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# The calcium-binding properties of pamidronate, a bone-resorption inhibitor

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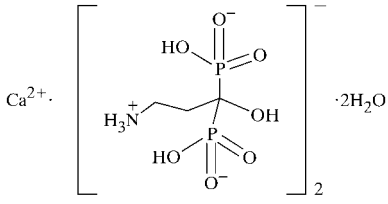
The title compound, calcium bis(3-ammonio-1-hydroxypropylidene-1,1-bisphosphonate) dihydrate, $Ca^{2+}\cdot 2C_3H_{10}NO_7P_2^{-}\cdot 2H_2O$, consists of calcium octahedra arranged in columns along the *c* axis and coordinated by hydrogen-bonded molecular anions. The $Ca^{2+}$ cation lies on a twofold axis. Pamidronate adopts a twisted conformation of the hydroxyalkylamine backbone that enables the formation of an intramolecular N—H···O hydrogen bond. The molecular anion is chelating monodentate as well as bidentate, with an O···O bite distance of 3.0647 (15) Å.

## Comment

*gem*-Bisphosphonates are commonly used in clinical practice as safe and efficacious therapeutic agents for the treatment of a number of bone disorders, such as osteoporosis, Paget's disease and hypercalcaemia associated with malignancy (Compston, 1994; Russell & Rogers, 1999; Rodan & Martin, 2000). These compounds have the $PO_3$ groups bridged by the geminal C atom, an atomic connectivity which, though chemically and enzymatically non-hydrolizable, resembles that of inorganic pyrophosphate. As has been recognized previously, these compounds are able to affect the growth of calcium hydroxyapatite crystals. In connection with this, the calcium salts of the bisphosphonates etidronate [calcium dihydrogen 1-hydroxyethane-1,1-diphosphonate dihydrate, $CaH_2EHDP\cdot 2H_2O$; Cambridge Structural Database (CSD; Allen *et al.*, 1983) refcode CAEHDP (Uchtman, 1972)] and clodronate [calcium dichloromethylene-1,1-diphosphonate pentahydrate, $CaH_2Cl_2MDP\cdot 5H_2O$; CSD refcode CAVKUF (Nardelli *et al.*, 1983)] have been studied crystallographically and their chelating capabilities unveiled. Subsequently, biological activity has been associated with the mechanism of action of the bisphosphonates (Felix & Fleisch, 1981). At present, it is known that the surface of bone is resorbed by specialized cells, so the bisphosphonates are incorporated, but not metabolized, by the osteoclasts, thus leading selectively to their loss of activity and death (Fisher *et al.*, 1999; Rogers *et al.*, 2000; Coxon *et al.*, 2001; van Beek *et al.*, 2002).

In a similar manner, bisphosphonates have been found to be inhibitors of diverse enzymes (Bau *et al.*, 1988; Smirnova *et al.*, 1988; Reiersen *et al.*, 1994; Atack & Fletcher, 1994; Gordon-Weeks *et al.*, 1999) and, as such, they are currently being investigated as herbicides (Chuiko *et al.*, 1999; Cromartie *et al.*, 1999) and antiparasitics (Docampo, 2001). In this latter context, molecular modelling work has been carried out to develop a new therapeutic agent for the treatment of American trypanosomiasis (Fernández, 2002). The basis of the design is one of the clinically used bisphosphonates, so to obtain experimental data on the conformation of the ligand in a complex with a divalent metal cation, possibly the true substrate for the enzyme, we undertook the single-crystal X-ray analysis of the title compound, (I), and the results are presented here.




(I)

In the molecular anion of (I) (Fig. 1), which can also be denoted $CaH_2PAM$, the geminal C1 atom is substituted with a pair of negatively charged $PO_3H^-$ groups, an OH group and an alkylamine lateral chain containing a tetrahedral N atom. As with the previously studied free acid, $H_3PAM$ (Shkol'nikova *et al.*, 1990), and the pentahydrated disodium salt, $Na_2HPAM$ (Vega *et al.*, 2002), (I) has a zwitterionic character, with atom N1 bearing the positive charge, but here the overall charge is −1, so the zwitterion forms a 2:1 complex with $Ca^{2+}$.

From Table 1, it is evident that the geometry around the P atoms is tetrahedral. The O—P—C bond angles are somewhat less than the ideal tetrahedral value [105.64 (7)–109.90 (7)°], while the O—P—O angles involving the two deprotonated O atoms are the largest in both groups; the P—O(deprotonated) distances indicate a double delocalized bond and the P—O(protonated) bonds are single. These are very similar to the geometric parameters found in the single $PO_3H^-$ group in $H_3PAM$, but they differ slightly from those in $Na_2HPAM$, where this group has an unequal distribution of the negative charge among the deprotonated O atoms (Vega *et al.*, 2002).

The P—C bond lengths are comparable in the three structures, with values of 1.848 (2) and 1.854 (2) Å in $H_3PAM$, 1.845 (4) and 1.869 (3) Å in $Na_2HPAM$, and 1.846 (2) and 1.851 (2) Å in (I). However, the P—C—P angle in (I) is 2° wider than in the other two compounds.

The mutual orientation of the $PO_3$ groups enables the formation of a planar 'W' arrangement of the O—P—C—P—O chain, where one protonated and one deprotonated O atom lies in the plane [O2—P1—C1—P2 164.6 (1)° and O6—P2—

C1—P1 165.6 (2)°]. A similar configuration is observed in $Na_2HPAM$ (171.4 and 161.0°), while in the plane of the 'W' in $H_3PAM$ (174.5 and 162.9°), there are two protonated O atoms.

The main structural difference between the three compounds is found in the conformation of the O—C—C—C—N backbone, which adopts a *gauche*− conformation in (I), as shown by the value of the C1—C2—C3—N1 torsion angle of −72.1 (2)°. However, in $H_3PAM$ (168.9°) and $Na_2HPAM$ (153.6°), this backbone is *trans*. In addition, the hydroxyl group in (I) is nearly 30° more inclined toward the lateral chain [O7—C1—C2—C3 34.7 (2)°] than in the other two structures [66.5 (1)° in $H_3PAM$ and 59.6 (1)° in $Na_2HPAM$]. Therefore, the twisted conformation of the backbone of pamidronate in the calcium salt, (I), facilitates an intramolecular N1—H5···O7 hydrogen bond (Table 2), which leads to a six-membered ring made up of all the hydroxyalkylamine atoms. However, this cannot be formed by $H_3PAM$ or $Na_2HPAM$, because the extended conformation of the backbone separates N1 from O7 by more than 4 Å.

The $Ca^{2+}$ cation lies on a twofold axis parallel to **b** (Fig. 2). The coordination sphere around the $Ca^{2+}$ cation is octahedral and consists of six phosphonyl O atoms, half of which are symmetry independent. The $Ca^{2+}$ cation lies in the plane defined by atoms O5, O3($1-x, -y, -z$) and their symmetry equivalents (r.m.s. deviation from the plane = 0.028 Å). There are two O1 atoms, at 2.2867 (12) Å above and below this plane, forming an O1···Ca···O1($1-x, y, -z-\frac{1}{2}$) angle of 167.72 (6)°. The Ca···O contact distances are between 2.2878 (12) and 2.3871 (12) Å (Table 1), and the O1···O5 bite distance is 3.0647 (15) Å.

The remaining phosphonyl O atoms, namely the deprotonated atom O6 and the protonated atoms O2 and O4, are not coordinated and there are no other contacts of less than 3.2 Å to indicate additional coordination to $Ca^{2+}$. The alcohol atom O7 is separated by *ca* 3.9 Å from the metal cation, and hence the pamidronate cannot function as a tridentate ligand; this is different from what is observed in $Na_2HPAM$.

The hydrate water molecule is located near the positive end of the zwitterion in (I), so, as with one of the water molecules in the disodium salt, it is not in the coordination sphere of the metal cation.

On inspecting Fig. 2, it is evident that the $Ca^{2+}$ cations are stacked in a columnar fashion (as with the $Na^+$ cations in the disodium salt) along the *c* axis, and this is sustained by a three-dimensional framework of hydrogen-bonded pamidronate ligands. The latter are disposed in the column in the manner expected from their zwitterionic character; the negative end faces the $Ca^{2+}$ cation in the centre, while the positive end is stretched outside.

The molecular anion is chelating bidentate, using one deprotonated O atom from each $PO_3$ group (O1 and O5) and, at the same time, it is chelating monodentate to a symmetry-related $Ca^{2+}$ cation *via* another deprotonated phosphonyl O atom (O3).

The intermolecular hydrogen-bonding scheme (Table 2) involves two O(phosphonyl)···O(phosphonyl) interactions [mean 2.543 (5) Å], one O(phosphonyl)···O(hydroxy) [2.765 (2) Å], two O(phosphonyl)···O(water) [mean 2.84 (14) Å] and two O(phosphonyl)···N [mean 2.967 (4) Å]. As with $H_3PAM$ and $Na_2HPAM$, atom N1 is a hydrogen-bond donor to a pair of phosphonyl O atoms, but due to the fact that it forms an intramolecular contact with atom O7, these interactions appear to be weaker (*ca* 0.2 Å longer) than in the other compounds. The remaining interaction occurs with the hydrate water in (I) and $Na_2HPAM$, or with a symmetry-related hydroxyl O atom in $H_3PAM$, and agrees well in the three structures, with N···O distances in the range 2.838 (2) [in (I)] to 2.878 (2) Å (in $H_3PAM$).

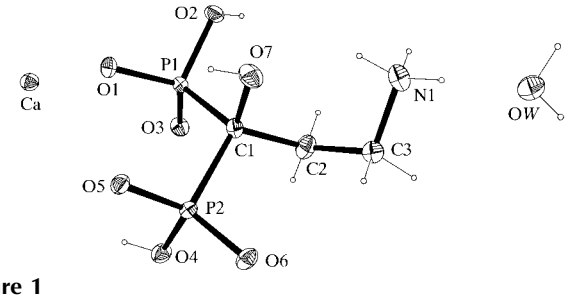



**Figure 1**
A view of (I), showing the atom-numbering scheme, with displacement ellipsoids drawn at the 50% probability level. H atoms are shown as small spheres of arbitrary radii.

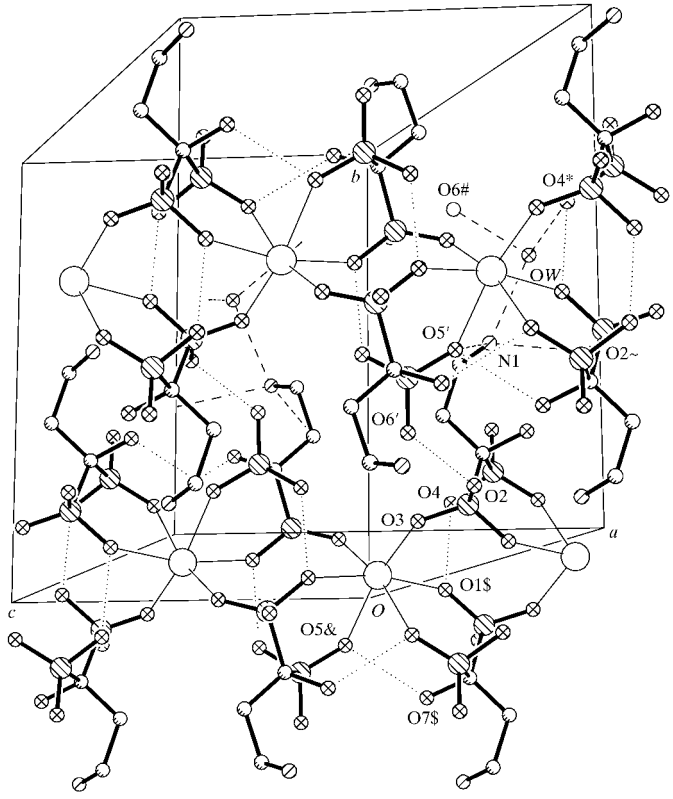



**Figure 2**
A simplified packing diagram for (I), showing the Ca coordination sphere (thin solid lines) and zwitterionic hydrogen bonds (dotted lines), as well as some involving N atoms and water (dashed lines). Atoms labelled with a dollar sign (\$), ampersand (&), hash (#), prime (′), tilde (~) or asterisk (*) are at symmetry positions ($1-x, -y, -z$), ($1-x, y, -z-\frac{1}{2}$), ($1-x, 1-y, -z$), ($x+\frac{1}{2}, \frac{1}{2}-y, z+\frac{1}{2}$), ($\frac{3}{2}-x, \frac{1}{2}-y, -z$) or ($x+\frac{1}{2}, y+\frac{1}{2}, z$), respectively.

A comparison of the crystal structure of (I) with those of $CaH_2EHDP{\cdot}2H_2O$ (Uchtman, 1972) and $CaH_2Cl_2MDP{\cdot}5H_2O$ (Nardelli *et al.*, 1983) shows good agreement concerning the calcium-chelating properties of the anions. Although the coordination number of the $Ca^{2+}$ cation differs, being 6 in (I), 7 in $CaH_2Cl_2MDP{\cdot}5H_2O$ and 8 in $CaH_2EHDP{\cdot}2H_2O$, and noting that, in the latter two compounds, the ligands act as dianions in 1:1 $Ca^{2+}$ complexes, other relevant features are common to all three compounds. First, the ligand is chelating monodentate and/or bidentate, but in no case is there tridentate chelation, as has invariably been seen with the corresponding $Na^+$ salts. Secondly, the bidentate Ca···O(phosphonyl) distances are within the narrow range 2.31–2.42 Å, a fact possibly related to the presence of the pair of monoprotonated $PO_3H^-$ groups attached to the geminal C atom. Lastly, and most importantly, they have an O···O bite distance of between 2.9 and 3.1 Å in common. As suggested by Nardelli *et al.* (1983), who observed that this atomic disposition compares well with that found in the O atoms more tightly bound to $Ca^{2+}$ in calcium hydroxyapatite, this explains the biological activity and argues against a tridentate calcium-chelating behaviour of the *gem*-bisphosphonates.

## Experimental

A sample of disodium pamidronate was obtained from Laboratorios Gador S.A., Buenos Aires, Argentina. The calcium salt was prepared as described by Uchtman (1972). A powdered sample of disodium pamidronate ($M_r$ = 369.11) was added to $CaHPO_4{\cdot}2H_2O$ ($M_r$ = 172.09; calcium hydrogen phosphate dihydrate; Riedel–de Haën, Germany) and then placed in an excess of water. Crystals of (I) suitable for X-ray diffraction were obtained by evaporating this solution in an oven at 315 K.

*Crystal data*

$Ca^{2+}{\cdot}2C_3H_{10}NO_7P_2^-{\cdot}2H_2O$
$M_r$ = 544.24
Monoclinic, $C2/c$
$a$ = 14.2921 (9) Å
$b$ = 14.2755 (9) Å
$c$ = 11.1465 (7) Å
$\beta$ = 119.0310 (10)°
$V$ = 1988.4 (2) Å$^3$
$Z$ = 4
$D_x$ = 1.818 Mg m$^{-3}$
Mo $K\alpha$ radiation
Cell parameters from 844 reflections
$\theta$ = 3.9–26.0°
$\mu$ = 0.72 mm$^{-1}$
$T$ = 120 (2) K
Prism, colourless
0.16 × 0.12 × 0.11 mm

*Data collection*

Bruker SMART-6000 CCD area-detector diffractometer
$\omega$ scans
Absorption correction: by integration (*XPREP* in *SHELXTL-NT*; Bruker, 1998)
$T_{min}$ = 0.876, $T_{max}$ = 0.915
10 866 measured reflections
3036 independent reflections
2778 reflections with $I > 2\sigma(I)$
$R_{int}$ = 0.016
$\theta_{max}$ = 30.5°
$h = -20 \rightarrow 20$
$k = -20 \rightarrow 20$
$l = -15 \rightarrow 15$

*Refinement*

Refinement on $F^2$
$R(F)$ = 0.035
$wR(F^2)$ = 0.100
$S$ = 1.06
3036 reflections
164 parameters
H atoms treated by a mixture of independent and constrained refinement
$w = 1/[\sigma^2(F_o^2) + (0.0541P)^2 + 4.9457P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.007
$\Delta\rho_{max}$ = 1.55 e Å$^{-3}$
$\Delta\rho_{min}$ = −0.43 e Å$^{-3}$

**Table 1**
Selected geometric parameters (Å, °).

| | | | |
|---|---|---|---|
| O1—P1 | 1.5220 (12) | O6—P2 | 1.5039 (13) |
| O2—P1 | 1.5660 (13) | Ca—O3$^{i}$ | 2.2878 (12) |
| O3—P1 | 1.4965 (12) | Ca—O1$^{ii}$ | 2.3080 (12) |
| O4—P2 | 1.5750 (13) | Ca—O5$^{ii}$ | 2.3871 (12) |
| O5—P2 | 1.5167 (12) | | |
| P1—C1—P2 | 112.55 (9) | O6—P2—O5 | 115.57 (7) |
| O3—P1—O1 | 116.73 (7) | O6—P2—O4 | 107.33 (7) |
| O3—P1—O2 | 112.45 (7) | O5—P2—O4 | 110.22 (7) |
| O1—P1—O2 | 104.92 (7) | | |
| O7—C1—C2—C3 | 34.65 (19) | C1—C2—C3—N1 | −72.1 (2) |

Symmetry codes: (i) $1-x, -y, -z$; (ii) $1-x, y, -\frac{1}{2}-z$.

**Table 2**
Hydrogen-bonding geometry (Å, °).

| $D$—H···$A$ | $D$—H | H···$A$ | $D$···$A$ | $D$—H···$A$ |
|---|---|---|---|---|
| C3—H9···O6 | 0.99 | 2.46 | 3.180 (2) | 129 |
| N1—H4···O$W$ | 0.90 (3) | 1.95 (3) | 2.838 (2) | 171 (3) |
| N1—H5···O7 | 1.00 (4) | 1.93 (4) | 2.692 (2) | 132 (3) |
| O4—H3···O1$^{i}$ | 0.79 (3) | 1.77 (3) | 2.5476 (18) | 167 (3) |
| O7—H1···O5$^{ii}$ | 0.94 (5) | 1.86 (5) | 2.7655 (19) | 161 (4) |
| O2—H2···O6$^{iii}$ | 0.68 (4) | 1.86 (4) | 2.5380 (18) | 174 (5) |
| N1—H6···O5$^{iii}$ | 0.91 (3) | 2.10 (3) | 2.970 (2) | 159 (3) |
| N1—H5···O2$^{iv}$ | 1.00 (4) | 2.20 (4) | 2.964 (2) | 133 (3) |
| O$W$—H11···O6$^{v}$ | 0.76 (4) | 1.95 (4) | 2.7010 (19) | 176 (4) |
| O$W$—H12···O4$^{vi}$ | 0.80 (4) | 2.18 (4) | 2.979 (2) | 171 (3) |

Symmetry codes: (i) $1-x, -y, -z$; (ii) $1-x, y, -\frac{1}{2}-z$; (iii) $\frac{1}{2}+x, \frac{1}{2}-y, \frac{1}{2}+z$; (iv) $\frac{3}{2}-x, \frac{1}{2}-y, -z$; (v) $1-x, 1-y, -z$; (vi) $\frac{1}{2}+x, \frac{1}{2}+y, z$.

The H atoms attached to C atoms were fixed at 0.99 Å from their hosts and refined using a riding model, with $U_{iso}(H) = 1.3U_{eq}(C)$. The other H atoms had their positional and displacement parameters freely refined. The highest positive peak in the Fourier difference map was 1.32 Å from atom H10.

Data collection: *SMART-NT* (Bruker, 1998); cell refinement: *SMART-NT*; data reduction: *SAINT-NT* (Bruker, 1998); program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *XP* in *SHELXTL/PC* (Sheldrick, 1991); software used to prepare material for publication: *PARST* (Nardelli, 1995) and *WinGX* (Farrugia, 1999).

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: OB1076). Services for accessing these data are described at the back of the journal.

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