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Complex Formation of Hypoxanthine and 6-Mercaptopurine with Cd(II) Ion

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Reaction of Cd(II) ion with hypoxanthine (H_2Y) and with 6-mercaptopurine (H_2MP) in dioxane-water (50%) leads to the formation of $CdY \cdot 2H_2O$ and $Cd(HMP)_2 \cdot 2H_2O$, respectively. In methanolic medium Cd(II) and H_2MP give $Cd(MP) \cdot H_2O$. These compounds have been characterized by chemical analysis, IR spectra and thermogravimetric analysis. The stability constant of CdY complex at 25 ± 0.1 °C and 1 M ionic strength with $NaClO_4$ in dioxane-water medium is $\log\beta = 10.25 \pm 0.05$.

[*Keywords: Complexes; Cadmium (II); Hypoxanthine; 6-Mercaptopurine*]

Komplexbildung von Hypoxanthin und 6-Mercaptopurin mit Cd(II)

Die Umsetzung von Cd(II)-Ionen mit Hypoxanthin (H_2Y) und 6-Mercaptopurin (H_2MP) in Dioxan-Wasser (50%) ergibt die Verbindungen $CdY \cdot 2H_2O$ und $Cd(HMP)_2 \cdot 2H_2O$. In Methanol entsteht aus Cd(II) und H_2MP $CdMP \cdot H_2O$. Die Verbindungen wurden durch chemische Analysen, IR-Spektren und thermogravimetrische Analysen charakterisiert. Die Stabilitätskonstante der Verbindung CdY bei 25 °C und bei einer Ionenstärke = 1 ($NaClO_4$) in Dioxan-Wasser wurde zu $\lg\beta = 10{,}25 \pm 0{,}05$ bestimmt.

Introduction

There are several studies on complex formation between divalent ions of biological interest [Cu(II), Zn(II), Ni(II), Co(II) etc.] and some derivatives of purine[1—5]. These studies are of great interest from a bioinorganic point of view owing to the role of purine derivatives in processes such as energy transfer and oxidative phosphorylation in which it can be assumed that the metallic ions participate through complex formation in such processes.

Cd(II) ion is a polluting and carcinogenic agent, and often can act substituting for Zn(II). Thus, it is of great interest to study the interaction of Cd(II) with derivatives of purine. The literature available on this subject presents gaps, while some data are hardly acceptable. *Griffith* et al.[6] obtained di-μ-Chloro-dichloro bis-(6-mercaptopurine) diaquo di-Cadmium (II); *Randhawa* et al.[7] studied the hypoxanthine interaction with Zn(II), Cd(II), and Hg(II) ions, observed formation of 1 : 1 and 1 : 2 complexes, and determined their stability constants at different temperatures and different ionic strengths. In the latter case, a 0.05 *M* hypoxanthine solution was prepared at 25 °C, which is in disagreement with the literature[8]. Our observations indicate that the maximum concentration of hypoxanthine at 25 °C is $5.4 \cdot 10^{-3}$ *M*. Therefore titrations in water are carried out at 45 °C[4] while at 25 °C other solvents are used, such as dioxane-water[3] where the solubility of hypoxanthine increases.

All these considerations have prompted us to undertake the study of complex formation between Cd(II) ion and hypoxanthine and 6-mercaptopurine in dioxane-water and in methanol at 25 °C.

Experimental

6-Mercaptopurine was supplied by Gayoso Wellcome S.A. and recrystallized from boiling water with charcoal and dried at 110 °C. Hypoxanthine ("Merck") was recrystallized according to the procedure of *Hitchings*[9]. The dioxane was purified using the method proposed by *Freiser* et al.[10]. All of the reagents were r.a. grade.

Synthesis of $CdY \cdot 2H_2O$. 50 ml of dioxane, 40 ml of 0.1 *M* $Cd(NO_3)_2 \cdot 4H_2O$, 5 ml of $NaClO_4$ 1 *M* and 5 ml of 1 *M* perchloric acid were added with stirring to 0.552 g of H_2Y. Then 12.3 ml of 1 *M* NaOH solution were added until a $pH = 6$ was reached. A precipitate was formed. It was filtered and washed with a large volume of dioxane-water (50%) solution and dried at 80 °C. A white solid was obtained. The yield was 88%.

Synthesis of $Cd(HMP)_2 \cdot 2H_2O$. 50 ml of dioxane, 40 ml of 0.05 *M* $Cd(NO_3) \cdot 4H_2O$, 5 ml of $NaClO_4$ 1 *M* and 5 ml of 1 *M* perchloric acid were added with stirring to 0.608 g of H_2MP. Then 14.4 ml of 1 *M* NaOH solution were added, until $pH = 4.1$ was reached. A white precipitate was formed. It was filtered and washed with dioxane-water (50%) solution and dried at 80 °C. The yield was 90%.

Synthesis of $Cd(MP) \cdot H_2O$. An aqueous solution of $Cd(NO_3)_2 \cdot 4H_2O$ ($4 \cdot 10^{-3}$ mol in 100 ml) containing $8 \cdot 10^{-3}$ mol of $HClO_4$, was added with stirring to an methanolic solution of H_2MP ($4 \cdot 10^{-3}$ mol in 100 ml). Then 13.1 ml of 0.1 *M* T_4N OH (Tetrabutylammonium hydroxide solution) were added until $pH = 6.8$ was reached. The white precipitate formed was filtered, washed with methanol and dried at 80 °C. The yield was 89%. The compound decomposes at 245 °C without melting.

Chemical Analysis. The Cd content was determined by AAS method, and by precipitation as cadmium anthranilate[11].

C, H and N were determined by Elemental Analyzer (Carlo Erba model M.O.V.). The N was confirmed by the *Kjeldhal* method[12].

The results are recorded in Table 1.

Table 1. *Analytical data of the compounds*

Compound	*MP** °C	% C Found (Calcd.)	% H Found (Calcd.)	% N Found (Calcd.)	% Cd Found (Calcd.)
$CdY \cdot 2H_2O$	305	21.24 (21.25)	1.88 (2.12)	19.63 (19.83)	39.53 (39.80)
$Cd(HMP)_2 \cdot 2H_2O$	303	26.34 (26.64)	2.16 (2.22)	24.32 (24.86)	24.49 (24.96)
$Cd(MP) \cdot H_2O$	—	20.83 (21.25)	1.37 (1.42)	19.56 (19.97)	39.73 (40.08)

* Decomposition temperature.

The potentiometric measurements of the *pH* were carried out with a Radiometer PHM 62 using a glass electrode, model G 2040 and a calomel electrode model K 4040, calibrated according to the usual procedures[13].

In the methanolic solution the *De Ligny* procedure was followed[14—17], with the above mentioned electrodes, although the calomel electrode was bathed in methanol with LiCl.

Solutions containing Cd(II) and chelating agent with the molar ratios and conditions indicated in Figs. 1, 2, and 3 were prepared.

The thermal stability of the obtained derivatives was studied in a Setaram Balance under nitrogen. The heating rate employed was 2.8 °C/min. ATG, DTG and ATD curves were recorded simultaneously. Electronic spectra were recorded on a Pye Unicam model SP-8-100 using 1 cm matched silica cells. IR spectra were recorded on a Perkin-Elmer 577 Spectrophotometer. Samples were prepared as CsI discs.

Results and Discussion

Dissociation constants of hypoxanthine and 6-mercaptopurine in the solvents used in this study were determined potentiometrically. Their values are given in Table 2; pK_1 refers to the dissociation of the protonated species (H_3A^+); pK_2 refers to the dissociation of HS in the case of 6-mercaptopurine and to that of the imine group of the imidazole ring in the case of hypoxanthine. Our values are in agreement with those reported elsewhere[3,4,18].

Potentiometric titrations of each ligand in the presence of Cd(II) are given in Figs. 1, 2, and 3. From Fig. 1 it is possible to derive the following equilibria

$$H_3Y^+ + H_2O = H_2Y + H_3O^+ \qquad m = 1$$

$$Cd^{2+} + H_2Y + 2H_2O = CdY_{(s)} + 2H_3O^+ \qquad m = 3$$

Table 2. *Acid dissociation constants of 6-mercaptopurine and hypoxanthine at 25.0 °C and 1 M* (NaClO$_4$) *ionic strength*

	Solvent	pKa_1	pKa_2	pKa_3
6-mercaptopurine	50% Dioxane	< 2	8.55 ± 0.03	—
	Methanol	< 2	10.36 ± 0.02	—
Hypoxanthine	50% Dioxane	< 2	9.60 ± 0.03	11.48 ± 0.02*

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* Spectrophotometrically.

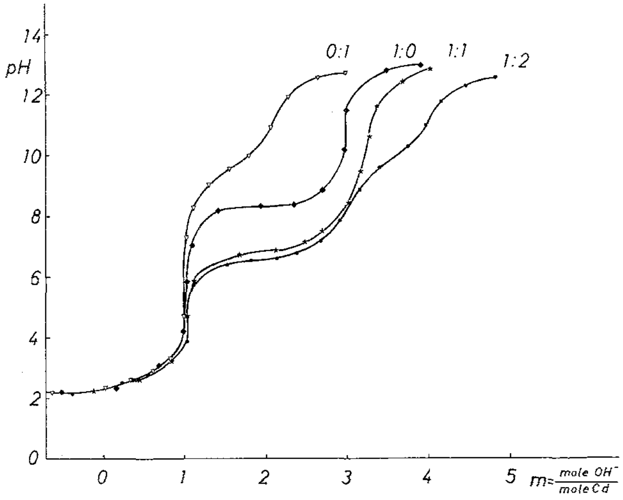


Fig. 1. Titration curves of Cd : H_2Y in dioxane-water (50%) solutions, Cd(II) 0.25 mmol; HClO$_4$ added, 0.42 mmol; V_t 100 ml; $T = 25 \pm 0.1$ °C

At H_2Y/Cd > 1 a third inflexion point is observed, which corresponds to the dissociation of excess acid.

From Fig. 2 the following equilibria are derived

$$H_3MP^+ + H_2O = H_2MP + H_3O^+ \qquad m = 1$$

$$Cd^{2+} + 2\,H_2MP + 2\,H_2O = Cd(HMP)_{2(s)} + 2\,H_3O^+ \qquad m = 3$$

For H_2MP/Cd relations > 2 a third inflexion point is observed corresponding to excess acid dissociation.

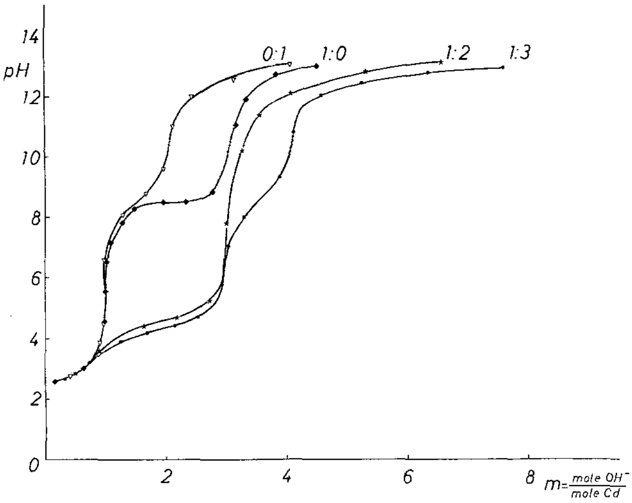


Fig. 2. Titration curves of Cd : H_2MP in dioxane-water (50%) solutions. Cd(II) 0.22 mmol; $HClO_4$ added, 0.50 mmol; V_t 100 ml; $T = 25 \pm 0.1$ °C

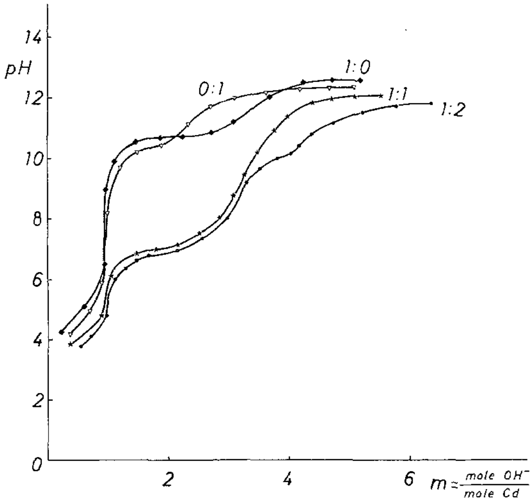


Fig. 3. Titration curves of Cd : H_2MP in methanolic solutions, Cd(II) 0.05 mmol; $HClO_4$ added, 0.22 mmol; V_t 100 ml; $T = 25 \pm 0.1$ °C

When a methanolic medium was used (Fig. 3) following equilibria are suggested:

$$H_3MP^+ + H_2O = H_2MP + H_3O^+ \qquad m = 1$$

$$Cd^{2+} + H_2MP + 2\,H_2O = CdMP_{(s)} + 2\,H_3O^+ \qquad m = 3$$

This is proved by the fact that the third inflexion point corresponding to excess acid dissociation is found for $m = 4$ in solution with $H_2MP/Cd = 2$.

From the potentiometric titrations it is possible to conclude that the Cd(II) ion has a different behaviour with 6-mercaptopurine in the different solvents used.

The stability constants reported in Table 3 for the complex Cd*Y* in the region of the titration curve between $a = 0.4$–0.8 [far from the precipitation point (Fig. 1)] have been calculated using the algebraic and *Rossotti-Rossotti*[19] (Fig. 4).

The constants of the Cd—*MP* compounds could not be evaluated because precipitation of the metal complex ocurred at lower *pH* values.

Table 3. *Complex formation constant of CdY at 25 °C*

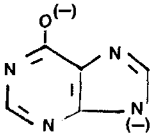
Ionic Strength	lg β (Algeb.)	lg β (*Rossotti-Rossotti*)
$NaClO_4$ (1 *M*)	10.20 ± 0.05	10.30

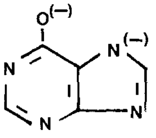
Properties of the Complexes

$CdY \cdot 2H_2O$. This compound is insoluble in water and in common organic solvents. It is soluble in HCl aqueous solutions. The study of its thermal stability shows a loss of weight (12.68%) from 65 °C until 175 °C, which corresponds to the dehydration of two water molecules. The anhydrous compound is thermally stable until 305 °C where endothermic decomposition begins.

The IR spectrum shows a broad band between 3 600 and 3 300 cm^{-1} which corresponds to O—H vibrations of water. The band at 3 180 cm^{-1} attributed to N—H stretching of the free ligand disappears upon complexation. The band at 1 585 assigned to N—H bending vibration[20] and the band at 705 cm^{-1} corresponding to the out-of-plane bending mode of O—H, also disappears. At the same time, the stretching band of C—O group, which for the free ligand appears at 1 160–1 150 is shifthed to 1 220 for the complex.

Based on the IR spectra, which do not show OH and NH vibrations, we can propose the following tautomeric structures for the Y^{2-} ion:





Since *Purnell* et al.[21] have indicated that N(7) is a softer base site than N(9), we can reasonabily expect that the very soft metal ion Cd^{2+} will coordinate at position 7 in the present complex.

$Cd(H\mathit{MP})_2 \cdot 2\,H_2O$. This compound is insoluble in water and in most common organic solvents. The study of its thermal stability shows a loss of weight (7.7%) between 45 °C and 200 °C, which corresponds to the elimination of two water molecules. The anhydrous compound is thermally stable until 303 °C, then an endothermic decomposition begins to take place.

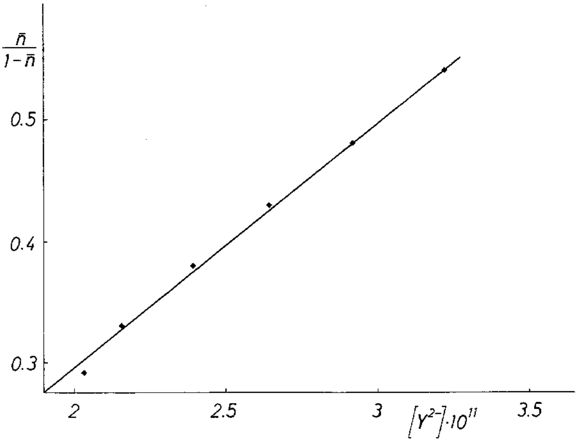


Fig. 4. *Rossotti-Rossotti* method for the determination of the Cd*Y* stability constant

The IR spectrum shows a broad band at 3 500–3 100 cm^{-1} with a maximum at 3 180 cm^{-1} due to the N—H stretching vibration. The band at 2 840 cm^{-1} assigned to vibrations of S—H of $H_2\mathit{MP}$ has disappeared. The band at 1 580 assigned to N—H bending modes is also present in the complex. The band at 290 cm^{-1}, not present in the IR spectrum of $H_2\mathit{MP}$, is assigned to the stretching vibration of S—Cd.

In agreement with these results and the bibliography[21] we conclude that Cd^{2+} is bonded through S to each $H_2\mathit{MP}$.

$Cd(\mathit{MP}) \cdot H_2O$. This compound is insoluble in water and in most common organic solvents. ATG and ATD curves show a weight loss (7.48%) from 40 °C until 200 °C, which corresponds to the dehydration of 1 water molecule. The anhydrous compound is thermally stable until 245 °C. Above this temperature decomposition starts.

Its IR spectrum shows a broad band at 3 500–3 300 cm^{-1} which is assigned to O—H vibrations of hydrated water. We notice the disappearance of the band at 1 580 (N—H bending vibration). The band at 2 840 cm^{-1}, which is assigned to the S—H vibration in the IR spectrum of H_2MP, also disappears. The band at 290 cm^{-1} is assigned to the stretching vibration of S—Cd.

The fact that N—H and S—H bands are not present in the IR spectrum of $CdMP \cdot H_2O$, as well as the set of data reported by *Hodgson*[22] and *Grinberg*[23], allow to propose that Cd is bound at N(7) and S(6) sites.

Acknowledgement

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