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Unusual structure of the dimeric 4-bromocalcimycin–Zn^{2+} complex

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The X-ray structure of [Zn(4-bromocalcimycin)$_2$·H_2O] complex shows two highly different conformations of the ligand in the dimeric association, unusual in this ionophore family.

Since its discovery, calcimycin or A23187[1] **1** (Fig. 1, a carboxylic polyether antibiotic with calcium carrier properties, has attracted considerable attention in biology as a tool for the study of calcium second messenger in living systems.[2] Its non-fluorescent 4-bromo derivative **2** was subsequently described and found suitable for the same application in the presence of fluorescent probes.[3]

Erdahl and colleagues[4] recently showed that **2** transports Zn^{2+} and Mn^{2+} with high selectivity over Ca^{2+} in phospholipid vesicles, and they made interesting findings concerning the stoichiometry of species involved in the transport. However, information on the structure of cation complexes with this specific ligand were lacking.

We recently undertook the preparation of crystalline adducts of **2** with various divalent cations suitable for X-ray analysis. Here we report the crystal structure obtained for a cation of topical biological interest, Zn^{2+}, which reveals unusual features. To obtain the complex, we used a H_2O–CH_2Cl_2 biphasic system in which the free acid dissolved in the organic layer was stirred with an aqueous $Zn(ClO_4)_2$ solution at pH 10.†

The crystal structure determination by X-ray diffraction was consistent with the neutral dicarboxylate complex [Zn(4-bromocalcimycin)$_2$·H_2O] (Fig. 2).‡ The zinc atom occupied the center of a slightly distorted octahedron (Table 1). Coordinations were provided by O(7) of a water ligand, O(1), N(2) and O(31), N(32) belonging respectively to the L1 and L2 benzoxazole-carboxylate moiety. Interestingly, as shown in Fig. 3 obtained from the crystal data, the conformation of L1 was such that O(6) of the ketopyrrole arm supplied the sixth liganding site, forming a complexing tripod. L1 and L2 were clearly not equivalent in the complex structure. The L2 benzoxazole ring is rotated by nearly 180° compared with L1, and the ketopyrrole arm is unfavourably positioned for participation in the scaffold (Fig. 2).

Octahedral arrangements of the same type were recently described for Zn(II) complexes containing more simple aromatic moities with nitrogen and carboxylate coordinating sites, but no such unsymmetrically bound ligand with a bidentate/tridentate arrangement has been described.[5]

The specific conformation adopted by L1 and L2 did not permit the two head-to-tail intermolecular chelations observed in the well-known calcimycin dimeric complexes[6–8] described for Ca^{2+}, Mg^{2+} and Fe^{2+}. One intermolecular hydrogen chelation remained for N(3)–H···O(31) and N(3)–H···O(32) (Table 1).

Also, the 3-aminomethyl substituent which was NH-chelated with the carboxylate group for calcimycin[6–8] was moved out of the aromatic plane owing to the presence of the bulky bromine in the 4 position, and the existing intramolecular hydrogen bonding was thus suppressed. We show that this steric hindrance has repercussions on the complexing properties for divalent cations, the 1:1 and 1:2 thermodynamic stability constants β_1^0 and β_2^0 in MeOH (25 °C) :

$$M^{2+} + A^- \rightleftharpoons MA^+ \ (1)\ \beta_1^0$$

$$M^{2+} + 2A^- \rightleftharpoons MA_2 \ (2)\ \beta_2^0$$

(A^-, carboxylate form of **1** or **2**; M^{2+} divalent cation) being lowered as follows for Zn^{2+}:[9]

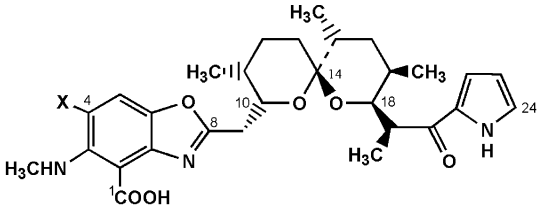

Fig. 1 X = H, calcimycin **1**; X = Br, 4-bromocalcimycin **2**.

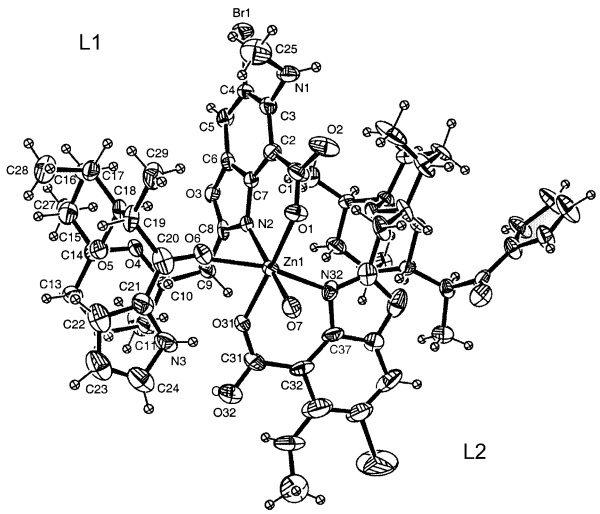

Fig. 2 ORTEP drawing of [Zn(4-bromocalcimycin)$_2$·H_2O] crystal structure with ligands L1 and L2 nomenclature.

Table 1 Selected bond lengths (Å) and angles (°)

Bond lengths		Angles	
Zn(1)–O(7)	2.103(6)	O(1)–Zn(1)–O(7)	88.3(2)
Zn(1)–O(31)	2.013(6)	O(7)–Zn(1)–N(2)	171.6(3)
Zn(1)–N(32)	2.147(8)	O(31)–Zn(1)–O(1)	174.2(3)
Zn(1)–O(1)	2.030(6)	N(32)–Zn(1)–O(6)	171.8(3)
Zn(1)–N(2)	2.147(7)	O(1)–Zn(1)–N(2)	85.5(3)
Zn(1)–O(6)	2.234(7)	O(1)–Zn(1)–O(6)	89.5(3)
		O(1)–Zn(1)–N(32)	98.5(3)
		O(31)–Zn(1)–N(32)	85.6(3)
		O(31)–Zn(1)–O(6)	86.3(3)
Intermolecular distances			
N(3)–H···O(31)	2.123		
N(3)–H···O(32)	2.451		

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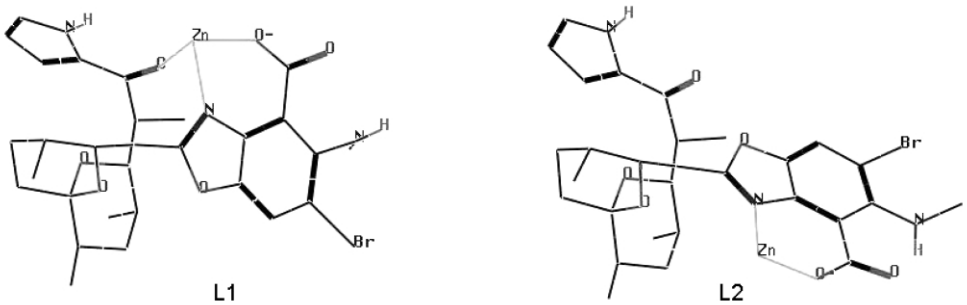

Fig. 3 A representation of L1 and L2 ligands structure coordinated with Zn^{2+} showing the specific benzoxazole rotation in L2.

calcimycin **1**: log β_1^0 = 8.24, log β_2^0 = 18.34

4-bromocalcimycin **2**: log β_1^0 = 6.72, log β_2^0 = 13.40

Interestingly, for both L1 and L2 the ketopyrrole arm adopted the preferential orientation already observed in the solid[6–8] and liquid state[10] for calcimycin with, in particular, the characteristic antiperiplanar position of H18 and H19.

All these observations suggest that in the complex formation, a 1 : 1 ($L1–Zn^+$) association occurs followed by the approach of the second ligand L2. The latter is unable to displace the remaining water ligand by its ketone function to give a final 1 : 2 supramolecular association. The molecular arrangement is thus not well designed towards the solvent attack in the reverse step. This may be related to the transport experiments in vesicles[4] where the authors state that Zn^{2+} is transported, in part, as a 1 : 1 complex in a pH-dependent stoichiometry.

In conclusion, we describe here what is to our knowledge the first solid state structure obtained with 4-bromocalcimycin. Furthermore, the Zn^{2+} complex studied shows an unusual supramolecular scaffold compared with known calcimycin Ca^{2+}, Mg^{2+} and Fe^{2+} dimeric arrangements and with other described carboxylate zinc complexes.

As recently stated,[11] zinc homeostasis studies in higher animals and humans have proved difficult. This work may therefore help gain a better understanding of the mechanism of cellular transport of zinc through biological membranes.

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Notes and references

† *Preparation*: A solution of 4-bromocalcimycin free acid, prepared from calcimycin as described[12] (100 mg, 0.17 mmol) in CH_2Cl_2 (17 mL) was stirred with 0.1 M aqueous $Zn(ClO_4)_2$ (50 mL). The pH was adjusted to *ca*. 10 by addition of tetrabutylammonium hydroxide. The mixture was stirred at 20 °C for 4 h under argon in the dark. The organic layer was then dried over anhydrous Na_2SO_4, filtered and evaporated. The solid residue was dissolved in EtOH (96.2 °C, Carlo Erba) and the solvent was left to evaporate at 20 °C for 1 week in the dark. Pale yellow crystals obtained proved suitable for X-ray analysis.

‡ *Structure analysis*: $ZnBr_2N_6O_{13}C_{58}H_{72}{\cdot}C_2H_5OH{\cdot}H_2O$, M_r = 1350.49, tetragonal, $P4_32_12$, $a = b$ = 15.4503(1), c = 52.1008(4) Å, V = 12437.07(15) $Å^{-3}$, Z = 8, D_x = 1.442 Mg m^{-3}, λ(MoKα) = 0.71073 Å, μ = 17.50 cm^{-1}, $F(000)$ = 5616, T = 110 K. The sample (0.35*0.22*0.20 mm) was studied on a NONIUS Kappa CCD with graphite monochromatized MoKα radiation. The cell parameters were obtained with Denzo and Scalepack[13] with 10 frames (psi rotation: 1° per frame). The data collection[14] ($2\theta_{max}$ = 60°, 1752 frames *via* 0.4° omega rotation and 28 s per frame, range *hkl*: h −19,19; k −13,13; l −52,66) gave 22439 reflections. The data reduction with Denzo and Scalepack[13] led to 13040 independent reflections of which 7989 with $I > 2.0\sigma(I)$. The structure was solved with SIR-97[15] which revealed the non hydrogen atoms. After anisotropic refinement, many hydrogen atoms may be found with a Fourier Difference. The whole structure was refined with SHELXL97[16] by the full-matrix least-square techniques (use of F square magnitude; x, y, z, β_{ij} for Zn, Br, N, O and C atoms, x, y, z in riding mode for H atoms; 768 variables and 7989 observations with $I > 2.0\sigma(I)$; calc. $w = 1/[\sigma^2(F_o^2) + (0.129P)^2 + 59P]$ where $P = (F_o^2 + 2F_c^2)/3$ with the resulting R = 0.097, R_w = 0.240 and S_w = 1.023 (residual around solvent molecules) $\Delta\rho < 2.5$ e $Å^{-3}$. The whole structure consists of the dimeric complex which crystallises with an ethanol molecule and a water molecule near a pyrrole ring. CCDC 195727. See http://www.rsc.org/suppdata/cc/b2/b210280n/ for crystallographic files in CIF or other electronic format.

Atomic scattering factors were from International Tables for X-ray Crystallography[17] and Ortep views realized with PLATON98.[18]

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