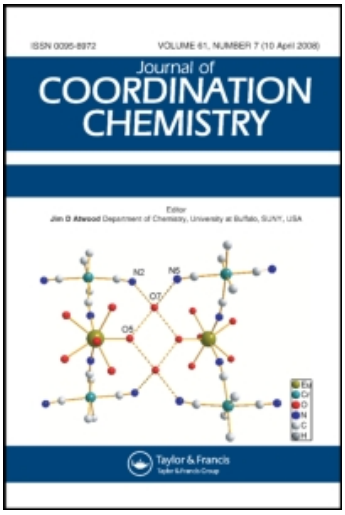

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Danuta Michalska[a]; Barbara Morzyk[b]; Walter Wojciechowski[a]
[a] Institute of Inorganic Chemistry and Metallurgy of Rare Elements, Technical University, Wroclaw, Poland [b] Institute of Chemistry, Pedagogical University, Czestochowa, Poland

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A POTENTIOMETRIC STUDY OF METAL ION COMPLEXES OF ANTINEOPLASTON A10, A NEW ANTITUMOUR AGENT

DANUTA MICHALSKA[1]*, BARBARA MORZYK[2] and WALTER WOJCIECHOWSKI[1]

[1]*Institute of Inorganic Chemistry and Metallurgy of Rare Elements, Technical University, 50-370 Wroclaw, ul. Smoluchowskiego 23, Poland*

[2]*Institute of Chemistry, Pedagogical University, 42-200 Czestochowa, Poland*

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3-(*N*-phenylacetylamino)-2,6-piperidinedione, Antineoplaston A10, is a new antitumour agent. In an attempt to explore its binding properties, the interaction of A10 with Ca(II), Mg(II), Cu(II), Zn(II), Co(II), Ni(II), Hg(II) and Ag(I) ions was investigated by potentiometric methods. Stability constants of 1:1 complexes (ML) were determined in aqueous methanol (50%, v/v) at 45.0°C and 0.1 M (KNO_3) ionic strength. It has been shown that complex formation is accomplished by release of a proton; binding with metal ions probably occurs at the deprotonated nitrogen atom of the 2,6-piperidinedione ring. It is concluded that the coordinating properties of Antineoplaston A10 are similar to those of uracil and thymine.

KEYWORDS: Antineoplaston A10, 3-(*N*-phenylacetylamino)-2,6-piperidinedione, antitumour, complexes, stability constants.

INTRODUCTION

3-(*N*-phenylacetylamino)-2,6-piperidinedione, Antineoplaston A10[1] is a new antitumour agent introduced recently to experimental chemotherapy.[2,3] It is a natural compound isolated from human urine and plasma.

It is interesting to note that Antineoplaston A10 contains the 2,6-piperidinedione ring, which is also present in several other anticancer drugs.[4] Theoretical studies[5,7] have revealed that the 2,6-piperidinedione group is very similar to uracil and thymine with respect to geometrical parameters and electron density distribution. Therefore, biological activity of 3-(*N*-phenylacetylamino)-2,6-piperidinedione may in part be related to structural and chemical resemblances with these pyrimidine bases.

Metal complexes of purine and pyrimidine bases play a dominant role in many biochemical systems, notably in energy transfer and oxidative phosphorylation and the structure of these complexes has been extensively studied.[8-11] Interaction of

* Author for correspondence.

metal ions with the structurally related drug, Antineoplaston A10, has not been investigated and it would be of interest to compare coordination behaviour. These studies seem to be important in elucidating the chemical features and binding properties of this drug.

EXPERIMENTAL

Materials

3-(*N*-phenylacetylamino)-2,6-piperidinedione was kindly provided by the Burzynski Research Institute, Houston, Texas. The compound was recrystallized from methanol/ethanol and infrared spectra were run to check purity. Because of its poor solubility in water, the compound was dissolved in hot methanol under reflux and aqueous methanol solutions (50%, v/v) were used for potentiometric titrations. Glutarimide was purchased from the Aldrich Chemical Co. Metal salts were of the analytical grade purity. Initial 0.1 M solutions of Cu(II), Ni(II), Co(II), Zn(II), Ca(II), Mg(II), Hg(II) and Ag(I) were prepared and the concentrations of metal ions determined according to the methods in refs. 12 and 13. Carbonate-free sodium hydroxide (0.10 M) was standardized by titration with potassium hydrogen phthalate.

Measurements

The potentiometric studies were performed for Antineoplaston A10 and glutarimide in aqueous methanol (50%, v/v). The experimental method consisted of the potentiometric titration of each ligand with standard sodium hydroxide solution in the absence and presence of the metal ions being investigated. An OP-211 pH meter (Radelkis, Budapest) with combination glass electrode was used to determine hydrogen ion concentration. The electrode system was calibrated according to ref. 14. The accuracy of the measured pH was ± 0.01. Since titrations were performed in mixed solvent, additional calibration of the electrode system has been necessary. According to the method described by Uitert and Haas[15] the absolute calibration coefficient, u^0_H, for the water-methanol mixture has been determined. Values of u^0_H show good agreement with those reported by Lahiri and Aditya[16] and Bates.[17] A linear relationship has been found between pH values and the concentration of hydrogen ions in the pH range 2.5–12 in the mixed solvent.

Titrations of the investigated systems were performed at 45.0 ± 0.1 °C and the ionic strength was maintained constant at 0.10 M (KNO_3). In the titrated samples the concentration of each ligand amounted to 5.0 mM, whereas the concentration of the metal ions was varied (2.5, 5.0 and 7.5 mM). All metal to ligand mol ratios were tested by at least three titrations.

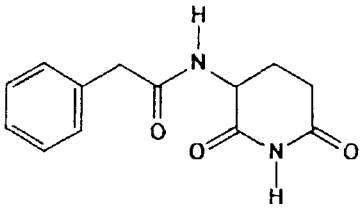
Calculations

The acid dissociation constant, K_a, measured for glutarimide and Antineoplaston A10, corresponds to the proton dissociation from the imido group. K_a has been calculated according to the equation

$$K_a = [H^+](aT_L + [H^+] - [OH^-])/\{T_L - (aT_L + [H^+] - [OH^-])\}$$

where a = mole of base added per mole of ligand and T_L = total ligand concentration.

The concentration of the bound ligand has been determined directly from the shifts in the titration curves according to refs. 10 and 18. The degree of formation ($\bar{n}$) and pL were computed as in our previous work.[19] Non-linear regression methods were used to obtain formation curves and stability constants of the metal-ligand complexes. Only those data were used in calculations which had been measured in homogeneous solution with no precipitation. Computations were performed on a PC486 with suitable programmes.



RESULTS AND DISCUSSION

IR and Raman studies on Antineoplaston A10[7] have shown that the 2,6-piperidinedione moiety exists in the diketo form. Therefore, the proton ionization should occur at the imido nitrogen in the ring. The acid dissociation constant, pK_a, measured for Antineoplaston A10 in the aqueous-methanol solution (50%, v/v), at 45°C and 0.10 M (KNO_3) ionic strength, is 11.3. The pK_a measured for glutarimide (2,6-piperidinedione) under the same experimental conditions, is 11.5. This value also corresponds to proton dissociation from the imido NH group.

Potentiometric studies were performed for Antineoplaston A10 as well as for glutarimide (Glu) in aqueous-methanol (50%, v/v) in the presence of Ca(II), Mg(II), Cu(II), Ni(II), Zn(II), Co(II), Hg(II) and Ag(I). It has been found that the pH titration curves obtained for Antineoplaston A10 in the presence of Ca(II) can be almost exactly superimposed on that of the free ligand. Similar effects have been observed for systems containing Antineoplaston A10 and Mg(II) as well as for glutarimide and these two metal ions. It follows from these data that neither Ca(II) nor Mg(II) forms any significant complexes with the investigated ligands.

A pronounced shift of the titration curves is observed in the case of Cu(II), Zn(II), Co(II), Ni(II), Hg(II) and Ag(I). Figure 1 shows titration curves measured for the systems A10-Ag(I) and A10-Hg(II). Analogous curves resulted from potentiometric studies of the corresponding systems Glu-Ag(I) and Glu-Hg(II). It should be noted that titration curves obtained for various metal to ligand molar ratios are very similar. This implies that only mononuclear species are formed in solution. Two inflexions are observed on each of these curves, the first at m about 1 and the second at m = 2. This indicates that the Ag(I) and Hg(II) ions are able to form ML and ML_2 complexes in a stepwise manner. It should be emphasized that similar results have been obtained in potentiometric studies of uracil and thymine with Hg(II);[10] deprotonation of imido nitrogen in these bases was followed by stepwise complex formation of the ML and ML_2 species.

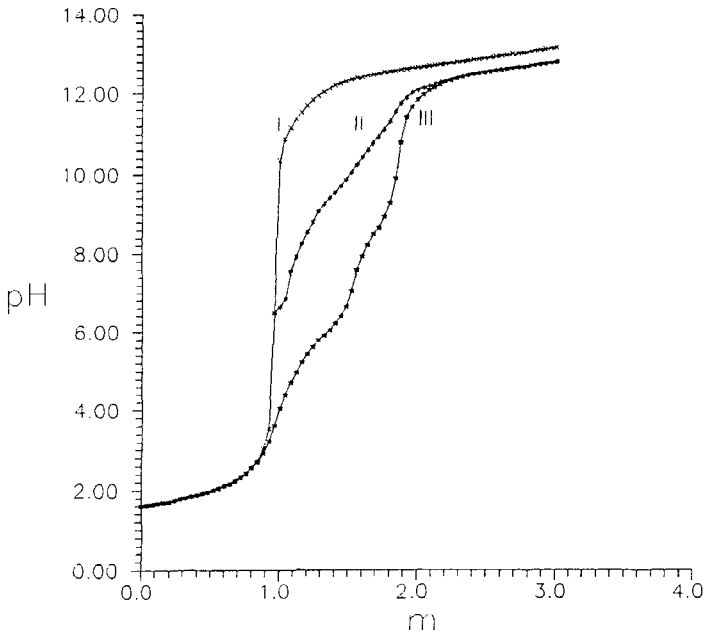


Figure 1 Potentiometric titration curves for Antineoplaston A10 and metal ions in aqueous methanol (50%, v/v): I = A10 free; II = A10 with Ag(I); III = A10 with Hg(II). The concentrations of the ligand and metal ions are 5.0 mM and 2.5 mM, respectively; *m* = mols of base added per mol of metal ion. For the free ligand titration, *m* corresponds to a on the abscissa.

These results clearly demonstrate that both mercury(II) and silver(I) are able to replace a proton from Antineoplaston A10. Unfortunately, in systems containing A10 or Glu and transition metal ions (Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+}) the titrations could not be completed due to a separation of a solid phase before the second inflection point was reached. Therefore, formation of the 1:1 (metal to ligand) complexes was assumed for these systems and calculations were performed well ahead of the precipitation point. Computed formation curves for the metal complexes of Antineoplaston A10 are shown in Figure 2. Calculated stability constants for the metal complexes are given in Table I. It appears from these data that the stabilities of the transition metal complexes of Antineoplaston A10 (as well as those of glutarimide complexes) follow the Irving-Williams order Ni~Co<Cu>Zn.

It is of interest to compare stabilities of the corresponding complexes of Antineoplaston A10, uracil, thymine and uridine since all these ligands should have a similar binding site; the imido nitrogen in A10 corresponds to the N(3) atom in the nucleic acid bases. For example, the A10-Cu complex is the most stable ($\log K_1 = \log_{CuL}^{Cu} = 8.1$) as compared to thymine-Cu ($\log K_1 = 5.8$),[11] uracil-Cu ($\log K_1 = 5.6$)[11] and uridine-Cu ($\log K_1 = 4.2$).[9] These values were determined under the same conditions of temperature (45°C) and ionic strength (0.1 M KNO_3). Decreasing stability of the above complexes is in accord with the decreasing basicity of the ligands A10>T>U>Urd. However, it should be noted that the measurements

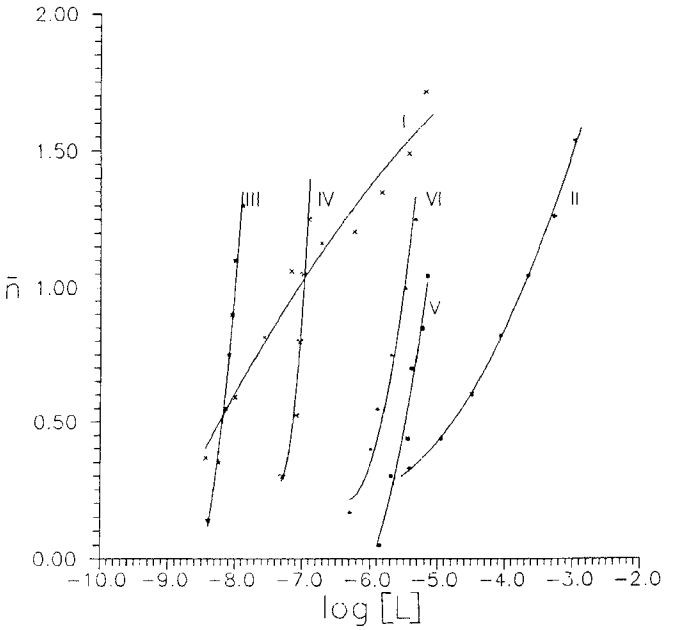


Figure 2 Formation curves of the metal complexes with Antineoplaston A10: I = A10 with Hg(II); II = A10 with Ag(I); III = A10 with Cu(II); IV = A10 with Zn(II); V = A10 with Ni(II); VI = A10 with Co(II).

Table I Stability constants of metal complexes of 3-(*N*-phenylacetylamino)-2,6-piperidinedione (Antineoplaston A10) and 2,6-piperidinedione (glutarimide) in aqueous methanol (50%, v/v) at 45 ± 0.1 °C, μ = 0.10 M KNO_3.

Ligand	pKa	Metal	log K^{M}_{ML}	log $K^{ML}_{ML_2}$	log $K^{M}_{ML_2}$
A10	11.31	Cu(II)	8.19 ± 0.01		
		Zn(II)	7.13 ± 0.07		
		Co(II)	5.87 ± 0.07		
		Ni(II)	5.55 ± 0.05		
		Hg(II)	8.23 ± 0.04	5.59 ± 0.04	13.82 ± 0.08
		Ag(I)	4.79 ± 0.01	3.00 ± 0.01	7.79 ± 0.02
Glutarimide	11.54	Cu(II)	7.96 ± 0.05		
		Zn(II)	7.07 ± 0.03		
		Co(II)	5.95 ± 0.02		
		Ni(II)	5.77 ± 0.02		
		Hg(II)	8.69 ± 0.01	7.08 ± 0.01	15.77 ± 0.02
		Ag(I)	5.37 ± 0.02	4.36 ± 0.02	9.73 ± 0.04

for A10 were performed in aqueous methanol, whereas those for T, U and Urd were in water. Change from the water to a less polar solvent causes a slight increase in stability of the complexes (Δlog K increases by 0.5–1).[19] Such conditions of solvent with effective dielectric constant lower than that of bulk water exists, for instance, in the active sites of enzymes,[8] where the drug may exert its biological

activity. It is interesting to note that the stability constant of the mercury(II) complex with Antineoplaston A10 ($\log \beta_2 = \log^{Hg}_{HgL_2} = 13.8$) is very similar to that of thymine-Hg(II)($\log\beta_2 = 14.3$) and uracil-Hg(II)($\log\beta_2 = 13.2$).[10]

Results from an X-ray crystal structure analysis of the 2:1 complex of 1-methylthymine with mercury(II) reveal that it contains a linear N(3)-Hg-N(3') fragment which links the two thymine moieties together.[20] Furthermore, crystallographic studies on (1-methyluracilato)Ag(I)[21] indicate that silver(I) is also linearly coordinated to two nitrogen atoms of two deprotonated uracil molecules. Since stabilities of the metal complexes of Antineoplaston A10 are similar to those of the corresponding metal complexes of uracil and thymine, it is concluded that the coordinating properties of these molecules should also be similar. In the case of Antineoplaston it has been determined that complex formation is accomplished by release of a proton; therefore, binding of the metal ion occurs at the deprotonated nitrogen atom of the 2,6-piperidinedione ring.

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