Histamine Complexation: Structural Studies of $[CaCl_4(H_2O)_2CaCl_2(H_2O)_2(histamine)_2]$ and Histamine Hydrobromide

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Single crystal *X*-ray studies of $[CaCl_4(H_2O)_2CaCl_2(H_2O)_2(histamine)_2]$ (**1**), $a = 8.437(3)$, $b = 11.964(5)$, $c = 6.767(4)$ Å, $\alpha = 92.26(4)$, $\beta = 66.04(3)$, $\gamma = 85.39(3)°$, $D_c = 1.58$ g cm^{-3}, $Z = 2$, triclinic space group $P\bar{1}$, $R/R_w = 4.8/7.7\%$, and histamine hydrobromide (**2**), $a = 17.647(6)$, $b = 9.363(4)$, $c = 4.686(1)$ Å, $\beta = 90.40(2)°$, $D_c = 1.65$ g cm^{-3}, $Z = 4$, monoclinic space group $P2_1/n$, $R/R_w = 5.1/6.2\%$, both show histamine [tautomeric form, 4-(2-aminoethyl)imidazole] to be protonated at the side-chain amine group. In (**1**) two histamine molecules are each bound in unidentate fashion to calcium *via* the nitrogen atom α to the side chain. There are two independent calcium atoms per asymmetric unit, both of which display octahedral geometry but co-ordination to different sets of atoms. Both (**1**) and (**2**) display *trans* conformation of amine and imidazole ring about the CH_2CH_2 bond of the side chain.

The role of histamine in inflammatory and anaphylactic reactions has long been established.[1] A typical allergic manifestation involves the release of preformed histamine from mast cells challenged with an appropriate antigen. Release is in some way mediated by IgE bound to the surface of the cell and by the presence of calcium. Histamine release can be effected in the absence of antigen upon direct introduction of calcium, but not magnesium, into the mast cell.[2] To approach an understanding of the mechanism of histamine release at a molecular level, it is important to establish the binding patterns of histamine and to investigate the possible direct involvement of calcium with histamine.

Structural evaluation of calcium binding to small molecules has shown that calcium may co-ordinate with 5 to 10 atoms, showing irregular geometries, preferring oxygen donor ligands, and displaying wide ranges of binding distances.[3] Attention has been focussed on the structural chemistry of calcium bound to carboxylate systems[4,5] and on calcium bound to ionophoric systems.[6] In biological systems, binding patterns may show subtle but significant differences from those seen under synthetic conditions, because biological systems offer multiple choices of complexation site, and patterns of calcium complexation to mixed ligands may differ from those involving a single type of ligand. Also the complexes of greater stability may be unimportant in a mechanistic pathway which must involve ready bond formation and bond breaking.

Early structural consideration of histamine focussed upon identification of one of the two tautomeric forms of histamine as the active tautomer.[7] Thus **A**, [4-(2-aminoethyl)imidazole] and **B**, [5-(2-aminoethyl)imidazole] (Figure 1) were considered. The addition of a proton to **A** or **B** might lead to cations **C**,**D** or **E**,**F**, respectively, with forms **C**,**D** being favoured owing to the interaction of side chain or ring unshared pairs in an intramolecular hydrogen bond.

However, molecular orbital calculations of preferred conformations of the side chain with respect to the CH_2–CH_2 bond suggested that the lowest energy conformation of ring and amine should be *trans*. This conformation would make the intramolecular hydrogen bond impossible. Positioning of ring and amine *gauche* with respect to the CH_2–CH_2 bond, while only slightly less favourable in energy (6.5 kcal), still does not permit an N–H ··· N interaction of less than 2.0 Å due to the geometry of the NH_3 group.[8] The picture was confused by the finding that histamine free base is of form **B**[9] (*trans* conformation of side chain) whereas the structure of the degradation product, histidine, shows it to be derived from form **A** with a *gauche* conformation of the side chain.[10]

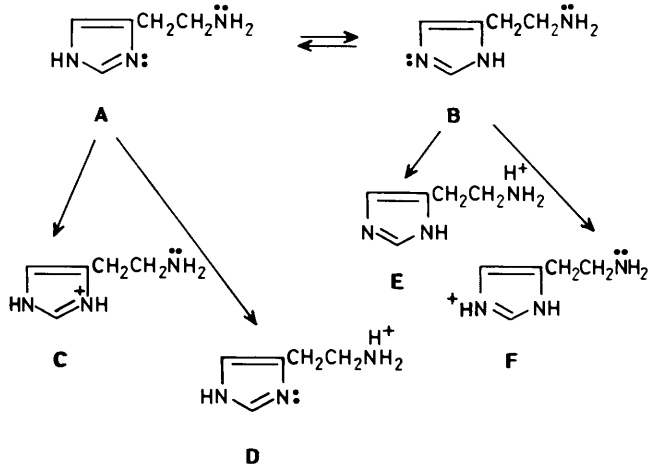

Figure 1. Tautomeric forms of histamine, **A** and **B**, and protonated forms, **C**, **D**, **E**, **F**

Structural studies of co-ordinated histamine, *e.g.* $[Cu(histamine)_2](ClO_4)_2$,[11] $[Cu(histamine)_2](BF_4)_2$,[12] $[Ni(H_2O)(histamine)_2ClO_4]ClO_4$,[13] and $[Ni(histamine)_3](ClO_4)_2$[14] show bidentate co-ordination of tautomer **A** with the side-chain amine and the ring nitrogen α to the side chain, each serving as an electron pair donor to the transition metal. The formation of a six-membered ring with metal and histamine is not possible for tautomer **B**.

Structural studies of protonated histamine have been limited to observations of the dication: histamine dihydrochloride,[15] histamine dihydrobromide,[16] $CoCl_4(histamine)$,[17] and histamine diphosphate monohydrate[18] show **C**, **F** with protonation of the primary side-chain amine. These are resonance structures which are not distinguishable crystallographically.

In an attempt to elucidate the possible involvement of histamine with calcium, we have isolated and characterized by single-crystal *X*-ray diffraction, two monocationic forms of histamine: $[CaCl_4(H_2O)_2CaCl_2(H_2O)_2(histamine)_2]$ (**1**) and histamine hydrobromide (**2**).

Experimental

Synthesis of $[CaCl_4(H_2O)_2CaCl_2(H_2O)_2(Histamine)_2]$.—Histamine (0.111 g, 1 mmol) was dissolved in water (20 ml) to which was added aqueous $CaCl_2$ (0.5M; 0.4 ml, 2 mmol Ca^{2+}).

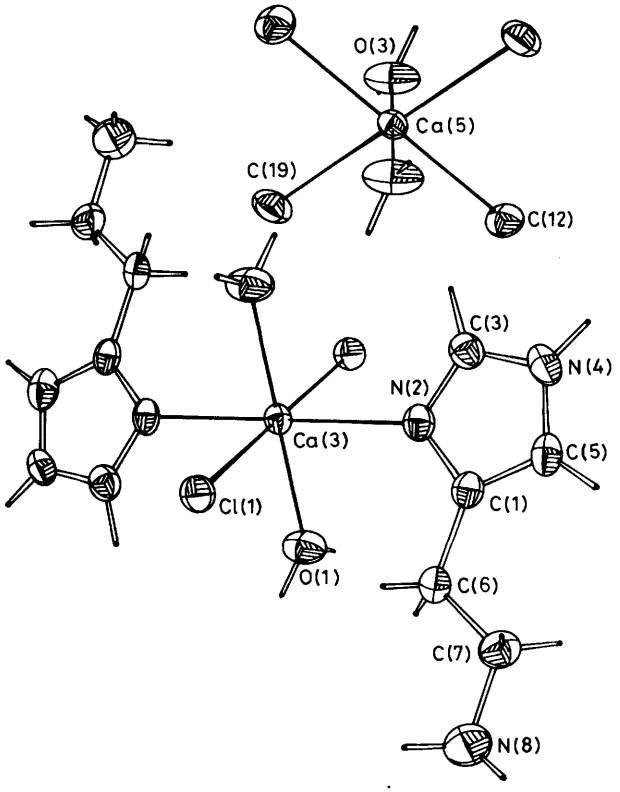

Figure 2. Projection view of $[CaCl_4(H_2O)_2CaCl_2(H_2O)_2(histamine)_2]$ (**1**)

The solution was set aside under nitrogen for 2 weeks to give a fine white precipitate. This was filtered off and the solution allowed to evaporate under nitrogen to near dryness to give colourless platelets. Single-crystal *X*-ray studies were carried out on the platelet form.

Synthesis of Histamine Hydrobromide.—Histamine (1.11 g, 0.1 mol) and $CaBr_2 \cdot 2H_2O$ (1.18 g, 5 mmol Ca^{2+}) were mixed in water (50 ml) and the solution was refrigerated. The fine white precipitate which appeared after 2 days was filtered off and the solution allowed to evaporate to near dryness whereupon clear plates formed after *ca.* 1 week. Single crystal *X*-ray data were collected on a crystalline plate.

Single-crystal X-ray Studies.—Crystals of $[CaCl_4(H_2O)_2 \cdot CaCl_2(H_2O)_2(histamine)_2]$ (**1**) and histamine hydrobrmide (**2**) were sealed in a capillary and mounted on a Syntex P3 automated diffractometer. Unit cell dimensions (Table 1) were determined by least-squares refinement of the best angular positions for 15 independent reflections ($2\theta > 15°$) during normal alignment procedures using molybdenum radiation ($\lambda = 0.710\,69$ Å). At room temperature using a variable scan rate, a θ—2θ scan mode, and a scan width of 1.2° below $K\alpha_1$ and 1.2° above $K\alpha_2$ to a maximum 2θ value of 116°, 3 178 data points were collected for (**1**) and 1 932 for (**2**). Backgrounds were measured at each side of the scan for a combined time equal to the total scan time. The intensities of three standard reflections were remeasured after every 97 reflections and as the intensities of these reflections showed less than 6% variation, corrections for decomposition were deemed unnecessary. Data were corrected for Lorentz, polarization, and background effects. After removal of redundant [(**1**) and (**2**)] and space group forbidden data (**2**), 2 219(1); 1 148(2) reflections were considered observed [$I > 3.0\sigma(I)$]. The structures were solved using a Patterson

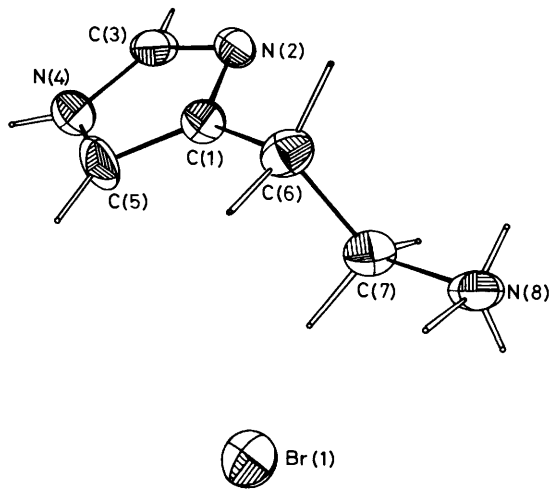

Figure 3. Projection view of histamine hydrobromide (**2**)

synthesis to locate the heavy atoms. Successive least-squares/-difference Fourier cycles allowed location of the remainder of the non-hydrogen atoms. Refinement of scale factor and positional and anisotropic thermal parameters for all non hydrogen atoms was carried out to convergence.[19] Hydrogen positional parameters were determined from a difference Fourier synthesis. These hydrogen positional parameters and the associated fixed isotropic thermal parameters ($U = 0.03$) were included in the final cycles of refinement but were held invariant. The final cycle of refinement [function minimized $\Sigma(|F_o| - |F_c|)^2$] led to a final agreement factor, R/R_w = 4.8/7.7%, (**1**); 5.1/6.2% (**2**) [$R = (\Sigma||F_o| - |F_c||/|F_o|) \times 100$]. Anomalous dispersion corrections were made for Cl and Ca in (**1**); for Br in (**2**). Scattering factors were taken from Cromer and Mann.[20] In the final cycles of refinement a weight = $1/\sigma|F|$ was applied.

Discussion

The complex formed by $CaCl_2$ and histamine, isolated from aqueous medium, shows two calcium atoms per asymmetric unit, each with a distinct co-ordination sphere. Ca(5) Lies on a centre of inversion and is bound to four chloride atoms and two water molecules [Ca–Cl, 2.755(13) Å av., Ca–O, 2.325(17) Å] in approximately octahedral geometry (Figure 2, Table 4, based on the positional parameters of Table 2). Ca(3) Is similarly centred on an inversion centre and is bound to two chloride atoms [Ca–Cl, 2.707(13) Å], two water molecules [Ca–O, 2.333(13) Å], and two histamine molecules *via* the nitrogen atom α to the side chain of the 4-(2-aminoethylimidazole) form [Ca–N, 2.441(13) Å], Thus protonated form, **D**, of tautomer **A** is present. The side chain shows a *trans* conformation of imidazole ring and ammonium group about bond C(6)–C(7) [dihedral angle 175.3(4)°] as predicted by Kier to be of lowest energy.[4] Each chloride atom shows hydrogen bonding to three protons. Each water hydrogen atom, the hydrogen atoms on N(4), and H(80) and H(81) of N(8) are involved in single hydrogen bonds. H(82) Is hydrogen bonded to two different chloride atoms in a bifurcated bond. All hydrogen bonding is intermolecular. {See results given in supplementary publication. This publication [SUP no. 56380 (5 pp.)] contains tables of thermal parameters and hydrogen bond lengths.*}

* For details of the Supplementary publications scheme, see Instructions for Authors (1986), *J. Chem. Soc., Perkin Trans. 1*, 1986, Issue 1. Copies of the structure factors are available on request from the Editorial office.

Table 1. Crystal data for $[CaCl_4(H_2O)_2CaCl_2(H_2O)_2(histamine)_2]$ (**1**) and histamine hydrobromide (**2**)

	(1)	(2)
Formula	$C_5H_{14}CaCl_3N_3O_2$	$C_5H_{10}BrN_3$
M	294.6	192.1
a	8.437(3) Å	17.647(6) Å
b	11.964(5)	9.363(4)
c	6.767(4)	4.686(1)
α	92.26(4)°	90.0°
β	66.04(3)	90.40(2)
γ	85.39(3)	90.0
V	620.2(4) Å^3	774.3(4) Å^3
$F(000)$	304	384
$\mu(Mo\text{-}K_\alpha)$	11.28 cm^{-1}	51.77 cm^{-1}
$\lambda(Mo\text{-}K_\alpha)$	0.710 69 Å	0.710 69 Å
D_c	1.58 g cm^{-3}	1.65 g cm^{-3}
Z	2	4
Obs. refl.	2 219	1 148
R/R_w	4.8/7.7%	5.1/6.2%
Space group	$P\bar{1}$	$P2_1/n$

Table 2. Positional parameters for $[CaCl_4(H_2O)_2CaCl_2(H_2O)_2(histamine)_2]$ (**1**)

Atom	x	y	z
Ca(3)	0.0000	0.0000	0.0000
Ca(5)	0.0000	0.5000	0.5000
Cl(1)	0.002 4(2)	−0.134 3(1)	0.667 1(2)
Cl(2)	−0.165 5(2)	0.409 0(1)	−0.110 9(2)
Cl(9)	0.322 3(2)	0.378 8(1)	0.379 6(2)
O(1)	0.022 2(5)	−0.152 0(3)	0.196 7(5)
O(3)	0.115 2(7)	0.619 7(3)	0.675 0(7)
C(1)	0.564 8(5)	−0.070 0(4)	0.216 3(6)
N(2)	0.682 3(5)	0.010 7(3)	0.167 1(6)
C(3)	0.586 0(6)	0.108 3(4)	0.214 1(7)
N(4)	0.415 0(5)	0.095 3(3)	0.290 0(6)
C(5)	0.399 8(6)	−0.017 5(4)	0.290 9(7)
C(6)	0.626 5(6)	−0.192 9(4)	0.184 1(7)
C(7)	0.477 3(7)	−0.265 4(4)	0.243 1(8)
N(8)	0.540 7(6)	−0.385 0(4)	0.227 0(8)
H(10)	0.0103	−0.1445	0.3241
H(11)	0.0616	−0.2221	0.1571
H(30)	0.0637	0.7000	0.7064
H(31)	0.2003	0.6200	0.7460
H(3)	0.6294	0.1895	0.1991
H(4)	0.3306	0.1528	0.3341
H(5)	0.2924	−0.0533	0.3357
H(60)	0.6894	−0.2093	0.0445
H(61)	0.7123	−0.2270	0.2501
H(70)	0.3940	−0.2435	0.3876
H(71)	0.4229	−0.2546	0.1410
H(80)	0.5930	−0.4028	0.2960
H(81)	0.4594	−0.4176	0.2595
H(82)	0.6232	−0.4013	0.0660

Table 3. Positional parameters for histamine hydrobromide (**2**)

Atom	x	y	z
C(1)	0.363 0(5)	0.443 1(10)	0.805 8(20)
N(2)	0.384 1(5)	0.575 7(8)	0.690 1(18)
C(3)	0.329 4(6)	0.609 3(11)	0.508 6(22)
N(4)	0.275 3(5)	0.507 5(11)	0.496 4(18)
C(5)	0.297 4(6)	0.401 7(11)	0.685 5(25)
C(6)	0.412 0(6)	0.366 8(10)	1.018 9(21)
C(7)	0.469 6(6)	0.274 7(10)	0.871 0(21)
N(8)	0.520 7(5)	0.204 8(8)	1.094 8(17)
Br(1)	0.114 7(1)	0.489 0(1)	0.121 1(2)
H(3)	0.342 1	0.701 7	0.3767
H(4)	0.2649	0.4700	0.3440
H(5)	0.2730	0.3189	0.7131
H(61)	0.4328	0.4507	1.1632
H(62)	0.3782	0.2858	1.0429
H(71)	0.4366	0.2012	0.7394
H(72)	0.5070	0.3140	0.7379
H(81)	0.5406	0.1502	1.0147
H(82)	0.5390	0.2771	1.1066
H(83)	0.4811	0.1359	1.2603

Table 4. Bond angles and distances for $[CaCl_4(H_2O)_2CaCl_2(H_2O)_2(histamine)_2]$ (1)

Ca(3)–C(11)	2.707(13)	N(2)–C(3)	1.321(6)
Ca(3)–N(2)	2.441(13)	C(3)–N(4)	1.339(6)
Ca(3)–O(1)	2.333(13)	N(4)–C(5)	1.360(7)
Ca(5)–C(12)	2.748(13)	C(5)–C(1)	1.360(6)
Ca(5)–C(19)	2.762(13)	C(1)–C(6)	1.501(7)
Ca(5)–O(3)	2.325(17)	C(6)–C(7)	1.511(8)
C(1)–N(2)	1.392(6)	C(7)–N(8)	1.470(7)

Cl(1)–Ca(3)–Cl(1)[a]	180.0	Cl(9)–Ca(5)–O(3)	81.8(5)
N(2)–Ca(3)–N(2)[b]	180.0	C(5)–C(1)–N(2)	108.8(4)
O(1)–Ca(3)–O(1)[c]	180.0	C(1)–N(2)–C(3)	105.4(4)
Cl(1)–Ca(3)–N(2)	89.3(5)	N(2)–C(3)–N(4)	111.3(4)
Cl(1)–Ca(3)–O(1)	92.9(4)	C(3)–N(4)–C(5)	107.9(4)
N(2)–Ca(3)–O(1)	91.3(5)	N(4)–C(5)–C(1)	106.6(4)
Cl(2)–Ca(5)–Cl(2)[d]	180.0	C(5)–C(1)–C(6)	130.2(4)
Cl(9)–Ca(5)–Cl(9)[e]	180.0	N(2)–C(1)–C(6)	120.9(4)
O(3)–Ca(5)–O(3)[e]	180.0	C(1)–C(6)–C(7)	111.7(4)
Cl(2)–Ca(5)–Cl(9)	97.2(5)	C(6)–C(7)–N(8)	110.4(4)
Cl(2)–Ca(5)–O(3)	88.1(5)		

[a] $-x, -y, 1-z$; [b] $1-x, -y, -z$; [c] $-x, -y, -z$; [d] $-x, 1-y, -z$; [e] $-x, 1-y, 1-z$.

Table 5. Bond angles (°) and distances (Å) for histamine hydrobromide (**2**)

C(1)–N(2)	1.390(9)	C(5)–C(1)	1.335(10)
N(2)–C(3)	1.315(10)	C(1)–C(6)	1.507(10)
C(3)–N(4)	1.329(10)	C(6)–C(7)	1.504(10)
N(4)–C(5)	1.363(10)	C(7)–N(8)	1.516(9)

C(5)–C(1)–N(2)	108.9(6)	C(5)–C(1)–C(6)	129.6(7)
C(1)–N(2)–C(3)	105.2(6)	N(2)–C(1)–C(6)	121.4(6)
N(2)–C(3)–N(4)	111.6(7)	C(1)–C(6)–C(7)	110.9(6)
C(3)–N(4)–C(5)	107.2(6)	C(6)–C(7)–N(8)	109.6(6)
N(4)–C(5)–C(2)	107.1(7)		

Calcium atoms are more than 6.7 Å apart as befits calcium atoms that are linked by networks of hydrogen bonding and not by carboxylate groups as in the solid state Ca(nicotinate)$_2$-$(H_2O)_2(H_2O)_3$ structure[21] (Ca–Ca 4.05 Å).

Histamine hydrobromide shows tautomeric form **D** of the histamine, protonated at the side-chain amine group. The side-chain conformation shows the NH_3^+ and imidazole rings to be *trans* about the C(6)–C(7) bond [dihedral angle 177.2(7)°] as in (**1**). Bromine is within hydrogen bonding distance of three hydrogen atoms; H(81), H(83), and H(4), and 3.120(1) Å from a second H(83). Thus H(83) is bifurcated between two bromide atoms. H(81) and H(4) display a single set of close unshared pairs (see Supplementary data). The nitrogen bearing an unshared pair of electrons, N(2), is 2.16 Å from H(82) of a second histamine group. Thus the packing of molecules into the unit cell of (**2**) serves to maximize hydrogen bonding opportunities.

Structures (**1**) and (**2**) offer a unique structural view of protonated form, **D**, of histamine whereas previous studies have

identified form **A** or side-chain protonated forms of resonance isomers **C**; **F**. Histamine forms **B** and **D** remain unobserved structurally.

Calcium complexes with histamine. In the solid-state form examined, calcium has formed a complex with unidentate histamine molecules of form 4-(2-aminoethylimidazole) protonated at the side-chain amine group. Thus calcium may have a direct structural involvement in histamine release. Further work will investigate the complexes formed by calcium and histamine with control of pH.

Acknowledgements

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