Acta Cryst. (1981). **B37**, 839–843

The Structure of Monopotassium Phosphoenolpyruvate

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(*Received* 25 *September* 1980; *accepted* 3 *November* 1980)

Abstract

$C_3H_4O_6P^-.K^+$, $M_r = 206{\cdot}10$, is orthorhombic, space group *Pbca* (from systematic absences), $a = 14{\cdot}538\,(4)$, $b = 13{\cdot}364\,(5)$, $c = 6{\cdot}880\,(6)$ Å, $U = 1383{\cdot}9$ Å^3, $D_x = 2{\cdot}07$ Mg m^{-3}, $Z = 8$, λ(Mo $K\alpha$) = 0·7107 Å, μ(MO $K\alpha$) = 1·015 mm^{-1}. The final R value is 0·042 for a total of 1397 reflections. The high energy P—O(13) and the enolic C(1)—O(13) bonds are 1·612 and 1·374 Å respectively. The enolpyruvate moiety is essentially planar. The orientation of the phosphate with respect to the pyruvate group in PEP.K is distinctly different from that in the PEP-cyclohexylammonium salt, the torsion angle C(2)—C(1)—O(13)—P being −209·1 in the former and −90° in the latter. The K^+ ion binds simultaneously to both the phosphate and carboxyl ends of the same PEP molecule. The ester O(13) is also a binding site for the cation. The K^+ ion is coplanar with the pyruvate moiety and binds to O(22) and O(13) almost along their lone-pair directions. The carbonyl O(22) prefers to bind to the K^+ ion rather than take part in the formation of hydrogen bonds usually observed in carboxylic acid structures.

Introduction

The high-energy phosphate compounds play a central role in the energy storage and transfer processes of the cell. Phosphoenolpyruvate (PEP) which belongs to this class of compounds has a free energy of hydrolysis which is among the highest known for naturally occurring phosphates. It has a major biological role as the phosphate donor in the biosynthesis of ATP from ADP and PEP. This reaction, catalysed by the enzyme pyruvate kinase, requires the presence of monovalent K^+ ions in addition to divalent Mg^{2+}. While Rb^+ and Tl^+ can replace K^+ ions as cofactors, Na^+ and Li^+ ions are found to be inhibitory. We have been investigating the molecular structures of substrates of this reaction in the presence of various metal ions by single-crystal X-ray analysis (Viswamitra, Hosur, Shakked & Kennard, 1976; Katti & Viswamitra, 1979; Hosur & Viswamitra, 1979). We report here the structure of PEP as its monopotassium salt.

Experimental

Crystals of PEP.K (original sample from Boehringer Mannheim GmbH) were grown by slowly diffusing acetone into 1 ml of the aqueous solution containing 8–10 mg of the compound. Single crystals with well developed prismatic faces were seen sticking to the walls of the test tube after about ten days. From Weissenberg and precession photographs, the space group of the crystals was uniquely identified as *Pbca*. The cell parameters obtained by photographic methods were later refined on a CAD-4 diffractometer. The density, measured by flotation in bromoform and acetone, was consistent with the presence of one PEP.K molecule in the asymmetric unit.

A crystal 0·30 × 0·20 × 0·70 mm was mounted in a Lindemann-glass capillary with a little mother liquor. X-ray intensities to a resolution of 0·76 Å were collected at 293 K on the κ axis diffractometer with graphite-monochromated Mo $K\alpha$ radiation in the $\omega/2\theta$ scan mode. Backgrounds for each reflection were measured on each side of the Bragg peak for $\frac{1}{8}$th of the scan angle. Corrections for Lorentz and polarization factors were applied.

Structure solution and refinement

The structure was solved with *MULTAN* (Main, Woolfson & Germain, 1971). Phases for 122 reflections with $|E_{\min}| = 1{\cdot}60$ were determined with three symbols. The phosphate group and the K^+ ion were identified in the E map computed with the best set of phases ($R = 0{\cdot}2536$, ABSFOM = 1·29, combined figure of merit = 2·00). The rest of the structure was developed by a difference synthesis based on this partial structure. Block-diagonal least-squares refinement with individual isotropic temperature factors reduced R to 0·15 and subsequently to 0·054 with the introduction of anisotropic thermal parameters. The

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difference map computed at this stage revealed the positions of all H atoms. R converged to 0·042 on further refinement with anisotropic temperature factors for non-H atoms and isotropic for H. The function minimized during the final cycles was $\sum w(|F_o| - k|F_c|)^2$ where $w = 1/(a + b|F_o| + c|F_o|^2)$, a = 2·00, b = 0·10 and c = 0·003. Scattering factors for non-H atoms were computed with the function developed by Cromer & Waber (1965). For H atoms they were taken from Stewart, Davidson & Simpson (1965).

Results and discussion

Tables 1 and 2 give the final positional parameters for the non-H and H atoms respectively.* Bond lengths and angles are listed in Table 3. Torsion angles involving non-H atoms are given in Table 4.

Molecular geometry

Phosphate group

The H atom attached to the phosphate group was clearly located near O(11) in a difference map. Since the other two terminal P—O bonds are nearly equal [P—O(12) = 1·482, P—O(14) = 1·493 Å], the single negative charge on the phosphate group is likely to be distributed between O(12) and O(14). The high-energy P—O(13) bond is 1·612 (6) Å and agrees with that found in the cyclohexylammonium salt of PEP (PEP.CHA) (Watson & Kennard, 1973), within experimental error. The O—P—O angles are distributed on either side of the tetrahedral value and the smallest O—P—O angle involves O(12) and O(13) coordinating the metal ion.

The conformation about the phosphate ester bond, P—O(13), is distinctly different in PEP.K from that in PEP.CHA, the hydroxyl O(11) being *gauche* to C(1) in the former (—46·5°) and *trans* in the latter (159·9°). The hydroxyl H(11), however, is found to assume the *gauche* geometry with respect to the ester O(13) in both compounds, the torsion angle H(11)—O(11)—P—O(13) being −72·8 in PEP.K and −29·5° in PEP.CHA.

Table 1. *Final positional and thermal parameters for the non-*H *atoms* (×10⁴) *with e.s.d.'s in parentheses for x,y,z*

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

	x	y	z	U_{eq} (Å^2)
P	3299 (1)	1498 (2)	4354 (3)	21
O(11)	3440 (4)	2603 (4)	4497 (9)	32
O(12)	2552 (4)	1094 (5)	5589 (9)	32
O(13)	4207 (4)	1020 (4)	5327 (9)	29
O(14)	3254 (5)	1236 (4)	2245 (9)	37
K	3568 (1)	4756 (2)	3910 (3)	30
C(1)	5104 (6)	1286 (6)	4967 (13)	25
C(2)	5690 (6)	1104 (6)	6714 (13)	28
O(21)	6544 (5)	1366 (6)	6458 (11)	43
O(22)	5388 (4)	778 (5)	8214 (9)	35
C(3)	5431 (7)	1640 (7)	3289 (15)	39

Table 2. *Final positional and thermal parameters for the* H *atoms* (×10³)

	x	y	z	U (Å^2)
H(11)	338	285	563	63
H(32)	607	188	319	9
H(31)	500	175	217	27
H(21)	708	133	753	45

Table 3. *Bond lengths* (Å) *and angles* (°) *with e.s.d.'s in parentheses*

C(3)—C(1)	1·335 (13)	C(3)—C(1)—C(2)	123·5 (8)
C(1)—C(2)	1·493 (12)	C(3)—C(1)—O(13)	125·8 (8)
C(2)—O(22)	1·203 (11)	C(2)—C(1)—O(13)	110·7 (7)
C(2)—O(21)	1·302 (11)	C(1)—C(2)—O(22)	122·8 (8)
C(1)—O(13)	1·374 (10)	C(1)—C(2)—O(21)	113·0 (7)
O(13)—P	1·612 (6)	O(22)—C(2)—O(21)	124·1 (8)
P—O(12)	1·482 (7)	C(1)—O(13)—P	126·8 (5)
P—O(11)	1·494 (6)	O(13)—P—O(11)	104·6 (3)
P—O(14)	1·493 (7)	O(13)—P—O(12)	102·6 (3)
		O(13)—P—O(14)	110·3 (4)
		O(11)—P—O(12)	115·0 (4)
		O(11)—P—O(14)	107·5 (4)
		O(12)—P—O(14)	116·1 (4)

Table 4. *Torsion angles* (°) *involving non-*H *atoms*

Average e.s.d. is 0·8°.

C(1)—O(13)—P—O(11)	−47·3
C(1)—O(13)—P—O(12)	−167·7
C(1)—O(13)—P—O(14)	68·0
C(2)—C(1)—O(13)—P	150·9
C(3)—C(1)—O(13)—P	−30·5
O(13)—C(1)—C(2)—O(21)	−178·6
C(3)—C(1)—C(2)—O(21)	2·7
O(13)—C(1)—C(2)—O(22)	−0·7
C(3)—C(1)—C(2)—O(22)	−179·4

The enolpyruvate group

The enolic C(1)—O(13) is 1·374 Å, significantly shorter than the 1·43 Å in PEP.CHA, suggesting partial double-bond character for the bond. The P—O(13)—C(1) angle (126·8°) is also significantly different from that in PEP.CHA (121·2°) and from the average (120·0°) for the P—O—C angle in phosphate monoesters (Newton & Campbell, 1974).

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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 35884 (10 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

C(2)—C(1)—O(13) is only 111°, which is considerably less than the standard value of 120° around an sp^2 C atom. In pyruvic acid (Harata, Sakabe & Tanaka, 1977), Na pyruvate (Tavale, Pant & Biswas, 1961) and PEP.CHA it is 123·8, 119·1 and 117·8° respectively.

Phosphate orientation with respect to the enolpyruvate group

The enolpyruvate system, comprising C(3), C(1), O(13), C(2), O(22) and O(21), is essentially planar. The least-squares plane and the atomic deviations are given in Table 5. The carboxyl H atom is *cis* planar with respect to the carbonyl O(22) [H(21)—O(21)—C(2)—O(22) = −0·7°]. The olefinic C(3) is *trans* planar with respect to O(22) [O(22)—C(2)—C(1)—C(3) = −179·4°], in contrast to the *cis* planar conformation (−7·2°) in PEP.CHA. The orientation of the phosphate with respect to the pyruvate moiety is very different in the two structures. The torsion angle C(2)—C(1)—O(13)—P is −209·1 in PEP.K and −90° in PEP.CHA. The consequent change in the molecular shape is shown in Fig. 1 which is a view of the molecules along the pyruvate plane.

Table 5. *Least-squares plane and deviations of atoms* (Å)

Average e.s.d. in atom deviations is 0·007 Å.

C(1)*	−0·003	C(3)*	0·016
C(2)*	0·011	O(13)*	−0·006
O(21)*	−0·015	K	−0·002
O(22)*	0·005		

Equation of the plane

$$0{\cdot}1960x - 0{\cdot}9285y - 0{\cdot}3153z = 1{\cdot}2168$$

* Atoms used to calculate mean plane.

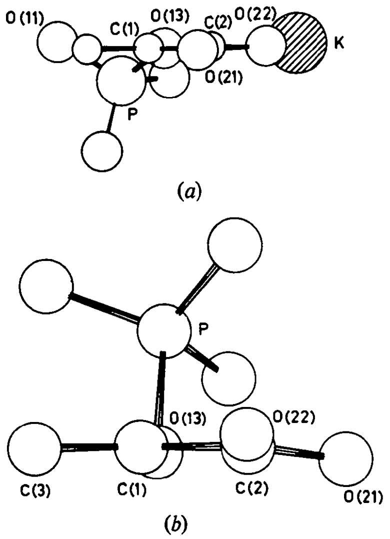

Fig. 1. Orientation of the phosphate group with respect to the pyruvate moiety in (*a*) PEP.K and (*b*) PEP.CHA. The view is along the bisector of C(3)—C(1)—C(2).

Potassium ion binding

K^+ binds to both the phosphate and carboxyl ends of the same PEP molecule. It directly coordinates to the phosphate O(12), the carboxyl O(22) and the phosphate ester O(13) as shown in Fig. 2(*a*). The K^+ ion has the right size (radius = 1·33 Å) to bind in this specific pattern (Fig. 2*a*). The cation is coplanar with the pyruvate moiety, the deviation being only 0·02 Å (Fig. 2*b*). Also, the angles K···O(22)—C(2) and

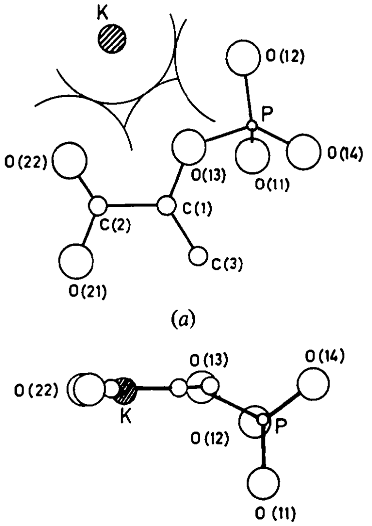

Fig. 2. View of PEP.K (*a*) perpendicular and (*b*) parallel to the pyruvate group. Van der Waals radii for K^+ and the ligating O atoms are shown in (*a*).

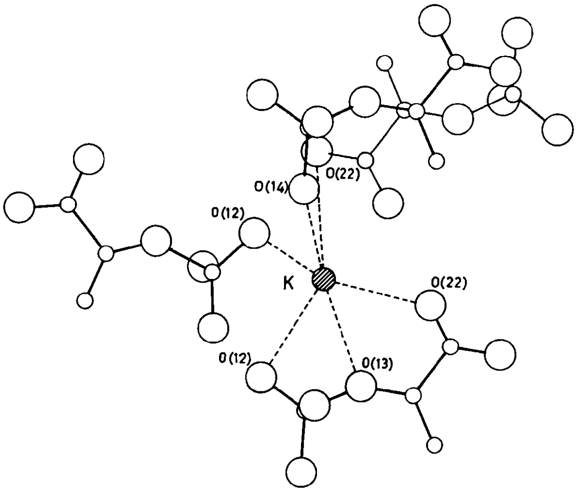

Fig. 3. Nature of cation binding and hydrogen bonding in PEP.K.

Table 6. *Geometry of the coordination polyhedron around the* K$^+$ *ion*

Average e.s.d. in distances is 0·006 Å and in angles 0·5°.

(*a*) Distances (Å)

		Symmetry code	Translation		
K···O(14)	2·689	(i)	0	0	0
K···O(12)	2·680	(ii)	0	0	0
K···O(22)	2·782	(i)	0	0	−1
K···O(13)	2·831	(i)	0	0	−1
K···O(22)	2·842	(iii)	1	0	1
K···O(12)	2·949	(i)	0	0	−1

(*b*) Angles (°)

O(14^{i})···K···O(12^{ii})	81·8	O(13^{i})···K···O(12^{i})	49·3
O(14^{i})···K···O(22^{i})	100·5	O(22^{iii})···K···O(12^{i})	172·7
O(14^{i})···K···O(13^{i})	128·2	O(12^{ii})···K···O(13^{i})	145·0
O(14^{i})···K···O(22^{iii})	74·6	O(12^{ii})···K···O(22^{iii})	72·8
O(14^{i})···K···O(12^{i})	112·7	O(22^{i})···K···O(22^{iii})	74·6
O(12^{ii})···K···O(22^{i})	145·4	O(22^{i})···K···O(12^{i})	104·1
O(12^{ii})···K···O(12^{i})	106·7	O(13^{i})···K···O(22^{iii})	127·4
O(22^{i})···K···O(13^{i})	56·2		

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

K···O(13)—C(1) are both about 120°. Thus the cation binding is almost along their electron lone-pair directions.

The binding of the K$^+$ ion to the ester O(13) (2·85 Å) is a feature not observed in the K$^+$ salts of other phosphate esters such as ADP.K (Katti & Viswamitra, 1979) and UDP.K$_2$ (Viswamitra, Post & Kennard, 1979). For Na$^+$ salts, however, there have been a few instances as in 3′-UMP.Na$_2$ (Viswamitra, Reddy, James & Williams, 1972), and Na.ApU (Seeman, Rosenberg, Suddath, Kim & Rich, 1976) where the ester O atom is found to take part in metal ligation. Three more O atoms from three different PEP molecules in the crystal complete the sixfold coordination round the K$^+$ ion as shown in Fig. 3. The coordination polyhedron is irregular with the dimensions given in Table 6.

Hydrogen bonding and molecular packing

There are two intermolecular hydrogen bonds in the structure as shown in Fig. 4. One involves the phosphate O(14) and O(11) [O(14)···H—O(11) = 2·49 Å], while the second is between the carboxyl O(21) and the phosphate O(12) [O(21)—H···O(12) = 2·53 Å]. Carboxylic acids generally form dimeric structures through hydrogen bonds between the carbonyl and hydroxyl O atoms (Leiserowitz, 1976), as for example, in pyruvic acid. The coordination of two K$^+$ ions to the carbonyl O atom prevents such an association in this structure.

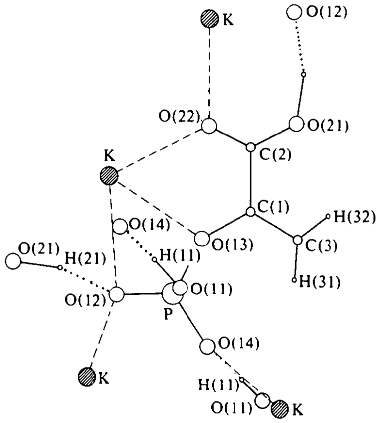

Fig. 4. Intermolecular interactions of the PEP molecule.

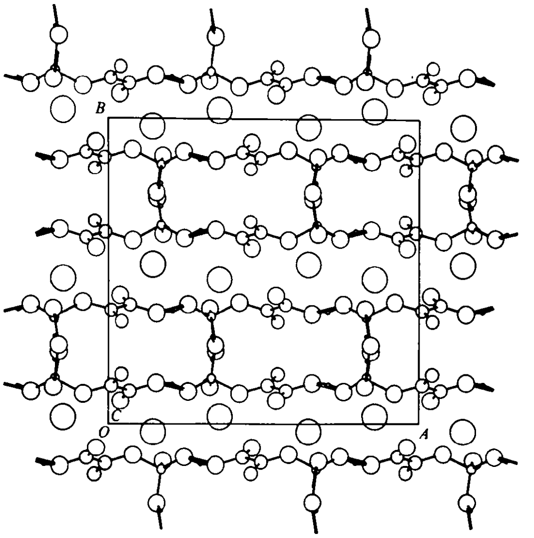

Fig. 5. Packing diagram viewed down **c**.

A packing diagram of the structure down **c** is given in Fig. 5. The hydrogen bond O(21) ···O(12) links the PEP molecules head-to-tail along the [110] direction and the other hydrogen bond O(14) ···O(11) links two adjacent chains. The packing of these double chains is completed through the coordination to the K$^+$ ions.

We thank the DST for financial support. MVH thanks the ICMR for the award of a Fellowship. We thank Mr S. K. Katti for help and discussion.

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