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## The Structure of Monosodium Phosphoenolpyruvate

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(Received 25 September 1980; accepted 3 November 1980)

### Abstract

$C_3H_4O_6P^- \cdot Na^+ \cdot H_2O$ ,  $M_r = 208.0$ , is monoclinic,  $Cc$ ,  $a = 11.423$  (2),  $b = 23.253$  (5),  $c = 6.604$  (1) Å,  $\beta = 123.63$  (1)°,  $U = 1460.6$  Å<sup>3</sup>,  $D_x = 1.89$  Mg m<sup>-3</sup>,  $Z = 8$ ,  $\lambda(\text{Mo } K\alpha) = 0.7107$  Å,  $\mu(\text{Mo } K\alpha) = 0.44$  mm<sup>-1</sup>,  $F(000) = 840$ . Final  $R = 0.063$  for 1697 reflections. The two crystallographically independent molecules of phosphoenolpyruvate (PEP) (*A* and *B*) are almost mirror images of each other, the mirror being the planar enolpyruvate group. The torsion angle C(3)–C(2)–O(1)–P(1) is 122.6 in *A* and –112.0° in *B*, in contrast to –209.1° in PEP.K. The enolic C(2)–O(1) has a partial double-bond character [1.401 (*A*), 1.386 Å (*B*)]. The high-energy P~O bond (1.595 and 1.610 Å) is comparable to that in PEP.K (1.612 Å). Na(1) has six nearest neighbours while Na(2) has only five. The Na<sup>+</sup> ions are involved in binding only the phosphates of different molecules, in contrast to the K<sup>+</sup> ion in PEP.K, which binds to both the phosphate and carboxyl ends of the same molecule. The planar carboxyl groups stack on each other at an average distance of 3.2 Å instead of forming hydrogen-bonded dimers usually found in carboxylate structures.

### Introduction

Phosphoenolpyruvate (PEP) is known to play an important role in glycolysis as a substrate in the pyruvate kinase catalysed reaction, where adenosine 5'-triphosphate is synthesized by a phosphate-group transfer from PEP to adenosine 5'-diphosphate. For this phosphorylation reaction to proceed, the presence of divalent Mg<sup>2+</sup> and monovalent K<sup>+</sup> cations is

obligatory (Mahler & Cordes, 1969). Replacement of K<sup>+</sup> by Na<sup>+</sup> is known to inhibit the reaction. Hence it would be of interest to study the structure of PEP when bound to Na<sup>+</sup> and compare it with that of its K<sup>+</sup> complex. We report here the structure of monosodium phosphoenolpyruvate (PEP.Na) monohydrate as obtained from single-crystal X-ray analysis. The conformation of PEP in the present structure is found to be significantly different from that in its K<sup>+</sup> salt (Hosur & Viswamitra, 1981).

### Experimental

Crystals of the monosodium salt of PEP (original sample from Boehringer Mannheim GmbH) were grown by slow diffusion of acetone into an aqueous solution of the compound. A single crystal 0.15 × 0.17 × 0.22 mm, with well developed prismatic faces, was used to determine the crystal data by rotation and Weissenberg photographs. The cell parameters were later refined on a CAD-4 diffractometer by least squares from 25 high-angle reflections. The density, measured by flotation in bromoform and acetone mixtures, indicated eight (PEP.Na.H<sub>2</sub>O) units in the cell.

Systematic absences  $hkl: h + k = 2n + 1$  and  $h0l: l = 2n + 1$  indicated space groups  $Cc$  or  $C2/c$ .

Mo  $K\alpha$  intensities to a  $2\theta$  limit of 56° (resolution = 0.76 Å) were collected on the diffractometer in the  $\omega/2\theta$  scan mode. Background counts were measured at the two edges of the Bragg peak for  $\frac{1}{2}$  of the total scan angle. There was no significant change in the intensities of the monitor reflections checked at regular intervals. The data consisted of 1697 unique reflections in the  $hkl$

and  $hk\bar{l}$  octants. These were corrected for Lorentz and polarization effects but not for absorption ( $\mu r \approx 0.08$ ).

### Structure solution and refinement

Initial attempts to solve the structure in  $C2/c$  were unsuccessful. The structure was solved in  $Cc$  by the application of direct methods and difference syntheses with *SHELX 76* (Sheldrick, 1976). The phosphates of the two crystallographically independent molecules were identified in the  $E$  map only after renormalizing the  $E$  values for  $l = 2n + 1$  reflections. A difference synthesis based on these two phosphates revealed the positions of enolpyruvate atoms and the  $\text{Na}^+$  ions. Block-diagonal least-squares refinement with individual isotropic temperature factors reduced  $R$  to 0.200 and later to 0.163 with the introduction of anisotropy for the thermal parameters. The two water molecules in the asymmetric unit were located in the difference map computed at this stage. The molecular and water H atoms were identified after further refinement and a difference synthesis at  $R = 0.069$ . The final  $R$  obtained by refinement with the weighting scheme [ $w = 1/\sigma(F)^2$ ] was 0.063. Scattering factors for the non-H atoms were computed with the Cromer & Waber (1965) constants, that for H was from Stewart, Davidson & Simpson (1965).

Table 1. Final positional and thermal parameters for the non-H atoms ( $\times 10^4$ ) with *e.s.d.*'s in parentheses for  $x, y, z$

$$U_{\text{eq}} = 1/(6\pi^2) \sum_i \sum_j \beta_{ij} \mathbf{a}_i \cdot \mathbf{a}_j$$

	$x$	$y$	$z$	$U_{\text{eq}} (\text{\AA}^2)$
<b>Molecule A</b>				
C(1)	4188 (8)	2867 (4)	5659 (16)	40
C(2)	4511 (6)	3384 (3)	5329 (11)	24
C(3)	5974 (7)	3622 (3)	6623 (11)	25
O(31)	6228 (5)	4102 (2)	6215 (9)	39
O(32)	6929 (6)	3266 (2)	8211 (9)	35
O(1)	3521 (5)	3801 (2)	3829 (8)	29
P(1)	2400 (2)	3744 (1)	970 (3)	21
O(2)	1672 (5)	4308 (2)	313 (9)	28
O(3)	3138 (5)	3579 (2)	-238 (9)	35
O(4)	1387 (5)	3244 (2)	576 (9)	33
<b>Molecule B</b>				
C(1)	-1537 (8)	2912 (3)	4829 (16)	39
C(2)	-1903 (6)	3437 (3)	3924 (12)	24
C(3)	-3400 (7)	3621 (3)	2207 (12)	24
O(31)	-3680 (5)	4103 (2)	1326 (9)	33
O(32)	-4318 (5)	3228 (2)	1808 (9)	33
O(1)	-956 (5)	3884 (2)	4554 (8)	26
P(1)	129 (2)	3915 (1)	3726 (3)	21
O(2)	751 (5)	4503 (2)	4478 (9)	32
O(3)	-637 (5)	3764 (2)	1088 (8)	30
O(4)	1216 (5)	3419 (2)	5097 (9)	31
Na(1)	2372 (3)	4961 (1)	3711 (6)	36
Na(2)	4357 (3)	416 (1)	3500 (6)	40
W(1)	4261 (6)	5082 (2)	3128 (10)	39
W(2)	7877 (6)	4998 (2)	9677 (10)	36

### Results and discussion

The final positional parameters for the non-H atoms are given in Table 1, those for H in Table 2.\* Bond lengths and angles are listed in Tables 3 and 4 respectively. The torsion angles are given in Table 5.

\* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 35883 (15 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. Final positional and thermal parameters for the H atoms ( $\times 10^3$ )

	$x$	$y$	$z$	$U (\text{\AA}^2)$
<b>Molecule A</b>				
H(1)	492	259	710	30
H'(1)	331	271	492	97
H(32)	788	330	735	35
H(4)	110	337	195	17
<b>Molecule B</b>				
H(1)	-41	283	671	69
H'(1)	-223	263	449	65
H(32)	-495	331	248	11
H(4)	96	337	699	35
H'W(1)	437	486	251	31
H'W'(1)	356	507	307	28
H'W(2)	721	519	769	50
H'W'(2)	772	502	1135	63

Table 3. Bond lengths ( $\text{\AA}$ )

	A	B
C(1)—C(2)	1.310 (11)	1.323 (10)
C(2)—C(3)	1.499 (11)	1.499 (11)
C(3)—O(31)	1.220 (9)	1.220 (8)
C(3)—O(32)	1.307 (9)	1.304 (10)
C(2)—O(1)	1.401 (8)	1.386 (9)
O(1)—P(1)	1.595 (5)	1.610 (6)
P(1)—O(2)	1.483 (5)	1.493 (6)
P(1)—O(3)	1.497 (7)	1.495 (5)
P(1)—O(4)	1.558 (6)	1.564 (6)

Table 4. Bond angles ( $^\circ$ )

	A	B
C(1)—C(2)—C(3)	124.9 (7)	123.2 (7)
C(1)—C(2)—O(1)	124.2 (7)	123.9 (7)
O(1)—C(2)—C(3)	110.8 (6)	112.8 (6)
C(2)—C(3)—O(31)	122.6 (7)	120.7 (7)
C(2)—C(3)—O(32)	113.3 (6)	114.0 (6)
O(31)—C(3)—O(32)	124.1 (7)	125.4 (7)
C(2)—O(1)—P(1)	125.7 (5)	123.4 (5)
O(1)—P(1)—O(2)	103.0 (3)	104.0 (3)
O(1)—P(1)—O(3)	109.4 (3)	108.7 (3)
O(1)—P(1)—O(4)	107.2 (3)	106.5 (3)
O(2)—P(1)—O(3)	117.4 (3)	117.8 (3)
O(2)—P(1)—O(4)	111.6 (3)	113.8 (3)
O(3)—P(1)—O(4)	107.8 (3)	105.4 (3)

Table 5. *Torsion angles (°) involving non-H atoms*

Average e.s.d. is 0.3°.

	<i>A</i>	<i>B</i>
C(1)—C(2)—C(3)—O(31)	178.2	-176.2
O(1)—C(2)—C(3)—O(31)	-5.6	7.0
C(1)—C(2)—C(3)—O(32)	-1.9	4.3
O(1)—C(2)—C(3)—O(32)	174.3	-172.4
C(1)—C(2)—O(1)—P(1)	-61.2	71.3
C(3)—C(2)—O(1)—P(1)	122.6	-112.0
C(2)—O(1)—P(1)—O(2)	-178.0	171.3
C(2)—O(1)—P(1)—O(4)	64.1	-68.2
C(2)—O(1)—P(1)—O(3)	-52.4	45.0

### Molecular geometry

There are two molecules of PEP in the asymmetric unit which will be referred to as *A* and *B*.

#### The phosphate group

As the compound is a monosodium salt, the phosphate groups of both *A* and *B* are expected to carry a single negative charge. The longest of the three bond distances from P to the terminal O atoms [P(1)—O(4) = 1.558 (*A*), 1.564 Å (*B*)] is close to the mean P—O(H) distance of 1.568 Å in putrescine diphosphate (Takusagawa & Koetzle, 1978). The other two [P(1)—O(2) = 1.483, P(1)—O(3) = 1.497 (*A*) and 1.493, 1.495 Å (*B*)] are almost equal. Hence it is likely that the single anionic charge is distributed between O(2) and O(3), and that the H atom is attached to O(4). The high energy P ~ O bond is 1.595 in *A* and 1.610 Å in *B*, which is comparable to that found in the monopotassium salt of PEP [1.612 (6) Å].

The H atom on O(4) of the phosphates in *A* and *B* was located in the difference map. It was found to take up a staggered position which is more prominent in *A* than in *B* [O(1)—P(1)—O(4)—H(4) = 49.4 (*A*), -21.9° (*B*)]. The hydroxyl O(4) is *gauche* with respect to C(2) in both molecules [O(4)—P(1)—O(1)—C(2) = 64.1 (*A*), -68.2° (*B*)], as also found in PEP.K (-46.5°). In contrast, it is *trans* in PEP-cyclohexylammonium salt (PEP.CHA) (Watson & Kennard, 1973), the torsion

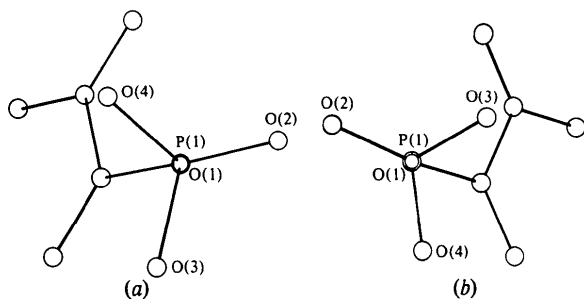


Fig. 1. View down the P(1)—O(1) bond of (a) molecule *A*, (b) molecule *B*.

angle being 159.9°. When viewed down P(1)—O(1), the phosphates in *A* and *B* are found to assume the normally preferred staggered conformation (Fig. 1).

The phosphate geometry of both molecules deviates significantly from that of a regular tetrahedral arrangement. The O—P—O angles are distributed on either side of 109.5°, the angle contained by the shortest bonds being the largest. The two independent phosphates *A* and *B* have similar bond lengths and angles, the maximum differences being for the angles O(2)—P(1)—O(4) [111.6 (*A*), 113.8 (*B*)] and O(3)—P(1)—O(4) [107.8 (*A*), 105.4° (*B*), e.s.d. = 0.3°].

#### The enolpyruvate group

*The enolic bond.* The enolic bond C(2)—O(1) is 1.401 in *A* and 1.386 Å in *B*. It is comparable to the average C(sp<sup>2</sup>)—O distance of 1.395 Å (Newton & Campbell, 1974). Hence the enolic bond has a considerable amount of double-bond character. In PEP.K, this bond length is 1.374 Å. The P—O—C angle is 125.7 in *A* and 123.4° in *B* (e.s.d. = 0.5°), considerably larger than the 120° normally observed in phosphate ester structures.

#### The enolpyruvate geometry

Results of mean-plane calculations for the enolpyruvate parts of the two PEP.Na molecules are given

Table 6. *Least-squares planes and deviations of atoms (Å)*

Average e.s.d. in the atom deviation is 0.007 Å.

	<i>A</i>	<i>B</i>
(I)		
C(1)	0.052	0.093
C(2)	0.039	0.047
C(3)	0.010	0.015
O(31)	0.030	0.041
O(32)	-0.041	-0.054
O(1)	-0.038	-0.049
(II)		
C(1)	-0.014	-0.011
C(2)	0.020	0.017
C(3)	-0.006	-0.005
O(1)	-0.004	-0.003
(III)		
C(2)	-0.001	-0.001
C(3)	0.001	0.003
O(31)	-0.001	-0.001
O(32)	-0.001	-0.001

#### Equations of planes

(IA)	0.5770x - 0.3571y - 0.7346z = 3.1528
(IB)	0.5715x - 0.0369y - 0.7610z = 6.2041
(IIA)	0.5743x - 0.3200y - 0.7535z = 2.9056
(IIB)	0.5914x - 0.2476y - 0.7674z = 5.7854
(IIIA)	0.5962x - 0.3780y - 0.7083z = 3.1398
(IIIB)	0.5359x - 0.3393y - 0.7731z = 6.3125

in Table 6. The angle between the normals to the planes formed by C(1), C(2), C(3), O(1) and C(2), C(3), O(31), O(32) is  $4.4^\circ$  in *A* and  $7.3^\circ$  in *B*, which compares with the  $3.5^\circ$  in pyruvic acid (Harata, Sakabe & Tanaka, 1977). This angle is  $18.1^\circ$  in Na pyruvate (Tavale, Pant & Biswas, 1961), the maximum observed in pyruvate structures studied so far. This angle is an indication of the flexibility for rotation about the central C(2)—C(3) bond, *i.e.* the degree of conjugation across it. In PEP.Na, the C(2)—C(3) bond is  $1.499 \text{ \AA}$  in both *A* and *B* (e.s.d. =  $0.011 \text{ \AA}$ ), suggesting partial double-bond character. It is significantly different from the  $1.579 \text{ \AA}$  in Na pyruvate, which is close to the C—C single-bond length. The C=O and C—O(H) lengths ( $1.22$  and  $1.31 \text{ \AA}$ ) in the carboxyl group compare well with those usually found in carboxylic acid structures (Leiserowitz, 1976). The carbonyl O atom is *trans* planar to the olefinic C atom [C(1)—C(2)—C(3)—O(31) =  $178.2$  (*A*),  $-176.2^\circ$  (*B*)] in both molecules. This is in contrast to the *cis* planar orientation in PEP.CHA ( $7.2^\circ$ ). The angle C(3)—C(2)—O(1) is  $110.8$  in *A* and  $112.8^\circ$  in *B*, considerably smaller than the  $120^\circ$  expected at an  $sp^2$  hybridized C atom.

#### Orientation of the phosphate relative to the enolpyruvate system

The orientation of the phosphate with respect to the enolpyruvate system in *A* is different from that in *B*, the torsion angle C(3)—C(2)—O(1)—P(1) being  $122.6$  in *A* and  $-112.0^\circ$  in *B*. This makes the molecular shapes of *A* and *B* almost mirror images of each other, the mirror being the planar enolpyruvate group (Fig. 2). This dihedral angle is  $-209.1$  in PEP.K and  $-90^\circ$  in PEP.CHA.

#### Na<sup>+</sup> ion coordination

There are two crystallographically independent Na<sup>+</sup> ions in the structure. The nature of their interactions with the surroundings is different. Na(1) has six nearest neighbours. Four of them are contributed by the phosphates of four different PEP molecules and the remaining two by the water molecule *W*(1) and its symmetry equivalent *W*(1)'. Na(2) is coordinated to

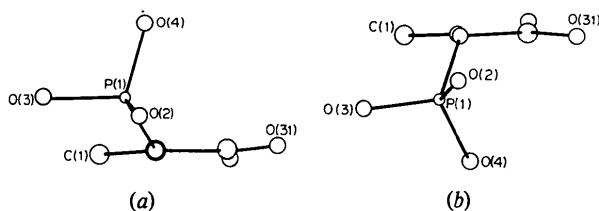


Fig. 2. View along the enolpyruvate plane of (a) molecule *A*, (b) molecule *B*.

five ligands. It binds to three independent PEP's and two waters *W*(2) and *W*(2)'. Somewhat similar six and five coordinations for Na<sup>+</sup> ions have been observed in 5'-dGMP.Na<sub>2</sub> (Young, Tollin & Wilson, 1974; Viswamitra & Seshadri, 1974). The ester O(1) of *A* is  $2.98 \text{ \AA}$  from Na(1) and that of *B* is  $2.92 \text{ \AA}$  from Na(2).

In PEP.K, the K<sup>+</sup> ion is almost in the plane of the enolpyruvate (deviation =  $0.02 \text{ \AA}$ ) and binds to the phosphate, ester and carboxyl O atoms of the same molecule. However, in the present structure, Na<sup>+</sup> is involved in bridging only the phosphate ends of different molecules. The Na<sup>+</sup> ion, because it is smaller (ionic radius =  $0.95 \text{ \AA}$ ; Pauling, 1960), is not able to link both the carboxyl and phosphate ends of the same molecule. It is probable that the differences in the molecular shapes of PEP.Na and PEP.K are brought about by the differences in their metal-ion binding patterns.

#### Molecular packing and hydrogen bonding

There are four intermolecular hydrogen bonds in the structure. Two of them link the PEP molecules along a

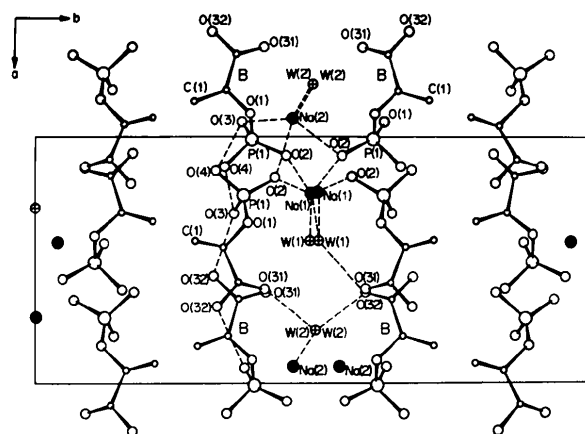


Fig. 3. Packing diagram down *c*.

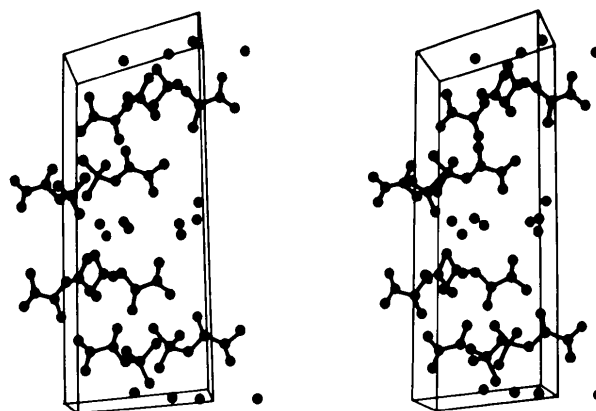


Fig. 4. Stereoview of the molecular packing.

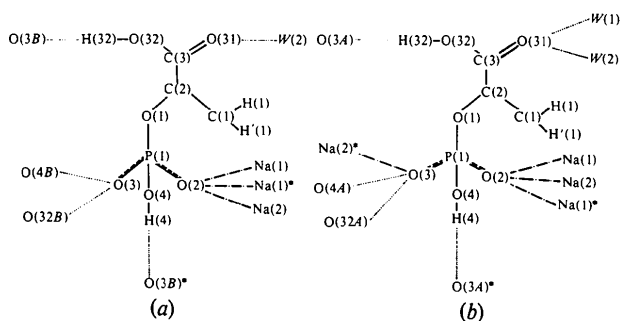


Fig. 5. Schematic diagram showing the molecular interactions of (a) molecule A, (b) molecule B. An asterisk marks atoms related by a different symmetry element of the space group.

Table 7. Sodium coordination and hydrogen bonding (Å)

Average e.s.d. is 0.008 Å.

		Symmetry code				
Na(1)...	O(2A)	2.448	1	0	0	0
Na(1)...	O(2B)	2.421	1	0	0	0
Na(1)...	O(2B)	2.658	3	0	1	-1
Na(1)...	O(2A)	2.363	3	0	1	0
Na(1)...	W(1)	2.409	1	0	0	0
Na(1)...	W(1)	2.490	3	0	1	0
Na(2)...	O(2B)	2.515	2	0	-1	0
Na(2)...	O(3B)	2.558	4	-1	0	0
Na(2)...	O(2A)	2.309	4	0	0	0
Na(2)...	W(2)	2.335	2	-1	-1	-1
Na(2)...	W(2)	2.418	4	-1	0	-1
O(3A)...	O(32B)	2.566	1	1	0	0
O(4B)...	O(3A)	2.638	1	0	0	1
O(32A)...	O(3B)	2.613	1	1	0	1
O(4A)...	O(3B)	2.791	1	0	0	0
O(31A)...	W(2)	2.889	1	0	0	0
O(31B)...	W(2)	2.841	3	-1	1	-1
O(31B)...	W(1)	2.844	3	1	1	-1

Symmetry code: (1)  $x, y, z$ ; (2)  $\frac{1}{2} + x, \frac{1}{2} + y, z$ ; (3)  $x, \bar{y}, \frac{1}{2} + z$ ; (4)  $\frac{1}{2} + x, \frac{1}{2} - y, \frac{1}{2} + z$ . The numbers following the symmetry code indicate cell translations of the atom.

[O(3A)...

structure is completed through metal-ion binding and the hydrogen bonds involving the water molecules (Table 7). Fig. 4 shows the molecular packing. Fig. 5 is a schematic diagram illustrating the molecular interactions of the two individual molecules A and B.

Carboxylic acids generally form dimeric structures through hydrogen bonds between the carbonyl and hydroxyl O atoms (Leiserowitz, 1976). Pyruvic acid shows this type of association in its crystal structure. In the present structure, however, the carboxyl groups are not involved in any dimer formation. Instead, they stack one above the other at an average distance of 3.2 Å.

We thank the DST for financial support. One of us (SKK) thanks the CSIR for the award of a Fellowship.

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