Acetazolamide Binding to Zinc(II), Cobalt(II) and Copper(II) Model Complexes of Carbonic Anhydrase

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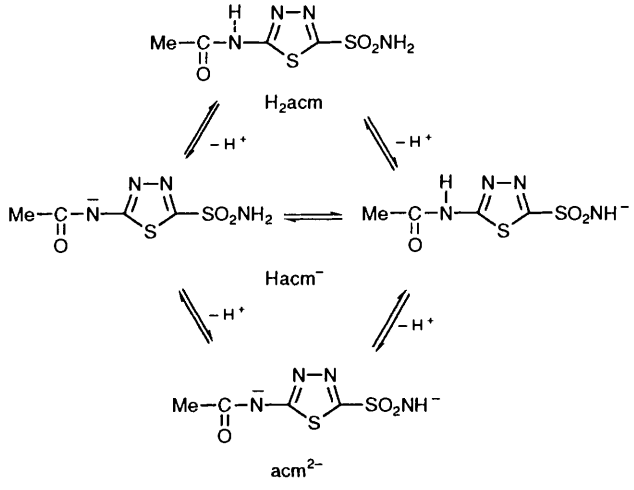
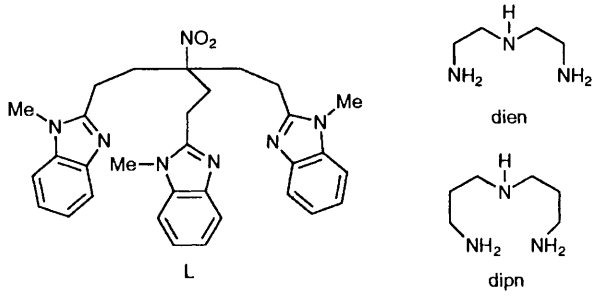
New zinc(II), cobalt(II) and copper(II) complexes with tris[2-(1-methylbenzimidazol-2-yl)ethyl]-nitromethane (L) have been prepared. The tris(benzimidazole) donors provided by the ligand simulate the environment of the zinc(II) site of carbonic anhydrase. The structural properties of the complexes deduced on the basis of their spectroscopic characteristics correlate with those of the metal derivatives of the enzyme, at least in the solid state, while the alkyl chains connecting the donor groups of L allow a more flexible co-ordination environment of the metal sites in solution, with the adoption of five-co-ordinate structures. The complexes bind the inhibitor acetazolamide {*N*-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]acetamide} in its monoanionic form through the deprotonated sulfonamide group and these ternary complexes model the carbonic anhydrase–acetazolamide adduct recently characterized by X-ray crystallography. In the case of copper(II) it was impossible to isolate the pure ternary complex, probably because of the ease of formation of a dinuclear Cu^{II}–L–OH^- complex under the reaction conditions, but the related ternary complexes with acetazolamide and diethylene- or dipropylene-triamine were readily obtained.

Carbonic anhydrase (CA) is the enzyme which catalyses the biological interconversion between CO_2 and HCO_3^-. The zinc(II) ion at the active site of the enzyme is bound to three imidazole groups and an apical water molecule, with an approximately tetrahedral geometry.[1] Most of the strong CA inhibitors are anions, *e.g.*. SCN^-, CN^- and I^-. On the other hand, neutral sulfonamides are even stronger inhibitors; among them *N*-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]acetamide (acetazolamide, H_2acm) (Scheme 1) is the most prominent.[2] These compounds have played an important role in physicochemical and enzymatic studies on CA. Moreover, it is known that among the metallocarbonic anhydrases only the zinc(II) and cobalt(II) enzymes are active, and that the binding of sulfonamides is metal dependent.[3] On the basis of an X-ray crystallographic study of the CA–acetazolamide complex at 1.9 Å resolution[4] it is accepted that the inhibitor acts as a monodentate ligand in the hydrophobic pocket of the CA active site.

Although the ligation of the zinc ion in the enzyme active centre has been modelled by tridentate ligands with nitrogen donors,[5–8] only a few model sulfonamide–metal complexes have been reported.[9–12] The paucity of appropriate chemical models for the enzyme–inhibitor complexes led us to initiate an investigation of the binding of acetazolamide to model complexes with ligands which can be considered as mimics of the CA active site. In this paper we report the zinc(II), copper(II) and cobalt(II) complexes of the tridentate ligand tris[2-(1-methylbenzimidazol-2-yl)ethyl]nitromethane (L), together with the adducts they form with acetazolamide. In the case of Cu^{II} the mixed-ligand complexes with diethylenetriamine (dien) or dipropylenetriamine (dipn) and acetazolamide were also obtained.



Scheme 1 Proton-dissociation equilibria undergone by the CA inhibitor acetazolamide



Experimental

General Methods.—All reagents were of the highest grade commercially available and used without further purification. The compound L was prepared as reported elsewhere.[13] Elemental analyses were by the microanalytical laboratory of the University of Milano. The IR spectra were obtained on a Mattson 5000 FTIR spectrometer. Solid-state EPR spectra were recorded on a Bruker ER 200D spectrometer operating at X-band frequencies, spectra in frozen solutions at 123 K using a Varian E-109 spectrometer at X-band frequencies and a V-4000 variable-temperature control apparatus. Diffuse reflectance

spectra were obtained on a Perkin-Elmer Lambda 15 spectrophotometer, ^{1}H NMR spectra on a Bruker AC-200 spectrometer operating at 200 MHz and electronic spectra in solution on a Hewlett-Packard 8452A diode-array spectrophotometer. Conductivity measurements were performed on an Amel model 133 conductimeter.

$[ML(OH_2)][ClO_4]_2$ (M = Zn, Cu *or* Co).—These complexes were prepared by adding the stoichiometric amount of the metal(II) perchlorate hexahydrate salt to an ethanolic solution of L. The precipitate thus formed was filtered off, washed with small amounts of ethanol, and dried under vacuum.

$[ZnL(OH_2)][ClO_4]_2$ (yield 43%) (Found: C, 46.7; H, 4.65; N, 11.9. $C_{31}H_{35}Cl_2N_7O_{11}Zn$ requires C, 45.6; H, 4.3; N, 12.0%): $\tilde{\nu}_{max}/cm^{-1}$ 1618m and 1491m [ν(ring)], 1543s [$\nu_{asym}(NO_2)$], 1340m [$\nu_{sym}(NO_2)$], 752s [δ(CH)], 1100s and 623s [ν(ClO_4)] (Nujol mull); $\delta_H[(CD_3)_2SO]$ 2.7–2.9 and 2.9–3.1 (m, 12 H, AA'BB' system, CH_2CH_2), 3.74 (s, 9 H, CH_3N), 7.1–7.3 and 7.4–7.6 (m, 12 H, benzimidazolyl H).

$[CoL(OH_2)][ClO_4]_2$ (yield 60%) (Found: C, 46.2; H, 4.7; N, 11.9. $C_{31}H_{35}Cl_2CoN_7O_{11}$ requires C, 45.9; H, 4.3; N, 12.1%): λ_{max}/nm (solid) 530 (sh), 575, 600 and 650 (sh); $\tilde{\nu}_{max}/cm^{-1}$ 1618m and 1491m [ν(ring)], 1543s [$\nu_{asym}(NO_2)$], 1338m [$\nu_{sym}(NO_2)$], 750s [δ(CH)], 1100s and 623s [ν(ClO_4)] (Nujol mull); $\delta_H[(CD_3)_2SO]$ 2.8 and 3.0 (12 H, CH_2CH_2), 3.8 (9 H, CH_3N), 7.2 and 7.5 (12 H, benzimidazolyl H).

$[CuL(OH_2)_3][ClO_4]_2$ (yield 73%) (Found: C, 43.6; H, 4.5; N, 11.8. $C_{31}H_{39}Cl_2CuN_7O_{13}$ requires C, 43.7; H, 4.3; N, 12.0%): λ_{max}/nm (solid) 340 (sh), 560 and 720 (sh); $\tilde{\nu}_{max}/cm^{-1}$ 1618m and 1492m [ν(ring)], 1539s [$\nu_{asym}(NO_2)$], 1336m [$\nu_{sym}(NO_2)$], 750s [δ(CH)], 1100s and 623s [ν(ClO_4)] (Nujol mull); Λ_M(MeCN) 248 S cm^2 mol^{-1}, concentration 1.1 × 10^{-3} mol dm^{-3}.

[Zn(Hacm)L][ClO_4].—To a solution of zinc(II) perchlorate hexahydrate (0.1 mmol) in MeOH (10 cm^3) was added a solution of acetazolamide (0.1 mmol) in MeOH (10 cm^3) containing 1 equivalent of methanolic sodium hydroxide. The resulting mixture was left to stir for several minutes and then a stoichiometric amount of L dissolved in ethanol (25 cm^3) was slowly added. The resulting suspension was stirred for about 3 h. The white solid obtained was filtered off, washed with ethanol and dried under vacuum (yield 32%) (Found: C, 44.7; H, 4.8; N, 16.2. $C_{35}H_{38}ClN_{11}O_9S_2Zn$ requires C, 45.6; H, 4.1; N, 16.7%): $\tilde{\nu}_{max}/cm^{-1}$ 1687s [ν(C=O)]; 1616s and 1493s [ν(ring)]; 1537m [$\nu_{asym}(NO_2)$], 1336w [$\nu_{sym}(NO_2)$]; 1300s [$\nu_{asym}(SO_2)$]; 1148s [$\nu_{sym}(SO_2)$], 1100vs and 625s [ν(ClO_4)] (Nujol mull); $\delta_H[(CD_3)_2SO]$ 2.15 (s, 3 H, CH_3, Hacm), 2.7–2.9 and 2.9–3.1 (m, 12 H, CH_2CH_2), 3.76 (s, 9 H, CH_3N), 7.1–7.3 and 7.4–7.7 (m, 12 H, benzimidazoyl H); Λ_M(MeCN) 115 S cm^2 mol^{-1}, concentration 4.0 × 10^{-4} mol dm^{-3}.

[Co(Hacm)L][ClO_4]·2H_2O.—A solution of $Co(ClO_4)_2$·6H_2O (0.2 mmol) in ethanol (10 cm^3) was added to an ethanolic solution of acetazolamide (0.2 mmol in 18 cm^3) containing 1 equivalent of sodium hydroxide. The mixture was stirred for about 5 min; then a solution of L (0.2 mmol) in ethanol (20 cm^3) was added dropwise. The resulting suspension was left to stir for 4 h. The violet product that precipitated was filtered off, washed with ethanol and dried under vacuum (yield 31%) (Found: C, 44.5; H, 4.6; N, 15.9. $C_{35}H_{42}ClCoN_{11}O_{11}S_2$ requires C, 44.2; H, 4.4; N, 16.2%): λ_{max}/nm (solid) 500 (sh), 565, 610 and 650 (sh); $\tilde{\nu}_{max}/cm^{-1}$ 1698m [ν(C=O)]; 1618m [ν(ring)]; 1542m [$\nu_{asym}(NO_2)$], 1336w [$\nu_{sym}(NO_2)$]; 1300m [$\nu_{asym}(SO_2)$]; 1140w [$\nu_{sym}(SO_2)$], 1100vs (br) and 625s [ν(ClO_4)] (Nujol mull); $\delta_H[(CD_3)_2SO]$ 2.1 (3 H, CH_3, Hacm), 2.8 and 2.9 (12 H, CH_2CH_2), 3.6 (9 H, CH_3N), 7.1 and 7.5 (12 H, benzimidazolyl H); Λ_M(MeCN) 143 S cm^2 mol^{-1}, concentration 1.2 × 10^{-3} mol dm^{-3}.

[Cu(Hacm)(dien)][ClO_4]·H_2O.—Acetazolamide (1 mmol) was dissolved in a 0.1 mol dm^{-3} solution (10 cm^3) of NaOH in MeOH. To this the stoichiometric amount of copper(II) perchlorate hexahydrate was added. The resulting mixture was left to stir for several minutes. Then diethylenetriamine (1 mmol) in methanol (10 cm^3) was added dropwise and with continuous stirring. The violet precipitate formed was filtered off, washed with MeOH and dried (yield 62%) (Found: C, 19.3; H, 4.1; N, 19.7. $C_8H_{20}ClCuN_7O_8S_2$ requires C, 19.0; H, 3.9; N, 19.4%): λ_{max}/nm (solid) 596; $\tilde{\nu}_{max}/cm^{-1}$ 3342m, 3288m and 3269m [ν(N–H)]; 1689s [ν(C=O)]; 1305s [$\nu_{asym}(SO_2)$]; 1154s [$\nu_{sym}(SO_2)$], 1100vs and 621s [ν(ClO_4)] (Nujol mull); Λ_M(MeCN) 118 S cm^2 mol^{-1}, concentration 1.2 × 10^{-3} mol dm^{-3}.

[Cu(Hacm)(dipn)][ClO_4].—Copper(II) perchlorate hexahydrate (2 mmol) was dissolved in 0.1 mol dm^{-3} NaOH (20 cm^3) in MeOH containing acetazolamide (2 mmol). To this mixture a solution of dipropylenetriamine (2 mmol) in MeOH (25 cm^3) was slowly added dropwise. After the addition of dipn (*ca.* 1 mmol) a small amount of a green solid formed, and this remained present throughout the whole addition of the triamine. This green precipitate was filtered off and the filtrate was concentrated to half volume under vacuum (yield 30%) (Found: C, 23.2; H, 4.5; N, 19.6. $C_{10}H_{22}ClCuN_7O_7S_2$ requires C, 23.2; H, 4.25; N, 19.0%): λ_{max}/nm (solid) 612; $\tilde{\nu}_{max}/cm^{-1}$ 3321m, 3307m and 3280m [ν(N–H)]; 1677s [ν(C=O)]; 1310s [$\nu_{asym}(SO_2)$]; 1152s [$\nu_{sym}(SO_2)$], 1100vs, 945m, and 621s [ν(ClO_4)] (Nujol mull); Λ_M(MeCN) 125 S cm^2 mol^{-1}, concentration 1.1 × 10^{-3} mol dm^{-3}.

Results and Discussion

Characterization of $[ML]^{2+}$ *Complexes.*—Among the various approaches followed for the synthesis of ligand systems reproducing the environment of the zinc site of CA, that leading to the tris(benzimidazole) unit of L is promising for its ease of preparation, possibility of further elaboration, and similarity with the tris(imidazole) donor set present in the enzyme.[2] The complexes of L were all obtained as adducts with one or more molecules of water. These are probably co-ordinated to the metal centres in the solid state, but may be replaced by donor solvent molecules in solution. On the other hand, the perchlorate counter ions do not seem to be co-ordinated, judging from the shape of the bands associated with these ions in the IR spectra of the complexes (at about 1100 and 625 cm^{-1}).[14] The IR absorptions associated with the vibration modes of the nitro group of L are little affected by co-ordination, suggesting that this group is not involved in metal binding.

The proton NMR spectrum of L undergoes only small changes upon co-ordination to zinc(II), probably because the ligand CH groups are substantially removed from the nitrogen-donor atoms to experience significant deshielding effects by the metal ion, and the chelate rings formed by the benzimidazole arms of the ligand are too large to impose drastic restrictions in the conformational mobility of the alkyl chains. The main effect caused on the NMR resonances of L in $(CD_3)_2SO$ by the paramagnetic cobalt(II) centre is broadening, which results in the loss of the multicomponent structure of the signals present in the spectra of free L and its zinc(II) complex. The broadening effect is more severe in CD_3CN and is accompanied by sizeable shifts that make difficult the spectral assignment.

The electronic spectral data for the $[ML]^{2+}$ complexes in solution are summarized in Table 1. The UV region is dominated by intense bands associated with the benzimidazole chromophores. For $[CuL]^{2+}$ additional weaker absorptions in the range 300–400 nm, likely ligand-to-metal charge transfer (l.m.c.t.) in origin, occur. The visible portion of the spectra for the complexes of both Cu^{II} and Co^{II} is indicative of five-co-ordinated species. For $[CuL]^{2+}$ the maximum near 730 nm observed in acetonitrile solution is in agreement with a structure intermediate between square pyramidal and trigonal bipyrami-

Table 1 Electronic spectral data in solution at room temperature

Complex	Solvent	λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})
$[ZnL]^{2+}$	MeCN	254 (18 700), 274 (19 350), 280 (18 800)
$[CoL]^{2+}$	MeCN	254 (18 750), 278 (18 800), 284 (16 650), 450 (sh) (20), 536 (50), 580 (70), 602 (75), 760 (12)
$[CuL]^{2+}$	MeCN	254 (22 100), 274 (23 400), 280 (22 000), 350 (480), 430 (sh) (230), 730 (85), 870 (sh) (75)
$[Zn(Hacm)L]^{+}$	MeCN–MeOH (1:1)	254 (19 200), 274 (22 600), 282 (22 300), 305 (sh) (3 200)
$[Co(Hacm)L]^{+}$	MeCN–MeOH (1:1)	252 (21 000), 278 (19 500), 284 (20 200), 305 (sh) (3 800), 484 (80), 560 (80), 610 (65), 790 (15)
$[Cu(Hacm)(dien)]^{+}$	MeCN–MeOH (1:1)	260 (12 450), 305 (sh) (5 000), 586 (110)
$[Cu(Hacm)(dipn)]^{+}$	MeCN–MeOH (1:1)	266 (18 000), 305 (sh) (5 900), 600 (105)

Table 2 The EPR spectral parameters for the copper(II) complexes in frozen solutions (123 K)

Complex	Solvent	$g_{\|\|}$	$g_{\perp}$	$10^{-4}\|A_{\|\|}\|$/cm^{-1}
$[CuL]^{2+}$	MeCN[a]	2.335	2.078	148
Cu^{II}–CA[b]	Aqueous buffer (pH 8.3)	2.32	2.07	140
$[Cu(Hacm)(dien)]^{+}$	MeCN	2.224	2.070	183
$[Cu(Hacm)(dipn)]^{+}$	MeCN–MeOH (1:1)	2.240	2.062	180
$[Cu(dien)]^{2+}$	MeCN–MeOH (1:1)	2.232	[c]	184
$[Cu(dipn)]^{2+}$	MeCN–MeOH (1:1)	2.224	2.053	188

[a] The parameters in frozen Me_2SO or MeOH solutions are almost coincident. [b] Ref. 15. [c] A broad unresolved signal, probably due to magnetically undiluted species, overlaps the mononuclear signal in the perpendicular region and makes inaccurate the estimate of $g_{\perp}$.

dal, and the spectrum is actually very similar to that of copper(II)-substituted carbonic anhydrase.[15] The structural similarity between Cu^{II}–CA and $[CuL]^{2+}$ is confirmed by the EPR data in frozen solution (Table 2). The axial spectrum ($g_{||} > g_{\perp}$), with reduced $|A_{||}|$ value, of the model complex is indeed reminiscent of that of the copper(II) enzyme.[15] The modest intensity of the absorption bands of $[CoL]^{2+}$ in the d–d region excludes that this cobalt(II) centre assumes a pseudo-tetrahedral geometry, as for Co^{II}–CA.[16] The spectral features are in fact consistent with a five-co-ordinate structure. However, we note that the solid-state reflectance spectrum of $[CoL(OH_2)][ClO_4]_2$ is different, and is more consistent with a pseudo-tetrahedral geometry. A similar situation is likely to hold for the $[ZnL]^{2+}$ complex. The solid-state IR spectra of the complexes of Zn^{II} and Co^{II} are, in fact, practically superimposable.

The electronic spectra of $[CoL]^{2+}$ and $[CuL]^{2+}$ show solvent dependence. This effect is only partly due to solvent co-ordination to the metal centre, as it is clearly shown by the near-UV spectrum of the copper(II) complex. This spectrum is very different in acetonitrile or methanol solutions. In the first case two absorptions of modest intensity occur at 350 ($\varepsilon \approx 500$) and 430 nm ($\varepsilon \approx 250$ dm^3 mol^{-1} cm^{-1}), as a shoulder. In methanol or mixed acetonitrile–methanol solutions, however, the spectrum exhibits a pair of much stronger bands, at 320 and 365 nm ($\varepsilon \approx 1000$ dm^3 mol^{-1} cm^{-1}). While the weaker

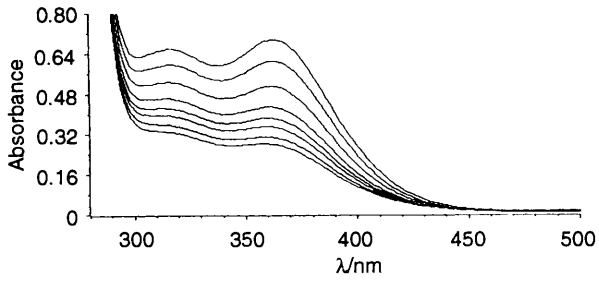


Fig. 1 Spectrophotometric titration of $[CuL]^{2+}$ (3×10^{-4} mol dm^{-3}) with methanolic sodium hydroxide (10^{-2} mol dm^{-3}) in methanol solution. The spectra, normalized for dilution, refer to solutions containing $[OH^-]:[Cu^{2+}]$ ratios of 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8 and 1.0:1, in the order of increasing near-UV absorption

absorptions observed in acetonitrile can be quite certainly assigned to benzimidazole $\pi(N) \rightarrow Cu^{II}$ l.m.c.t. transitions, as it has been for other copper(II)–benzimidazole complexes,[17] the spectra in alcoholic solutions resemble those of dinuclear di-μ-hydroxo-bridged copper(II) complexes.[17b] These result from partial dissociation of bound water, according to the equilibrium (1) as shown by the full development of the near-UV

$$2[CuL(OH_2)]^{2+} \longrightarrow [\{CuL(OH)\}_2]^{2+} + 2H^+ \quad (1)$$

bands upon addition of stoichiometric amounts of sodium hydroxide to the methanolic solution of the complex ($\varepsilon \approx 2000$ dm^3 mol^{-1} cm^{-1} for the final solution) (Fig. 1). The origin of these absorptions is thus $OH^- \rightarrow Cu^{II}$ l.m.c.t.,[17b,18] but the presence of a pair of l.m.c.t. bands suggests the existence of hydroxo bridges, and hence a dinuclear $[\{CuL(OH)\}_2]^{2+}$ complex. This interpretation is supported by the observation that the addition of slightly more than the stoichiometric amount of hydroxide to $[CuL]^{2+}$ leads to complete depletion of the copper(II) EPR signal in the frozen solution. The behaviour of $[CuL]^{2+}$ thus seems to parallel that of other copper(II) complexes with tridentate ligands, which give dinuclear bis(μ-hydroxo) bridged compounds,[8b,19] in some cases exhibiting strong antiferromagnetic interactions. Since proton-dissociation equilibria of bound water molecules are of some importance in the context of CA model systems, the relevant chemistry displayed by the complexes of L will be studied in detail separately.

Mixed Complexes $[M(Hacm)(triamine)][ClO_4]$.—The ternary complexes were obtained by reaction of the metal perchlorate salt, the triamine, and the sodium salt of acetazolamide. The need to operate in a mixed solvent is determined by the different solubility properties of the ligands (acetazolamide and triamine). The order of addition of the reagents is probably important to get the mixed-ligand complexes; all attempts to bind the acetazolamide ligand to the preformed $[M(triamine)]^{2+}$ complex led only to partial formation of the ternary complex. Also, acetazolamide must be present in its deprotonated form, since binding was not observed when the neutral compound was used in the reaction. We were unable to isolate the ternary complex $[Cu(Hacm)L]^{+}$ in sufficiently pure form under several reaction conditions. As will be discussed below, there is spectral evidence that this complex is formed to some extent in solution, but the material isolated in most instances had a composition closer to that of binary complexes of Cu^{II} and L. Solubility problems, incomplete complex formation, and possibly steric effects make difficult the isolation of the ternary species. On the other hand, we could easily obtain the ternary complexes of copper(II) with acetazolamide and the less sterically demanding triamines dien and dipn.

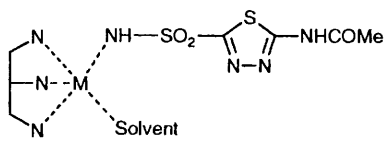
The IR spectra of the complexes $[M(Hacm)(triamine)][ClO_4]$ are very important to establish the site of co-ordination

of the $Hacm^-$ residue. In general, the spectra exhibit the ν(C=O) band of this ligand at slightly higher frequency than for free acetazolamide (1678 cm^{-1}), but lower than for $Hacm^-$ (1704 cm^{-1}).[20a] This effect can be related to a loss of the hydrogen-bonding interactions occurring in free acetazolamide.[20a,21] The sulfonamide $\nu_{asym}(SO_2)$ and $\nu_{sym}(SO_2)$ stretching frequencies (at 1321 and 1176 cm^{-1}) undergo marked shift to lower energy on complex formation, as it has been reported for bis(amino) complexes of type $[M(Hacm)_2(NH_3)_2]$.[20] The recently reported crystal structure of $[Zn(Hacm)_2(NH_3)_2]$ unequivocally showed that this spectral feature is associated with binding of the acetazolamide anion through the deprotonated sulfonamide nitrogen atom,[11] thus supporting the previous deduction based on the crystal structure of the CA–inhibitor complex.[4] It is more difficult to give accurate assignments for the ν(NH) stretching vibrations of the acetamido and sulfonamido groups of bound acetazolamide, since broad absorption bands due to H_2O, or additional bands due to NH groups of the triamine, are usually present in the IR spectra. The IR bands near 1100 and 625 cm^{-1}, typically associated with the perchlorate group, show some splitting in the spectra of the derivatives containing aliphatic triamines. This probably arises from hydrogen-bonding interactions of the anion with some of the several NH groups of the triamine, while co-ordination of perchlorate to the metal centre seems much less likely in these ternary complexes. Conductivity measurements indicate that the acetazolamide ligand remains co-ordinated to the metal in the ternary complexes in solution, since the Λ_M values found are those typical for 1:1 electrolytes.[22]

Deprotonation of acetazolamide produces stepwise spectral changes in its electronic spectrum. Upon adding 1 equivalent hydroxide to acetazolamide in MeOH–MeCN (1:1) a new absorption band at 305 nm ($\varepsilon \approx 4000$ dm^3 mol^{-1} cm^{-1}) appears, flanking the more intense UV band of the heterocyclic ring at 268 nm ($\varepsilon \approx 6800$ dm^3 mol^{-1} cm^{-1}). With a molar ratio of acetazolamide:hydroxide 1:2 an intense UV band at 302 ($\varepsilon \approx 11\,700$) and a weaker band at 248 nm ($\varepsilon \approx 4500$ dm^3 mol^{-1} cm^{-1}) replace the original absorption band of the thiadiazole chromophore. The difference in pK_a of the two dissociable protons of acetazolamide (7.19 and 8.65 in water, 7.52 and 9.41 in ethanol–water)[23] enables the changes occurring in the first step probably to be attributed to deprotonation of the sulfonamide NH_2 group and those in the second step to deprotonation of the amide NH group, which is directly bound to the heterocyclic ring.

The electronic spectra of the ternary $[M(Hacm)(triamine)]^+$ complexes exhibit the UV marker of the acetazolamide monoanionic ligand at 305 nm. This occurs as a poorly defined shoulder on the intense benzimidazole-ring absorptions at higher energy for the complexes of L but the whole UV spectral features of the $Hacm^-$ ligand are apparent in the spectra of the complexes $[Cu(Hacm)(dien)]^+$ and $[Cu(Hacm)(dipn)]^+$, even though contributions from amine→Cu^{II} l.m.c.t. transitions are clearly present in the near-UV region. The band at 305 nm develops to a large extent ($\Delta\varepsilon \approx 3000$ dm^3 mol^{-1} cm^{-1}) also on addition of 1 equivalent OH^- to a solution of Cu^{II}:H_2acm:L (1:1:1) in MeOH–MeCN (1:1) and this is accompanied by partial depletion of the absorption at 360 nm due to the Cu^{II}–L–OH^- complex discussed above. A corroboration that the acetazolamide ligand is at least partially co-ordinated to Cu^{II} in this system comes from the visible spectrum of the resulting solution (λ_{max} 660 nm, $\varepsilon \approx 65$ dm^3 mol^{-1} cm^{-1}), which cannot be obtained as a simple combination of those of $[CuL]^{2+}$ (λ_{max} 710 nm, $\varepsilon \approx 80$ dm^3 mol^{-1} cm^{-1}) and $[\{CuL(OH)\}_2]^{2+}$ (λ_{max} 604 nm, $\varepsilon \approx 70$ dm^3 mol^{-1} cm^{-1}). Formation of the ternary complex in the solid state may be disfavoured, though, and even freezing of the solution gives EPR-silent material, very likely the dinuclear Cu^{II}–L–OH^- complex.

For $[Co(Hacm)L]^+$ the electronic spectra in the visible region indicate that the complex assumes a different stereochemistry in the solid state and in solution. The solid-state spectrum is in fact consistent with a pseudo-tetrahedral geometry, while that in solution is clearly indicative of five-co-ordination, based both on the intensity of the bands and the presence of a weak absorption near 800 nm.[16] The same situation probably holds for $[Zn(Hacm)L]^+$ and it is important to emphasize that in the acetazolamide adducts of CA[4] and Co^{II}–CA[16] the metal centre is pseudo-tetrahedral. The ligand L is clearly too flexible to maintain a rigid geometry in solution. Of the two possibilities to achieve five-co-ordination, indicated in structures **A** and **B**, we strongly prefer that involving monodentate co-ordination by $Hacm^-$, based on the solvent dependence observed for the visible spectrum of $[Co(Hacm)L]^+$. The acetazolamide anion has been found occasionally to act as a chelating ligand, but this occurs in polynuclear structures where the two nitrogen atoms of the thiadiazole ring act as a bridge between pairs of metal atoms.[9b]



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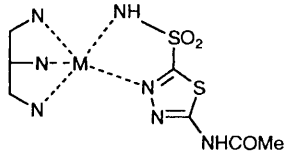

B

More similarity exists between the solid-state and solution structures of the two mixed-ligand copper(II) complexes $[Cu(Hacm)(dien)]^+$ and $[Cu(Hacm)(dipn)]^+$. The visible absorption band of these complexes undergoes only small changes on dissolution and for the dien complex even the EPR parameters measured from the powder spectrum ($g_{\|} = 2.20$, $g_{\perp} = 2.06$) are rather similar to the data obtained from the frozen-solution spectra (Table 2) {for $[Cu(Hacm)(dipn)][ClO_4]$ the powder EPR spectrum displays only a broad isotropic signal centred at $g = 2.08$}. In general, both the optical and EPR data for the two ternary complexes are consistent with tetragonal symmetry, with only weak axial interaction with the metal. The d–d bands of $[Cu(dien)]^{2+}$ and $[Cu(dipn)]^{2+}$ (at 604 and 608 nm, respectively, in MeOH–MeCN, 1:1) undergo a blue shift on binding $Hacm^-$, while the EPR parameter $|A_{\|}|$ remains large for the ternary complexes. These data indicate that $Hacm^-$ behaves as a monodentate equatorial ligand to the copper(II) triamine centres, in agreement with expectation based on the interaction between a dipositive copper(II) complex with a tridentate ligand and a monoanionic exogenous ligand.[24]

In conclusion, the present investigation has shown that it is possible to obtain ternary complexes which can be considered as analogues of the adducts formed by CA and the inhibitor acetazolamide. All the available spectral evidence indicates that this inhibitor binds as a monodentate ligand, through the deprotonated sulfonamide group, to metal centres containing additional tridentate nitrogen ligands. It seems reasonable to assume that the more variable co-ordination behaviour of acetazolamide observed in mixed-ligand complexes with structurally simpler mono- or bi-dentate amines depends to a large extent on the limited steric requirements imposed by these auxiliary ligands.

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