

Cobalt(II) and Iron(II) Complexes of 6-Mercaptopurine

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Abstract

Cobalt(II) and iron(II) complexes of 6-mercaptopurine (mpH) were synthesized in nonaqueous solution, their compositions and physicochemical properties were characterized by elemental analysis, IR, DTA and magnetic susceptibility measurements. For the iron(II) complex the Mössbauer and mass spectra data were also recorded. The novel complexes have the stoichiometric formulas $\text{Co}(\text{mp})(\text{Ac})(\text{H}_2\text{O})$ ($\text{Ac} = \text{acetate}$) and $\text{Fe}(\text{mpH}_2)(\text{OH})\text{SO}_4$ respectively, and both display significant activity against the L-615 tumor system in mice.

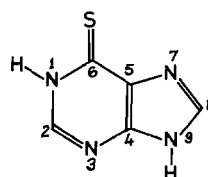
Introduction

For many decades, drugs used for cancer chemotherapy were predominantly derived from organic and biochemical preparations. When in 1969 Rosenberg [1] discovered the strongly cell-mitosis-depressing, broad spectrum, inorganic anticancer agent Cisplatin (*i.e.* *cis*-dichlorodiammineplatinum), the use of metal coordination compounds in the search for new drugs for cancer treatment started to evolve. In recent years, numerous interesting results have attracted the attention of inorganic chemists [2–7].

There are possibilities for improving therapeutic results and suppressing undesirable side effects of currently used antitumor drugs by using the coordination compounds of certain transition metals, some promising results have been reported in the literature [5].

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Although 6-mercaptopurine (I) has been used as an antimetabolite against leukemia, as yet few reports are available on the isolation and characterization of its metal complexes [8–11]. This paper presents results for the novel title complexes which have been studied in our laboratory.



I

Experimental

Synthesis of Complexes

The metal complexes were prepared as follows: A hot solution of the metal salt in DMF was added slowly under stirring to the ligand solution in equimolar quantity. The reaction mixture, the pH of which was adjusted to 6 for Co(II) complex and around 3 for Fe(II) complex, was refluxed for 2 h. After cooling, the crystalline solids thus separated were washed with cold water, ethanol and ether, and finally dried *in vacuo*. When preparing the ferrous complex the reaction was undertaken under nitrogen atmosphere in order to prevent the oxidation of the bivalent iron.

Both complexes are coloured, stable in air and sparingly soluble in most solvents. The results of

TABLE I. Analytical Data of Complexes.

Complex	Colour	Found (Calc.), %				Metal	
		C	H	N	S	Total	Due to sulphate
$\text{Co}(\text{mp})(\text{Ac})(\text{H}_2\text{O})$	Greyish violet	29.04	2.97	20.14			20.98
		(29.29)	(2.81)	(19.51)			(20.53)
$\text{Fe}(\text{mpH}_2)(\text{OH})\text{SO}_4$	Light yellow	18.67	1.80	17.07	19.74	10.54	17.51
		(18.63)	(1.88)	(17.39)	(19.91)	(9.95)	(17.35)

TABLE II. Selected IR Data of Complexes (ν_{\max} in cm^{-1}).

Co(II) complex	Assignment	Fe(II) complex	Assignment
3390s,br	$\nu(\text{O}-\text{H})$ (aqua)	3165s, 3114s	$\nu(\text{O}-\text{H})$
1605vs	$\nu(\text{C}=\text{O})$	2766m	$\nu(\text{N}-\text{H})$
1389s	$\nu(\text{C}=\text{O})$	1776w	$\nu(\text{C}=\text{S})$
663m	$\delta(\text{O}-\text{C}-\text{O})$, wagging	1620vs	$\nu(\equiv\text{N}-\text{H}) + \nu(\text{C}=\text{N})$
618w	$\delta(\text{O}-\text{C}-\text{O})$, wagging	1108vs	$\nu(\text{SO}_4)$
554w	Chelate ring deformation	817w	$\delta(\text{Fe}-\text{O}-\text{Fe})$
387w,br	$\nu(\text{Co}-\text{O})$ (aqua)	612w	$\nu(\text{SO}_4)$
318w	$\nu(\text{Co}-\text{S})$	548w	Chelate ring deformation
252w	$\nu(\text{Co}-\text{N})$	486w	Metal-ligand vibration
		272w	$\nu(\text{Fe}-\text{N})$

Abbreviations: s, strong; vs, very strong; m, medium; w, weak; br, broad.

elemental analyses are shown in Table I. The iron content was analyzed by atomic absorption in the presence of potassium cyanide [12]. Cobalt was analyzed gravimetrically by hydrogen reduction. Sulphur was determined as barium sulphate after oxidative decomposition with mixed acids.

Physicochemical Measurements

The infrared spectra of complexes and ligand have been recorded on IFS-113V IR spectrophotometer between 3900–400 cm^{-1} by KBr disc and between 670–100 cm^{-1} by Nujol mull. Magnetic susceptibility of the solid complexes was measured at room temperature using Faraday's method, employing Mohr's salt as the calibrant ($\chi_g = 31.82 \times 10^{-6}$ cgs units at 25 °C). DTA studies were undertaken with a differential thermobalance Model 4.1 (Beijing Optical Instrument Works) under nitrogen atmosphere. Mössbauer and mass spectra of the iron complex were obtained using an Elscint AME50 spectrometer and a Hitachi M-80 mass spectrometer, respectively. Elemental analyses were performed by the Analytical Laboratory, Shanghai Institute of Organic Chemistry, Academia Sinica.

Results and Discussion

By comparison of the IR spectra of the complexes with those of the free ligand, the salient data of diagnostic value are presented in Table II.

The Co(II) complex exhibits distinct bands at 1605 and 1389 cm^{-1} assignable to $\nu(\text{C}=\text{O})$ of the coordinated acetate [13]; this is further supported by the appearance of wagging modes of acetate around 663 and 618 cm^{-1} [14]. A broad band around 3390 cm^{-1} is attributed to $\nu(\text{O}-\text{H})$ of coordinated water, its strong broad absorption masks the characteristic vibrational mode of $\nu(\text{N}-\text{H})$ at ca. 2800

cm^{-1} . On complex formation, the new peak observed at 554 cm^{-1} may be assigned to chelate ring deformation [15]. A few weak absorptions in the far IR region (387 and 318 cm^{-1}) are probably caused by the Co–O (aqua) and Co–S stretching vibrations respectively [16], and the peak at 252 cm^{-1} is tentatively assigned to the $\nu(\text{Co}-\text{N})$ mode [17].

Owing to the poor solubility of the Co(II) complex in most solvents, it is impossible to produce an electronic spectrum to provide structural information.

In the spectrum of the Fe(II) complex the peaks found in the region 3165–3114 cm^{-1} are assignable to $\nu(\text{O}-\text{H})$, which shifts to the lower frequency as compared to the free OH ligand (band usually covering 3500–3400 cm^{-1}). This observation suggests bridging ligation of hydroxyl groups in the dimeric complex. The bands observed at 1108 and 612 cm^{-1} are assigned to the outer sphere sulphate groups [13]. The very strong band at 1620 cm^{-1} can be attributed to combination absorption arising due to $\nu(\text{C}=\text{N})$ [8] and the presumed characteristic band of protonated heterocyclic nitrogen [18]. On complex formation, the new band occurring at 817 cm^{-1} is tentatively assigned to the Fe–O–Fe bent bridge vibration [19]. The peaks observed at 548 and 486 cm^{-1} probably originate from chelate ring deformation [15] and metal–ligand vibration. The presence of the band at 272 cm^{-1} may be attributed to possible $\nu(\text{Fe}-\text{N})$ absorption [17].

The thermal behaviour of the metal complexes was investigated by differential thermal analysis. The DTA curve of the Co(II) complex shows a distinct endothermic peak around 70 °C which corresponds to the loss of coordinated water from the inner sphere. This observation is further supported by the weight loss determination of the solid complex at 110 °C (Found: 6.12%; Calc.: 6.27%). In the case of the ferrous complex there is no definite

TABLE III. Magnetic Moments of Complexes.

Complex	$\chi_g \cdot 10^6$ ^a cgs unit	Magnetic Moment, B.M.		
		μ_{eff} (298 K) ^b	Spin-only value	Range of literature data [13]
Co(mp)(Ac)(H ₂ O)	28.2	4.45	3.88	4.30–5.20
Fe(mpH ₂)(OH)SO ₄	36.1	5.32	4.90	5.10–5.70

^a χ_g represents the magnetic susceptibility per gram of sample. Diamagnetic corrections made by using Pascal's constants [13].

^b $\mu_{\text{eff}} = 2.84[(\chi_g \cdot \text{Mol. wt.} - \chi_d)T]^{1/2}$, where χ_d denotes the

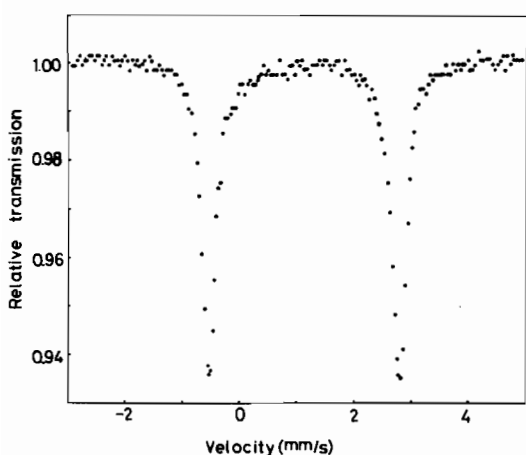


Fig. 1. Mössbauer Spectrum of ferrous complex.

thermal effect in the low temperature region and the only endotherm occurs up to 465 °C, indicating thermal decomposition.

The magnetic moments of the complexes at room temperature were calculated from the magnetic susceptibilities determined by Faraday's method and are presented in Table III.

Although the magnetic moments for spin-free complexes of Co(II) and Fe(II) are higher than the spin-only values, they are both within the range of literature data. However, according to Figgis [13] tetrahedral cobaltous complexes should exhibit moments closer to the spin-only value than their octahedral complexes, *i.e.* the moments should lie nearer to the lower limit of the literature data (4.30–5.20 B.M.). It seems reasonable to expect a similar relationship for the high spin ferrous complex. Consequently, this feature may be used to differentiate between six and four (*viz.* octahedral and tetrahedral) coordinate complexes. Accordingly both of the title complexes can be assigned to the tetrahedral configuration.

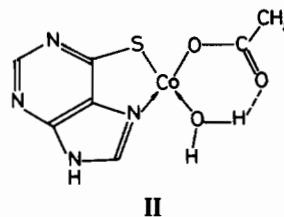
To obtain further evidence of the electronic structure of iron complex, the Mössbauer spectrum was recorded for the polycrystalline sample at room temperature. The spectrum is characterized by a

single quadrupole doublet (Fig. 1) with the parameters ($\delta = 1.13 \text{ mm s}^{-1}$, $\Delta = 3.39 \text{ mm s}^{-1}$) which is in good agreement with values for high spin ferrous compounds [20].

The mass spectrum of the ferrous complex gives molecular ions ($M^+ = 644$) only in lower abundance (3%). The main fragment ions are m/z 207(13)*, 300(20), 392(8) and 484(6), which can be considered as the iron-mercaptopurine fragments represented by the formula $[(M^+/2 - \text{SO}_4 - \text{OH}) + nG - 1]$, where G is the mass of the matrix glycerol, and $n = 0, 1, 2$ or 3. The other major fragments in the spectrum are m/z 225(20), 317(12), 409(3) and 501(2), which are generated from the parent complex ions by homolytic cleavage and can be represented by the general formula $[(M^+/2 - \text{SO}_4) + nG - 1]$, where n is between zero and three.

Owing to the poor volatility of the ferrous complex, mass spectral data would appear insufficient to substantiate a dimeric structure, however, they are good enough to prove the stoichiometric composition within the inner sphere.

With respect to the evidence above, the structures for the novel complexes are tentatively proposed as follows:



According to the structure shown in **II** the mpH ligand takes its usual anionic form (mp^-) to chelate Co(II) through N(7) with thiolato-sulphur as the potential binding sites, the aqua ligand and acetate anion just fit the remaining free coordination positions. The preceding IR evidence, (a broad band at around 3390 cm^{-1}) suggests that H-bonding inter-

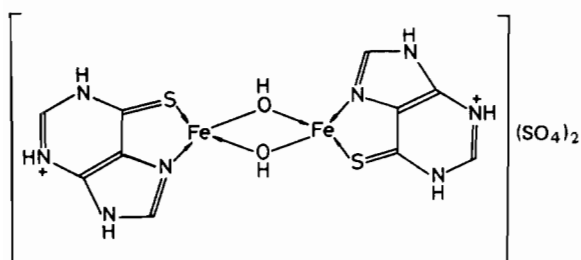
*The figures in parentheses denote the relative abundances of the corresponding fragment ions.

TABLE IV. Screening Data for mpH and Its Metal Complexes in L-615 Test System in Mice.

Compound	Dosage, mg/kg	% ILS ^a
mpH·H ₂ O	30	450
Co(mp)(Ac)(H ₂ O)	30	451
[Fe(mpH ₂)(OH)] ₂ (SO ₄) ₂	30	289

^a% ILS = % increase in lifespan of treated over control mice.

action exists between the water molecule and the carboxylate oxygen of acetate.



III

As regards the likely structure III of the ferrous complex, it is worth pointing out that the 6-mercaptopurine would react with the Fe(II) as a bidentate ligand in the preferred keto tautomeric form I, and furthermore that it enters the inner sphere as the monoprotonated cation (mpH₂⁺) in acidic media. It is obvious that if the N(7) coordinates with Fe(II) on complex formation, N(9) originally bonds with hydrogen before chelation and the hydrogen of SH group tautomerizes to N(1), so that the unique possible position for quaternization is at N(3). A crystallographically similar complex of Cu(I) has been reported in the literature [21]. In general, the sulphato-group is incapable of entering the first coordination sphere, and thus the iron(II) is surrounded by hydroxyl and mercaptopurinium bidentate ligands. It follows that coordination number four could be attained only if the ferrous complex would take the dimeric structure. An analogous guanine complex reported, namely [Fe(guH)(OH)]₂(SO₄)·4H₂O [22], lends support to our structural assignment.

Biological Activity

The novel complexes were evaluated for antitumor activity in mice bearing L-615 leukemia and compared with the activity of free ligand mpH. From the data listed in Table IV it can be seen that the Co(II) complex is almost as effective as an anti-

tumor agent as mpH itself, whereas the Fe(II) complex is considerably less active.

Acknowledgements

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References

- B. Rosenberg, L. Van Camp, J. E. Trosko and V. H. Mansour, *Nature, (London)*, 222, 385 (1969).
- S. E. Livingstone and A. E. Mihkelson, *Inorg. Chem.*, 9, 2545 (1970).
- M. Das and S. E. Livingstone, *Inorg. Chim. Acta*, 19, 5 (1976).
- M. Das and S. E. Livingstone, *Br. J. Cancer*, 37, 466 (1978).
- M. Gosálvez, M. F. Blanco, C. Vivero and F. Vallee, *Eur. J. Cancer.*, 14, 1185 (1978).
- H. Köpf and P. Köpf-Maier, *Angew. Chem., Int. Ed. Engl.*, 18, 477 (1979).
- P. J. Sadler, *Chem. Br.*, 18, 182 (1982).
- A. K. Ghosh and S. Chatterjee, *J. Inorg. Nucl. Chem.*, 26, 1459 (1964).
- S. Kirschner, Y.-K. Wei, D. Francis and J. C. Bergman, *J. Med. Chem.*, 9, 369 (1966).
- D. Colaitis and J. Brigando, *C.R. Acad. Sc. Paris*, 264c, 1035, 1101 (1967).
- M. Das and S. E. Livingstone, *Br. J. Cancer*, 38, 325 (1978).
- D. Banerjee (ed.), 'Coordination Chemistry-20', Pergamon, Oxford, 1980, p. 81.
- J. Lewis and R. G. Wilkins (eds.), 'Modern Coordination Chemistry', Interscience, New York, 1960.
- E. N. Sakuntala, G. Srivastava and R. C. Mehrotra, *Indian J. Chem.*, 20A, 243 (1981).
- K. Ueno and A. E. Martell, *J. Phys. Chem.*, 60, 1270 (1956).
- D. M. Adams, 'Metal-Ligand and Related Vibrations: A Critical Survey of the IR and Raman Spectra of Metallic and Organometallic Compounds', Arnold, London, 1967.
- C. M. Mikulski, L. Mattucci, Y. Smith, T. B. Tran and N. M. Karayannis, *Inorg. Chim. Acta*, 80, 127 (1983).
- G. J. Pouchert, 'The Aldrich Library of Infrared Spectra', Aldrich Chemical Co., 1970, p. 953.
- W. M. Reiff, G. J. Long and W. A. Baker, Jr., *J. Am. Chem. Soc.*, 90, 6347 (1968).
- K. Burger, 'Coordination Chemistry: Experimental Methods', Butterworths, London, 1973, p. 155.
- A. L. Shoemaker, P. Singh and D. J. Hodgson, *Acta Crystallogr., Sect. B.*, 32, 979 (1976).
- B. Jeżowska-Trzebiatowska, A. Antonów, H. Kozłowski and T. Cukierda, *Bull. Acad. Pol. Sci., Ser. Sci. Chim.*, 22, 1087 (1974).