# Conformation of the Choline Phosphate Zwitterion. Crystal and Molecular Structure of Cyclopentylphosphorylcholine Monohydrate, $[(CH_3)_3N^+CH_2CH_2O](c\text{-}C_5H_9O)P(O)\overline{O}\cdot H_2O$

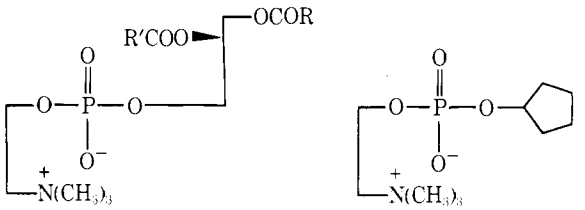
**Raghupathy Sarma,[1a] Fausto Ramirez,*[1b] Poojappan Narayanan,[1a,b] Brian McKeever,[1a] Hiroshi Okazaki,[1b] and James F. Marecek[1b]**

*Contribution from the Biochemistry and Chemistry Departments of the State University of New York at Stony Brook, Stony Brook, New York 11794.* *Received December 27, 1977*

**Abstract:** Cyclopentylphosphorylcholine (CPPC) was synthesized from cyclopentanol and choline chloride, $(CH_3)_3N^+CH_2CH_2OH\overline{Cl}$, by the cyclic enediol phosphate method. CPPC crystallizes from moist methanol/acetone in the monoclinic system, space group $P2_{1/c}$. There are four molecules of the monohydrate, $C_{10}H_{22}O_4NP\cdot H_2O$, in the unit cell ($Z = 4$). The cell dimensions measured at −40 °C are $a = 12.688$ (3), $b = 9.782$ (3), $c = 11.034$ (5) Å, $\beta = 94.33$ (3)°; $\rho_{calcd} = 1.27$ g cm$^{-3}$, $\rho_{obsd} = 1.24$ g cm$^{-3}$ (at 25 °C). Intensities for 2292 reflections were measured at 25 and at −40 °C on an Enraf-Nonius CAD-4 automatic diffractometer by $\theta/2\theta$ scan techniques. The structure was solved by direct methods (MULTAN program), and refined by full matrix least-squares techniques to a final $R$ value of 7.8% on $F$ based on 2174 independent reflections. The ethane skeleton of choline is nearly staggered, with the nitrogen and oxygen atoms in a gauche relationship, and one of the methyl groups of the trimethylammonium ion in between the N and O atoms producing a close C···O contact (2.95 Å). The overall conformation of the molecule gives rise to a large intramolecular separation between the alkyltrimethylammonium cation and the phosphoryl oxygen anion (av N···O = 5.41 Å). The intermolecular separation between opposite charges is significantly shorter [N···O (symmetry related) = 3.80 Å]. The nonbonded intermolecular interactions are of the hydrophobic–hydrophobic and hydrophobic–hydrophilic types. Adjacent molecules are linked by a H-bonding network, in which the water molecule donates a H atom to one phosphoryl oxygen in each of the two neighboring molecules. It is suggested that in the crystalline state, and possibly also in fluid phases, including media of relatively low polarity, intermolecular associations between choline phosphate zwitterions are quite strong as a result of the inability of opposite charges to approach each other intramolecularly.

The choline phosphate zwitterion constitutes the polar head group of the lecithins (phosphatidylcholines, Scheme I),

Scheme I



lecithin 1

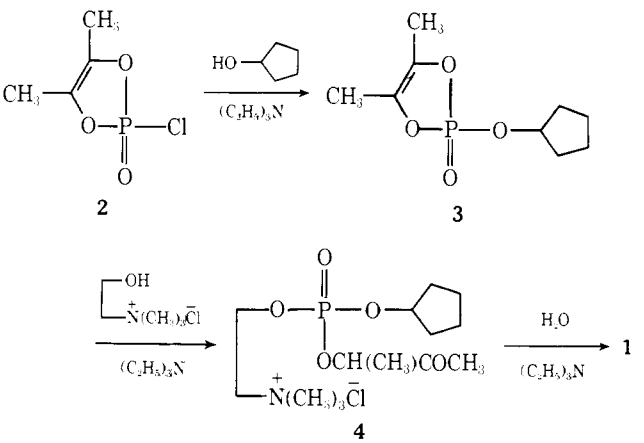
the ubiquitous component of biological membranes, and also forms part of the coenzyme-like compound cytidine diphosphate choline (CDP choline) which plays a key role in the biosynthesis of the lecithins.[2] This phospholipid is being extensively utilized as the source of bilayers in studies of membrane models by the black-film and vesicle techniques.[3a] Consequently, detailed knowledge of the conformation of the choline phosphate zwitterion is desirable.[3b,c] This paper describes the synthesis of cyclopentylphosphorylcholine (CPPC, **1**) and the study of its monohydrate $CPPC\cdot H_2O$, by x-ray crystallographic techniques.

The literature contains several reports on x-ray crystallographic structures relevant to the present investigation. Two of these compounds are diesters, and one is a monoester, of the choline phosphate zwitterion: *sn*-glycero-3-phosphorylcholine,[4] *sn*-glycero-3-phosphorylcholine·$CdCl_2\cdot 3H_2O$,[5] and phosphorylcholine·$CaCl_2\cdot 4H_2O$,[6] respectively; complete structural parameters, however, are not as yet available for the last two complexes.[5,6] Information has also been obtained on the structure of a significantly different type of choline ester, acetylcholine chloride,[7] bromide,[8] and iodide.[9] The conformation of these ion pairs, $\overline{X}(CH_3)_3N^+CH_2CH_2OCOCH_3$, has been scrutinized in relation to the problem of cholinergic agonists, e.g., muscarine, in neurotransmission.[10] Among derivatives of the phosphorylethanolamine zwitterion, $^+NH_3\cdot CH_2CH_2OP(O)(OR)\overline{O}$, two structures are known, the parent monoester (R = H)[11] and 1,2-di-*O*-lauroyl-DL-glycero-3-phosphorylethanolamine,[12] a member of the cephalin family of lipids.[2] Finally, among diesters of pyrophosphoric acid, the structure of CDP choline $Na^+$ has been elucidated recently.[13]

## Experimental Section

**Cyclopentylphosphorylcholine Monohydrate.** A solution of cyclopentanol (3.61 g, 41.9 mmol) and triethylamine (4.24 g, 1 molar equiv) in anhydrous diethyl ether (20 mL) was added dropwise to a stirred ether solution (100 mL) of 1,2-dimethylethenylenephosphorochloridate[14,15] (**2**) (Scheme II) (7.06 g, 1 molar equiv) at 20 °C. After 1

Scheme II



h at 20 °C, the alkylammonium salt was filtered and washed with ether. The combined ether solution was evaporated. The residue was dissolved in anhydrous acetonitrile (100 mL), and the solution was treated with choline chloride (5.85 g, recrystallized from anhydrous ethanol and dried under vacuum) and triethylamine (4.24 g) at 20 °C.

0002-7863/78/1500-4453$01.00/0 

The mixture was stirred for 24 h at 20 °C, diluted with water (200 mL), treated with triethylamine (4.24 g), and stirred for 24 h at 20 °C. The resulting solution was evaporated under vacuum at 40 °C, the residue was dissolved in chloroform (7 mL), and the solution was applied to a column (4 × 52 cm) containing silica gel 60 (400 g, packed in $CHCl_3$). Elution with $CHCl_3$ (2 L) and with mixtures of $CHCl_3/CH_3OH$ (99/1, 1 L; 95/5, 2 L; 90/10, 1 L; 80/20, 1 L) removed impurities. The desired product appeared in 2 L of methanol. Evaporation of the methanol gave anhydrous cyclopentylphosphorylcholine (**1**, 7.9 g, 75% yield) according to the $^1H$ NMR spectrum. This material (4.0 g) was dissolved in methanol (20 mL) containing 1 molar equiv of water (0.29 mL). The solution was evaporated, and the residue was redissolved in methanol (5 mL). Addition of acetone (10 mL) to the solution afforded the monohydrate, CPPC·$H_2O$, as rectangular-shaped prisms after 24 h at 20 °C. The sample for analysis was kept for 1 h at 20 °C (0.2 mm). It melted with decomposition at 255 °C, and had the following NMR parameters: $\delta$ $^{31}P$ −2.0 ($CDCl_3$), −1.2 ($D_2O$) ppm to high field of $H_3PO_4$ = O; $\tau$ $^1H$ 8.27, 6.74, 6.26, 5.70, and 5.10 ppm vs $Me_4Si$ = 10.

Anal. Calcd for $C_{10}H_{22}O_4NP·H_2O$: C, 44.6; H, 8.9; N, 5.2; P, 11.5; $H_2O$, 6.7. Found: C, 44.5; H, 9.0; N, 5.1; P, 11.4; $H_2O$, 6.4 (Karl Fischer method).

**Characterization of the Intermediate Cyclopentyloxy-3-oxo-2-butoxyphosphorylcholine Chloride (4).** The reaction of choline chloride with cyclopentyl-1,2-dimethylethenylene phosphate[16] (**3**) was carried out in acetonitrile as described above. The solution was evaporated, and the residue was crystallized from dichloromethane/diethyl ether (1/1) at −20 °C to yield the triester **4**: mp 123–124 °C; main $^1H$ NMR signals $\tau$ 8.55, $J$ = 7 Hz ($CH_3CH$), 7.80 ($CH_3CO$), 6.60 ($CH_3N$).

Anal. Calcd for $C_{14}H_{29}O_5NPCl$: C, 47.0; H, 8.2; P, 8.7; Cl, 9.9. Found: C, 46.8; H, 8.4; P, 8.5; Cl, 10.2.

**Crystal Data at −40 °C.** $C_{10}H_{24}O_5NP$: monoclinic; $P2_{1/c}$; $a$ = 12.688 (3), $b$ = 9.782 (3), $c$ = 11.034 (5) Å; $\beta$ = 94.33 (3)°, $V$ = 1365.3 Å$^3$ (mol wt 269.4); $Z$ = 4 (one molecule in the asymmetric unit). Density at 25 °C; $\rho_{calcd}$ 1.27 g cm$^{-3}$; $\rho_{obsd}$ 1.24 g cm$^{-3}$ (flotation in $n$-hexane–$CCl_4$).

**Data Collection and Structure Determination.** Crystals of approximately 0.2 × 0.2 × 0.5 mm in size were mounted and sealed in glass capillaries, and diffraction data were collected on an Enraf-Ninius CAD-4 automatic diffractometer, using nickel-filtered Cu K$\alpha$ radiation, by $\theta/2\theta$ scan method, initially at room temperature (25 °C). Subsequently, the data were recollected at low temperature (−40 °C) using another crystal of similar size. The temperature at the crystal was maintained at −40 ± 3 °C by controlling the flow rate of liquid nitrogen; the temperature was monitored by a thermocouple placed near the crystal and connected to a chart recorder. The scan range was 1° and the scan speed was 1.5° min$^{-1}$. Intensities of three standard reflections were monitored after every 100 reflections, in order to check for significant changes in intensities; no such changes were observed. Intensities for 2292 reflections within $\theta \leq 65°$ were measured, out of which 2174 had their intensities greater than 4$\sigma$ (estimated from the measured intensities). An empirical absorption correction was applied by measuring the intensity of a strong reflection at $\chi \approx 90°$, for values of $\phi$ at every 10°[17]. Lorentz and polarization corrections were applied as usual but no extinction corrections were applied.

The structure analysis was carried out in two stages. The diffraction data collected at room temperature were used to solve the structure by direct methods. A Fourier map calculated from one of the best solutions of MULTAN[18] revealed the locations of the phosphate group and the choline moiety (except the methyl groups). The remaining atoms were identified from subsequent Fourier maps. In the course of refinements all the carbon atoms of the cyclopentane ring had large thermal parameters (average B ~20 Å$^2$). In order to minimize the thermal disorder, the diffraction data were measured at low temperature (−40 °C), and these data were used to refine further the structure determined earlier. Unit weights were given for all observed reflections in the initial stages of refinements. Prior to the anisotropic refinement of the structure, the weights ($W = 1/\sigma^2$) were determined from an analysis of the variance. For $F_o \leq 10.0$, $\sigma$ = 1.8, and for $F_o > 10.0$, $\sigma = \{10.0 - 0.0625(140. - F_o)\}$ were used. Few cycles of anisotropic full-matrix least-squares refinements (ORFLS) reduced the $R$ value to 10%. All the hydrogen atom peaks were located from a difference-Fourier map computed at this stage. Their positions were accepted as correct from their relevant bond lengths and angles. In the later stages of refinements the positional and thermal parameters of nonhydrogen atoms were refined anisotropically and those of the hydrogen atoms isotropically. Four reflections which had their $\Delta F/\sigma > 4.0$ were not included in the final refinements, but were included in the final structure factor calculation. The refinements were terminated at $R$ = 7.8% ($R_w$ = 11.0%). The final parameters are presented in Tables V and VII, and the structure amplitudes in Table VIII. (See paragraph at end of paper regarding supplementary material.)

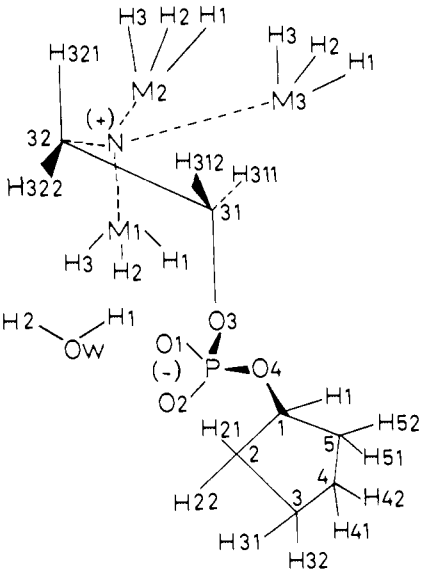



**Figure 1.** Schematic diagram and numbering of atoms of cyclopentylphosphorylcholine monohydrate. Atoms O(3), C(31), C(32), and H321 are in one plane.

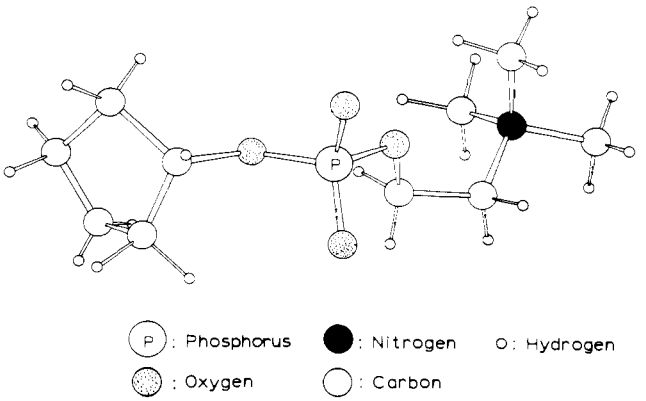



**Figure 2.** One molecule of cyclopentylphosphorylcholine monohydrate.

## Discussion of Results

**Molecular Structure of Cyclopentylphosphorylcholine Monohydrate.** Figure 1 gives the numbering system of the atoms in the compound. Figure 2 shows a drawing of the molecule, and Figure 3 displays the molecular packing. The interatomic distances and bond angles for nonhydrogen atoms are given in Table I, and the pertinent data concerning hydrogen atoms in Table VI. Some nonbonded distances are indicated in Table II. Several torsional angles are gathered in Table III, and data for several best least-squares planes are shown in Table IV.

The overall conformation of the molecule is reflected in the values of the torsional angles given in Table III. Note in particular that the ethane skeleton of choline is nearly staggered. Atoms O(3) and N occupy gauche positions on the skeleton, and are separated by 3.16 Å, which is not far from the distance allowed by the corresponding sum of van der Waals[19] (VW)

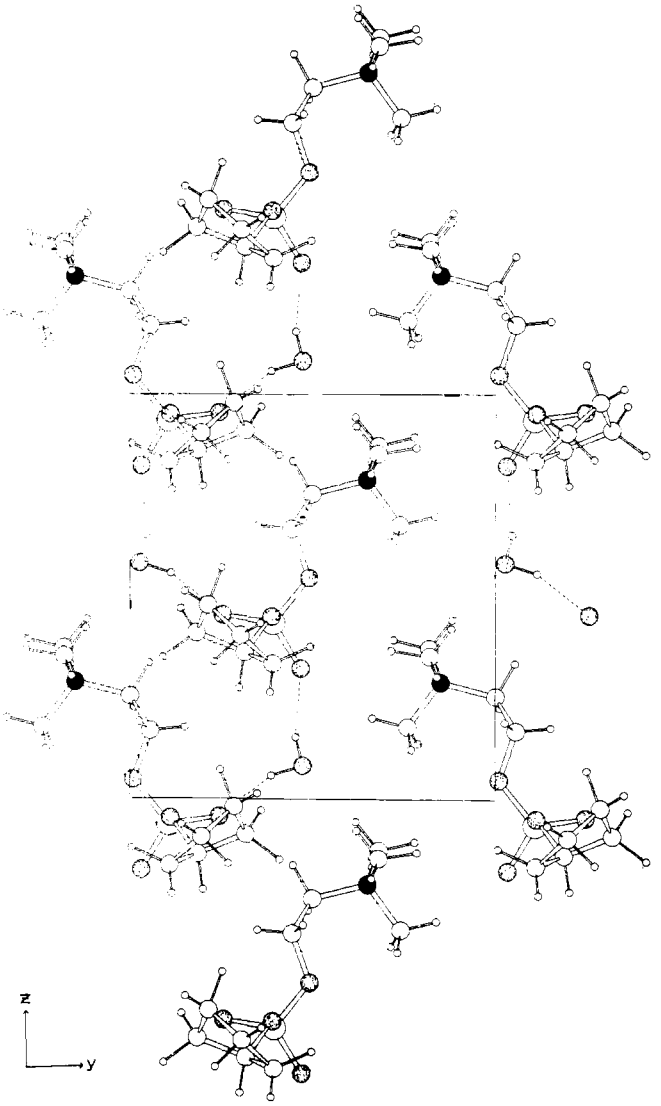



Figure 3. Molecular packing viewed along the $x$ axis.

Table I. Bond Distances (Å) and Angles (deg)[a,b]

| | | | |
|---|---|---|---|
| | Distances | | |
| P-O(1) | 1.482 | C(M2)-N | 1.488 |
| P-O(2) | 1.490 | C(M3)-N | 1.496 |
| P-O(3) | 1.601 | C(31)-C(32) | 1.501 |
| P-O(4) | 1.595 | C(1)-C(2) | 1.443 |
| C(1)-O(4) | 1.459 | C(2)-C(3) | 1.447 |
| C(31)-O(3) | 1.429 | C(3)-C(4) | 1.445 |
| C(32)-N | 1.519 | C(4)-C(5) | 1.508 |
| C(M1)-N | 1.504 | C(5)-C(1) | 1.612 |
| | Angles | | |
| | In Phosphate | | |
| (O(1)-P-O(2) | 118.1 | O(2)-P-O(4) | 111.2 |
| O(1)-P-O(3) | 110.3 | O(3)-P-O(4) | 99.3 |
| O(1)-P-O(4) | 110.3 | P-O(3)-C(31) | 119.5 |
| O(2)-P-O(3) | 106.0 | P-O(4)-C(1) | 119.3 |
| | In Choline | | |
| O(3)-C(31)-C(32) | 111.5 | C(32)-N-C(M2) | 111.5 |
| C(31)-C(32)-N | 117.3 | C(32)-N-C(M3) | 107.2 |
| C(32)-N-C(M1) | 110.8 | C(M1)-N-C(M2) | 110.0 |
| | | C(M1)-N-C(M3) | 108.3 |
| | | C(M2)-N-C(M3) | 109.0 |
| | In Cyclopentanol | | |
| O(4)-C(1)-C(2) | 110.2 | C(2)-C(3)-C(4) | 107.0 |
| O(4)-C(1)-C(5) | 111.4 | C(3)-C(4)-C(5) | 106.1 |
| C(1)-C(2)-C(3) | 102.3 | C(4)-C(5)-C(1) | 101.3 |
| | | C(5)-C(1)-C(2) | 105.6 |

[a] The esd's for phosphorus bond distances are 0.003 Å, and for bond angles 0.3°; for C-O, C-N, and C-C bond distances 0.007 Å, bond angles 0.6°. [b] Bond distances and angles involving hydrogen atoms are included with the supplementary material.

radii (2.9 Å). One methyl group C(M1) is in between O(3) and N, and the distance O(3)···C(M1) is shorter (2.95 Å) than the sum of VW radii (3.4 Å). The distance O(3)···H1(M1) is 2.49 Å while the corresponding sum of VW radii is 2.6 Å. This type of short intramolecular contact has been observed in *sn*-glycero-3-phosphorylcholine,[4] and also in acetylcholine ion pairs.[7-9]

The bonds in the cyclopentane show large variations, from 1.443 to 1.612 Å. The deviations from the standard C($sp^3$)-C($sp^3$) bond distance are attributed to the disorder of the ring. The cyclopentane ring could not be located with certainty using the data collected at room temperature. All the atoms in the ring showed high thermal disorder ($B \sim 30$ Å$^2$). Some disorder of the ring persists even at −40 °C. Attempts to collect data at very low temperature (∼−130 °C) were abandoned because the crystals deteriorated at these temperatures. The final difference Fourier map in the cyclopentane region was not "clean". Though the cyclopentane hydrogen atoms could be discerned from the peak heights and geometric considerations, there were other spurious peaks in the vicinity of the ring carbon atoms with electron densities comparable to the hydrogen atom peaks. The highest of them was found around the C1-C5 bond, closer to C1. Refinements with partial occupancies for ring carbon atoms were not carried out.

The cyclopentane ring is symmetrically puckered with C(2) and C(3) equally displaced on either side of the best five atom planes of the ring. The best four atom planes (not shown) are through C(1), C(3), C(4), C(5) and C(1), C(2), C(4), C(5), and the deviations of C(2) and C(3), respectively, from these planes are −0.56 and 0.54 Å, the dihedral angle between these planes being 16°.

The phosphate group is a highly distorted tetrahedron. The ∠O(1)-P-O(2) angle is significantly larger (118°) than the ∠O(3)-P-O(4) angle (99°), while the P-O bond distances involving the former phosphoryl oxygens are shorter than those involving the latter ester oxygens, as would be expected from a higher degree of p-d $\pi$ bonding associated with negatively charged vs. neutral oxygen atoms attached to four-coordinate phosphorus. The small ∠O-P-O angle is interesting, since such values have been previously observed inside the ring of saturated[20-22] and unsaturated[23] five-membered cyclic phosphates and aryl phosphates,[24] which are high-energy phosphates.

The most interesting features of the structure relate to the charge separation and the nature of the nonbonded interactions. The negatively charged phosphoryl oxygens and the positively charged nitrogen atom are widely separated *within the same molecule* ($O_p$···N = 5.4 Å). This distance is significantly larger than that which separates the oppositely charged atoms *in adjacent molecules* ($O_p$···N = 3.8 Å). The nonbonded intermolecular interactions are of the hydrophobic-hydrophobic and hydrophobic-hydrophilic types, as can be seen from the data in Table IIC. Adjacent molecules of the zwitterion, CPPC, are linked together by the H-bonding network depicted in Figure 4, in which one water molecule donates one hydrogen atom to a phosphoryl oxygen in each of the two molecules, O(1) and O(2) (symmetry related).

A comparison of the data now available for alkylphosphorylcholine zwitterions discloses that the torsion angles about the central bond of the chain O-C-C-N are very similar in CPPC·$H_2O$ and in the anhydrous *sn*-glycero-3-phosphorylcholine[4] (∼75°). It should also be noted that possible differences could be expected in intramolecular conformations and

**Table II.** Some Nonbonded Distances

| | | | |
|---|---|---|---|
| A. Significant Intramolecular Distances <3.5 Å | | | |
| O(3)···H1(M1) | 2.49 | O(3)···O(4) | 2.435 (min) |
| O(3)···C(M1) | 2.950 | O(1)···O(2) | 2.548 (max) |
| O(3)···N | 3.160 | | |
| B. Intramolecular O···N Distances >3.5 Å | | | |
| O(4)···N | 5.238 | O(2)···N | 5.458 |
| O(1)···N | 5.354 | | |

C. Intermolecular Distances <4.0 Å

| atom in molecule 1 | atom in Mo molecule 2 | distance, Å | symmetry operation | | |
|---|---|---|---|---|---|
| O(1) | C(M3) | 3.249 | $-x$, | $y - 1/2$, | $-z + 3/2$ |
| O(1) | C(32) | 3.334 | $x$, | $-y$, | $z - 1/2$ |
| O(1) | C(M1) | 3.459 | $-x$, | $-y + 1$, | $-z + 1$ |
| O(1) | C(31) | 3.793 | $x$, | $-y + 1/2$, | $z - 1/2$ |
| O(2) | C(M1) | 3.297 | $x$, | $-y + 3/2$, | $z - 1/2$ |
| O(2) | C(32) | 3.457 | $-x$, | $-y + 1$, | $-z + 1$ |
| O(2) | C(M3) | 3.476 | $-x$, | $-y + 1$, | $-z + 1$ |
| O(2) | C(M2) | 3.597 | $x$, | $-y + 3/2$, | $z - 1/2$ |
| O(2) | C(M3) | 3.749 | $x$, | $-y + 3/2$, | $z - 1/2$ |
| O(2) | N | 3.762 | $x$, | $-y + 3/2$, | $z - 1/2$ |
| O(2) | N | 3.836 | $-x$, | $-y + 1$, | $-z + 1$ |
| O(2) | C(M1) | 3.890 | $-x$, | $-y + 1$, | $-z + 1$ |
| O(4) | C(4) | 3.685 | $-x + 1$, | $-y + 1$, | $-z + 1$ |
| W | C(M2) | 3.270 | $x$, | $-y + 1/2$, | $z - 1/2$ |
| W | C(M1) | 3.278 | $x$, | $y - 1$, | $z$ |
| W | C(M3) | 3.598 | $-x$, | $y - 1/2$, | $-z + 3/2$ |
| W | C(M3) | 3.635 | $x$, | $-y + 1/2$, | $z - 1/2$ |
| W | C(32) | 3.752 | $x$, | $-y + 1/2$, | $z - 1/2$ |
| W | N | 3.752 | $x$, | $-y + 1/2$, | $z - 1/2$ |
| C(M1) | C(M3) | 3.508 | $x$, | $-y + 3/2$, | $z - 1/2$ |
| C(M2) | C(3) | 3.715 | $-x + 1$, | $y + 1/2$, | $-z + 3/2$ |
| C(M2) | C(4) | 3.793 | $x$, | $-y + 1/2$, | $z - 1/2$ |

**Table III.** Some Torsional Angles ($\tau$)[a]

| bond system | $\tau$, deg |
|---|---|
| O(3)-C(31)-C(32)-H(321) | 166 |
| O(3)-C(31)-C(32)-N | −73.7 |
| P-O(3)-C(31)-C(32) | −146.5 |
| O(4)-P-O(3)-C(31) | −67.0 |
| C(1)-O(4)-P-O(3) | −177.1 |
| C(2)-C(1)-O(4)-P | −100.3 |
| C(5)-C(1)-O(4)-P | 142.7 |
| C(31)-C(32)-N-C(M1) | 61.3 |
| C(31)-C(32)-N-C(M3) | 179.3 |

[a] The torsion angle $\tau$ for a four-atom system, A-B-C-D, is the twist of atom A with respect to atom D viewed along the central bond B→C, and is considered positive if the rotation of D is clockwise.

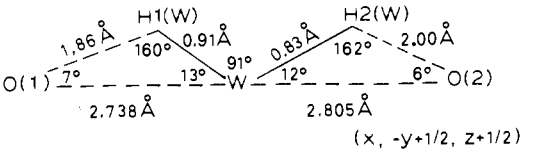



**Figure 4.** Details of the hydrogen bonding in CPPC·$H_2O$.

**Table IV.** Coefficients *A, B, C, D* of the Equation to Plane, $AX + BY + CZ - D = 0$, Where *X, Y, Z* Are Coordinates Relative to Orthogonal Axes. Deviations (Å) of Individual Atoms from Planes; Root Mean Square Deviations in Parentheses

| | |
|---|---|
| plane 1: | O(3)-C(31)-C(32)-H(321) (0.069)<br>0.603; 0.758; 0.249; 6.205<br>H(321) = −0.079; C(32) − 0.078; C(31) = 0.058;<br>O(3) = −0.057;<br>P = −0.867; O(4) = 0.102; C(1) = 0.210; C(3) = 0.309;<br>C(2) = −0.834; C(5) = 1.127; C(4) = 1.254 |
| plane 2: | O(4)-O(3)-C(31)-C(32)-H(321) (0.064)<br>0.571; 0.783; 0.247; 6.263<br>H(321) = −0.077; C(32) = 0.097; C(31) = 0.031;<br>O(3) = −0.062;<br>O(4) = 0.011 |
| plane 3: | C(1)-C(2)-C(3)-C(4)-C(5) (0.174)<br>−0.245; 0.555; 0.795; 3.649<br>C(1) = 0.146; C(2) = −0.242; C(3) = 0.235; C(4) = −0.125;<br>C(5) = −0.014 |

intermolecular associations in the hydrated $CdCl_2$ and $CaCl_2$ complexes of choline phosphate zwitterions,[5,6] since the metal ions are coordinated to the phosphoryl oxygens in those structures. Zwitterions and ion pairs derived from choline are not strictly comparable since they have different charge distributions; nevertheless, it is interesting that there are similarities between the conformations of both systems as shown by the respective O-C-C-N (74 vs. ~85°) and X-O-C-C (X = PO, 147° vs. X = CO, ~150°) torsional angles in CPPC·$H_2O$ vs. acetylcholine halides.[7-9]

## Conclusions

The distance between the positively charged nitrogen and the negatively charged phosphoryl oxygen, O(1) or O(2) in CPPC·$H_2O$, can be altered by rotations about three types of bonds. Rotation about bond C(31)-C(32) is hindered primarily by steric interactions involving the N and O(3) atoms, and the gauche placement of these two atoms on a nearly staggered ethane skeleton probably represents their closest possible approach. Rotations about bonds O(3)-C(31) and P-O(3) are hindered mainly by groups present on the N atom and on the phosphate ester, O(4). These effects result in a

**Table V.** Fractional Coordinates and Thermal Parameters ($\times 10^5$) for Nonhydrogen Atoms[a,b]

| atom | $x$ | $y$ | $z$ | $B_{11}$ | $B_{22}$ | $B_{33}$ | $B_{12}$ | $B_{13}$ | $B_{23}$ |
|---|---|---|---|---|---|---|---|---|---|
| P | 18 953 (8) | 39 299 (10) | 43 576 (9) | 2.48 (4) | 1.63 (4) | 2.36 (4) | 0.07 (1) | −0.10 (1) | −0.09 (1) |
| O1 | 14 569 (25) | 25 496 (29) | 45 496 (28) | 3.44 (13) | 2.02 (11) | 3.52 (13) | −0.27 (4) | 0.05 (5) | −0.12 (5) |
| O2 | 15 138 (27) | 46 887 (32) | 32 383 (28) | 4.57 (15) | 2.63 (12) | 2.77 (13) | 0.38 (5) | 0.01 (5) | 0.21 (5) |
| O3 | 16 782 (25) | 49 087 (29) | 54 749 (26) | 4.02 (14) | 2.00 (11) | 2.54 (12) | 0.34 (4) | −0.09 (5) | −0.08 (4) |
| O4 | 31 549 (25) | 38 903 (35) | 45 130 (31) | 3.13 (14) | 3.43 (14) | 4.05 (15) | −0.03 (5) | −0.16 (5) | −0.53 (6) |
| W | 20 891 (34) | 2 601 (35) | 58 334 (31) | 7.64 (23) | 2.47 (13) | 3.29 (14) | 0.18 (7) | 0.59 (7) | 0.11 (5) |
| N | 12 709 (25) | 64 964 (32) | 78 888 (30) | 2.44 (12) | 1.58 (12) | 2.35 (13) | −0.14 (4) | −0.10 (5) | 0.02 (5) |
| C31 | 19 268 (37) | 44 414 (45) | 66 898 (39) | 4.19 (21) | 2.32 (16) | 2.59 (16) | 0.34 (7) | −0.15 (7) | 0.21 (6) |
| C32 | 11 653 (32) | 49 966 (38) | 75 435 (36) | 3.18 (17) | 1.37 (14) | 2.50 (15) | −0.12 (5) | −0.25 (6) | 0.02 (6) |
| CM1 | 10 988 (37) | 73 929 (40) | 67 847 (38) | 4.00 (19) | 1.64 (14) | 2.59 (16) | 0.02 (6) | 0.12 (7) | 0.16 (5) |
| CM2 | 23 195 (34) | 67 936 (48) | 85 302 (43) | 2.62 (16) | 2.99 (18) | 3.56 (19) | −0.32 (7) | −0.21 (7) | −0.25 (7) |
| CM3 | 4 262 (36) | 68 065 (48) | 87 239 (41) | 3.26 (18) | 3.00 (18) | 3.11 (18) | 0.06 (7) | 0.41 (7) | −0.10 (7) |
| C1 | 37 306 (48) | 31 427 (75) | 36 287 (73) | 3.84 (24) | 5.90 (34) | 8.00 (41) | 0.21 (11) | −0.56 (12) | −1.49 (15) |
| C2 | 40 546 (53) | 18 234 (83) | 41 088 (84) | 4.25 (28) | 6.07 (37) | 10.04 (52) | −0.65 (13) | 0.35 (15) | −0.50 (17) |
| C3 | 50 430 (80) | 21 389 (93) | 47 926 (83) | 8.68 (48) | 7.35 (46) | 7.39 (46) | −1.19 (19) | −1.10 (18) | 1.17 (18) |
| C4 | 56 234 (47) | 30 394 (78) | 40 459 (86) | 2.98 (23) | 5.95 (34) | 10.84 (54) | −0.51 (11) | −0.30 (13) | 0.25 (17) |
| C5 | 48 077 (59) | 39 185 (71) | 33 533 (70) | 6.16 (34) | 5.11 (31) | 6.61 (35) | −0.46 (13) | 0.41 (13) | 0.57 (13) |

[a] Numbers in parentheses are estimated standard deviations in the least significant digits. [b] Anisotropic thermal parameters are in the form $T = \exp[-1/4(h^2a^{*2}B_{11} + \cdots + 2hka^*b^*B_{12} + \cdots)]$.

relatively large separation between the two opposite charges within the molecule, which presumably represents a relatively high energy situation. In the crystalline state, the molecules associate in such a way that oppositely charged groups come as close together as is sterically permissible and a water molecule provides an additional link between two negatively charged phosphates. It seems possible that the picture disclosed by x-ray crystallography may also apply to this type of compound in fluid phases, including media of relatively low polarity,[25] since the difficulties in bringing opposite charges into close proximity within the molecule should induce relatively strong intermolecular associations as a means of reducing the overall energy of the system.

Extrapolation of this hypothesis to the more complex lecithin molecule must consider the effect of these strong intermolecular associations between choline phosphate zwitterions on the powerful lipid–lipid interactions which operate among diglyceride moieties.[2,3,12] The physicobiochemical properties of phosphatidylcholine, including questions related to membrane permeability, are quite different from those of the acidic phospholipids exemplified by phosphatidylinositol,[2] phosphatidylglycerol,[2] and cardiolipin.[2,26] The acidic phospholipids are ion pairs, $(R^IO)(R^{II}O)P(O)\bar{O}M^+$ where $R^I$ and $R^{II}$ are derived, respectively, from the hydrophobic and hydrophilic alcohols, $R^IOH$ and $R^{II}OH$, which comprise the phospholipid diester molecule. Evidently, acidic phospholipids are not capable of engaging in the same type of strong intermolecular associations resulting from the presence of a zwitterion in the polar head group. In the sense discussed here, therefore, strong intermolecular associations should result from lipid–lipid interactions *and* zwitterion–zwitterion interactions in phosphoglycerides and phosphosphingolipids which are based on choline, i.e., lecithins and sphingomyelins. Strong intermolecular associations should result only from lipid–lipid interactions in the acidic phospholipids. The phospholipids based on ethanolamine, and on *N*-methyl- and *N*,*N*-dimethylethanolamine, are in a class by themselves, to the extent that their zwitterion structures depend on the presence of a proton on nitrogen, rather than on the quaternary ammonium cation typical of choline. A third type of ethanolamine derivative, *N*-acetylphosphatidylethanolamine, lacks altogether the ability to form a zwitterion due to a decrease in basicity of the nitrogen function, and consequently the properties of these phospholipids should reflect this structural difference with respect to phosphatidylethanolamines and -choline.

**Acknowledgment.** We are grateful to Dr. S. T. Rao of the University of Wisconsin (Madison) for the use of the programs, and to Drs. H. L. Carrell and W. Stallings of the Institute for Cancer Research, Philadelphia, Pa., for their help in obtaining computer-plot diagrams.

**Supplementary Material Available:** Table VI, bond distances and angles involving hydrogen atoms; Table VII, hydrogen atoms positions; Table VIII, structure factors (17 pages). Ordering information is given on any current masthead page.

## References and Notes

(1) (a) Department of Biochemistry, State University of New York at Stony Brook. (b) Department of Chemistry, State University of New York at Stony Brook. The support of this research by the National Science Foundation (Grant CHE76-16785) and by the National Institute of General Medical Sciences (Grant GM-20672) is gratefully acknowledged.
(2) G. B. Ansell, R. M. C. Dawson, and J. N. Hawthorne, Ed., "Form and Function of Phospholipids", 2nd ed, Elsevier, Amsterdam, 1973, pp 19, 69, 128.
(3) (a) M. K. Jain, "The Bimolecular Lipid Membrane", Van Nostrand-Reinhold, Princeton, N.J., 1972; (b) S. J. Kohler and M. P. Klein, *Biochemistry*, **16**, 519 (1977); (c) H. Hauser, M. C. Phillips, R. A. Levine, and R. J. P. Williams, *Nature (London)*, **261**, 390 (1976).
(4) S. Abrahamsson and I. Pascher, *Acta Crystallogr.*, **21**, 79 (1966).
(5) (a) M. Sundaralingam and L. H. Jensen, *Science*, **150**, 1035 (1965); (b) A. Fitzgerald, M. Sundaralingam, and L. H. Jensen, Abstracts, American Crystallographic Association, Spring Meeting, 1974, Vol. 2, No. M10, 98.
(6) J. McAlister, D. Fries and M. Sundaralingam, Abstracts, American Crystallographic Association, Summer Meeting, 1973, Vol. 1, No. Q8, 197.
(7) J. K. Herdklotz and R. L. Sass, *Biochem. Biophys. Res. Commun.*, **40**, 583 (1970).
(8) T. Svinning and H. Sørum, *Acta Crystallogr., Sect. B*, **31**, 1581 (1975).
(9) S. Jagner and B. Jensen, *Acta Crystallogr., Sect. B*, 2757 (1977).
(10) (a) R. W. Baker, C. H. Chotia, P. Pauling, and T. J. Petcher, *Nature (London)*, **230**, 439 (1971); (b) C. H. Chotia, R. W. Baker, and P. Pauling, *J. Mol. Biol.*, **105**, 517 (1976).
(11) J. Kraut, *Acta Crystallogr.*, **14**, 1146 (1961).
(12) P. B. Hitchcock, R. Mason, K. M. Thomas, and G. G. Shipley, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 3036 (1974).
(13) M. A. Viswamitra, T. P. Seshadri, M. L. Post, and O. Kennard, *Nature (London)*, **258**, 497 (1975).
(14) (a) F. Ramirez, H. Okazaki, and J. F. Marecek, *Synthesis*, 637 (1975); (b) F. Ramirez, H. Okazaki, J. F. Marecek, and H. Tsuboi, *ibid.*, 819 (1976).
(15) I. P. Gozman, *Izv. Akad. Nauk SSSR*, 2362 (1968).
(16) F. Ramirez, J. F. Marecek, and H. Okazaki, *J. Am. Chem. Soc.*, **98**, 5310 (1976).
(17) A. C. T. North, D. C. Phillips, and F. S. Mathews, *Acta Crystallogr., Sect. A*, **24**, 351 (1968).
(18) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. A*, **27**, 368 (1971).
(19) Covalent bond (van der Waals) radii (Å): H, 0.30 (1.2); C, 0.77 (1.6); N, 0.70 (1.5); O, 0.66 (1.4); P, 1.10 (1.9); $CH_3$, (2.0). L. Pauling, "The Nature of the Chemical Bond", 2nd ed, Cornell University Press, Ithaca, N.Y., 1945, pp 164, 189.

(20) T. A. Steitz and W. N. Lipscomb, *J. Am. Chem. Soc.*, **87**, 2488 (1965).
(21) M. G. Newton, J. R. Cox, Jr., and J. A. Bertrand, *J. Am. Chem. Soc.*, **88**, 1503 (1966).
(22) F. Ramirez, J. S. Ricci, Jr., O. P. Madan, J. F. Marecek, and H. Tsuboi, *J. Am. Chem. Soc.*, **99**, 5135 (1977).
(23) D. Swank, C. N. Caughlan, F. Ramirez, O. P. Madan, and C. P. Smith, *J. Am. Chem. Soc.*, **89**, 6503 (1967).
(24) G. D. Smith, C. N. Caughlan, F. Ramirez, S. Glaser, and P. Stern, *J. Am. Chem. Soc.*, **96**, 2698 (1974).
(25) G. H. Brown, J. W. Doane, and V. D. Neff, "A Review of the Structure and Physical Properties of Liquid Crystals", CRC Press, Cleveland, Ohio, 1971.
(26) F. Ramirez, P. V. Ioannou, J. F. Marecek, G. H. Dodd, and B. T. Golding, *Tetrahedron*, **33**, 599 (1977).

# Structural Studies of Tetracyclines. Crystal and Molecular Structures of Anhydrotetracycline Hydrobromide Monohydrate and 6-Demethyl-7-chlorotetracycline Hydrochloride Trihydrate[1]

**Gus J. Palenik,*[2] M. Mathew, and R. Restivo**

*Contribution from the Center for Molecular Structure, Department of Chemistry, University of Florida, Gainesville, Florida 32611, and the Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada.* *Received August 20, 1977*

**Abstract:** The crystal and molecular structures of two tetracyclines, anhydrotetracycline hydrobromide (I) and 6-demethyl-7-chlorotetracycline hydrochloride (II) have been determined by x-ray diffraction techniques. Aromatization of the C ring of tetracyclines gives I but also destroys any useful therapeutic value. In contrast II has excellent antibacterial properties. The crystals of I monohydrate are orthorhombic, space group $P2_12_12_1$ with unit cell dimensions of $a = 19.562$ (2), $b = 16.586$ (2), and $c = 6.796$ (2) Å. Crystals of II as the trihydrate are monoclinic, space group $P2_1$, with cell dimensions of $a = 9.219$ (1), $b = 11.634$ (1), $c = 11.240$ (1) Å; $\beta = 102.39$ (4)°. Although the conformation of I is similar to that of II, significant differences are observed. In addition, various bond lengths are affected by the aromatization of the C ring. These differences may account for the different antibacterial spectrum of the two compounds. The conformation of II, except for the orientation of the amide group, is virtually identical with the conformations found in all therapeutically useful tetracyclines. The amide group in II has the same orientation as in tetracycline free base but is rotated 180° relative to that found in the other derivatives. The difference in hydration and crystal packing of II relative to the other tetracyclines suggests that the conformation of II is very stable and is most likely the conformation found at the site of biological activity.

Tetracyclines (Figure 1) are a group of hydronaphthacenes,[3] some of which are important antibiotics. When we initiated our program, in spite of the importance of these drugs,[6] there were x-ray crystal structures for only two active tetracyclines.[7,8] However, a comparison of both therapeutically active and inactive drugs is necessary to elucidate the conformational requirements for biological activity. The resulting knowledge of the possible conformations for active tetracyclines is required for an understanding of the mechanism of biological action on a molecular level.

Aromatization of the C ring of tetracyclines occurs easily by removal of a molecule of water to give anhydrotetracyclines.[9] The anhydro derivatives have only minimal activity against staphylococcus aureus and are not of any therapeutic significance.[10] We undertook a crystal structure determination of anhydrotetracycline hydrobromide, henceforth ANTC·HBr, to study the changes in the molecule which resulted from aromatizing the C ring.

In contrast to ANTC, 7-chlorotetracycline hydrochloride, henceforth 7-CLTC·HCl, 5-hydroxytetracycline hydrochloride, henceforth 5-HTC·HCl, and 6-demethyl-7-chlorotetracycline hydrochloride, henceforth 6-DM-7-CLTC·HCl, are widely used antibiotics. Crystals of 7-CLTC·HCl and 5-HTC·HCl[7] were found to be isomorphous and the two cations had identical conformations. The question was raised as to whether the observed conformations were a result of crystal packing forces. Therefore, when we found that 6-DM-7-CLTC·HCl crystallized in a completely different arrangement and with a different degree of hydration, we decided to determine the crystal structure. If the conformations of the cations in 7-CLTC·HCl and 5-HTC·HCl were a result of crystal packing forces, then we might expect a different conformation in 6-DM-7-CLTC·HCl. The present report provides a detailed description of our structural studies of ANTC·HBr and 6-DM-7-CLTC·HCl, together with a comparison of these results with recently reported structural studies of other tetracyclines.[11-15]

## Experimental Section

Yellow, acicular crystals of both compounds were grown from saturated methanol solutions by slow evaporation of the solvent. The pH of the solutions had been adjusted to ~1.5 with either HBr or HCl to prevent epimerization. Preliminary Weissenberg and precession photographs indicated that ANTC·HBr crystallized in the orthorhombic space group $P2_12_12_1$ ($D_2^4$) and that 6-DM-7-CLTC·HCl was monoclinic with space group $P2_1$ ($C_2^2$) or $P2_1/m$ ($C_{2h}^2$). The latter seemed unlikely since with 2 molecules of 6-DM-7-CLTC·HCl per unit cell the molecule would be required to have a mirror plane of symmetry. The subsequent intensity statistics were consistent with the choice of $P2_1$ ($C_2^2$).

Small, approximately equidimensional crystals were used for the measurement of cell constants and intensity data using a General Electric XRD-6 diffractometer. The crystal sizes and other pertinent data are summarized in Table I. The cell dimensions were obtained by a least-squares procedure from the $2\theta$ values of Cu K$\beta$ ($\lambda = 1.39217$ Å) peaks. The intensity measurements were made using previously described techniques[16] to a $2\theta$ limit of 135° (Cu K$\alpha$ radiation) in both cases. Only those measurements in which the intensity was greater than or equal to 1.2 times the appropriate background were considered

0002-7863/78/1500-4458$01.00/0 