

the interaction of adriamycin and daunorubicin with Pd(II). In this communication we report the results of a detailed potentiometric and spectroscopic investigation undertaken to characterize Pd(II)-anthracycline complexes. Their stability constants have been determined as well as their interaction with DNA, their antitumor activity and their ability to catalyze the flow of electrons from NADH to molecular oxygen when they are inserted into the NADH-NADH dehydrogenase system.

Physico-chemical characterization

The addition of PdCl_4^{2-} to Adr (or Dr) at 1:1 molar ratio yields a complex with the concomitant release of two protons ($\text{pK}=2.4$). Our potentiometric and spectroscopic titrations strongly suggest that in this complex the four ligands of Pd(II) are i) one carbonyl and one phenolate oxygen forming a six membered chelate, ii) the deprotonated amine of the sugar portion, and iii), depending on pH, either a water molecule or OH^- group. The kinetics of formation of this complex is rather slow and follows a second order rate law with $k_2 = 3.9 \text{ s}^{-1} \text{ M}^{-2}$. The value of the constant of formation defined as $K = \frac{[\text{Pd}(\text{AdH}, \text{NH}_2)]}{[\text{Pd}][\text{AdH}, \text{NH}_2]}$ is 1.3×10^{22} . (AdH, NH_2)

stands for adriamycin with the anthraquinone moiety half deprotonated and the sugar amine deprotonated. Similar results have been obtained with daunorubicin.

Similar complexes are obtained when either $\text{Pd}(\text{NH}_3)_4^{2+}$ or $\text{cis-Pd}(\text{NH}_3)_2\text{Cl}_2$ is added to adriamycin instead of PdCl_4^{2-} ; however in that case the fourth position of the square of coordination is occupied by an amine group.

Interaction with DNA

The complex has been added to DNA at molar ratio $[\text{Nucleotide}]/[\text{Complex}] \approx 10$. A very slow evolution of the CD spectrum of the system is observed: in two weeks about 25% of the complex has disappeared suggesting that due to the high affinity of Adr and Pd^{2+} for DNA the complex partially dissociates.

Antitumor activity

The *in vitro* inhibition of P 388 leukemia cell growth by the complexes compares with that induced by the free drugs. They display antitumor activity against P 388 leukemia; no significant differences from the free drugs in terms of therapeutic efficacy were observed.

Effect of the complex on superoxide production by NADH dehydrogenase

Adr and Dr increased superoxide formation by NADH dehydrogenase in a dose-dependent fashion that appeared to follow saturation kinetics. On the contrary the Pd(II)-anthracycline complexes do not increase superoxide formation over control level.

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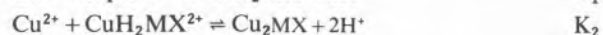
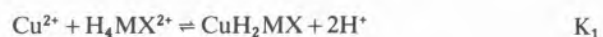
COPPER(II) BINDING BY MITOXANTRONE

The compound 1,4-dihydroxy-5,8-bis(2[(2-hydroxyethyl)amino]ethylamino)-9,10-anthracenedione dihydrochloride (Mitoxantrone (H_2MX), or No-

vantrone) has been developed as an antineoplastic agent and will soon be available commercially. The molecule is structurally similar to adriamycin and daunomycin and consequently, is thought to behave similarly. Because one of the major side effects of adriamycin is cardiotoxicity and the cardiotoxicity is thought to be associated with metal binding by the adriamycin molecule [1], we have been investigating the metal binding properties of the mitoxantrone molecule. However, there is also evidence that cardiotoxicity is not metal ion related [2]. Because of its biological abundance initial metal binding studies were made using Cu(II). For comparison, information for Cu-adriamycin and Cu-bleomycin complexes is available as is information about the distribution of copper in biological fluids [3-5]. Potentiometric titrations of the doubly charged form of mitoxantrone show that the first pK_a for the molecule is 6.8 and the second is 7.8 which agrees with earlier results [6]. There is almost no UV-vis spectrophotometric change associated with the first deprotonation, but a large change associated with the second. (Measured at 610 nm the extinction coefficient for the protonated form is 1.8×10^4 and for the deprotonated form 1.1×10^4). The differences in pK_a and the spectrophotometric results imply that the deprotonations occur at two different sites. Because of the lack of spectrophotometric change, the first probably occurs on the ethylenediamine side arm and the second with the large spectrophotometric change at a hydroxyl proton on the hydroxyanthraquinone ring.

Results of a spectrophotometric (700 to 600 nm) titration of the mitoxantrone molecule with Cu^{2+} at pH 4.8 show that little copper binds. However, if the titration is repeated at pH 7.2 then the absorbance changes show that two coppers bind per mitoxantrone molecule. Job's studies at pH 7 also show that two coppers bind per drug molecule. Because the binding was so strong in the concentration ranges used for these studies, no binding constants could be determined. Solid Cu_2MX complex was isolated using tetraphenyl borate as the counterion and analysis results confirm that two coppers bind per mitoxantrone molecule and show that the complex has a +1 charge. The complex with a +1 charge at pH 7.2 was also found from potentiometric titration curves. Com-

parison of titration curves for mitoxantrone alone and the same concentration of mitoxantrone containing two equivalents of copper showed that the copper binds strongly to the mitoxantrone even at low pH and each copper that binds releases two hydrogen ions. Using the titration data equilibrium constants were calculated according to the following equations:



The calculated values of K_1 and K_2 are 3.65×10^{-7} and 1.7×10^{-5} respectively. A fifth proton is also lost from the complex with a pK_D of 5.9 (potentiometrically) and 5.5 (spectrophotometrically). There is a large spectrophotometric change associated with this deprotonation. Copper binding constants for various antibiotics and blood serum components are given in Table 1. At pH 7 competition studies done spectrophotometrically show

Table 1

Compound	Complex	Log β	Ref.
Mitoxantrone(MX)	Cu_2MX^+	17.8 ^a	this work
Adriamycin(Adria)	Cu-Adria	12.08	[3]
Bleomycin(Bleo)	Cu-Bleo	12.63	[4]
Human Serum Albumin(HSA)	Cu-HSA	16.2	[5]
Histidine(his)	$Cu(his)_2$	17.5	[5]

$$a) \text{ pH } 7.2 \quad \beta = K_1 K_2 K_D \frac{([H^+]^2 + [H^+] K_{a1} + K_{a1} K_{a2})}{[H^+]^5 K_{a1} K_{a2}}$$

that the mitoxantrone binds copper more strongly than CH_3CN , imidazole, and pyridine but less strongly than bipyridine.

Electrochemical, EPR and X-ray structure determinations will be attempted to further characterize the Cu_2MX complex.

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PYRIDOXAL ISONICOTINOYL

HYDRAZONE:

A PROMISING AGENT FOR CHELATION THERAPY OF IRON OVERLOAD

The chemistry of pyridoxal isonicotinoyl hydrazone (PIH) has been investigated with emphasis on the chemical characteristics pertinent to the assessment of its value as a biological iron chelator. In acidic environments, PIH is remarkably stable, with a decomposition of $\leq 3\%$ after 72 hr at pH 2, 37°C. The acid dissociation constants of the several ionisable groups present in PIH have been determined by potentiometric titration and the formation of the iron complexes studied by potentiometry and UV-Vis spectrophotometry. The systems are quite complex due to the number of dissociable protons in both the free and coordinated ligands, the high affinity of these compounds towards iron(III) and the formation of sparingly soluble species at pH 5.

At pH 7.4, $[\text{Fe}^{3+}] = 10^{-6} \text{ M}$ and a 1000-fold excess of ligand, PIH has a pM value of 27.7 which, when compared to 25.6 for transferrin, indicates that PIH is thermodynamically capable of removing iron from transferrin.

The distribution of the complex species as a function of pH shows that in each case a significant fraction is present as the electrically neutral Fe(L)(HL) at pH 7.4. A model of the coordina-

tion geometry of this species, supported by spectroscopic data, is proposed.

The affinity of PIH for iron(II) is significantly lower than for iron(III), as indicated by a formation constant of 7.0 for $[\text{Fe(II)(HL)}_2]$ compared to 12.47 for $[\text{Fe(III)(HL)}_2]$.

PIH therefore possesses many chemical features desirable in an effective iron chelating drug. Furthermore, this chelating agent has been reported to be effective in several cellular and animal bioassays and is currently a highly promising pharmacological agent for the treatment of iron overload.



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ASCORBATE OXIDASE, DIAMINE OXIDASE AND THEIR USE IN DIAGNOSIS OF COPPER DEFICIENCY IN PLANTS

Copper deficiency in soils and crops is a cause of concern in many agricultural industries. In general, copper deficiency leads to significant decreases in crop yield and plant fertility. However, it is often difficult to diagnose deficiency without complex laboratory procedures and at a sufficiently early stage in the plant's growth to allow remedial measures to be adopted, *e.g.* by including copper compounds in fertiliser. Analysis of the range of copper compounds present in plants,