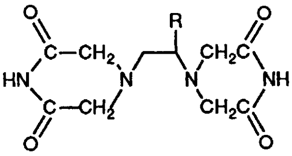
Copper(II) Complexes of Hydrolysis Products of the Anticancer Bis(3,5-dioxopiperazin-1-yl)alkanes. Displacement of Co-ordinated Carboxylate Ligands by Deprotonated Amide Groups in Basic Solution

Niamh Nic Daeid, Kevin B. Nolan* and Leo P. Ryan
Department of Chemistry, Royal College of Surgeons in Ireland, St. Stephen's Green, Dublin 2, Ireland

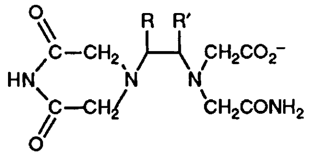
The copper(II) complexes of the diacid diamide $^-O_2CCH_2(H_2NOCCH_2)NCH_2CHRN(CH_2CONH_2)CH_2CO_2^-$ (R = H, L^3; Me, L^4) which have in-plane $2N_{amine}$, $2\ O_{carboxylate}$ ligands at pH 6 undergo amide group deprotonation in basic solution to give $CuN(amine)_2N^-(amide)_2$ chromophores resulting from the displacement of the in-plane carboxylate ligands by the superior σ-donating, deprotonated, amide nitrogens. The concentration pK_a values for the deprotonations at 25 °C, I = 0.1 mol dm^{-3} are 9.19 ± 0.02 and 9.87 ± 0.02 for [CuL^3], and 8.60 ± 0.02 and 9.81 ± 0.02 for [CuL^4]. The unexpectedly large differences in the first pK_a values is attributed to a steric interaction between the methyl substituent and an axially co-ordinated CH_2CONH_2 group, in the complex of L^4 which is relieved as a result of the first ionisation step.

A number of bis(3,5-dioxopiperazin-1-yl)alkanes have in the last few decades been investigated with some success as antitumour agents and the propane derivative L^1 (R = Me) has been marketed for such use as the antitumour drug Razoxane.[1,2] Initially the design of these drugs was based on the rationale that they are sufficiently lipophilic to penetrate intracellular sites, where they undergo hydrolytic metabolism of either one or both of the imide groups producing powerful chelating agents which interfere with metalloenzymes essential for tumour cell growth.

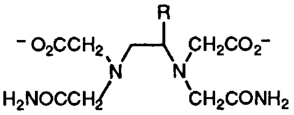
We have previously investigated the hydrolysis of Razoxane *in vitro*.[3] While this compound undergoes slow base-catalysed hydrolysis (k_2 = 1.8 dm^3 mol^{-1} min^{-1}, 25 °C, I = 0.3 mol dm^{-3}, 25 °C), the reaction is markedly enhanced in the presence of copper(II) (*e.g.* at 0.053 mol dm^{-3} metal ion concentration k_2 = 4 × 10^8 dm^3 mol^{-1} min^{-1} under the same reaction conditions) and the product contains the monohydrolysed one-ring-open ligand product L^2. Bis(hydrolysed) derivatives of a number of these diimides, *e.g.* L^3 and L^4 have been isolated and the stability constants of their complexes with a number of metal ions reported.[4,5] In this paper we report the synthesis and properties of copper(II) complexes of the hydrolysis products L^3 and L^4 and the results of potentiometric and spectrophotometric investigations of the reactions of these complexes with base.



L^1



L^2 R = H, R′ = Me or R = Me, R′ = H



L^3 R = H
L^4 R = Me

Experimental

Preparation of Complexes.—[CuL^3]·HCl·H_2O. The diacid diamide of ethylenediaminetetraacetate (edta) *i.e.* L^3 (0.25 g, 0.87 mmol) was suspended in methanol (20 cm^3) and heated to 65 °C whilst stirring for 10 min. An aqueous solution (8 cm^3) of $CuCl_2$·2H_2O (0.3 g, 1.79 mmol) was added dropwise to the methanol suspension whereupon a blue solution immediately formed. After cooling in a refrigerator overnight a green crystalline material was deposited. This was collected by suction filtration, washed with methanol then diethyl ether and finally air dried. Yield 0.27 g, 78% (Found: C, 29.9; H, 4.5; N, 13.9. $C_{10}H_{17}ClN_4O_6$·H_2O requires C, 29.6; H, 4.7; N, 13.8%). Main IR bands (Nujol mull) at 3450, 3340, 3170, 1706, 1675 (sh), 1652 and 1560 cm^{-1}.

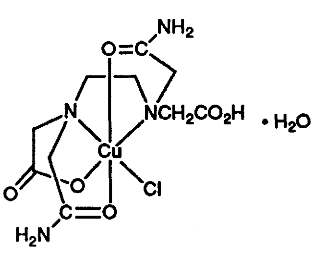
[CuL^3]·3.5H_2O. A stirring aqueous suspension (20 cm^3) of L^3 (0.25 g, 0.87 mmol) was treated with triethylamine (0.25 cm^3, 1.79 mmol) whereupon a colourless solution formed. This was treated dropwise with a solution of $CuCl_2$·2H_2O (0.15 g, 0.86 mmol) in methanol (10 cm^3), with stirring. The resulting blue solution was stored in a refrigerator and after about 1 week a blue crystalline compound was obtained. This was collected by suction filtration, then air dried and stored in a vacuum desiccator over P_2O_5. Yield 0.31 g, 87% (Found: C, 28.8; H, 5.4; N, 13.5. $C_{10}H_{16}N_4O_6$·3.5H_2O requires C, 28.9; H, 5.6; N, 13.5%). Main IR bands (Nujol mull) at 3450, 3360, 3300, 3160, 1675 and 1570 (br) cm^{-1}.

[CuL^4]·2H_2O. This compound was obtained from D,L-1,2-bis(3,5-dioxopiperazin-1-yl)propane, L^1 (R = Me) by a literature method.[5] Yield 5.7 g, 58% (Found: C, 32.4; H, 5.4; Cu, 15.5; N, 13.7. $C_{11}H_{18}CuN_4O_6$·2H_2O requires C, 32.9; H, 5.5; Cu, 15.8; N, 13.9%). Main IR bands (KBr disc) at 3460, 3400, 3300, 3100, 1680 and 1625 cm^{-1}.

Instrumentation.—Potentiometric studies were carried out on a Mettler DL25 Automatic Titrator fitted with a Mettler DG III combined electrode. The pH measuring system was calibrated using pH 4 and 7 buffers. The double-jacketted reaction vessel was thermostatted at 25.0 °C by water circulating from a constant-temperature water-bath. In the potentiometric studies

Table 1 Visible spectra of copper(II) complexes with edta and related ligands in aqueous solution at 25.0 °C, $I = 0.1$ mol dm^{-3} $NaClO_4$

Complex	pH	λ_{max}/nm	ε/dm^3 mol^{-1} cm^{-1}
$[CuL^3]\cdot 3.5H_2O$	0.8	765	40.9
	6.0	740	50.1
	>11.6	636	117
$[CuL^4]\cdot 2H_2O$	0.8	774	45.6
	6.0	735	65.5
	>11.5	631	126
$[Cu(edtam)]^{2+}$	<6	774	45.2
	10.3	639.2	118
$[Cu(edta)]^{2-}$	>4.3	730	87.3
	2.7	739	50.1
	<1.7	754	34.9



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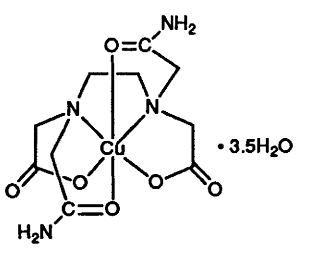

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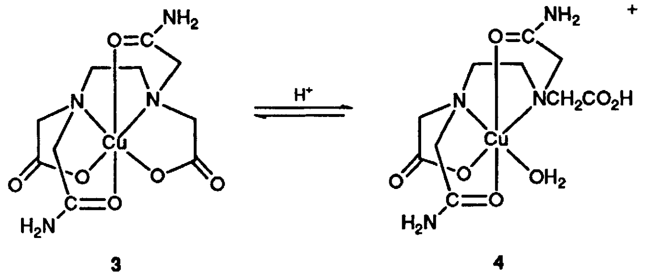

3 4

Scheme 1

an aqueous solution (25.0 cm^3) of the copper(II) complex, 1.87×10^{-3} mol dm^{-3} in the case of $[CuL^3]\cdot 3.5H_2O$ and 3.58×10^{-3} mol dm^{-3} in the case of $[CuL^4]\cdot 2H_2O$, which also contained 0.1 mol dm^{-3} $NaClO_4$ was titrated against standard 0.1 mol dm^{-3} NaOH. In order to obtain values of p[H] from the pH meter readings, p(H), so that concentration pK_a values could be calculated, a standard 0.1 mol dm^{-3} acid solution adjusted to an ionic strength of 0.1 mol dm^{-3} by the addition of $NaClO_4$ was titrated with standard 0.1 mol dm^{-3} NaOH at 25 °C and p(H) plotted against p[H].[6] In the pH range 2–12 the linear relationship (1) was found to hold. The potentiometric

$$\mathrm{p(H)} = 0.970\,\mathrm{p[H]} + 0.126 \qquad (1)$$

data for the complexes were analysed using the SUPERQUAD computer program.[7]

Visible spectra were recorded on a Philips 8730 UV/Vis scanning spectrophotometer and infrared spectra on a Philips PU 9714 spectrophotometer.

Results and Discussion

Three solid copper(II) complexes of hydrolysed products of bis(3,5-dioxopiperazin-1-yl)alkanes have been synthesised. One of these *i.e.* $[CuL^4]\cdot 2H_2O$ had previously been isolated and its crystal structure determined.[5,8] The structure consists of discrete molecules of the complex with the metal in a tetragonally distorted octahedral environment comprised of four short bonds, M–N 2.005 and 2.036 Å, M–O (carboxylate) 1.954 and 1.972 Å and two long bonds, M–O (amide) 2.298 and 2.387 Å. Two complexes of the diacid diamide of edta *i.e.* (L^3) were isolated under conditions of different basicity. Hence the addition of the diacid diamide to copper(II) chloride in methanol led to the isolation of the complex $[CuL^3]\cdot HCl\cdot H_2O$. The IR spectrum of this complex has a distinct C=O stretching band at 1706 cm^{-1} which is not present in the spectrum of either $[CuL^4]\cdot 2H_2O$ or $[CuL^3]\cdot 3.5H_2O$ and which is indicative of an un-ionised, unco-ordinated, carboxylic acid group.[9,10] When the preparation was carried out in the presence of the sterically hindered base triethylamine the complex $[CuL^3]\cdot 3.5H_2O$ was obtained. The infrared spectrum of this complex in the carbonyl stretching region is similar to that of $[CuL^4]\cdot 2H_2O$ with ν(C=O) bands at 1675 and 1570 cm^{-1} due to the co-ordinated amide and carboxylate groups respectively.[9,10] The likely solid-state structures for the complexes of (L^3) are represented in **1** and **2**.

The visible spectra of the complexes in aqueous solution each consists of a broad band with pH-dependent λ_{max} values. These values with their respective molar absorption coefficients are shown in Table 1 and are compared with the corresponding values for the related copper(II) ethylenediaminetetraacetamide (edtam) and edta complexes. The spectral changes for solutions of $[CuL^3]\cdot 3.5H_2O$ and $[CuL^4]\cdot 2H_2O$ between pH 6.0 and 0.8 correspond to protonation and decomplexation of a carboxylate ligand as shown in Scheme 1 for $[CuL^3]$. A potentiometric titration of $[CuL^3]\cdot HCl\cdot H_2O$ which has structure **4** in aqueous solution against 0.1 mol dm^{-3} NaOH gave a pK_a^c value* for ionisation of the carboxylic acid group as 1.70 ± 0.10 at 25.0 °C, $I = 0.1$ mol dm^{-3} $NaClO_4$.

The complexes behave as dibasic acids in the pH range 7–11, giving upon the addition of base intensely coloured blue solutions. These deprotonation reactions were monitored both potentiometrically and spectrophotometrically at 25.0 °C, and ionic strength 0.1 mol dm^{-3} ($NaClO_4$). For $[CuL^3]$ the values obtained for pK_a* were pK_{a1}^c = 9.19 ± 0.02 and pK_{a2}^c = 9.87 ± 0.02 and for $[CuL^4]$, pK_{a1}^c = 8.60 ± 0.02 and pK_{a2}^c = 9.81 ± 0.02. The equilibria involved in the ionisation steps are shown in Scheme 2 and the species distribution curves for the complexes and their conjugate bases are presented in Fig. 1(*a*) and (*b*). The values of the ionisation constants as well as the hypsochromic shifts of ≈100 nm obtained in the visible spectra during deprotonation, Fig. 2(*a*) and (*b*), are consistent with amide group deprotonation and concomitant equatorial co-ordination of the deprotonated amide nitrogens to the metal ion.[11–13] These observations are consistent with the fact that no copper(II) complexes with axially co-ordinated deprotonated amide ligands have yet been reported even in situations where this is geometrically favoured.[14] The displacement of the carboxylate groups by the deprotonated amide groups from the equatorial positions and the shifts in λ_{max} are consistent with a previous report that a deprotonated amide (peptide) nitrogen is a stronger σ donor and is higher in the spectrochemical series than carboxylate.[15]

Similar wavelength shifts are observed as a result of peptide group deprotonation and complexation in copper(II) peptide and related complexes.[11–13] Amide group deprotonation and co-ordination in the copper(II) complex of *N,N′*-bis(β-carbamoylethyl)ethylenediamine, $[Cu(bcen)]^{2+}$, gives rise to shifts in λ_{max} from 645 to 580 nm in the production of $[Cu(bcen)H_{-1}]^+$ and from 580 to 535 nm in the production of $[Cu(bcen)H_{-2}]$.[12] The pK_a values for these ionisations are reported as 8.12 and 9.45 at 25 °C, $I = 0.2$ mol dm^{-3} $NaNO_3$. Although the λ_{max} are longer in the case of $[CuL^3]$ and $[CuL^4]$ the shifts on deprotonation of the amide groups are comparable. The different positions of λ_{max} are probably due to stronger

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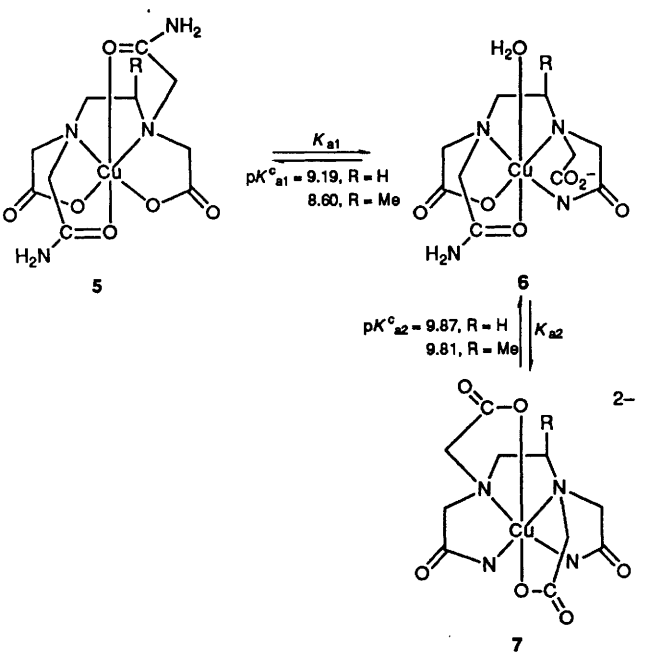
* The pK_a^c values given are concentration pK_a values based on hydrogen-ion concentrations calculated from the pH meter readings using equation (1).



Scheme 2

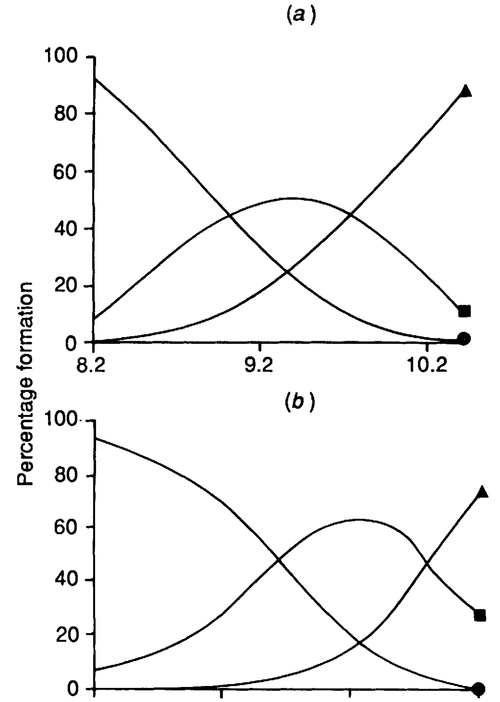


Fig. 1 Species distribution curves for (*a*) [CuL^3] and (*b*) [CuL^4] and their conjugate bases at 25.0 °C, I = 0.1 mol dm^{-3} NaClO$_4$. Species: [CuL] (●), [CuLH$_{-1}$] (■) and [CuLH$_{-2}$] (▲), where L = L^3 (*a*) or L^4 (*b*)

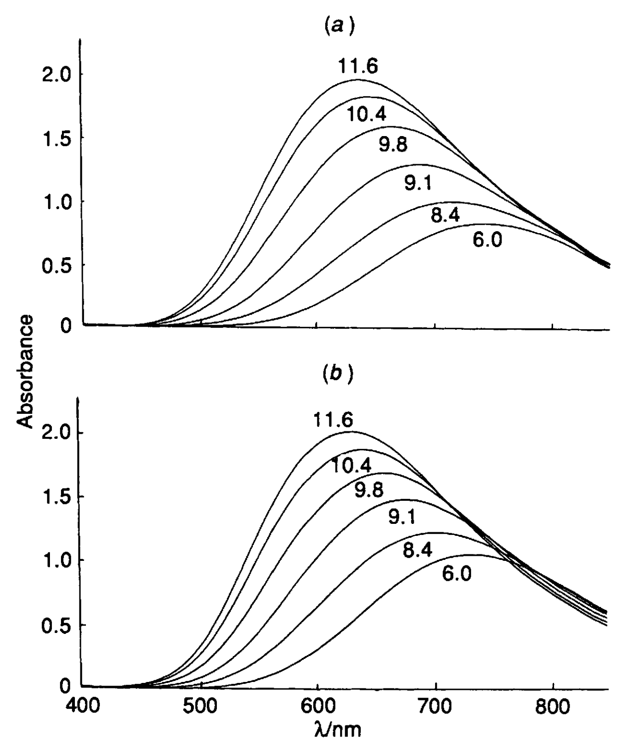
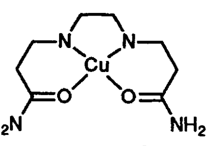


Fig. 2 Spectra of 0.017 mol dm^{-3} (*a*) [CuL^3] and (*b*) [CuL^4] at 25.0 °C, I = 0.1 mol dm^{-3} NaClO$_4$ at the pH values indicated



[Cu(bcen)]$^{2+}$

axial co-ordination by the amide groups in [CuL^3] and [CuL^4] than by aqua ligands in solutions of [Cu(bcen)]$^{2+}$. Increased axial co-ordination has previously been shown to produce red shifts in the visible spectra of six-co-ordinate complexes of copper(II).*^{,15,16} The different tetragonalities of [CuL^3] and [CuL^4] relative to [Cu(bcen)]$^{2+}$ are likely to originate from the fact that while the equatorial N atoms in the first two complexes are tertiary those in the last are secondary. The effects of type of ligand, co-ordination number and geometry on the electronic spectra of copper(II) complexes have been thoroughly reviewed.[17] The fact that the pK_a values for the [Cu(bcen)]$^{2+}$ system are lower than those for [CuL^3] and [CuL^4] may be explained by the higher positive charges (+2 and +1) on the conjugate acids of the former system as opposed to the latter (neutral and −1). The positions of λ_{max} before and after amide group deprotonation in [Cu(edtam)]$^{2+}$, a CuN$_2$O(amide)$_4$ chromophore which changes to CuN$_2$N$^-$(amide)$_2$O(amide)$_2$ on ionisation, Table 1,[18] are very similar to those reported for [CuL^3] and [CuL^4] in this paper.

Since the pK_{a2} values of [CuL^3] and [CuL^4] are almost identical we assume that this ionisation step involves very similar acidic sites and conclude that in [CuL^4] it refers to the amide group remote from the methyl substituent, Scheme 2. The difference of 0.59 in the pK_{a1} values is due to the methyl substituent and is more likely to have its origin in steric rather than electronic effects. In structure **5** there is steric interference between the methyl substituent and the methylene group of the adjacent co-ordinated CH$_2$CONH$_2$. Deprotonation of this amide group gives a conjugate base species **6** in which it occupies an in-plane position having displaced a carboxylate group, with a water molecule axially co-ordinated and the displaced carboxylate either unco-ordinated as shown, or axially co-ordinated *trans* to the H$_2$O ligand (this occurs if it displaces the un-ionised amide ligand, a situation which might be expected in view of it being favourably positioned to do so as well as it being a superior σ donor). The ensuing relief of steric strain in [CuL^4] as a result of this ionisation may account for its enhanced acidity relative to [CuL^3]. The existence of steric hindrance in [CuL^4] is confirmed by the crystal structure of

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* We are grateful to both referees for pointing this out.

$[CuL^4]\cdot2H_2O$ which shows that the methyl group and the adjacent CH_2CONH_2 are on the same side of the CuN_2O_2 plane and interact. The pK_{a2} values of the complexes are almost identical since the methyl group in **6** is unencumbered as it is in the conjugate base species **7**, in which the methylene group of the axially co-ordinated $CH_2CO_2^-$ which is nearer to the methyl substituent is on the opposite side of the CuN_2O_2 plane from it. That species **7** contains axial carboxylate ligands is to be expected in view of its superior σ-donor ability relative to H_2O, an expectation previously substantiated in copper(II) peptide complexes with carboxylate side chains axially co-ordinated.[19] The greater separation of pK_a values in $[CuL^4]$ ($\Delta pK_a = 1.21$) compared to $[CuL^3]$ ($\Delta pK_a = 0.68$) results in the appearance of two distinct isosbestic points in the spectra of the former complex, one at 790 nm corresponding to the first ionisation step, the other at 715 nm corresponding to the second.

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