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The Structure, Conformation and Metal Coordination of Choline Phosphate Calcium Chloride Tetrahydrate. A Model for Membrane Surface Interactions

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Abstract. $C_5H_{13}NO_4P^-.Ca^{2+}.Cl^-.4H_2O$, triclinic, $P\bar{1}$, $a = 5{\cdot}797\,(1)$, $b = 7{\cdot}473\,(1)$, $c = 16{\cdot}428\,(2)$ Å, $\alpha = 87{\cdot}591\,(2)$, $\beta = 73{\cdot}268\,(4)$, $\gamma = 90{\cdot}457\,(4)^\circ$, $Z = 2$. The structure was solved by direct methods and refined by full-matrix least squares to an R index of 0·09 using 1855 intensities measured on a Picker diffractometer. The O—C—C—N atomic sequence exhibits the characteristic *gauche* (±57°) conformation despite the presence of the divalent metal ion. The calcium ion is tightly coordinated by three water oxygens and three phosphate oxygens. The chloride ion sits in a positively charged pocket formed by the quaternary ammonium groups. An intricate network of hydrogen bonds involving the phosphate and water oxygens interconnects the choline phosphate molecules and the calcium coordination sphere. This structure provides a model for the mode of interaction of calcium and water with the phospholipids at the surface of biological membranes.

Introduction. As part of a program of research in these laboratories on structural and conformational properties of phospholipids and their constituents, we report the structure of choline phosphate calcium chloride tetrahydrate. Choline phosphate forms the head group of phosphatidylcholine (lecithin) which is one of the more abundant components of biological membranes. This structural study was undertaken to determine the effect of calcium on the conformation of the O—C—C—N function and to provide insights into the mode of interaction of calcium and water molecules with phospholipids.

Choline phosphate was crystallized as the calcium chloride tetrahydrate complex from an aqueous ethanol solution. Weissenberg, oscillation and precession photographs indicated that the crystals were triclinic. Intensity data were collected on a Picker diffractometer with Cu $K\alpha$ radiation in the $\omega/2\theta$ scan mode. Due to problems of crystal decay in the X-ray beam, the data were collected in two sets ($hkl, h\bar{k}l$ and $hk\bar{l}, h\bar{k}\bar{l}$) using two different crystals. 2912 intensities were measured, of which 2338 were unique. After correction for Lorentz and polarization effects, the two data sets were scaled based on 287 pairs of common reflections and merged. The $R_{\rm sym}[=\sum(F_i - F_{\rm av})/\sum F_{\rm av}]$ for the common reflections was 0·085. 1855 reflections with $I > 1{\cdot}5\sigma(I)$, where $\sigma(I)$ is the standard deviation computed from counting statistics, were considered to be observed and were used in the subsequent structure analysis. Intensity statistics clearly indicated the space group to be $P\bar{1}$.

The structure was solved by direct methods using the program *MULTAN* (Main, Woolfson & Germain, 1971). The initial E map revealed the positions of all the atoms of the choline phosphate molecule as well as the calcium and chloride ions. An electron density map phased on these atoms yielded the positions of the four water oxygens in the asymmetric unit.

Several cyles of full-matrix least-squares refinement minimizing $w(F_o - F_c)^2$ with isotropic temperature factors for the 17 nonhydrogen atoms lowered the crystallographic R index $[=\sum(|F_o| - |F_c|)/\sum|F_o|]$ to 0·11. Inclusion of the 21 H atoms obtained by difference electron density maps and further refinement of the nonhydrogen atoms reduced the R index to 0·09.‡ The shift/σ ratios for the atomic parameters were all less than 0·10. The final difference Fourier map was essentially featureless and contained no peaks larger than 0·3 e Å^{-3}. Weights were applied for the reflections in the final stages of refinement by the method of Hughes (1941) and are given by $w = 1$ for $F_o < 12{\cdot}8$ and $w = 12{\cdot}8/F_o$ for $F_o \geq 12{\cdot}8$. Scattering factors from Cromer & Waber (1965) were used for the heavy atoms and from Stewart, Davidson & Simpson (1965) for the H atoms.

Discussion. The positional parameters for the atoms are listed in Table 1. Standard deviations in the

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‡ Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 34513 (11 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

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Table 1. *Positional parameters for the nonhydrogen atoms* ($\times 10^4$) *and for hydrogen atoms* ($\times 10^3$) *and their estimated standard deviations in choline phosphate calcium chloride tetrahydrate*

	x	*y*	*z*
Ca	4197 (2)	6762 (1)	4248 (1)
P	9466 (3)	3763 (2)	3963 (1)
Cl	5675 (4)	7453 (3)	923 (1)
N	8851 (11)	2403 (7)	1338 (4)
O(1)	9482 (9)	3098 (6)	3035 (3)
O(2)	9494 (8)	2127 (6)	4543 (3)
O(3)	7119 (8)	4719 (6)	4345 (3)
O(4)	11701 (8)	4940 (6)	3749 (3)
C(1)	7541 (13)	2001 (10)	2970 (4)
C(2)	6914 (13)	2486 (10)	2164 (4)
C(3)	10311 (16)	784 (10)	1285 (5)
C(4)	7687 (18)	2418 (12)	625 (5)
C(5)	10505 (15)	4025 (10)	1185 (5)
O(*W*1)	1314 (9)	7427 (7)	2516 (3)
O(*W*2)	6533 (8)	7018 (7)	2767 (3)
O(*W*3)	2767 (8)	9489 (6)	3666 (3)
O(*W*4)	6975 (10)	8652 (6)	4639 (3)
H(11)	832	82	294
H(12)	608	180	356
H(21)	587	159	206
H(22)	565	371	231
H(31)	1105	84	169
H(32)	946	26	136
H(33)	1184	77	67
H(41)	678	341	66
H(42)	653	133	78
H(43)	900	240	12
H(51)	1121	421	159
H(52)	1194	393	53
H(53)	938	521	129
H(*W*11)	167	665	267
H(*W*12)	250	721	195
H(*W*21)	613	715	226
H(*W*22)	809	708	273
H(*W*31)	224	935	327
H(*W*32)	171	1040	401
H(*W*41)	791	819	501
H(*W*42)	780	997	450

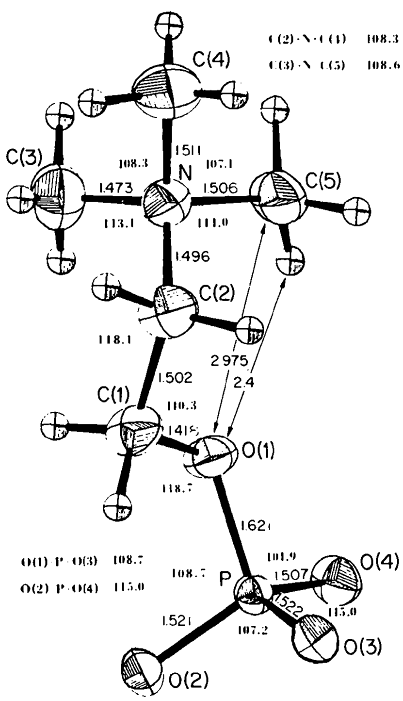

Fig. 1. A thermal-ellipsoid plot showing the bond distances (Å) and angles (°), and the molecular conformation of the choline phosphate molecule. The average standard deviations for all bond distances and angles not involving the P atom are 0·010 Å and 0·7° respectively and for those involving P are 0·005 Å and 0·2°.

positional parameters for Ca, Cl and P are approximately 0·001 to 0·002 Å and for O, N and C are 0·005 to 0·008 Å. Fig. 1 depicts the thermal ellipsoids (Johnson, 1965) of the nonhydrogen atoms of choline phosphate and the bond distances and angles. The most notable feature about the molecular dimensions is the angle C(1)–C(2)–N of 118·1° which appears to be significantly larger than the values (av. 114·6°) found in the other known choline head groups (Sundaralingam, 1972). This angle in the choline group is considerably larger than those observed in the ethanolamine group (av. 111·6°) due to the presence of the bulky quarternary ammonium group.

The major conformational features of the choline phosphate molecule are shown in Fig. 1. The relevant torsion angles for the molecule are given in Table 2. It is interesting that the conformation of the O(1)–C(1)–C(2)–N group is *gauche* (±57°) despite the presence of the highly charged calcium and chloride ion matrix of the crystal lattice. This conformation results in close intramolecular contacts between O(1) and the quarternary N atom of 2·98 Å and between O(1) and C(5) of 2·97 Å. The O(1)···N distance is approximately equal to the allowed van der Waals contact; however, the O(1)···C(5) distance is less than the van der Waals limit by 0·4 Å (Pauling, 1960). Rotation has occurred about the C(2)–N bond to remove the methyl groups from their ideally staggered position with respect to the C(1)–C(2) bond (see Table 2) in order to partially relieve the close contact between O(1) and C(5). The *gauche* conformation is apparently stabilized by the electrostatic attraction between the positively charged ammonium group and the negatively charged phosphate group (Sundaralingam, 1968). The phosphate oxygens O(2), O(3) and O(4) are staggered with respect to C(1). Similar conformational features have been observed for the other phospholipid head groups whose structures are known (Sundaralingam, 1972; DeTitta & Craven, 1973) and for the recently determined structure of the phospholipid dilauroylphosphatidyl ethanolamine (Elder, Hitchcock, Mason & Shipley, 1977). It has been noted that a large number of muscarinic and cholinergic effectors containing the O–C–C–N function also display similar conforma-

Table 2. *Torsion angles (°) in choline phosphate calcium chloride tetrahydrate*

The estimated standard deviations are given in parentheses.

O(2)–P–O(1)–C(1)	61·1 (6)	O(1)–C(1)–C(2)–N	57·0 (9)
O(3)–P–O(1)–C(1)	−55·3 (6)	C(1)–C(2)–N–C(3)	43·8 (9)
O(4)–P–O(1)–C(1)	−177·1 (5)	C(1)–C(2)–N–C(4)	164·0 (7)
P–O(1)–C(1)–C(2)	142·5 (5)	C(1)–C(2)–N–C(5)	−78·6 (9)

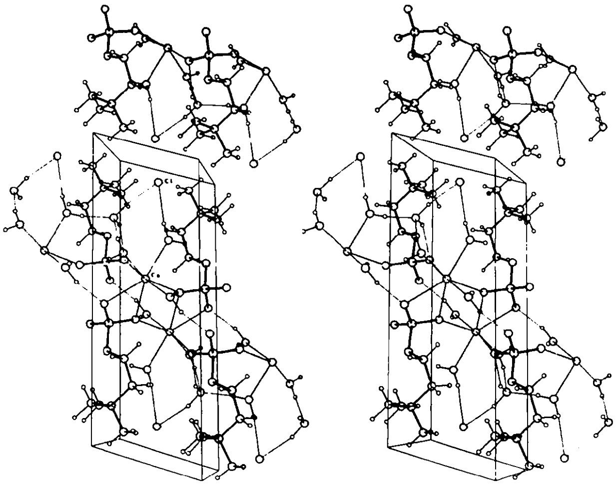

Fig. 2. A stereoscopic diagram viewed down the *a* axis; the hydrogen bonds and calcium coordination are shown by broken lines.

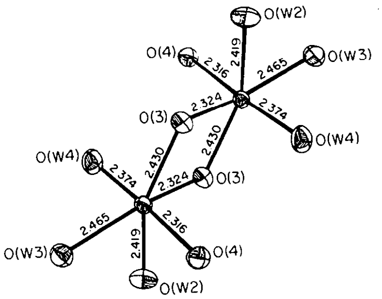

Fig. 3. The inner calcium coordination sphere showing the Ca—O distances. Only the six closest contacts are shown (see text). The average standard deviation for these distances is 0·005 Å.

Table 3. *Hydrogen-bond distances in the crystal structure of choline phosphate calcium chloride tetrahydrate*

The average standard deviation for these distances is 0·01 Å.

D–H···*A*	*D*–*A*
O(*W*1)–H(*W*11)···O(4)(II)*	2·750 Å
O(*W*1)–H(*W*12)···Cl(I)	3·070
O(*W*2)–H(*W*21)···Cl(I)	3·212
O(*W*2)–H(*W*22)···O(*W*1)(III)	2·695
O(*W*3)–H(*W*31)···O(*W*1)(I)	2·792
O(*W*3)–H(*W*32)···O(2)(IV)	2·879
O(*W*4)–H(*W*41)···O(2)(V)	2·806
O(*W*4)–H(*W*42)···O(2)(VI)	2·945

* The symmetry operations applied to the acceptor atoms are: (I) [1;0,0,0], (II) [1;−1,0,0], (III) [1;1,0,0] (IV) [1;−1,1,0], (V) [2;2,1,1], (VI) [1;0,1,0]. In this representation, the first number in the square brackets corresponds to the symmetry operations: (1) x,y,z or (2) $\bar{x},\bar{y},\bar{z}$. The remaining three numbers are the subsequent translations along x, y, and z respectively.

tional features (Sundaralingam, 1968; Baker, Chothia, Pauling & Petcher, 1971).

Fig. 2 shows a stereoview of the contents of the unit cell in addition to molecules from neighboring unit cells. It can be seen that the centrosymmetrically related calcium ions are at the centers of two capped octahedra having a common edge formed by the centrosymmetrically related phosphate oxygens O(3). Each capped octahedron is composed of the three water oxygens O(*W*2), O(*W*3), and O(*W*4), and a somewhat weaker ligand provided by a second oxygen O(2) of one of the phosphate groups (Fig. 2). The Ca···O distances for the six stronger ligands range between 2·32 and 2·46 Å (Fig. 3) while that of the seventh ligand is 2·63 Å.

The chloride ion is located in a positively charged pocket surrounded by the quaternary ammonium groups of symmetry-related molecules illustrating the delocalized nature of the positive charge over the quaternary ammonium group. A somewhat analogous situation was noted in the structure of puromycin hydrochloride where a chloride ion is in close contact with the N(6)-dimethyl groups of the protonated adenine base (Sundaralingam & Arora, 1972). The chloride ion is bridged to the calcium ion through a hydrogen bond to water molecules *W*(2) (Fig. 2). A second hydrogen bond to the chloride ion is provided by water molecule *W*(1) which in turn is linked to another water and a phosphate oxygen in the calcium coordination sphere.

The degree of hydration found here for the phospholipid head group is higher than in any of the previously elucidated structures, and is most probably due to the presence of the metal ion. The intricate hydrogen-bond network (Fig. 2 and Table 3) involving the calcium and chloride ions, the water molecules and the phosphate groups provides an interesting model for the tight shell of water known to be bound to the membrane surface (Keith, Snipes & Chapman, 1977).

Local-anesthetic molecules are found to bind to the surface of the biological membrane with inhibition of calcium binding (Blaustein & Goldman, 1966). Crystallographic studies have suggested that these molecules are involved in a strong hydrogen bond to the phosphate group of the phospholipids (Sax & Pletcher, 1969). The binding of calcium to the phosphate observed in this structure thus supports the idea that the local anesthetics compete for the calcium binding sites on the phosphate group.

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Ethyl Ester of (*Z*)-2-Ferrocenyl-2-methylcyclopropanecarboxylic Acid

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Abstract. $C_{17}H_{20}FeO_2$, $M_r = 312{\cdot}2$, triclinic, $P\bar{1}$, $a = 7{\cdot}580\,(2)$, $b = 11{\cdot}121\,(3)$, $c = 10{\cdot}187\,(3)$ Å, $\alpha = 100{\cdot}48\,(5)$, $\beta = 103{\cdot}85\,(5)$, $\gamma = 111{\cdot}85\,(5)^\circ$, $d_m = 1{\cdot}39$, $Z = 2$, $d_x = 1{\cdot}40$ Mg m^{-3}, $V = 738{\cdot}2$ Å^3. The structure was solved by heavy-atom methods and refined to $R_F = 0{\cdot}033$ for 2512 reflexions. X-ray analysis confirmed the *Z* configuration of the title compound. The cyclopentadienyl rings in the ferrocenyl skeleton are parallel within 1·3° and twisted about 3·8° from the eclipsed conformation.

Introduction. Although most ferrocene derivatives have been explored, little is known about the chemistry of the ferrocenylcycloalkanes. This work is an example of an X-ray study of the structural properties of the smallest ring, *i.e.* cyclopropane, combined with a ferrocenyl substituent. The X-ray investigations were undertaken in order to determine unequivocally the configuration and the preferable conformation of the ethyl ester of (*Z*)-2-ferrocenyl-2-methylcyclopropanecarboxylic acid.

Crystals suitable for X-ray analysis were obtained by slow cooling of a saturated petroleum ether solution. The crystal chosen was cut and formed into the shape of a barrel 0·2 mm in diameter and height. The measurements were performed on a Syntex $P2_1$ four-circle diffractometer with Mo $K\alpha$ ($\lambda = 0{\cdot}71069$ Å) graphite-monochromated radiation. The data set ($2\theta_{\max} = 60^\circ$, θ–2θ scan, scan speeds varying linearly between 2·0 and 29·30° min^{-1}) contained 3033 reflexions, of which 2518 with $I > 3\sigma(I)$ were used in the calculations. Intensities were corrected for Lorentz and polarization effects but not for absorption ($\mu_l = 1{\cdot}05$ mm^{-1}).

Structure analyses attempted in space groups $P1$ and $P\bar{1}$ showed the latter to be correct. Firstly, as the piezo effect was ambiguous, space group $P1$ was chosen on the basis of *E* statistics. The structure was solved by heavy-atom methods and refined by full-matrix least squares with anisotropic thermal parameters for non-hydrogen atoms to $R = 0{\cdot}049$. The H atoms were easily located from a difference map, and were included in the calculations with fixed parameters. The *R* value fell to 0·033 but convergence in the refinement process could not be reached and a model of the molecular

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