

The C6-H, CH_2S, SCH_2 and 2'CH resonances of the ligand appear as singlets at 7.93, 3.65, 3.14 and 2.41 ppm, respectively. In the complex $Pt(AMMPTA)_2(OH)_2 \cdot H_2O$, C6-H resonance appears as a doublet with almost equal intensity at 7.69 and 7.57 ppm. This observation is ascribed to a mixture of two isomers which may be resolved into one isomer with many recrystallizations [19]. The CH_2S, SCH_2 resonances moved downfield to 4.05 and 3.86 ppm, respectively. Thus, the proton NMR has provided additional support for complexation of Pt(II) *via* the carbonyl carbon and the sulfur donor atom. The $2'CH_3$ resonance appears as a doublet with equal intensity at 2.33 and 2.27 ppm, respectively. Again, this observation is due to a mixture of two isomers. In the complex $Pd(AMMPTA)_2Cl_4 \cdot H_2O$, C6-H and $2'CH_3$ resonances remained unaffected while CH_2S and SCH_2 resonances were unobservable, implying a possible coordination of Pd(II) *via* the sulfur donor atom of the ligand.

Infrared Spectra

Although the ligand and the complexes show many absorption bands, only the significant ones will be discussed here. In the Pt-complex, the assymetric stretching vibration band for COO–Pt(II) occurs at 1640 cm^{-1}. This observation is in excellent agreement with the earlier work [20]. In both Pt(II) and Pd(II) complexes, the bands at 1195 and 785 cm^{-1} indicate the involvement of sulfur in the coordination [21]. Another marked difference between the Pt(II) and Pd(II) complexes is the band at 347 cm^{-1} which is ascribed to Pd–Cl. Platinum(II) complex, on the contrary, did not show Pt–Cl in agreement with the elemental analysis. The band between 1700 and 1600 cm^{-1} is abnormally broad in both Pt(II) and Pd(II) complexes compared to the free ligand. This broad band is tentatively assigned to the existence of hydrogen bonding of the form $\overset{+}{N}H- \cdots \bar{X}$ [14–17].

In conclusion, the results in the present study clearly show that Pt(II) is bound to the ligand through the carbonyl carbon and the sulfur donor atom whereas Pd(II) is weakly bound *via* sulfur donor atom. This is an interesting observation in view of the fact that nitrogen donor atom has always been the coordination site in all the metal complexes of thiamine and its alkylpyrimidine derivatives reported thus far [9, 15–17]. The size of the metal ion and the nature of the ligand especially the location of the

potential donor sites relative to each other in ligands having a wide variety of coordination sites are very important in complex formation.

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