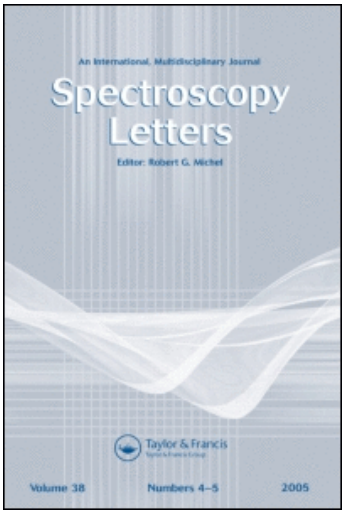

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Spectroscopic Study of the Interaction of Mitoxantrone with Copper(I), Copper(II), and Neocuproine

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SPECTROSCOPIC STUDY OF THE INTERACTION OF MITOXANTRONE WITH COPPER(I), COPPER(II), AND NEOCUPROINE

KEY WORDS: Mitoxantrone, copper(I), copper(II), neocuproine, bis(2,9-dimethyl-1,10-phenanthroline)copper complexes, charge-transfer complexes, visible spectroscopy, antitumor activity.

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ABSTRACT

Both reductive and oxidative mode of metabolism have been hypothesized for the antitumor agent mitoxantrone. This work aims to better understand the redox properties of mitoxantrone in the presence of the physiological redox couple, copper(I)/copper(II), by means of polarography and spectrophotometry. The first quasi-reversible one-electron reduction of mitoxantrone to the semiquinone and the second reduction to the hydroquinone have been shown to be considerably affected by copper. Visible spectra of mitoxantrone–copper mixtures in nitrogen and oxygen purged solutions taken in a one-week period exhibit varying degrees of complexation and oxido-reduction.

When copper(II), neocuproine and mitoxantrone are mixed in a certain ratio in pH 7.4 phosphate buffer solution,

two new peaks at 584 and 632 nm emerge in the spectra indicating a ternary charge transfer complex.The complex stoichiometry was established as Cu(II):neocuproine:mitoxantrone = 2:4:1 by Jŏb's method of continuous variations.The ternary complex is sensitive to the relative concentrations of mitoxantrone and copper(II),an excess of the latter giving rise to complete redox products.This complex may be important in modelling the drug's oxidative mode of antitumor action.

INTRODUCTION

The antineoplastic activities of certain chemotherapeutic agents such as anthracycline antibiotics and other related quinonic compounds have previously been correlated with their in vivo reduction by cytochromes,followed by generation of free radicals[1].Mitoxantrone,1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione abbreviated as Mx,was designated as an anthraquinone derivative which was expected to mimic anthracyclines.Its activity and potency are markedly enhanced by 1,4-hydroxylation, probably due to internal H-bonding contribution of the α-hydroxyl groups bringing about the stabilization of the semiquinone structure[2].

As with anthracyclines,there is some doubt over the relevance of the redox properties of the substituted anthraquinones with respect to cytotoxicity[3].Reduced levels of cardiotoxicity and in vivo lipid peroxidation were observed with mitoxantrone compared to those of anthracyclines[4]. Mx shows a more negative reduction potential than daunorubicin both in one- and two-electron reductions.The quinone-semiquinone couples at pH 7.0 for ametantrone(the dehydroxylated

analouge of Mx) and daunorubicin[5] are -0.348 V and -0.305 V, respectively.For the quinone-hydroquinone couples at pH 7.1, Mx and daunorubicin exhibit half-wave potentials($E_{1/2}$) of -0.775 V and -0.625 V vs. SCE,respectively[6].Therefore mitoxantrone's mode of action may be different from the one ascribed to most anthracyclines such as daunorubicin.

In a broad sense,quinonic antitumor agents may be visualized as cytotoxic compounds which may undergo extensive oxidation-reduction cycles catalyzed by physiological redox couples Fe(II)/(III) and Cu(I)/(II).Such a hypothesis necessitates the reversibility of their electron transfer capabilities[7],a phenomena correlated to antitumor activity[8]. Hence we have dealt with the spectrophotometric and polarographic behaviour of Mx,in the presence of cuprous and cupric salts,with a special emphasis to the reversibility of their electrode reactions.It is noteworthy that the electronic spectroscopy of Mx and its analogues has been relatively little investigated until the end of the 1980s.

EXPERIMENTAL

Materials and Methods

Mitoxantrone hydrochloride was synthesized in the department laboratories according to the procedure of Murdock et al.[9],and crystallized from pure ethanol.All other chemicals (Merck) were analytical grade and were used without further purification.

Solutions Used

1.0×10^{-4} M Mx solutions were prepared by dissolving solid Mx in deaerated distilled water,N_2 was bubbled through, and the solutions were kept under N_2 prior to spectrophotometric and polarographic measurements.

Copper(II) and copper(I) solutions in 1.0×10^{-4} M concentrations were prepared in the Mx solution from $CuCl_2 \cdot 2H_2O$ and CuCl salts such that solid cupric chloride was added directly to the Mx solution while the corresponding amount of cuprous chloride was dissolved in minimal volume of concentrated HCl, made slightly acidic with NaOH, and rapidly added to the Mx solution all operations being carried out under N_2. As the solutions of Cu(I) salts and complexes are air sensitive[10], the solutions were nitrogenated and kept in stoppered flasks. Nitrogen purging was repeated in each sampling before recording the spectra on day one, two and eight.

2,9-Dimethyl-1,10-phenanthroline("neocuproine" further abbreviated as Nc) was prepared in 2.0×10^{-3} M concentration in 96% ethanol.

Apparatus

The molecular absorption spectra were recorded with a Hitachi 220A UV-Visible spectrophotometer, and a PO 4 Radiometer polarographic cell with two electrode(calomel and dropping mercury electrode) assembly was used for polarographic measurements.

RESULTS AND DISCUSSION

The pH of the freshly prepared Mx solution was 5.6, while the cupric and cuprous mixtures of Mx exhibited pH between 4.7 and 4.9. The spectra of the mixture solutions in nitrogenated media recorded on the first, second and eighth days are presented in Figures 1 and 2. Thus, unlike Cu(II)-smetantrone spectra which were recorded a few minutes after the reagents were mixed in Tarasiuk et al.'s work[11] the

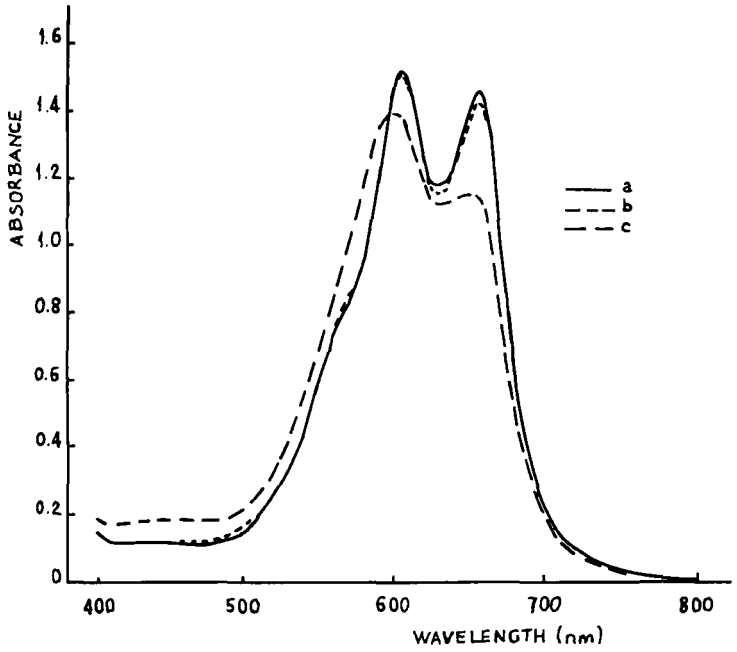

FIG.1: The spectra of Cu(II)-mitoxantrone equimolar mixtures at a final concentration of 1.0×10^{-4} M under nitrogen atmosphere on the 1st.,2nd. and 8th. days. a)Day 1, b)Day 2, c)Day 8.

interacting constituents were given sufficient time to reach equilibrium.The spectrum of the copper-Mx mixture,i.e., initially prepared with Cu(I),obtained on the eighth day (See Fig.2) shows a striking similarity to that of Cu(II)-ametantrone in the presence of an equimolar concentration of NADH[11].Mx and its dehydroxylated analogue(having H-atoms instead of the 1.4-dihydroxo substituents),ametantrone(AMET), possess similar chromophores giving rise to the visible band system due to the amino-ring charge-transfer transition[12]. NADH acting as the electron donor might have reduced copper

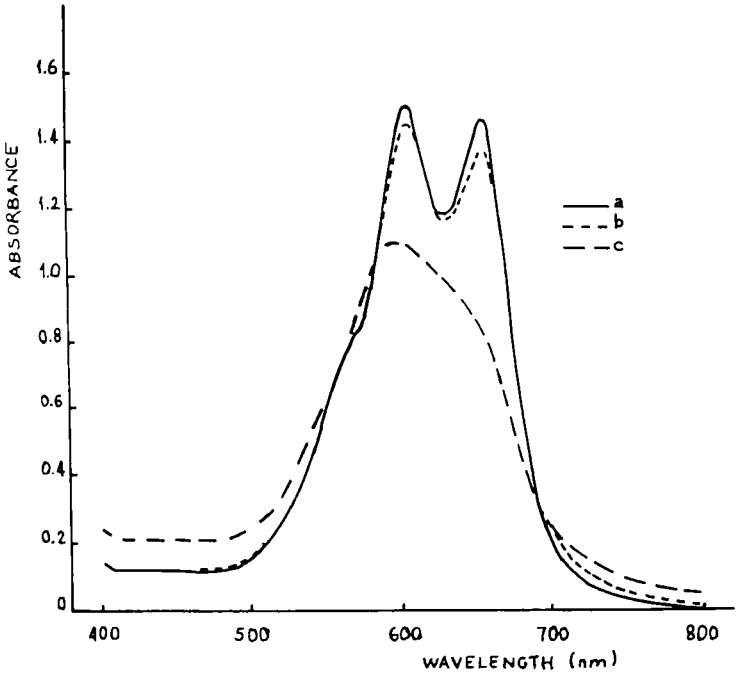

FIG.2: The spectra of Cu(I)-mitoxantrone equimolar mixtures at a final concentration of 1.0×10^{-4} M under nitrogen atmosphere on the 1st., 2nd. and 8th. days. a) Day 1, b) Day 2, c) Day 8.

to form essentially a cuprous complex with AMET, with the subsequent disappearance of the longest wavelength band of AMET and the merging of the two visible bands into a single broad band of smaller wavelength[11]. Cuprous complexation with Mx of this work might have produced a similar change in the spectrum of the parent compound(Mx) as observed in Figure 2. In accord with the suggestions of Kolodziejczyk and Garnier-Suillerot[13] for copper-AMET complexation, the most probable sites of Mx for copper coordination are the four nitrogen atoms of the aminoethanolamine side chains.

In order to observe the effect of atmospheric exposition on the mixtures,the solutions were separated into two portions at the end of the second day measurements.Nitrogen was bubbled through portion A solutions,and these were kept under nitrogen. Portion B solutions were not deaerated.On the eighth day the shoulder at 562 nm had disappeared for all solutions and the following spectral changes were observed:
Portion A: For Cu(II),the peaks(on day one at 658 and 608 nm) had shifted to 652 and 601 nm,respectively.For Cu(I),the 658 nm peak had transformed into a shoulder,while the 658 nm peak had been converted into a broad band at 597 nm.
Portion B: For Cu(II),the peak at 658 nm had transformed into a shoulder at 656 nm,and the 608 nm peak had shifted to 597 nm.

For pH between 5.6 and 7.0,the character of the mitoxantrone spectrum is virtually the same,but optical absorbances are slightly decreased.Below pH 3.9,sharp decreases occur in extinction.Above pH 7.0,the 658 nm peak transforms into a shoulder,while the extinction of the 608 nm peak decreases. Starting from pH 8.0,the 608 nm peak and 562 nm shoulder merge into a single broad band.

Mx solutions decompose when exposed to air at high pH which might be analogic to the observation that quinonic antitumor antibiotics are more susceptible to oxidation in their anionic forms[14].

Polarographic data show that the first wave of pure mitoxantrone solutions appearing at a half-wave potential of $E_{1/2}$ = -0.105 V may correspond to a quasi-reversible one-electron reduction to the semiquinone,but becomes drawn-out on standing of the Mx solutions.This wave is shifted to

-0.03 V with both Cu(II) and Cu(I), corresponding to a quasi-reversible one-electron reduction with the former, and assuming a drawn-out shape with the latter. The second wave of Mx at $E_{1/2}$ = -0.79 V is considered as a reversible one-electron reduction to the hydroquinone; both the reversibility of the electrode reaction and the half-wave potential are relatively independent of the changes in the medium. The reversible process is retained with Cu(II)-Mx mixtures, but assumes a quasi-reversible character with Cu(I)-Mx at $E_{1/2}$ = -0.73 V. Polarographic data confirm spectroscopic findings concerning the higher degree of interaction of Mx with Cu(I) than with Cu(II).

The initial one-electron reduction potential of Mx is in the range of most biological redox systems such as the cytochrome enzymes[15], but the reduction process has been found to be quasi-reversible and too dependent on the changes of media. Similar results were obtained by Li et al.[16] where the one-electron reduction of Mx by cyclic voltammetry in dimethylformamide medium was shown to be quasi-reversible in nature, the cathodic wave of this reduction being sensitive to the identity of substituents in the alkylamino-side chains of the Mx structure. Although the second reduction step of Mx to the hydroquinone is reversible, its potential is highly unfavourable to account for a bioreductive mode of metabolism of the drug.

Mx has been shown to exhibit a reduction potential more negative than that of 5-iminodaunorubicin at -0.67 V[17], the latter of which has been proven not to undergo <u>in vivo</u> metabolic reduction[18].

When Mx is treated with horseradish peroxidase and hydrogen peroxide, the characteristic absorption of Mx at 658 and 608 nm decreases; further reaction results in the appearance of a new absorption band with a maximum of 586 nm[19], which may be attributed to the formation of cyclic primary metabolite of Mx[20], the structural formula of which is shown in Figure 3. The enzymatic conversion of Mx to the primary metabolite is irreversible, but the latter may continue to undergo peroxidase action in the presence of hydrogen peroxide, and the resulting oxidized iminoquinone may be reduced by biologically important reducing agents such as ascorbic acid, cysteine and glutathione[20], the latter processes being fully reversible (See Figure 3). As shown by Fisher et al.[21] the Mx-derived air-stable free radical generated by the horseradish peroxidase catalyzed H_2O_2 oxidation of Mx is capable of cross-linking with plasmid DNA. The most probable source of the electrophilic cation radical[19] is the one-electron oxidation of the aromatic nitrogen atoms of the Mx side chain[22]. Hence oxidative mode of metabolism for the anticancer drug Mx is more likely and should be investigated in detail.

As shown with AMET, copper is coordinated to AMET through the N-atoms of the side chains[11] to form a complex which is not stable in the presence of cytochrome c reductase. However, Tarasiuk et al.[11] do not expect this complexation to give rise to a significant change in the redox properties of the parent drug as this type of complexation does not block the quinone function of AMET. This reasoning bears the assumption that Mx and AMET owe their redox properties to their enzymatic reduction to the semiquinone radical and the hydroquinone. On the other hand, if the oxidative mode of action prevails,

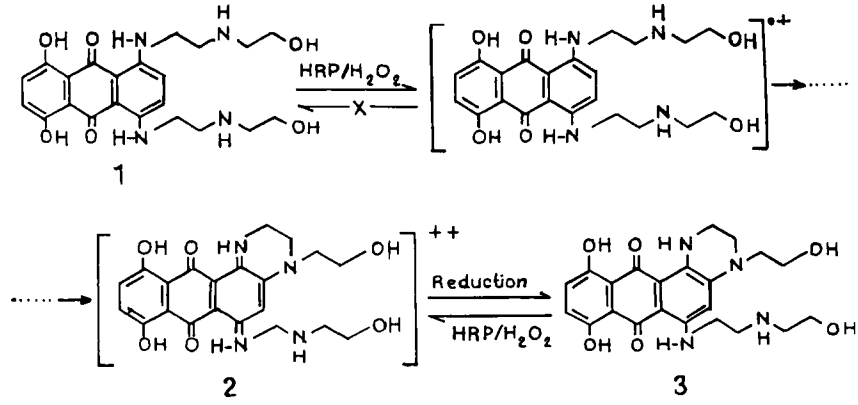

FIG.3: Horseradish peroxidase-catalyzed oxidation of mitoxantrone. 1:Mitoxantrone, 2:Oxidized iminoquinone form of mitoxantrone, 3:Cyclic primary metabolite of mitoxantrone.

the nitrogen atoms of the alkylamino-side chains are very important in the production of the cyclic metabolite,and their blockage by copper ions dramatically changes the redox behaviour of the parent drug,as shown in this work.

Ternary complexes of Cu(II) with α,α'-diimines and oxygen-donor ligands have attracted attention because of their extraordinary stability and their ability to serve as model compounds for metal-enzyme-substrate complexes[23,24]. With neocuproine(2,9-dimethyl-1,10-phenanthroline) as the diimine and acetylacetonate,acetate,oxalate,malonate or succinate as the oxygen-donor ligand(L),Cu(I) has been shown to form a ternary complex of the form $[Cu(Nc)L]$[25].

When Cu(II),Nc and Mx are mixed in a certain ratio in pH 7.4 phosphate buffer solution,two new peaks at 584 and 632 nm emerge in the spectra indicating a ternary charge transfer complex.A similar ternary complex does not form

with Cu(I).Job's method of continuous variations at 632 nm by mixing 2.0×10^{-4} M equimolar solutions in appropriate proportions yielded a mole ratio of Cu(II):Mx as 2:1 using a fixed Cu(II):Nc ratio of 1:3.When Cu:Mx was kept constant at 2:1,Job's method yielded a Cu:Nc ratio 1:2.Thus the empirical formula for the ternary complex was established as $Cu_2^{II}(Nc)_4Mx$ .The ternary complex is quite stable in aqueous solution,but does not form in the presence of either Cu(II) or Mx excess.The spectra of probable binary mixtures and of the ternary complex are shown in Figure 4.Excessive Cu(II) in the presence of Nc gives rise to a complete oxidation of Mx which may be followed by the disappearance of Mx bands[26]. The probable products of this latter redox process are $Cu^{I}(Nc)_2^+$ and the two electron oxidized iminoquinone form of Mx.

The ternary charge-transfer complex may be visualized as an intermediary stage of the complete oxidation of Mx which is brought about by an excess of bis-(neocuproine)copper(II) chloride.The redox chemistry of copper involves rate enhancement in some cases by an inner-sphere mechanism via chelation at neighboring nitrogen centers[27],which may account for the greatly increased oxidation rate of Mx with $Cu(Nc)_2^{2+}$ rather than Cu^{2+} alone.Further,the empirical formula of the ternary complex preceding the full oxidation of Mx suggests a hexa-coordinate copper rather than the tetra-coordinate copper(I) in the $Cu(Nc)_2^+$ cation; a change in the coordination number of copper usually accompanies the Cu(II) $\rightleftharpoons$ Cu(I) redox chemistry[28].

The ternary charge transfer complex may be important in modelling the drug's oxidative mode of antitumor action by

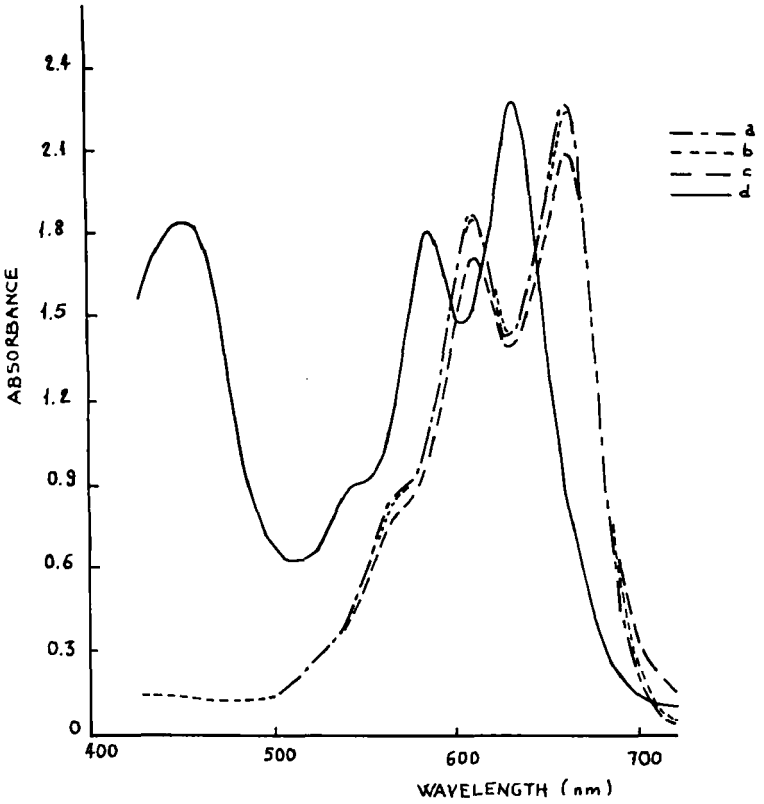

FIG.4: Spectra of binary and ternary mixtures of Cu(II)-neocuproine-mitoxantrone.
a) 10 ml of 2.0×10^{-4} M mitoxantrone+6 ml of 96% ethanol +4 ml of distilled water.
b) 10 ml of 2.0×10^{-4} M mitoxantrone+6 ml of 2.0×10^{-3} M neocuproine in 96% ethanol+4 ml of distilled water.
c) 10 ml of the mixture solution containing 2.0×10^{-4} M mitoxantrone and 4.0×10^{-4} M $CuCl_2$+6 ml of 96% ethanol+4 ml of distilled water.
d) Ternary complex solution:10 ml of the mixture solution containing 2.0×10^{-4} M mitoxantrone and 4.0×10^{-4} M $CuCl_2$+6 ml of 2.0×10^{-3} M neocuproine in 96% ethanol +4 ml of distilled water.

review of the relevant cancer chemotherapy literature due to the following:

i) By chelating copper with the 2,9-dimethyl-1,10-phenanthroline (Nc) rather than phenanthroline(phen) itself,the oxidizing power of the Cu(II)-Nc complex is dramatically enhanced,e.g., the standard potentials of the $Cu(Nc)_2^{2+/+}$ and $Cu(phen)_2^{2+/+}$ couples are 0.603 and 0.035 V,respectively[29].

ii) The ability of the primary metabolite(one-electron oxidized cyclic form) of Mx to undergo reversible charge-transfer interactions and redox reactions may play a vital role in the drug's mode of action[20].

iii) A charge-transfer complex in the excited state may effectively supress certain tumors[30].

iv) Cu-neocuproines are capable of DNA cleavage through the formation of free radicals[29],and Mx is a potent antitumor agent[4] which may undergo enzymatic oxidative activation.The advantages of these two agents may be efficiently combined in a Mx-to-Cu charge-transfer complex which may form and dissociate reversibly.

REFERENCES

1. Douglas,K.T.Anticancer Drugs:DNA Intercalation and Free Radical Attack.Chemistry and Industry(London) 1984; 21:766-71.

2. Murdock,K.C.,Child,R.G.,Fabio,P.F.,Angier,R.B.,Wallace, R.E.,Durr,F.E.,Citarella,R.V.Antitumor Agents:1.1,4-Bis-[(aminoalkyl)amino]-9,10-anthracenediones.J.Med.Chem. 1979;22:1024-30.

3. Neidle,S.,Waring,M.J.Molecular Aspects of Anticancer Drug Action,London and Basingstoke:McMillan Press,1983.

4. Durr,F.E.Seminars in Oncology:New Perspectives in Chemothrapy: Focus on Novantrone,XI,Suppl.1,1984:3-10.

5. Pullman,B.A Few Considerations on Quinones as Antitumor Agents.Int.J. of Quantum Chem.:Quantum Biology Symp., 1986;13:95-105.

6. Showalter,H.D.H.,Fry,D.W.,Leopold,R.,Lown,J.W.,Plambeck,J.A. Reszka,K. Design,Biochemical Pharmacology,Electrochemistry and Tumor Biology of Antitumor Anthrapyrazoles.Anticancer Drug Design 1986;1:73-85.

7. Apak,R.,Baykut,F. The Properties of Antitumor Active Metal Complexes.Proceeding of the Chemistry-86 National Symp., Ankara University,Ankara,1986;82.

8. Apak,R. An Approach to Antitumor Metal Complexes in the Light of Electron-Transfer Reactions.NATO/ARW on:Mixed Valency Systems,poster presentation,Crete,Greece,1990.

9. Durr,F.E.,Murdock,K.C. 1,4-Bis(substituted amino)-5,8-dihydroxyanthraquinones and leuco bases thereof.U.S.Pat. 4,197,249,1980.

10. Goodwin,J.A.,Stanbury,D.M.,Wilson,L.J.,Scott,R.A. In: Karlin,K.D.,Zubieta,J.eds.Biological and Inorganic Copper Chemistry,Guilderland,New York,1985;Vol.II:11-25.

11. Tarasiuk,J.,Kolodziejczyk,P.,Borowski,E., Enzymatic Studies of the Effect of Cu(II) on Oxygen Radical Production Stimulated by Daunorubicin and Ametantrone.Biochem.Pharmacol. 1990;39:1405-10.

12. Lin,S.,Struve,W.S. Solvatochromism and Time-Resolved Fluorescence of the Antitumor Agent Mitoxantrone and Its Analogues in Solution and in DNA.J.Phys.Chem.,1991; 95:2251-6.

13. Kolodziejczyk,P.,Garnier-Suillerot,A. Circular Dichroism Study of the Interaction of Mitoxantrone,Ametantrone and Their Pd(II) Complexes with Deoxyribonucleic Acid.Biochem. Biophys.Acta 1988;926:249-57.

14. Orlov,V.S.,Bogdanov,G.N. Oxidation of Anthracycline Antibiotics. Antibiotiki(Moscow) 1984;29:748.(CA 102,274 s,1985).

15. Baykut,G.,Baykut,D.,Baykut,F. An Approach to Cancer Development Based on Cellular Electron Transfer Mechanisms. Chim.Acta Turc. 1983;11:161-71.

16. Li,C-Y.,Jeng,J. Kinetic Study of One-Electron Reduction of Aromatic Quinones with Aminoalkylamino Side Chains in N,N-Dimethylformamide by Square Wave Voltammetry and Allied Techniques.Electrochimica Acta 1991;36:269-76.

17. Lown,J.W.,Chen,H.H.,Plambeck,J.A.,Acton,E.M. Diminished Supedoxide Anion Generation by Reduced 5-Iminodaunorubicin Relative to Daunorubicin and the Relationship to Cardiotoxicity of the Anthracycline Antitumor Agents.Biochem. Pharmacol. 1979;28:2563-8.

18. Peters,J.H.,Gordon,G.R.,Kashiwase,D.,Acton,E.M. Metabolic Disposition of 5-Iminodaunorubicin in the Rat.Cancer Res. 1984;44:1453-9.

19. Reszka,K.,Kolodziejczyk,P.,Lown,J.W. Horseradish peroxidase-Catalyzed Oxidation of Mitoxantrone:Spectrophotometric and Electron Paramagnetic Resonance Studies.Free Rad.Biol.Med. 1986;2:25-32.

20. Lown,J.W.,Reszka,K.,Kolodziejczyk,P.,Wilson,W.D. Molecular Mechanisms of Carcinogenic and Antitumor Activity.(Chagas, C.,Pullman,B.,eds.),Pontif.Acad.Scient.Scrip.Varia 1986; 243-74.

21. Fisher,G.R.,Patterson,L.H. DNA Strand Breakage by Peroxidase-Activated Mitoxantrone.J.Pharm.Pharmacol.1991;43:65-8.

22. Reszka,K.,Kolodziejczyk,P.,Lown,J.W. Enzymatic Oxidative Activation and Transformation of the Antitumour Agent Mitoxantrone.Free Rad.Biol.Med.1988;5:13-20.

23. Kwik,W-L.,Ang,K-P.,Chen,G. Complexes of (2,2'-bipyridyl) Copper(II) and (1,10-phenanthroline)Copper(II) with Some Amino Acids.J.Inorg.Nucl.Chem.1980;42:303-13.

24. Kwik,W-L.,Ang,K-P. Preparation and Characterization of Some Ternary Complexes of Copper(II) with o-phenanthroline and Oxygen Donor Ligands.Aust.J.Chem.1978;31:459-63.

25. Kwik,W-L.,Ang,K-P. Ternary Complexes of Copper(I) with 2,9-Dimethyl-1,10-Phenanthroline and Some Oxygen Donor Ligands.J.Chem.Soc.Dalton Trans.1981;452-5.

26. Tütem,E.,Apak,R.,Baykut,F. Spectrophotometric Determination of Trace Amounts of Copper(I) and Reducing Agents with Neocuproine in the Presence of Copper(II).Analyst 1991; 116:89-94.

27. Dockal,E.R.,Everhart,E.T.,Gould,E.S. Electron Transfer Through Organic Structural Units.IX.Reductions of Carboxylato Pentammine Cobalt(III) Complexes with Copper(I).J.Am.Chem.Soc. 1971;93:5661-7.

28. Goodwin,J.A.,Stanbury,D.M.,Wilson,L.J.,Eigenbrot,C.W., Scheidt,W.R. Molecular Structures and Electron-Transfer Kinetics for Some Pentacoordinate Cu(I)/Cu(II) Redox-Active Pairs.J.Am.Chem.Soc.1987;109:2979-91.

29. Tamilarasan,R.,McMillin,D.R.,Liu,F. Metal DNA Chemistry: Excited State Modelities for Studying the Binding of Copper Phenanthrolines to DNA.(Tullius,T.D.,ed.)ACS Symp.Series, Washington,D.C.,1989;48-58.

30. Szent-Györgyi,A. Protometabolism.International Quantum Chem.: Quantum Biol.Symp.1984;11:63-7.

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