Copper(II) complexes of aminolysis products of the anticancer bis(3,5-dioxopiperazin-l-yl)alkanes in aqueous solution

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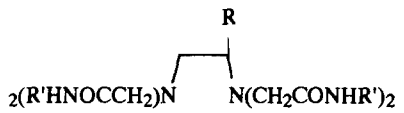
(Received October 13, 1992)

Abstract

The behaviour in aqueous solution of the copper(II) complexes of tetraamides of 1,2-diaminoethane-*N,N,N',N'*-tetraethanoic acid (I, R=H, R'=H or Me) and 1,2-diaminopropane-*N,N,N',N'*-tetraethanoic acid (I, R=Me, R'=H or Me) is described. Potentiometric and spectrophotometric titrations confirm that on the addition of base ionization of the equatorially coordinated amide groups occurs. The concentration pK_a values for the copper(II) complexes of ligands **I** at 25 °C, I=0.1 mol dm^{-3} $NaClO_4$ are 6.33±0.02 and 7.88±0.02 for R=R'=H; 6.78±0.02 and 8.66±0.02 for R=H, R'=Me; 6.33±0.02 and 7.83±0.02 for R=Me, R'=H; 6.76±0.02 and 8.56±0.02 for R=R'=Me. The ionization processes are accompanied by hypsochromic shifts of ~130 nm in the electronic spectra characteristic of two amide group ionizations with a change from O to N$^-$ coordination. In contrast to the corresponding copper(II) diacid diamide complexes in which the pK_a values of axially coordinated amide groups are lowered because of steric interaction between the methyl substituent, R, and the methylene group attached to the axial amide, in the tetraamide complexes where the ionization processes involve equatorially coordinated amide groups the methyl substituent R has little or no effect on pK_a values.

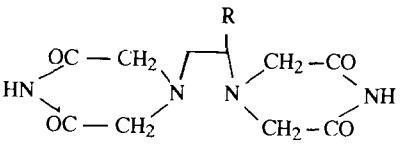
Introduction



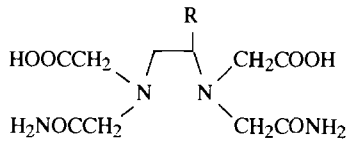
R = H or Me; R' = H or Me.

I

In recent publications we have described the chemistry of copper(II) complexes of bis(3,5-dioxopiperazin-l-yl)alkanes (**II**) and their hydrolysis products [1, 2]. These compounds are anticancer drugs of which Razoxane (**II**, R=Me) is the most widely used and which were originally designed on the rationale that they are sufficiently lipophilic to penetrate intracellular sites where they undergo hydrolytic metabolism to products which deactivate metalloenzymes necessary for tumour cell growth [3]. We have established that (i) the hydrolysis of Razoxane (**II**, R=Me) is accelerated greatly in the presence of copper(II); (ii) in the copper(II) complexes of the bis(acid-amide) hydrolysis products **III**, in-plane coordination through the amino and carboxylate groups occurs at low pH but the carboxylate groups are displaced by deprotonated amide groups at high pH; (iii) in the copper(II) complex of **III** (R=Me) there is steric hindrance between the methyl substituent and the methylene group of the adjacent axially coordinated ethanamide substituent which is relieved on ionization of the amide group and its concomitant movement to an in-plane position. This is reflected in a lower pK_a for this complex compared to the complex of the unsubstituted (R=H) hydrolysis product in which there is no such steric hindrance.



II



III

In this paper we report the behaviour in aqueous solution of copper(II) complexes of the amides (**I**) which we have found in the course of our studies are aminolysis products of the diimides (**II**).

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0020-1693/93/$6.00

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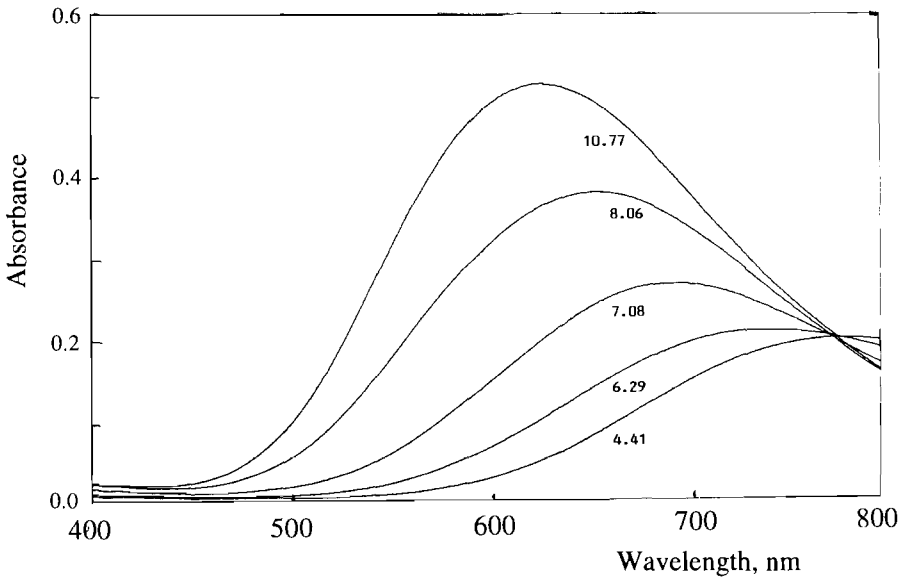


Fig. 1. Visible spectra of aqueous solutions of Cu–(**I**)$^{2+}$ (R = Me, R′ = H) at 25.0 °C, I = 0.1 mol dm^{-3} $NaClO_4$ and at the pH values indicated.

TABLE 1. Visible spectra in aqueous solution (0.1 M $NaClO_4$) for copper(II)–tetraamide complexes (**V**)

Complex	pH	λ_{max} (nm)	ϵ (dm^3 mol^{-1} cm^{-1})
R = R′ = H	4.57	765	31
	10.96	636	103
R = H, R′ = Me	4.07	773	47
	10.24	642	130
R = Me, R′ = H	4.41	760	39
	10.77	629	108
R = R′ = Me	4.66	760	44
	10.46	629	135

Experimental

Synthesis

1,2-Diaminoethane-N,N,N′,N′-tetraethanamide (I, R = R′ = H)

This was synthesized from the tetraacid via the tetramethyl ester as previously described [4]. *Anal.* Found: C, 41.7; H, 7.0; N, 28.9. Calc. for $C_{10}H_{20}N_6O_4$: C, 41.7; H, 7.0; N, 29.1%.

1,2-Diaminoethane-N,N,N′,N′-tetra-N″-methylethanamide (I, R = H, R′ = Me)

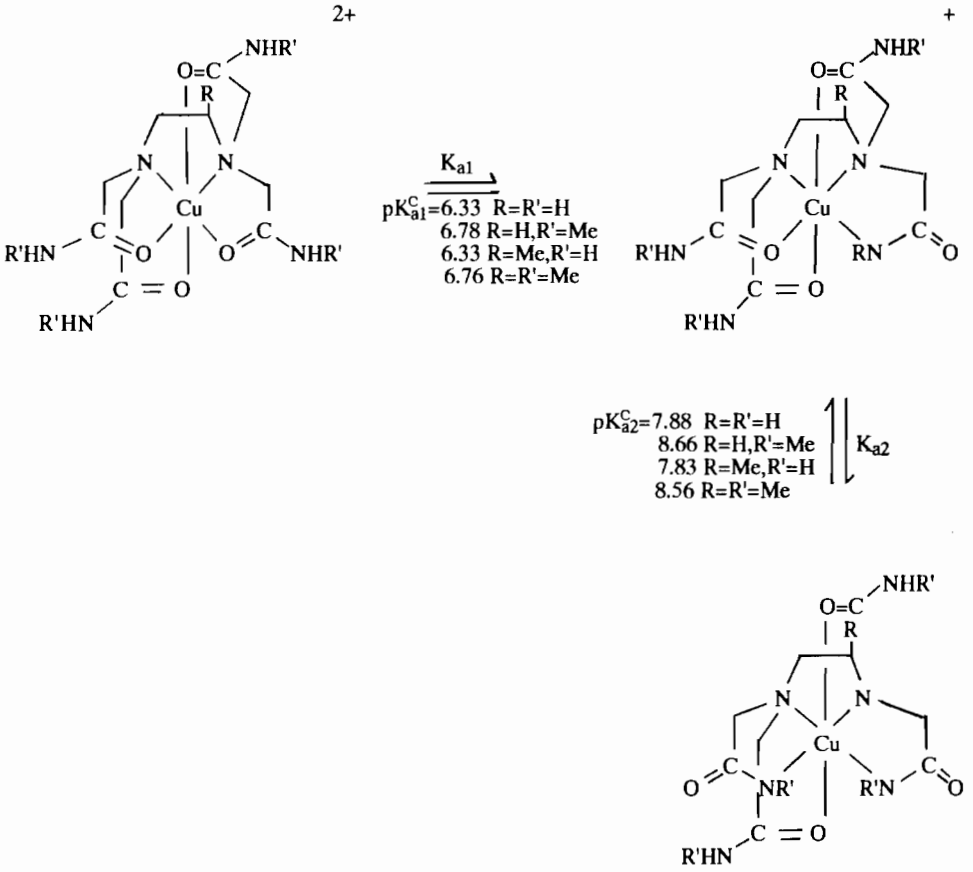
To a solution of 1,2-diaminoethane-*N*,*N*,*N*′,*N*′-tetramethylethanoate (3 g, 8.6 mmol) in dry methanol (25 cm^3) was added dropwise a 30% solution of methylamine in ethanol (17 cm^3, 164 mmol). The resulting solution was stirred for 1 h at room temperature, then refluxed for 3 h and finally the solvent removed under reduced pressure, leaving a solid residue. The residue was suspended in acetone at room temperature, filtered and dried. *Anal.* Found: C, 48.3; H, 8.4; N, 24.1. Calc. for $C_{14}H_{28}N_6O_4$: C, 48.8; H, 8.1; N, 24.4%. m.p. 215–217 °C. ^{1}H NMR in D_2O (ppm): 3.23 s (8H, NCH_2CON); 2.63 (4H, NCH_2CH_2N); 2.73 s (12H, $CONCH_3$). IR spectrum, KBr disc: ν(NH) 3350, 3100 cm^{-1}, ν(CO) 1650, $\delta(NH_2)$ 1550 cm^{-1}.

1,2-Diaminopropane-N,N,N′,N′-tetraethanamide (I, R = Me, R′ = H)

A stirred suspension of bis(3,5-dioxopiperazin-l-yl)propane (**II**, R = Me) (1 g, 3.7 mmol) in dry methanol (90 cm^3) was cooled in an ice bath and ammonia gas bubbled through the suspension. After about 10 min a colourless solution formed and this was stirred at room temperature. After about 1 h the amide precipitated from solution. The precipitate was filtered, recrystallized for a minimum volume of hot water and dried in an oven for 3 h. *Anal.* Found: C, 43.7; H, 7.3; N, 28.2. Calc. for $C_{11}H_{22}N_6O_4$: C, 43.7; H, 7.3; N, 27.8%. m.p. 132–135 °C. ^{1}H NMR in D_2O (ppm): 1–1.12 d (3H, CH_3); 3.24 s (4H, $-CH_2CON-$ near central CH_3); 3.30 s (4H, $-CH_2CON-$ remote from central CH_3); 2.5–3.1 broad (N–CH_2CH–N). IR spectrum, KBr disc: ν(NH) 3420, 3380, 3300, 3160 cm^{-1}, ν(CO) 1665, $\delta(NH_2)$ 1615 cm^{-1}.

1,2-Diaminopropane-N,N,N′,N′-tetra-N″-methylethanamide (I, R = R′ = Me)

A stirred suspension of bis(3,5-dioxopiperazin-l-yl)propane (1 g, 3.7 mmol) in dry methanol (90 cm^3) was treated dropwise with a 30% methylamine solution in ethanol (8 cm^3, 77 mmol). The suspension was stirred



Scheme 1.

for 1 h and a yellow solution formed. This was refluxed for 3 h, cooled and the solvent removed under reduced pressure. The oily residue was triturated with acetone (25 cm^3), the white product collected by filtration and air dried. *Anal.* Found: C, 50.3; H, 8.4; N, 23.7. Calc. for $C_{15}H_{30}N_6O_4$: C, 50.3; H, 8.4; N, 23.5%. m.p. 195–197 °C. ^{1}H NMR in D_2O (ppm): 1–1.08 d (3H, C–CH_3); 2.80 s (12H, –$CONCH_3$); 3.20 s (4H, NCH_2CON near central CH_3); 3.26 s (4H, NCH_2CON remote from central CH_3). IR spectrum, KBr disc: ν(NH) 3450, 3300, 3220, 3100 cm^{-1}, ν(CO) $\delta(NH_2)$ 1560 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 an aqueous solution (25.0 cm^3) of the copper(II) complex 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 0.01 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]. The potentiometric data for the complexes were analyzed using the SUPERQUAD computer program [5]. Visible spectra were recorded on a Philips 8730 UV–Vis scanning spectrophotometer, IR spectra on a Philips PU 9714 spectrophotometer and ^{1}H NMR spectra on a Jeol PMX 60 spectrometer.

Results and discussion

The amides of EDTA (**I**, R = H; R′ = H, Me) used in this study were obtained by treating the tetramethylester (from EDTA tetraacid and thionyl chloride in methanol) with NH_3 or methylamine. The amides of 1,2-diaminopropane-*N*,*N*,*N*′,*N*′-tetraethanoic acid (**III**, R = Me, R′ = H or Me) which have not previously been reported were obtained by treating a suspension of the diimide **I** (R = Me) in methanol with ammonia or methylamine. Attack by ammonia on the imide carbonyl group causes ring opening and leads directly to the tetraamide. Attack by methylamine or the imide

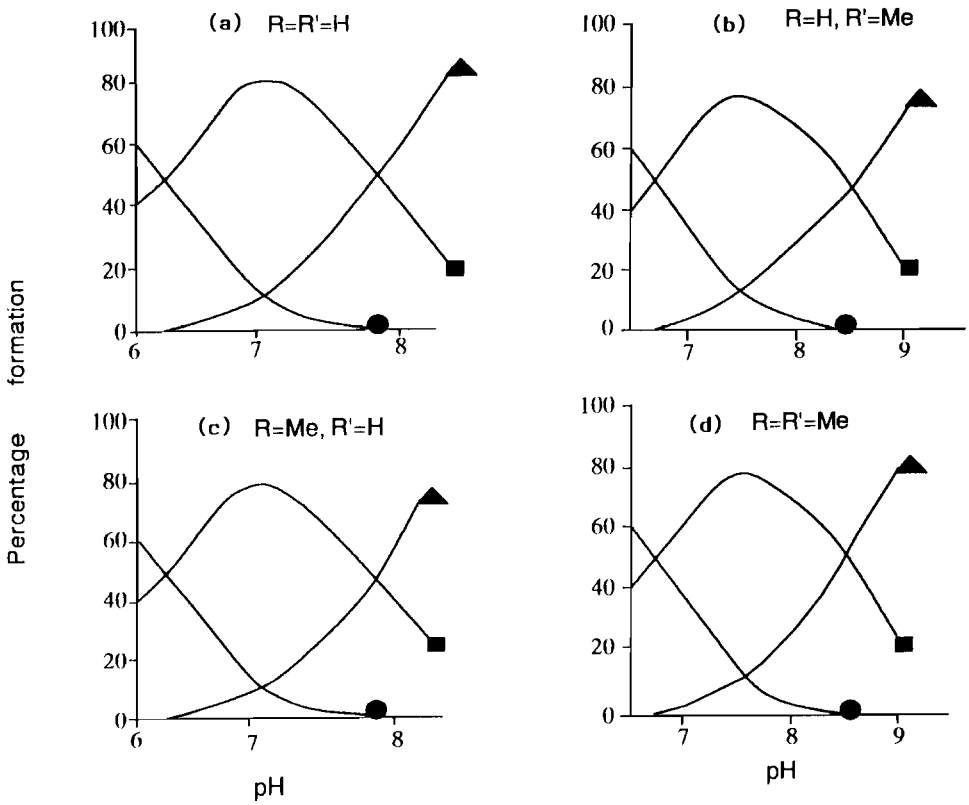
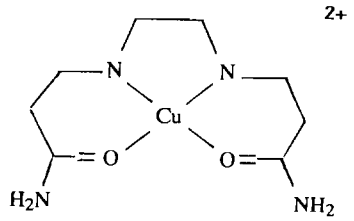


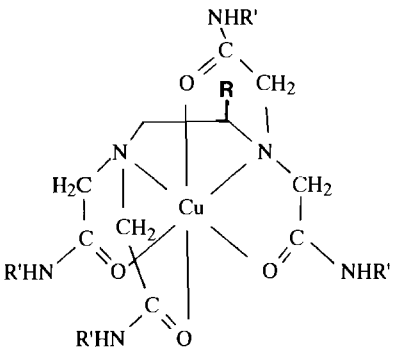
Fig. 2. Species distribution curves for Cu-(**I**)$^{2+}$ (●), and the conjugate bases Cu-(**I**)H_{-1}^{+} (■), Cu-(**I**)H_{-2} (▲), at 25.0 °C, I=0.1 mol dm^{-3} $NaClO_4$.

carbonyl group however should lead initially to a mixed amide, i.e. $-N(CH_2CONH_2)CH_2CONHMe$ but this evidently undergoes amide exchange in the presence of a vast excess of methylamine under reflux conditions to give the observed product, i.e. $-N(CH_2CONHMe)_2$. The amides all gave satisfactory microanalytical results and ^1H NMR and IR spectroscopic data.

Aqueous solutions containing equimolar amounts of Cu(II) and the tetraamide ligands contain a broad absorption band at 760–775 nm. This band is some 100 nm at longer wavelength than that for aqueous solutions of the copper(II) complex of *N,N'*-bis(β-carbamoylethyl)ethylenediamine (**IV**) [6] and suggests that in the tetraamide complexes amide groups are axially coordinated to the metal (**V**).



IV



V

Increased axial coordination has been shown to red shift the absorption band in the visible spectra of complexes of copper(II) [7]. Similar axial amide group coordination has previously been suggested for copper(II) complexes of the diacid diamide ligands (**III**) [2]. The effects of ligand, coordination number and geometry on the electronic spectra of copper(II) complexes have been thoroughly reviewed [8].

In a potentiometric titration with 0.1 mol dm^{-3} NaOH a solution containing equimolar amounts (2.0×10^{-3} mol dm^{-3}) of copper(II) chloride and the amide ligand at ionic strength 0.1 mol dm^{-3} $NaClO_4$ consumed two

moles of base per mole of complex in the pH range 5–10. This corresponds to the deprotonation of the two equatorially coordinated amide groups in each complex. Evidence for this is obtained from the visible spectra of the solutions in which hypsochromic shifts of ~130 nm occur on gradually raising the pH from 5 to 10, Fig. 1 and Table 1. This is typical of the shifts observed in copper(II) peptide and related complexes when two amide groups equatorially coordinated to copper undergo deprotonation with concomitant coordination of deprotonated amide groups to the metal [9].

The pK_a values for the ionizations were obtained from the potentiometric data using the SUPERQUAD program. The pK_a values and the ionization processes to which they correspond are shown in Scheme 1, and the species distribution curves for the complexes and their conjugate bases in Fig. 2(a)–(d). The ionization steps are attributed to the equatorial amide groups since on the basis of the Jahn–Teller effect these are coordinated more strongly to copper(II) than the axial groups [9]. Additionally no copper(II) complexes containing axially coordinated deprotonated amide ligands have yet been reported even in situations where this was geometrically favourable [10].

The pK_{a1} values of the *N*-methyl amides (R' = Me) are about 0.4 units higher than those of the corresponding unsubstituted amides (R' = H), the decreased acidity resulting from the introduction of the electron releasing methyl substituents into the groups undergoing ionization. The pK_{a2} values follow a similar pattern although the difference, i.e. ~0.7 units is more pronounced. The pK_a values for both ionization steps are largely independent of whether the substituent R on the central carbon is H or CH_3. This is in contrast to our results for the diacid diamide complexes (**III**) in which we observed large differences between the pK_{a1} values for the R = H and R = CH_3 complexes. This we attributed to steric interaction between the methyl substituent R and the methylene group of the axially coordinated amide undergoing ionization. Since this ionization step is accompanied by movement of the deprotonated amide group into the equatorial plane the process results in relief of strain and consequently a reduction in pK_{a1}. In the system presently being described it is the equatorial and not the axial amide groups which are undergoing ionization and steric effects such as those described are not relevant. The pK_a values reported herein for the tetraamide **I**, R = R' = H, i.e. $pK_a = 6.33 \pm 0.02$ and 7.88 ± 0.02 ($pK_a = 6.47 \pm 0.02$ and 8.01 ± 0.02) agree well with those previously reported for this amide [4].

In the complexes of the unsymmetrical ligands in which R = Me assignment of the ionization steps was made by comparison with the corresponding diacid diamide complex (**III**) also a dibasic acid in which the lower of the two pK_a values was attributed with justification to the amide group closer to R.

Acknowledgements

We thank the Research Committee of the Royal College of Surgeons in Ireland and the Irish Science and Technology Agency (EOLAS) for financial support, Professor D. A. Brown, Department of Chemistry, University College Dublin for useful discussions and the Microanalysis Laboratory at the same address for carrying out analytical work.

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