# Chelating Properties of Copper(II) Aminoanthracyclines: an ESR Study

L. COSTA, F. MORAZZONI, R. SCOTTI

*Dipartimento di Chimica Inorganica e Metallorganica, Via Venezian 21, 20133 Milan (Italy)*

F. ANGELUCCI, A. SUARATO

*R. S. Chimico Farmitalia-Carlo Erba, Via dei Gracchi 35, 20146 Milan (Italy)*

and V. MALATESTA

*Centro Ricerche G. Donegani, Via Fauser 4, 28100 Novara (Italy)*

(Received July 25, 1989)

## Abstract

Electron spin resonance was successfully used to characterize the Cu(II) chelating properties of 4-demethoxy-6(11)-deoxy-6(11)-amino daunorubicins.

The $C_6$–$NH_2$ group of 6-amino daunorubicin coordinates Cu(II) yielding a mononuclear complex, while 11-amino daunorubicin forms a polymeric $(CuL)_n$ derivative, with both $C_{11}$–$NH_2$ and $C_6$–OH coordinated to the metal.

The tendency of both the amino substituted anthracyclines to undergo molecular stacking in the presence of Cu(II) is discussed.

## Introduction

Copper(II) and iron(III) chelates of anthracyclines have been studied for a long time to ascertain whether:

– the anthracycline action mechanism does involve transition metal centers [1]

– the metal chelation could modify the therapeutic effects of the drug [2]

We have recently found that the cardiotoxicity on isolated rat atria induced by the doxorubicin (DXR)/Cu(II) derivative, $(CuDXR)_n$, is significantly lower than that of the free drug, while antitumor effects are comparable [3]. From our study it appears that the polymeric structure of the complex is responsible for the decrease of the drug cardiotoxicity. Therefore the ability of anthracyclines to form multimolecular aggregates (dimers, oligomers, etc) of large dimensions, either in the presence or in the absence of metal coordination centers and/or because of the chemical modifications of the parent (DXR) molecular structure warrants investigation.

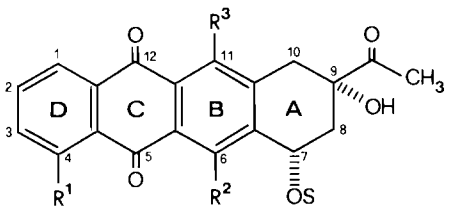
The copper(II) chelates so far studied have revealed two types of anthracycline intermolecular interaction:

The first type, stable in those bisbidentate drug molecules (DXR and DXR-like anthracyclines) where Cu(II) acts as bridging center, gives polymeric Cu(II) complexes [4]. These adducts are characterized by a strong magnetic exchange coupling between the Cu(II) centers.

The other, where the anthracycline ligand is stacked up, forms at high drug/metal molar ratio ($r$) and does not involve magnetic interaction between the Cu(II) centers [5].

We will refer to the products of the first interaction as $(CuL)_n$ type complexes and to those of the second one as $[Cu(L)_x]_n$. For the Cu(II) complex of the 5-imino daunorubicin we have recently described the occurrence of a dimeric L–Cu–L–Cu–L form, whose triplet electronic state of Cu(II) was recognized [6].

The present paper reports the spectromagnetic characterization of the Cu(II) complexes of 4-demethoxy-11-deoxy-11-amino daunorubicin and 4-demethoxy-6-deoxy-6-amino daunorubicin (Scheme 1). As the *ab initio* molecular orbital calculations of these two anthracyclines have been recently carried out [7], we will attempt a correlation between the results of the two investigations.




$R^1 = H$ $R^2 = NH_2$ $R^3 = OH$ 4-demethoxy-6-deoxy-6-$NH_2$-DNR

$R^1 = H$ $R^2 = OH$ $R^3 = NH_2$ 4-demethoxy-11-deoxy-11-$NH_2$-DNR

Scheme 1.

0020-1693/90/$3.50

## Experimental

The synthesis of the anthracyclines was carried out as described in ref. 8. Cu(II) complexes were obtained by mixing a $CuCl_2$ ($10^{-3}$ M) aqueous solution with an anthracycline aqueous solution of identical concentration. The solutions were mixed in the appropriate amount to obtain the desired anthracycline/Cu molar ratio ($r$).

ESR spectra were recorded at −150 °C on a Varian E-109 spectrometer, equipped with an automatic temperature control; a small amount of ethylene glycol (1%) was added before recording the spectra in order to obtain good quality glasses.

## Results and Discussion

*4-Demethoxy-11-deoxy-11-amino daunorubicin/Cu(II), r = 1*

The substitution of the $[Cu(H_2O)_6]^{2+}$ spectrum with that of an anthracycline Cu(II) derivative takes place starting at pH 6.5. The broadening of the resonances (Fig. 1(a)) is indicative of a strong exchange spin coupling between the Cu(II) centers, and the intermolecular interaction in this complex can be thought as originating within the polymeric structure $(CuL)_n$. The magnetic parameter values, though difficult to determine exactly, are in the range ($g_\parallel \simeq 2.2$, $g_\perp \simeq 2$, $A_\parallel \simeq 180$ G) characteristic of the Cu/anthracycline derivatives [4]. The drug belongs to the anthracycline class otherwise referred to as doxo-like anthracyclines [4].

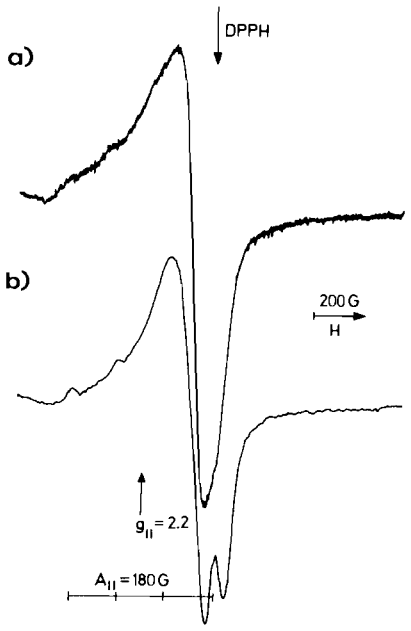



Fig. 1. ESR spectra, recorded in aqueous frozen solution, at −150 °C of: (a) 4-demethoxy-11-deoxy-11-amino daunorubicin/Cu(II), (b) 4-demethoxy-6-deoxy-6-amino daunorubicin/Cu(II).

*4-Demethoxy-6-deoxy-6-amino daunorubicin/Cu(II), r = 1*

For pH > 6.5 the spectrum corresponds to that of a tetragonal Cu(II) complex whose magnetic parameters are characteristic of a $CuL_2$ species (L = anthracycline) [4]. The magnetic exchange between the $Cu^{2+}$ centers (Fig. 1(b)) is very poor. The parallel component of the $g$ tensor ($g_\parallel = 2.21$) is well resolved ($A_\parallel = 180$ G) and corresponds to that peculiar of the Cu(II) monomeric complexes of the anthracycline class otherwise referred to as epi-doxo like [4].

The coordination of the amino anthracyclines here reported with Cu(II) reveals that the 6-amino derivative has a greater tendency to stabilize monomeric coordination compounds. We would in general expect that the $NH_2$ group behaves as a more efficient chelating site than the phenolic OH group. This oversimplified anticipation does, however, ignore the electronic interactions $NH_2$ may be involved in.

Our results show that the strength of the nitrogen binding site is dependent on which OH (i.e. $C_6$–OH or $C_{11}$–OH) is substituted.

If it is the more acidic $C_{11}$–OH, the strength becomes comparable to that of $C_6$–OH ($pK_a$ higher by 1.5 units than the corresponding $C_{11}$–OH) and a polymeric metal derivative of $(CuL)_n$ type is obtained; when $C_6$–OH is substituted with $NH_2$ the strength is much higher and the coordination site is most likely $C_6$–$NH_2$. We must also note that $C_6$–$NH_2$ and $C_{11}$–$NH_2$ have different hybridization [7]: the $sp^2$ hybridization of $C_{11}$–$NH_2$ decreases its basic and coordination properties to equal that of the hydroxo group at $C_6$; it is not unreasonable to suggest that, because of the $sp^3$ hybridization of $C_6$–$NH_2$, the coordination site is most likely the $C_6$–$NH_2$ group.

The $[(CuL_x)]_n$ structure was never observed in either of the amino anthracycline derivatives, not even at high $r$ values. The only intermolecular interaction noticed (in the 11-amino derivative) is that which couples the spin of several Cu(II) centers through a bridging anthracycline; thus in general the amino anthracyclines, as well as the 5-imino daunomycin [6], show low or no tendency to stacking in the presence of Cu(II).

This conclusion could be of some interest in the development of relationships between the anthracycline structure and their biological activiy. In fact if we consider that the ability to form $[Cu(L_x)]_n$ derivatives follows the order doxorubicin, daunorubicin > 5-imino daunorubicin > 4-demethoxy-6(11)-deoxy-6(11)-amino daunorubicins, and that the cardiotoxic side effects generally observed after drug administration were found stronger in both

doxo and daunorubicin than in the 5-imino daunorubicin [9], we would expect a further decrease of cardiotoxocity in the aminoderivatives of daunorubicin [10].

## Acknowledgement

The authors thank the Italian CNR, special project 'Oncology', grant 88.00766.44.115.00635, for financial support.

## References

1 J. L. Zweier, *Biochim. Biophys. Acta, 839* (1985) 209; K. B. Wallace, *Toxicol. Appl. Pharmacol., 86* (1986) 69.
2 H. Beraldo, A. Garnier-Suillerot, L. Tosi and F. Lavelle, *Biochemistry, 24* (1985) 284.
3 E. Monti, L. Paracchini, F. Piccinini, V. Malatesta, F. Morazzoni and R. Supino, *Cancer Chemother. Pharmacol.*, in press.
4 V. Malatesta, F. Morazzoni, A. Gervasini and F. Arcamone, *Anticancer Drug Design, 1* (1985) 53; F. Morazzoni, A. Gervasini and V. Malatesta, *Inorg. Chim. Acta, 136* (1987) 111.
5 V. Malatesta, A. Gervasini and F. Morazzoni, *Inorg. Chim. Acta, 136* (1987) 81.
6 V. Malatesta, F. Morazzoni, L. Pellicciari-Bollini, R. Scotti and G. Speranza, *Inorg. Chim. Acta, 152* (1988) 145.
7 V. Malatesta, G. Ranghino and F. Morazzoni, *J. Mol. Struct.*, in press.
8 F. Angelucci, M. Gigli and S. Penco, *U.S. Patent N 973 109* (filed 14/7/1987) (pending).
9 N. C. Love and J. I. Smallwood, *Biochem. Pharmacol., 5* (1985) 61.
10 F. Piccinini, E. Monti and F. Morazzoni, work in progress.