Structure of Tris(cyclohexylammonium) Phosphoenolpyruvate Monohydrate and Crystal Chemistry of Phosphoenolpyruvates

Mohamed Souhassou,[a]† Peter M. Schaber[a,b] and Robert H. Blessing[a]*

[a]*Hauptman–Woodward Institute (formerly the Medical Foundation of Buffalo), 73 High Street, Buffalo, NY 14203, USA, and* [b]*Department of Chemistry, Canisius College, 2001 Main Street, Buffalo, NY 14208, USA*
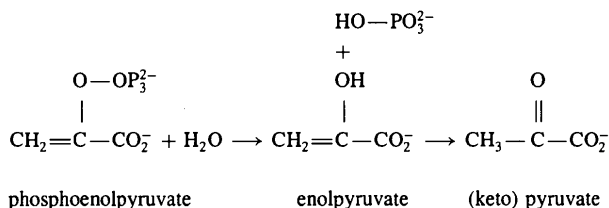
Abstract

The crystal structure of $(C_6H_{11}NH_3^+)_3 \cdot Pep^{3-} \cdot H_2O$, where $Pep^{3-} = (O^-)_2P(O){-}O{-}C(CH_2){-}CO_2^-$, is reported and the systematic structural variations among 19 crystallographic occurrences of H_3Pep, H_2Pep^-, $HPep^{2-}$ and Pep^{3-} species, which are important phosphate donors in the ATP cycle of bioenergetics, are reviewed. Tris(cyclohexylammonium) phosphoenolpyruvate monohydrate, $(C_6H_{11}NH_3^+)_3 \cdot [O_3POC(CH_2)CO_2]^{3-} \cdot H_2O$, $M_r = 483.6$, m.p. 418–420 K; $T = 296\,(1)$ K; orthorhombic, $P2_12_12_1$; $a = 16.7042\,(5)$, $b = 24.4881\,(6)$, $c = 6.38910\,(10)$ Å; $V = 2613.49\,(11)$ Å^3, $Z = 4$, $D_x = 1.23$, $D_m = 1.22$ mg mm^{-3}, $\mu = 0.14$ mm^{-1} for $\lambda(Mo\,K\alpha) = 0.7107$ Å; $F(000) = 1056$ e; $R(|F|) = 0.0608$ for 6056 hkl and $\overline{hkl}$ data with $(\sin\theta)/\lambda \le 0.65$ Å^{-1}.

1. Introduction

In the ATP cycle that forms the molecular basis of bioenergetics, phosphoenolpyruvate (Pep) is an important phosphate donor. Indeed, among the metabolic phosphates that participate in the ATP cycle, Pep has the most favorable free energy of hydrolysis or 'phosphate group transfer potential' [$\Delta G^0 = -61.96$ kJ mol^{-1} at pH $= 7$, $a(H_2O) = 1$, 1 M total phosphate and with excess Mg^{2+} (Jencks, 1968; Mahler & Cordes, 1971; Lehninger, 1975)]. This is in part because enzymic hydrolysis of Pep is 'pulled along' by rapid, subsequent, nonenzymic tautomerization of pyruvate.

$$
\begin{array}{ccccc}
 & & HO{-}PO_3^{2-} & & \\
 & & + & & \\
O{-}OP_3^{2-} & & OH & & O \\
| & & | & & \| \\
CH_2{=}C{-}CO_2^- + H_2O & \longrightarrow & CH_2{=}C{-}CO_2^- & \longrightarrow & CH_3{-}C{-}CO_2^-
\end{array}
$$

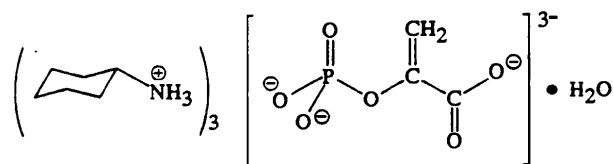phosphoenolpyruvate enolpyruvate (keto) pyruvate

---

† Permanent address: Laboratoire de Cristallographie et Modelisation des Matériaux Minéraux et Biologiques (LCM³B), URA CNRS no. 809, Université Henri Poincaré, Nancy I, Faculté des Sciences, Boîte Postale no. 239, 54506 Vandoeuvre-lès-Nancy CEDEX, France.

Phosphoenolpyruvic acid is tribasic and crystal structures are known for the free acid, H_3Pep, as well as for various salts with its mono-, di- and trianions, H_2Pep^-, $HPep^{2-}$ and Pep^{3-}. Table 1 lists these structures – almost all of which have been determined or refined by Professor Tadeusz Lis and his co-workers at the University of Wroclaw. There are four crystallographically distinct occurrences of the H_3Pep molecule, 11 of the H_2Pep^- monoanion, two of the $HPep^{2-}$ dianion and two of the Pep^{3-} trianion. This paper reports the second occurrence of the trianion.

2. Experimental work and crystal structure analysis

Experimental details are summarized in Table 2. Colorless rectangular parallelepiped shaped crystals of tris(cyclohexylammonium) phosphoenolpyruvate mono-

hydrate were obtained by recrystallizing the commercially available material (Sigma Chemical Co.) by vapor diffusion of acetonitrile into a concentrated aqueous solution. The melting point of the crystals was determined using a Fisher–Johns melting point apparatus and their mass density was determined by flotation in acetone–chloroform. A crystal of approximate dimensions $0.5 \times 0.2 \times 0.2$ mm was used for the X-ray analysis.

X-ray data were measured as $\omega/2\theta$ scan profiles for $|h| \le 21$, $|k| \le 31$, $|l| \le 8$, and $(\sin\theta)/\lambda \le 0.65$ Å^{-1} using Nb-filtered Mo radiation on a Siemens (née Nicolet née Syntex) P3 diffractometer. Preliminary axial rotation photographs on the diffractometer verified that the lattice is primitive. Lattice parameters were determined by least-squares fit to the optimized setting angles of 50 reflections with $15 < 2\theta < 28°$. Scan widths were $\Delta\omega = [\theta(K\alpha_2) + 0.325°] - [\theta(K\alpha_1) - 0.325°]$, which corresponded to *ca* twice the base width

$(C_6H_{11}NH_3^+)_3.Pep^{3-}.H_2O$

Table 1. *Crystallographic data for phosphoenolpyruvates*

Compound	Space group	a (Å) / α (°)	b (Å) / β (°)	c (Å) / γ (°)	Z	References
Free acid $H_2O_3POC(CH_2)CO_2H$	$P\bar{1}$	5.905 (5) / 104.70 (8)	8.135 (8) / 97.72 (8)	14.095 (15) / 100.99 (9)	4	(a)
Free acid–ammonium salt $H_2O_3POC(CH_2)CO_2H.-$ $HO_3POC(CH_2)CO_2H^-.NH_4^+$	$P2_1$	7.618 (4)	22.321 (10) / 90.14 (4)	8.311 (3)	4	(b)
Monosodium monohydrate $HO_3POC(CH_2)CO_2H^-.Na^+.H_2O$	Cc	11.423 (2)	23.253 (5) / 123.63 (1)	6.604 (1)	2×4	(c)
		11.426 (7)	23.274 (22) / 123.66 (5)	6.620 (5)		(d)
Monopotassium $HO_3POC(CH_2)CO_2H^-.K^+$	$Pbca$	14.538 (4)	13.364 (5)	6.880 (6)	8	(e)
		14.534 (8)	13.863 (6)	6.883 (3)		(f)
Monocyclohexylammonium $HO_3POC(CH_2)CO_2H^-.C_6H_{11}NH_3^+$	$P2_1/c$	13.218 (5)	8.117 (3) / 109.90 (2)	12.977 (5)	4	(g)
		13.48 (1)	8.104 (6) / 112.81 (6)	12.98 (1)		(h)
	$P2_12_12_1$	5.892 (4)	12.22 (1)	18.12 (2)	4	(h)
Calcium dihydrate $[HO_3POC(CH_2)CO_2H^-]_2.Ca^{2+}.2H_2O$	$P\bar{1}$	5.424 (4) / 83.00 (8)	5.594 (6) / 85.73 (6)	12.078 (9) / 85.18 (7)	1	(i)
Magnesium dihydrate $[HO_3POC(CH_2)CO_2H^-]_2.Mg^{2+}.2H_2O$	$P\bar{1}$	5.172 (3) / 85.30 (4)	5.33 (3) / 86.90 (4)	12.184 (6) / 86.56 (4)	1	(j)
Manganous dihydrate $[HO_3POC(CH_2)CO_2H^-]_2.Mn^{2+}.2H_2O$	$P\bar{1}$	5.277 (3) / 83.74 (4)	5.443 (3) / 86.35 (4)	12.090 (6) / 86.96 (4)	1	(j)
Zinc dihydrate $[HO_3POC(CH_2)CO_2H^-]_2.Zn^{2+}.2H_2O$	$P\bar{1}$	5.184 (3) / 84.69 (7)	5.341 (5) / 86.72 (6)	12.124 (9) / 86.36 (7)	1	(j)
Bis(cyclohexylammonium) methanol hemisolvate $[H_{0.5}O_3POC(CH_2)CO_2H_{0.5}]^{2-}.-$ $[HO_3POC(CH_2)CO_2]^{2-}.-$ $(C_6H_{11}NH_3^+)_4.CH_3OH$	$C2/c$	27.065 (15)	15.405 (6) / 112.30 (6)	21.291 (16)	8	(k)
Tris(cyclohexylammonium) monohydrate $O_3POC(CH_2)CO_2^{3-}.-$ $(C_6H_{11}NH_3^+)_3.H_2O$	$P2_12_12_1$	16.7042 (5)	24.4881 (6)	6.38910 (10)	4	(l)
Silver barium trihydrate $O_3POC(CH_2)CO_2^{3-}.Ag^+Ba^{2+}.3H_2O$	$P2_1/a$	6.587 (3)	23.811 (9) / 90.57 (3)	6.610 (3)	4	(m)

References: (a) Weichsel & Lis (1989b); Weichsel, Lis & Kuczek (1989); (b) Weichsel, Lis & Kuczek (1991); (c) Katti, Hosur & Viswamitra (1981); (d) Lis (1991); (e) Hosur & Viswamitra (1981); (f) Lis (1987); (g) Watson & Kennard (1973); (h) Weichsel & Lis (1989a); (i) Lis & Kuczek (1991); (j) Lis (1992); (k) Weichsel & Lis (1990a); (l) this work; (m) Weichsel & Lis (1990b).

of the reflection peaks, and the scan speed was a constant $\Delta\omega/\Delta t = 1.00°$ min^{-1}, which corresponded to ~ 90 reflection measurements per hour. Inspection of profile plots showed $h00$, $h = 2n$, $0k0$, $k = 2n$, and $00l$, $l = 2n$, to be the conditions for possible reflection. With the chosen scan widths, the scan profiles of several dozen high-angle $h10$, $h01$, $h11$, $1k0$, $0k1$ and $1k1$ reflections were found to include tails from neighboring reflection peaks. During data reduction, the affected scans were modified to remove the intruding peak tails using the interactive profile processing program *VIEW* (Blessing, 1989b, and references therein).

The 2,12,1, 11,2,0, 403 and 582 reflections were remeasured at 50 reflection intervals as reference intensity monitors; their intensities decreased smoothly by 2.6% over the 225 h of X-ray exposure with random variance $\sigma^2(|F|^2) = \sigma^2_{count} + (p|F|^2)^2$ and $p = 0.014$. After time-dependent scaling, averaging the 19449 symmetry-allowed equivalent or repeated measurements over point group 222 gave 6045 unique data with $R_{merge} = [\Sigma(|F^2| - \langle|F|^2\rangle)^2/\Sigma(|F|^2)^2]^{1/2} = 0.0241$; averaging over mmm gave 3456 unique data with $R_{merge} = 0.0262$. Absorption corrections were not necessary because all transmission factors would have exceeded $A_{min} = \exp(-\mu t_{max}) = 0.93$. The merged unique data were subjected to a Bayesian statistical treatment (French & Wilson, 1978) to improve the estimates of $|F|^2$, $\sigma(|F|^2)$, $|F|$ and $\sigma(|F|)$ for the weak reflections, and all the unique data, including approximately one-third of them which had $|F|^2 < 3\sigma(|F|^2)$, were included in the structure analysis.

Table 2. *Experimental details*

Crystal data

Chemical formula	$3C_6H_{14}N^+.C_3H_2O_6P^{3-}.H_2O$
Chemical formula weight	483.6
Cell setting	Orthorhombic
Space group	$P2_12_12_1$
a (Å)	16.7042 (5)
b (Å)	24.4881 (6)
c (Å)	6.38910 (10)
V (Å^3)	2613.49 (11)
Z	4
D_x (Mg m^{-3})	1.23
D_m (Mg m^{-3})	1.22
Density measured by	Flotation in acetone/chloroform
Radiation type	Mo $K\alpha$
Wavelength (Å)	0.7107
No. of reflections for cell parameters	50
θ range (°)	7.5–14
μ (mm^{-1})	0.14
Temperature (K)	296 (1)
Crystal form	Rectangular parallelepiped
Crystal size (mm)	0.5 × 0.2 × 0.2
Crystal color	Colorless

Data collection

Diffractometer	Siemens P3
Data collection method	$\omega/2\theta$ profiles
Absorption correction	None
No. of measured reflections	19449
No. of independent reflections	6045
No. of observed reflections	4030
Criterion for observed reflections	$|F|^2 > 3\sigma(|F|^2)$
R_{int}	0.0241
θ_{max} (°)	27.5
Range of h, k, l	$0 \rightarrow h \rightarrow 21$
	$0 \rightarrow k \rightarrow 31$
	$0 \rightarrow l \rightarrow 8$
No. of standard reflections	4
Frequency of standard reflections	Every 50 reflections
Intensity decay (%)	2.6

Refinement

Refinement on	F
R	0.0608
wR	0.0391
S	1.42
No. of reflections used in refinement	6045
No. of parameters used	475
H-atom treatment	All H-atom parameters refined
Weighting scheme	$w = 1/\sigma^2(|F_o|)$
$(\Delta/\sigma)_{max}$	0.2
$\Delta\rho_{max}$ (e Å^{-3})	0.34
$\Delta\rho_{min}$ (e Å^{-3})	−0.37
Extinction method	Zachariasen (1967)
Extinction coefficient	0.53 (6) ×10^{-4}
Source of atomic scattering factors	Cromer & Waber (1974) for C, N, O and P and Stewart, Davidson & Simpson (1965) for H. Corrections for anomalous dispersion from Cromer & Ibers (1974)

Table 3. *Fractional atomic coordinates and equivalent isotropic displacement parameters (Å^2)*

$$B_{eq} = (8\pi^2/3)\Sigma_i\Sigma_j U_{ij}a_i^* a_j^* \mathbf{a}_i.\mathbf{a}_j.$$

	x	y	z	B_{eq}
N1	0.28764 (12)	0.55166 (9)	0.6411 (4)	2.56 (9)
N3	1.06724 (14)	0.58877 (10)	0.6778 (4)	2.68 (10)
N2	0.32170 (15)	0.73546 (9)	0.1180 (4)	3.20 (11)
O2	0.25449 (10)	0.63649 (7)	0.0990 (3)	3.48 (8)
O3	0.17379 (10)	0.58203 (6)	0.3585 (3)	3.22 (8)
O4	0.17891 (10)	0.55329 (6)	−0.01888 (24)	2.52 (7)
O5	0.00010 (10)	0.68708 (7)	−0.1586 (3)	3.45 (8)
O6	−0.03840 (10)	0.73881 (7)	0.1123 (3)	3.51 (8)
Ow	0.89987 (15)	0.72134 (11)	0.5234 (4)	4.78 (12)
P	0.18456 (4)	0.600426 (23)	0.13582 (10)	2.060 (23)
O1	0.10321 (10)	0.63406 (7)	0.0709 (3)	2.87 (7)
C1	0.07330 (13)	0.68053 (9)	0.1568 (4)	2.33 (9)
C2	0.00621 (4)	0.70434 (10)	0.0249 (4)	2.60 (10)
C3	0.09822 (17)	0.70357 (11)	0.3305 (5)	3.02 (11)
C4	0.35628 (14)	0.58976 (10)	0.6623 (4)	2.58 (10)
C5	0.41119 (17)	0.57105 (12)	0.8378 (5)	3.26 (13)
C6	0.48164 (19)	0.60996 (15)	0.8610 (6)	4.28 (15)
C7	0.52652 (19)	0.61667 (16)	0.6552 (6)	4.90 (17)
C8	0.47089 (20)	0.63381 (16)	0.4809 (6)	4.62 (16)
C9	0.40095 (17)	0.59464 (14)	0.4580 (4)	3.54 (13)
C10	0.25850 (17)	0.77432 (11)	0.0437 (5)	3.34 (12)
C11	0.28892 (22)	0.83244 (13)	0.0523 (6)	4.23 (15)
C12	0.22711 (23)	0.87165 (15)	−0.0299 (7)	5.20 (20)
C13	0.2013 (3)	0.85642 (16)	−0.2462 (8)	6.57 (23)
C14	0.1712 (3)	0.79882 (16)	−0.2554 (10)	7.4 (3)
C15	0.23290 (21)	0.75846 (13)	−0.1743 (6)	4.44 (16)
C16	0.99847 (14)	0.55166 (10)	0.6369 (4)	2.57 (10)
C17	0.94925 (17)	0.54512 (12)	0.8334 (5)	3.38 (13)
C18	0.87878 (22)	0.50736 (15)	0.7930 (6)	4.85 (17)
C19	0.82859 (18)	0.52759 (15)	0.6127 (7)	5.14 (18)
C20	0.87860 (20)	0.53474 (16)	0.4160 (6)	4.69 (16)
C21	0.94982 (19)	0.57223 (14)	0.4533 (5)	3.66 (14)

value near $+1$ or -1 if anomalous dispersion due to resonant scattering is experimentally significant. The f^0 values were those given by the analytical approximations (Cromer & Waber, 1974) to the spherically averaged free-atom P, O, N and C form factors and the spherically contracted H-atom form factor (Stewart, Davidson & Simpson, 1965). The f' and f'' anomalous dispersion corrections were those tabulated for Mo $K\alpha$ (Cromer & Ibers, 1974). Even though the resonant scattering was quite weak in the present case, our data were numerous and accurate enough for the η refinement to determine the chirality of the $P2_12_12_1$ arrangement in the crystal of its achiral component molecules.

The structure refinement converged to $R = \Sigma|\Delta|/\Sigma|F_o| = 0.0608$, $wR = (\chi^2/\Sigma w|F_o|^2)^{1/2} = 0.0391$ and $z = [\chi^2/(n-m)]^{1/2} = 1.42$ for $n = 6045$ data and $m = 475$ parameters. The refined parameters included the extinction parameter $g = 0.53(6) \times 10^{-4}$, the chirality parameter $\eta = +0.96(20)$, anisotropic mean-square displacements for the non-H atoms and positions and isotropic mean-square displacements for the H atoms. Initial positions for the H atoms were found in a difference electron-density map at an intermediate stage of the refinement. In the last refinement cycle $|\delta|/\sigma < 0.2$ and in a final difference map $-0.37 < \Delta\rho < +0.34$ e Å^{-3}.

The crystal structure was determined by direct methods using the mmm averaged data and refined by full-matrix least-squares fit to the 222 averaged data. The fit minimized $\chi^2 = \Sigma w\Delta^2$, where $w = \sigma^{-2}(|F_o|)$ and $\Delta = |F_o| - k^{-1}y^{1/2}|F_c|$. The quantity $y = [1 + g(p_2/p_1^2)Lp|F_c|^2]^{-1/2}$ is an isotropic extinction correction (Zachariasen, 1967) and the F_c model also included the chirality/polarity parameter η (Rogers, 1981), which is defined by $f = f^0 + f' + i\eta f''$ and is expected to refine to a

Table 4. *Valence geometry* in crystals of* $(C_6H_{11}NH_3^+)_3.O_3POC(CH_2)CO_2^{3-}.H_2O$

Bond lengths (Å)		Valence angles (°)		O···O (Å)	Conformation angles (°)	
P—O2	1.483 (2)	O2—P—O3	115.0 (1)	2.519	O2—P—O1—C1	−62.8 (6)
P—O3	1.503 (2)	O2—P—O4	113.4 (1)	2.512	O3—P—O1—C1	61.6 (6)
P—O4	1.522 (2)	O2—P—O1	108.2 (1)	2.534	O4—P—O1—C1	178.6 (6)
P—O1	1.642 (2)	O3—P—O4	112.3 (1)	2.513	P—O1—C1—C3	−10.4 (5)
O1—C1	1.359 (3)	O3—P—O1	106.9 (1)	2.528	O—O1—C1—C2	168.9 (5)
C1—C3	1.313 (4)	O4—P—O1	99.5 (1)	2.417	O1—C1—C2—O5	−15.9 (6)
C3—H1	0.94 (3)	P—O1—C1	128.5 (2)		O1—C1—C2—O6	163.0 (6)
C3—H2	0.97 (3)	O1—C1—C3	125.8 (2)		C3—C1—C2—O5	163.5 (6)
C1—C2	1.518 (3)	H1—C3—H2	123.7 (2)		C3—C1—C2—O6	−17.6 (6)
C2—O5	1.251 (3)	O1—C1—C2	111.6 (2)			
C2—O6	1.257 (3)	C1—C2—O5	116.8 (2)			
		C1—C2—O6	116.7 (2)		⟨|N—C—C—C|⟩	179 ± 1
⟨N—H⟩	0.95 ± 9	O5—C2—O6	126.5 (2)		⟨|C—C—C—C|⟩	55.6 ± 8
⟨N—C⟩	1.490 ± 8					
⟨C—C⟩	1.513 ± 8	⟨H—N—H⟩	108±3			
⟨C—H⟩	0.99 ± 5	⟨H—N—C⟩	110±2			
Ow—Hw1	0.83 (4)	Hw1—Ow—Hw2	100 (4)			
Ow—Hw2	0.85 (4)					

* The values labeled with angle brackets are means and e.s.d.'s from the means for the three $C_6H_{11}NH_3^+$ cations. Along with the O—P—O valence angles, the corresponding O···O distances are given.

Local programs (Blessing, 1989b, and references therein) were used for the experimental data reduction and error analysis. The *NRCVAX* programs (Gabe, Lee & LePage, 1985) were used for the structure analysis. The *MULTAN* procedure (Germain, Main & Woolfson, 1970) as implemented in *NRCVAX* was used for the structure determination. The *ORTEP* program (Johnson, 1970) was used to prepare the crystal structure drawings.

3. Results and discussion for $(C_6H_{11}NH_3^+)_3.Pep^{3-}.H_2O$

Atomic coordinates are given in Table 3, valence geometries in Table 4 and hydrogen-bond geometries in

Table 5.* The unit cell and the phosphoenylpyruvate trianion and its hydrogen-bonded surroundings are illustrated in Fig. 1.

3.1. Crystal packing and hydrogen bonding

Fig. 1 shows that the phosphoenolpyruvate trianions, the ammonium groups of the cyclohexylammonium monocations and the water molecules are linked by hydrogen bonds into an intricate three-dimensional network and this network of charged and polar groups
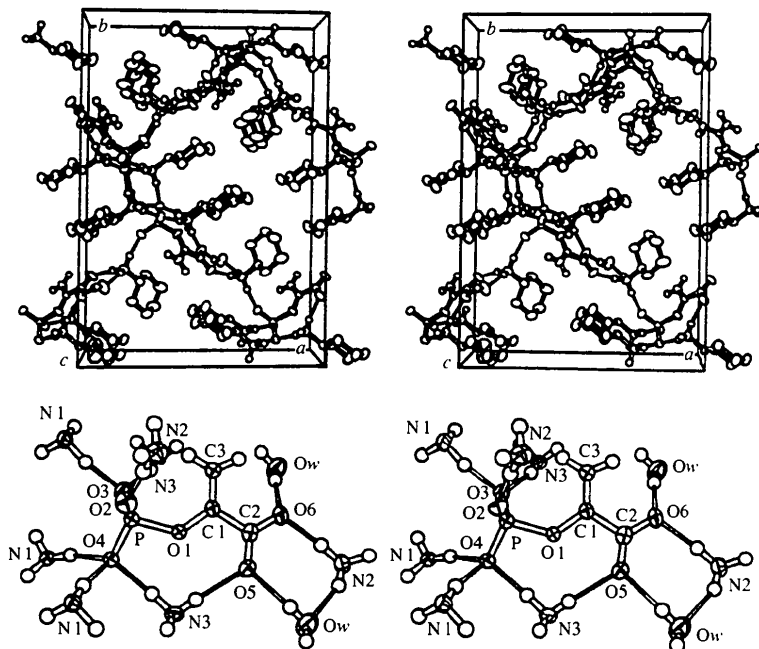
Fig. 1. Stereoscopic drawings of the unit cell viewed along [001] and of the anion and its hydrogen-bonded surroundings viewed perpendicular to the C3—C1—O1 plane of the $H_2C=C-O$ enol group. Non-H atoms are represented as 50% equiprobability ellipsoids; H atoms are represented as spheres of radius 0.15 Å. The cyclohexyl H atoms are omitted from the unit-cell diagram and the cyclohexyl groups of the cylcohexylammonium cations are omitted from the hydrogen-bonded anion diagram.

Table 5. *Hydrogen-bond distances (Å) and angles (°) in crystals of* $(C_6H_{11}NH_3^+)_3.O_3POC(CH_2)CO_2^{3-}.H_2O$*

			H—O	O···H—O	O···O
			H—N	O···H—N	O···N
C—O···H—O					
C—O···H—N					
P—O···H—N	Geometry	O···H			
C2—O5···Hw2—Ow ⎫	Trigonal	1.96	0.85	158	2.763
C2—O5···H33—N3 ⎭	planar	2.00	0.86	169	2.854
C2—O6···Hw1—Ow ⎫	Trigonal	2.03	0.83	169	2.854
C2—O6···H22—N2 ⎭	planar	1.80	1.04	173	2.833
P—O2···H21—N2	Angular	1.71	0.97	174	2.674
P—O3···H31—N3 ⎫	Trigonal	1.89	0.84	168	2.712
P—O3···H11—N1 ⎭	planar	1.65	1.09	169	2.726
P—O4···H13—N1 ⎫	Tetrahedral	1.90	0.95	162	2.822
P—O4···H32—N3 ⎬		1.81	1.03	170	2.827
P—O4···H12—N1 ⎭		1.96	0.88	168	2.832
H₂O···H23—N2	Trigonal pyramidal	2.00	0.90	155	2.841

*E.s.d.'s average 0.03 Å for O···H, H—O and H—N; 3° for O···H—O and O···H—N; 0.003 Å for O···O and O···N.

encloses large channels occupied by the nonpolar cyclohexyl groups. The channels run parallel to [001] and have an approximately rectangular cross section with their walls approximately parallel to (110) and

(1̄10). The arrangement of ammonium groups and water molecules that surround and hydrogen bond to the anion suggests a solvation structure that isolates the anion from neighboring anions and aggregates of cyclohexyl groups.

Dimensions of the hydrogen-bonding structure shown in Fig. 1 are given in Table 5. Each carboxylate oxygen accepts two hydrogen bonds, one from a water molecule and one from an ammonium group. The phosphate ester oxygen is not involved in hydrogen bonding and the other three phosphate oxygens accept one, two and three hydrogen bonds, respectively, all from ammonium groups. The water molecule accepts one hydrogen bond from an ammonium group, and donates in two hydrogen bonds that bridge between the carboxylate oxygens of neighboring anions. As Fig. 1 shows, the N—H hydrogen bond donors surrounding the phosphate group have a chiral arrangement with three hydrogen bonds to O4—P, two to O3—P, one to O2—P and none to O1—P. The P atom is, therefore, effectively a chiral center in the crystal, and the weak

H₃PEP MOL. A
(a)

H₃PEP MOL. B
(a)

H₃PEP. MOL. A
IN H₃PEP.H₂PEP⁻.NH₄⁺
(b)

H₃PEP MOL. B
IN H₃PEP.H₂PEP⁻.NH₄⁺
(b)

H₂PEP⁻ MOL. A
IN H₃PEP. H₂PEP⁻.NH₄⁺
(b)

H₂PEP⁻ MOL. B
IN H₃PEP.H₂PEP⁻.NH₄⁺
(b)

H₂PEP⁻ MOL. A
IN H₂PEP⁻.Na⁺.H₂O
(d)

H₂PEP⁻ MOL. B
IN H₂PEP⁻.Na⁺.H₂O
(d)

H₂PEP⁻ IN H₂PEP⁻.K⁺
(f)

H₂PEP⁻ IN ORTHORHOMBIC
H₂PEP⁻.C₆H₁₁NH₃⁺
(h)

H₂PEP⁻ IN MONOCLINIC
H₂PEP⁻.C₆H₁₁NH₃⁺
(h)

H₂PEP IN
(H₂PEP⁻)₂.Ca²⁺.2H₂O
(i)

H₂PEP IN
(H₂PEP)₂.Mg²⁺.2H₂O
(j)

(H₀.₅PEPH₀.₅)²⁻ IN
(HPEP²⁻)₂(C₆H₁₁NH₃)₄
.CH₃OH
(k)

HPEP²⁻ IN
(HPEP²⁻)₂(C₆H₁₁NH₃⁺)₄
.CH₃OH
(k)

PEP³⁻ IN
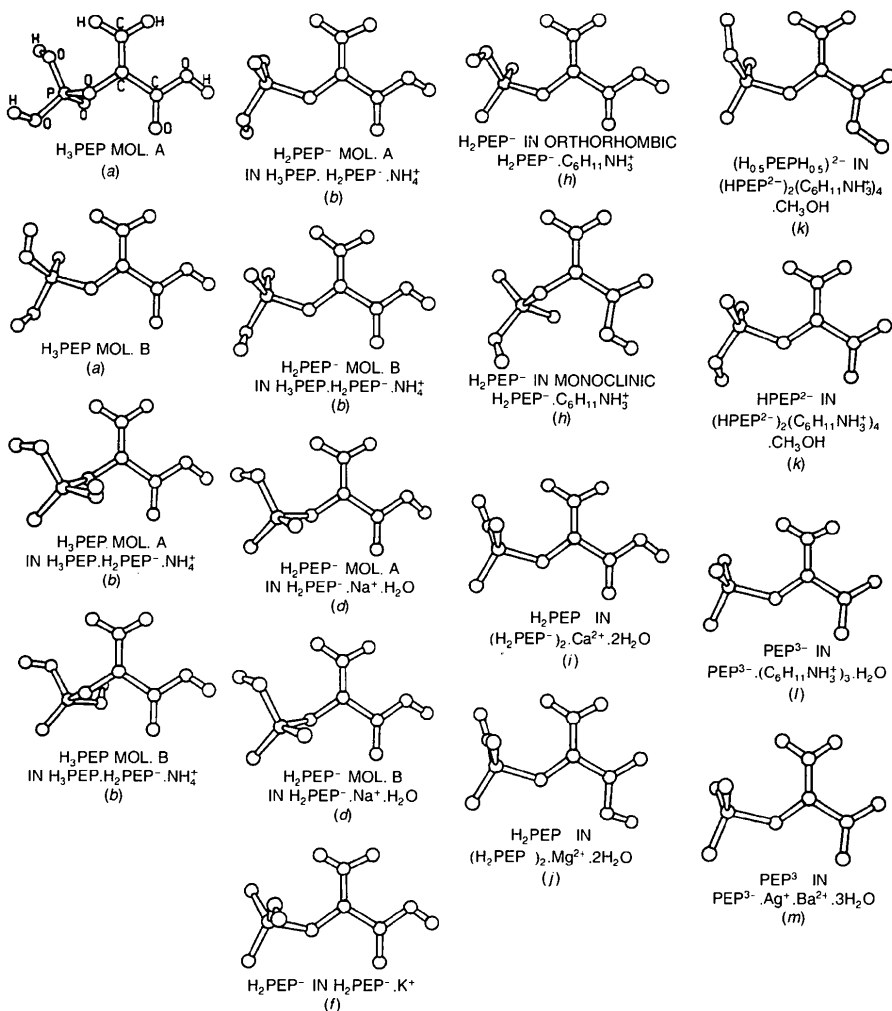PEP³⁻.(C₆H₁₁NH₃⁺)₃.H₂O
(l)

PEP³⁻ IN
PEP³⁻.Ag⁺.Ba²⁺.3H₂O
(m)

Fig. 2. Illustrations of the H₃Pep, H₂Pep⁻, HPep²⁻ and Pep³⁻ conformations observed in the crystals listed in Tables 1 and 6. Only 17 of the 19 Pep structures are illustrated, because the H₂Pep⁻ anions have the same conformation in the isomorphous Mg²⁺.2H₂O, Mn²⁺.2H₂O and Zn²⁺.2H₂O crystals. In each case, the view is perpendicular to the plane of the H₂C=C—O enol group, with the C—CH₂ group vertical, the phosphate ester group to the left and the pyruvate carboxylate group to the right. The letter codes in parentheses beneath each illustration denote the references cited in Table 1.

Table 6. *Conformation angles (°) in 19 crystallographically distinct phosphoenolpyruvate structures (e.s.d.'s average 0.8°)*

$\varphi_1 = C—C—C—O = H_2C=C(OPO_3)—C(O)—O$ for the enolpyruvate group, $\varphi_1 = C—C—O—P = O_2C—C(CH_2)—O—PO_3$ for the phosphate ester group and $\varphi_3 = C—O—P—O = O_2C(CH_2)C—O—PO_2—O$ with the maximum of the three possible $|\varphi_3|$.

		φ_1	φ_2	φ_3	Reference
H_3Pep	A	-3.9	-114.7	176.0	(a)
	B	-3.8	-154.1	162.1	
$H_3Pep.H_2Pep^-.NH_4^+$					
H_3Pep	A	3.0	101.9	-164.5	(b)
	B	-4.8	-101.4	163.7	
H_2Pep^-	A	13.4	175.0	-174.8	
	B	-10.1	-176.8	173.1	
$H_2Pep^-.Na^+.H_2O$	A	-2.1	123.0	-177.5	(c)
	B	-3.1	111.5	-171.2	
$H_2Pep^-.K^+$		2.7	150.9	-167.4	(d)
$H_2Pep^-.C_6H_{11}NH_3^+$	(I)	14.9	-155.8	180.0	(e)
	(II)	-7.5	-89.7	158.9	
$(H_2Pep^-)_2.Ca^{2+}.2H_2O$		5.6	163.8	-173.6	(f)
$(H_2Pep^-)_2.Mg^{2+}.2H_2O$		5.5	164.5	-178.0	(g)
$(H_2Pep^-)_2.Mn^{2+}.2H_2O$		7.7	165.7	-176.9	
$(H_2Pep^-)_2.Zn^{2+}.2H_2O$		5.5	165.9	-179.6	
$(HPep^{2-})_2.(C_6H_{11}NH_3^+)_4.CH_3OH$					
$[H_{0.5}PepH_{0.5}]^{2-}$		5.2	-172.0	-178.2	(h)
$HPep^{2-}$		11.0	-178.2	-176.0	
$Pep^{3-}.(C_6H_{11}NH_3^+)_3.H_2O$		-17.6	168.9	178.6	(i)
$Pep^{3-}.Ag^+Ba^{2+}.3H_2O$		-5.0	163.9	-166.9	(j)

References: (a) Weichsel & Lis (1989b); Weichsel, Lis & Kuczek (1989); (b) Weichsel, Lis & Kuczek (1991); (c) Lis (1991); (d) Lis (1987); (e) Weichsel & Lis (1989a); (f) Lis & Kuczek (1991); (g) Lis (1992); (h) Weichsel & Lis (1990a); (i) this work; (j) Weichsel & Lis (1990b).

Mo $K\alpha$ resonant scattering by phosphorus was strong enough to determine the chirality of the hydrogen-bonded structure.

The P—O bond lengths provide a particularly clear example of the effects of hydrogen bonding on the bond strengths in P—O acceptor groups. Hydrogen bonding weakens the partial double-bond character and strengthens the charge-separated single-bond character in the acceptor groups

$$\{P=O\cdots H—N \longleftrightarrow P—O^-\cdots H—N\}$$
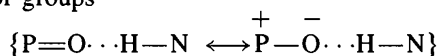
and the acceptor P—O bond lengths increase from 1.48 to 1.50 to 1.52 Å (Table 4) as the number of hydrogen bonds accepted increases from one to two to three (Table 5). This effect of the number of bonds accepted is evidently more important than the strength of the individual bonds: As the number of bonds accepted increases from one to two to three, the individual hydrogen bonds become somewhat weaker and longer, and the O···N distances increase from 2.67 Å to an average of 2.72 Å to an average of 2.83 Å (Table 5).

As the P—O bond lengths vary from 1.48 to 1.64 Å, the O—P—O valence angles vary from 115 to 100°, with the shorter P—O bonds corresponding to the larger O—P—O interbond angles (Table 4). As in other PO_4 structures, most of this variation is due to an off-center displacement of the central P atom within a relatively rigid tetrahedron of O atoms in close van der Waals contact. Table 4 shows that five of the six O···O

contact distances along the tetrahedron edges are close to the 2.52 Å average O···O for other PO_4 tetrahedra (Baur, 1974; Blessing, 1988, 1989a); the sixth O···O distance is ~0.1 Å shorter than the other five and corresponds to the O4—P—O1 group, which has the two longest P—O bonds and the smallest O—P—O angle.

## 4. Crystal chemistry of phosphoenolpyruvates

Although Pep is thermodynamically unstable towards hydrolysis under biological conditions, it is kinetically stable enough that all of its conjugate acid–base forms – H_3Pep, H_2Pep^-, $HPep^{2-}$ and Pep^{3-} – have been crystallized from aqueous solution over a much wider range of pH than the biological range. The 19 different crystallographic observations (Table 1) of the Pep species are illustrated in Fig. 2.

4.1. *Relative strengths of the three acidic functions of H_3Pep*

The H-atom positions found in the crystal structures (Fig. 2) show that $K_{a1}(—OPO_3H_2) > K_{a2}(—CO_2H) \geq K_{a3}(—OPO_3H^-)$. Evidence that the third acid ionization constant is only slightly smaller than the second is provided by the occurrence in crystals of $(C_6H_{11}NH_3^+)_2.HPep^{2-}.0.5MeOH$ of an $HPep^{2-}$ dianion, $[H_{0.5}O_3POC(CH_2)CO_2H_{0.5}]^{2-}$, in which the acid proton is disordered between the phosphate and carboxylate groups (Weichsel & Lis, 1990a).

4.2. *Conformational variations*

Table 6 and Fig. 2 show that there is little conformational variation in the enolpyruvate groups of the Pep molecules. The dihedral angle between the planes of the enol group and the carboxylate group corresponds to the conformation angle

$$\varphi_1 = C—C—C—O = H_2C=C(OPO_3)—C(O)—O,$$
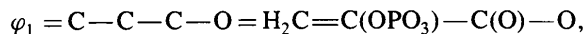
which maintains values close to zero so that the π-bond systems of the enol and carboxylate groups remain conjugated. In the 14 cases illustrated in Fig. 2, in which the carboxylate group is a protonated carboxylic acid group, the conjugated enol $H_2C=C$ group and carboxylic C=O group have an *anti* orientation in 11 cases and a *syn* orientation in only three.

In the phosphate ester groups of the Pep molecules the P—O—C ester links exhibit considerable conformational flexibility and the conformation angles

$$\varphi_2 = C—C—O—P = O_2C—C(CH_2)—O—PO_3$$

and

$$\varphi_3 = C—O—P—O = O_2C(CH_2)C—O—PO_2—O$$
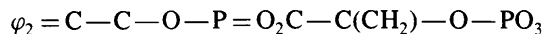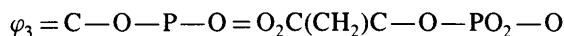
vary from 180° by up to ~90 and ~20°, respectively. The most compactly folded conformation is that of the

Table 7. *Bond lengths (Å) in the phosphate ester groups (e.s.d's average 0.004 Å)*

		P=O	P—O(H)	P—O(C)	(P)O—C	(P—O)	Reference
H₃Pep	A	1.493	1.553 1.534	1.578	1.400	1.534	(a)
	B	1.481	1.529 1.533	1.583	1.389	1.532	
H₃Pep.H₂Pep⁻.NH₄⁺							
H₃Pep	A	1.472	1.520 1.547	1.590	1.396	1.532	(b)
	B	1.469	1.531 1.535	1.594	1.389	1.537	
H₂Pep⁻	A	1.499 1.501	1.529	1.614	1.387	1.536	
	B	1.483 1.496	1.547	1.609	1.369	1.534	
H₂Pep⁻.Na⁺.H₂O	A	1.486 1.499	1.570	1.606	1.391	1.540	(c)
	B	1.485 1.506	1.568	1.618	1.398	1.544	
H₂Pep⁻.K⁺		1.490 1.493	1.545	1.622	1.382	1.537	(d)
H₂Pep⁻.C₆H₁₁NH₃⁺	(I)	1.484 1.500	1.567	1.610	1.377	1.540	(e)
	(II)	1.492 1.499	1.560	1.604	1.377	1.539	
(H₂Pep⁻)₂.Ca²⁺.2H₂O		1.477 1.499	1.573	1.616	1.383	1.541	(f)
(H₂Pep⁻)₂.Mg²⁺.2H₂O		1.477 1.496	1.564	1.615	1.390	1.538	(g)
(H₂Pep⁻)₂.Mn²⁺.2H₂O		1.474 1.495	1.568	1.618	1.380	1.539	
(H₂Pep⁻)₂.Zn²⁺.2H₂O		1.474 1.496	1.564	1.615	1.378	1.537	
(HPep²⁻)₂.(C₆H₁₁NH₃⁺)₄.CH₃OH							
[H₀.₅PepH₀.₅]²⁻		1.494 1.496	1.527	1.627	1.375	1.536	(h)
HPep²⁻		1.481 1.487	1.558	1.634	1.382	1.540	
Pep³⁻.(C₆H₁₁NH₃⁺)₃.H₂O		1.483 1.503 1.522		1.642	1.359	1.538	(i)
Pep³⁻.Ag⁺Ba²⁺.3H₂O		1.493 1.497 1.521		1.662	1.375	1.543	(j)
Sample size		35	21	19	19	19	
Minimum		1.469	1.520	1.578	1.359	1.532	
Maximum		1.522	1.573	1.662	1.400	1.544	
Range		0.053	0.053	0.084	0.041	0.012	
Sample mean		1.492	1.548	1.614	1.383	1.538	
Sample standard deviation		0.012	0.017	0.020	0.010	0.003	

References: (a) Weichsel & Lis (1989b); Weichsel, Lis & Kuczek (1989); (b) Weichsel, Lis & Kuczek (1991); (c) Lis (1991); (d) Lis (1987); (e) Weichsel & Lis (1989a); (f) Lis & Kuczek (1991); (g) Lis (1992); (h) Weichsel & Lis (1990a); (i) this work; (j) Weichsel & Lis (1990b).

monoanion in the monoclinic crystals of $C_6H_{11}NH_3^+.H_2Pep^-$. In general, folded conformations seem to be possible for only the neutral H_3Pep or singly charged H_2Pep^- species and phosphate–carboxylate like-charge repulsions seem to force the $HPep^{2-}$ dianions and Pep^{3-} trianions into fully extended conformations.

4.3. Valence geometric variations

Bond lengths and valence angles in the Pep phosphate ester groups are given in Tables 7 and 8, and bond lengths in the enolpyruvate moieties are given in Table 9. The sample of 19 structures is large enough to show statistically significant correlations of

the variations in valence geometry, and the correlations that involve the phosphate ester link are illustrated in Fig. 3. The least-squares straight lines plotted in Fig. 3 are those that minimize the sum of squares of the perpendicular, rather than the vertical, distances of the data points from the lines. Fig. 3 also gives the values of

$$p(n, r) = (1/\pi^{1/2})\{\Gamma[(\nu + 1)/2]/\Gamma(\nu/2)\}$$

$$\times \int_{|r|}^{1}(1 - x^2)^{(\nu-2)/2}dx, \quad \nu = n - 2,$$

the probability of no correlation in samples of size n with correlation coefficients

Table 8. *Valence angles (°) in the phosphate ester groups (e.s.d.'s average 0.4°)*

		O=P=O	O=P—OH	O=P—OC	HO—P—OH	HO—P—OC	P—O—C	Reference
H_3Pep	A		110.0	111.8	111.3	103.3	121.6	(a)
			115.0			104.6		
	B		113.0	110.2	106.0	102.8	125.8	
			115.0			109.0		
$H_3Pep.H_2Pep^-.NH_4^+$								
H_3Pep	A		112.9	107.9	106.1	108.2	120.3	(b)
			117.0			104.2		
	B		112.6	108.0	105.9	108.2	120.4	
			117.7			103.7		
H_2Pep^-	A	115.8	113.0	107.5		107.5	123.3	
			108.8	102.0				
	B	115.4	113.0	107.4		107.4	124.0	
			109.1	101.9				
$H_2Pep^-.Na^+.H_2O$	A	117.7	111.8	103.0		107.3	125.2	(c)
			106.8	109.8				
	B	117.6	113.8	104.4		105.8	123.4	
			105.1	109.5				
$H_2Pep^-.K^+$		115.8	115.4	101.5		105.9	125.6	(d)
			108.1	109.5				
$H_2Pep^-.C_6H_{11}NH_3^+$	(I)	117.6	107.6	103.8		106.5	124.8	(e)
			110.9	109.9				
	(II)	115.7	108.4	108.2		100.4	120.0	
			112.8	110.3				
$(H_2Pep^-)_2.Ca^{2+}.2H_2O$		119.9	107.9	102.4		104.0	124.0	(f)
			111.4	109.8				
$(H_2Pep^-)_2.Mg^{2+}.2H_2O$		120.5	107.4	102.3		103.5	123.3	(g)
			111.3	110.3				
$(H_2Pep^-)_2.Mn^{2+}.2H_2O$		120.3	107.5	102.5		103.9	123.3	
			111.0	110.1				
$(H_2Pep^-)_2.Zn^{2+}.2H_2O$		120.7	107.0	102.3		103.9	123.1	
			111.1	110.3				
$(HPep^{2-})_2.(C_6H_{11}NH_3^+)_4.CH_3OH$								
$[H_{0.5}PepH_{0.5}]^{2-}$		116.5	112.5	108.1		106.7	127.3	(h)
			111.2	100.6				
$HPep^{2-}$		117.7	112.4	107.1		100.4	122.6	
			109.0	108.6				
$Pep^{3-}.(C_6H_{11}NH_3^+)_3.H_2O$		115.0		108.2			128.5	(i)
		113.4		106.9				
		112.3		99.5				
$Pep^{3-}.Ag^+Ba^{2+}.3H_2O$		116.0		98.8			127.1	(j)
		114.1		107.2				
		113.6		105.1				
Sample size		19	34	36	4	21	19	
Minimum		112.3	105.1	98.8	105.9	100.4	120.0	
Maximum		120.7	117.7	111.8	111.3	109.0	128.5	
Range		8.4	12.6	13.0	5.4	8.6	8.5	
Sample mean		116.4	111.0	106.2	107.3	105.1	123.9	
Sample standard deviation		2.6	3.0	3.7	2.7	2.4	2.4	

References: (a) Weichsel & Lis (1989b); Weichsel, Lis & Kuczek (1989); (b) Weichsel, Lis & Kuczek (1991); (c) Lis (1991); (d) Lis (1987); (e) Weichsel & Lis (1989a); (f) Lis & Kuczek (1991); (g) Lis (1992); (h) Weichsel & Lis (1990a); (i) this work; (j) Weichsel & Lis (1990b).

$$r = \langle (x - \langle x \rangle)(y - \langle y \rangle) \rangle / \left[\langle (x - \langle x \rangle)^2 \rangle \langle (y - \langle y \rangle)^2 \rangle \right]^{1/2},$$
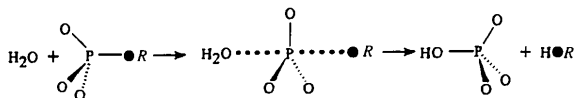$$-1 \leq r \leq +1$$

(Bevington, 1969). As Fig. 3(a) indicates, since $p(n, r)$ depends on both the sample size and correlation coefficient, the quantity $p(n, r)$ is a more informative statistic than r alone and can indicate probable correlation between data with $|r|$ values as small as ~0.5 if n is sufficiently large.

Fig. 3(a) suggests that there is a slight overall expansion of the phosphate group due to O···O like-charge repulsions, as the phosphate anionic charge increases from zero to three, and Figs. 3(b), (c) and

(d) show that the bond lengths and valence angle in the P—O—C ester link are mutually correlated with the anionic charge. As the anionic charge increases from zero to three, the ester P—O bond lengthens by ~0.07 Å, the ester O—C bond shortens by 0.03 Å and the ester P—O—C angle opens by ~5°. These changes act to relieve like-charge repulsion between the phosphate and carboxylate anionic sites at the two ends of the Pep molecules. Again, lengthening of the P—O(C) bonds is accomplished mainly by off-center displacements of the central P atoms in the PO₄ tetrahedra, and, as the P—O(C) bonds lengthen, the corresponding average values of the three O—P—O(C) valence angles decrease by several

degrees from ~107 to ~104°, as shown in Fig. 3(e). These behaviors manifest a structural flexibility in the various Pep species that is consistent with the hypothesis (see, *e.g.*, Westheimer, 1987, and references cited therein) that phosphate ester hydrolysis occurs by scission of the P—O(C) bond rather than the (P)O—C bond and passes through a trigonal bipyramidal five-coordinate transition state or a trigonal planar, monomeric metaphosphate PO_3^- intermediate.

$$H_2O + \overset{O}{\underset{O}{\overset{|}{P}}} -\bullet R \longrightarrow H_2O\cdots\overset{O}{\underset{O\quad O}{\overset{|}{P}}}\cdots\bullet R \longrightarrow HO-\overset{O}{\underset{O}{\overset{|}{P}}} + H\bullet R$$

4.4. Postscript

Just after we concluded our analyses, we learned that the crystal structures of two more Pep salts, NH_4H_2Pep and $(NH_4)_3Pep.H_2O$, had been determined recently (Weichsel & Lis, 1994). As should be expected, the valence geometries in these two new structures conform to the trends demonstrated by the earlier results we analyzed. In the monoammonium salt the H_2Pep^- monoanion has a conformation similar to that in KH_2Pep and in the triammonium salt, which represents a third crystallographic occurrence of the Pep^{3-} trianion, the conformation of the anion is extended as in $(C_6H_{11}NH_3)_3Pep.H_2O$ and $AgBaPep.3H_2O$.
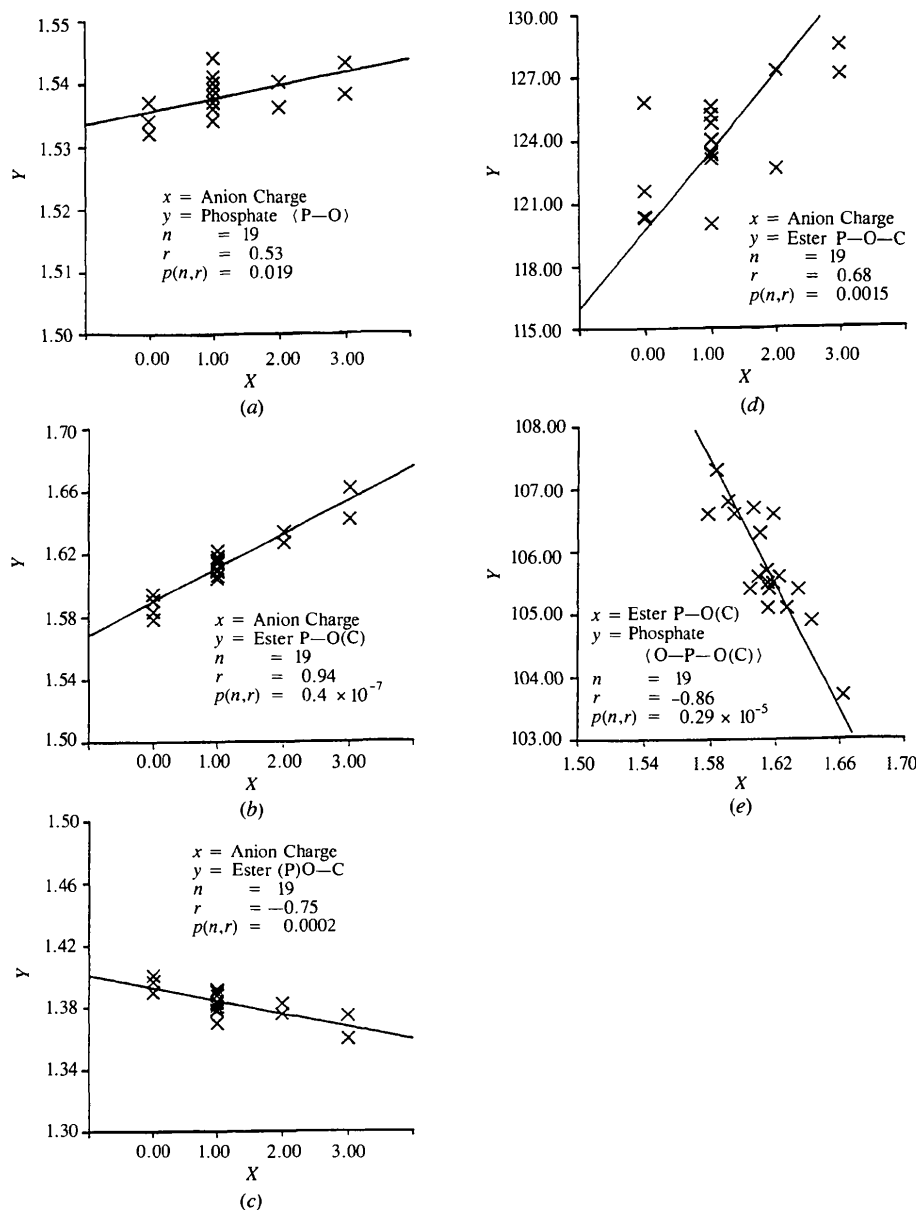

Fig. 3. Correlation plots for the valence geometries of the H_3Pep, H_2Pep^-, $HPep^{2-}$ and Pep^{3-} species listed in Tables 7–9: (a) anion-averaged P—O bond length *versus* anion charge; (b) phosphate ester P—O(C) bond length *versus* anion charge; (c) phosphate ester (P)O—C bond length *versus* anion charge; (d) phosphate ester P—O—C valence angle *versus* anion charge; (e) anion-averaged O—P—O(C) valence angle *versus* P—O(C) bond length.

Table 9. *Bond lengths (Å) in the enolpyruvate groups (e.s.d.'s average 0.004 Å)*

		(P)O—C	C=C(H₂)	C—C(O₂)	C=O	C—O(H)	(C—O)	Reference
H₃Pep	A	1.400	1.310	1.483	1.216	1.309	1.262	(a)
	B	1.389	1.309	1.490	1.214	1.309	1.262	
H₃Pep.H₂Pep⁻.NH₄⁺								
H₃Pep	A	1.396	1.305	1.488	1.209	1.317	1.263	(b)
	B	1.389	1.320	1.486	1.208	1.307	1.258	
H₂Pep⁻	A	1.387	1.306	1.491	1.206	1.313	1.260	
	B	1.369	1.304	1.494	1.203	1.313	1.258	
H₂Pep⁻.Na⁺.H₂O	A	1.391	1.312	1.497	1.210	1.322	1.266	(c)
	B	1.398	1.315	1.499	1.214	1.304	1.259	
H₂Pep⁻.K⁺		1.382	1.318	1.497	1.211	1.318	1.264	(d)
H₂Pep⁻.C₆H₁₁NH₃⁺	(I)	1.377	1.315	1.503	1.207	1.308	1.258	(e)
	(II)	1.377	1.317	1.490	1.212	1.309	1.260	
(H₂Pep⁻)₂.Ca²⁺.2H₂O		1.383	1.320	1.486	1.237	1.296	1.266	(f)
(H₂Pep⁻)₂.Mg²⁺.2H₂O		1.390	1.320	1.484	1.246	1.283	1.264	(g)
(H₂Pep⁻)₂.Mn²⁺.2H₂O		1.380	1.319	1.487	1.247	1.289	1.268	
(H₂Pep⁻)₂.Zn²⁺.2H₂O		1.378	1.318	1.487	1.249	1.269	1.259	
(HPep²⁻)₂.(C₆H₁₁NH₃⁺)₄.CH₃OH								
[H₀.₅PepH₀.₅]²⁻		1.375	1.324	1.504	1.217	(1.273)	1.245	(h)
HPep²⁻		1.382	1.316	1.515	1.233 1.252		1.242	
Pep³⁻.(C₆H₁₁NH₃⁺)₃.H₂O		1.359	1.313	1.518	1.251 1.257		1.254	(i)
Pep³⁻.Ag⁺Ba²⁺.3H₂O		1.374	1.329	1.515	1.250 1.254		1.252	(j)
Sample		19	19	19	22	15	19	
Minimum		1.359	1.304	1.483	1.203	1.269	1.242	
Maximum		1.400	1.329	1.518	1.257	1.322	1.268	
Range		0.041	0.025	0.035	0.054	0.053	0.026	
Sample mean		1.383	1.315	1.496	1.227	1.285	1.259	
Sample standard deviation		0.010	0.006	0.011	0.020	0.014	0.007	

References: (a) Weichsel & Lis (1989b); Weichsel, Lis & Kuczek (1989); (b) Weichsel, Lis & Kuczek (1991); (c) Lis (1991); (d) Lis (1987); (e) Weichsel & Lis (1989a); (f) Lis & Kuczek (1991); (g) Lis (1992); (h) Weichsel & Lis (1990a); (i) this work; (j) Weichsel & Lis (1990b).

References

Baur, W. H. (1974). *Acta Cryst.* B30, 1195–1215.

Bevington, P. R. (1969). *Data Reduction and Error Analysis for the Physical Sciences*, pp. 119–133, 310–312. New York: McGraw-Hill.

Blessing, R. H. (1988). *Acta Cryst.* B44, 334–340.

Blessing, R. H. (1989a). *Acta Cryst.* B45, 200.

Blessing, R. H. (1989b). *J. Appl. Cryst.* 22, 396–397.

Cromer, D. T. & Waber, J. T. (1974). In *International Tables for X-ray Crystallography*, edited by J. A. Ibers and W. C. Hamilton, Vol. IV, Table 2.3.1, pp. 148–151. Birmingham: Kynoch Press. (Present distributor Kluwer Academic Publishers, Dordrecht).

Cromer, D. T. & Waber, J. T. (1974). In *International Tables for X-ray Crystallography*, edited by J. A. Ibers and W. C. Hamilton, Vol. IV, Table 2.2B, pp. 99–102. Birmingham: Kynoch Press. (Present distributor Kluwer Academic Publishers, Dordrecht).

French, S. & Wilson, K. (1978). *Acta Cryst.* A34, 517–525.

Gabe, E. J., Lee, F. L. & LePage, Y. (1985). In *Crystallographic Computing 3*, edited by G. M. Sheldrick, C. Krüger & R. Goddard, pp. 164–174. Oxford: Clarendon Press.

Germain, G., Main, P. & Woolfson, M. M. (1970). *Acta Cryst.* A26, 274–285.

Hosur, M. V. & Viswamitra, M. A. (1991). *Acta Cryst.* B37, 839–843.

Jencks, W. P. (1968). In *Handbook of Biochemistry*, edited by H. A. Sober, pp. J144 ff. Cleveland, Ohio: The Chemical Rubber Co.

Johnson, C. K. (1970). *ORTEPII*. Report ORNL-3794, revised. Oak Ridge National Laboratory, Tennessee, USA.

Katti, S. K., Hosur, M. V. & Viswamitra, M. A. (1981). *Acta Cryst.* B37, 834–838.

Lehninger, A. L. (1975). *Biochemistry*, 2nd ed. New York: Worth Publishers, Inc.

Lis, T. (1987). *Acta Cryst.* C43, 1898–1900.

Lis, T. (1991). *Acta Cryst.* C47, 429–431.

Lis, T. (1992). *Acta Cryst.* C48, 424–427.

Lis, T. & Kuczek, M. (1991). *Acta Cryst.* C47, 1598–1600.

Mahler, H. R. & Cordes, E. H. (1971). *Biological Chemistry*, 2nd ed. New York: Harper and Row.

Rogers, D. (1981). *Acta Cryst.* A37, 734–741.

Stewart, R. F., Davidson, E. R. & Simpson, W. T. (1965). *J. Chem. Phys.* 42, 3175–3181.

Watson, D. G. & Kennard O. (1973). *Acta Cryst.* B29, 2358–2364.

Weichsel, A. & Lis, T. (1989*a*). *Acta Cryst.* C**45**, 1177–1180.

Weichsel, A. & Lis, T. (1989*b*). Collected Abstracts, Symposium on Organic Crystal Chemistry and XXXIst Polish Crystallographic Meeting, Poznan-Rydzyna, August 1989. Faculty of Chemistry, Adam Mickiewicz University , 60–780 Poznan, Grunwaldzka 6, Poland.

Weichsel, A. & Lis, T. (1990*a*). *Acta Cryst.* C**46**, 472–476.

Weichsel, A. & Lis, T. (1990*b*). *Acta Cryst.* C**46**, 962–965.

Weichsel, A. & Lis, T. (1994). *Polish J. Chem.* **68**, 2079–2096.

Weichsel, A., Lis, T. & Kuczek, M. (1989). *Carbohydr. Res.* **194**, 63–70.

Weichsel, A., Lis, T. & Kuczek M. (1991). *Acta Cryst.* C**47**, 2164–2167.

Westheimer, F. H. (1987). *Science*, **235**, 1173–1178.

Zachariasen, W. H. (1967). *Acta Cryst.* **23**, 558–564.