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THE CRYSTAL AND MOLECULAR STRUCTURE OF PHOSPHOENOLPYRUVIC ACID

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ABSTRACT

Phosphoenolpyruvic acid crystals, obtained by slow concentration of an aqueous solution, are triclinic, space group $P\bar{1}$, with a = 5.905(5), b = 8.135(8), c = 14.095(15) Å, α = 104.70(8), β = 97.72(8), γ = 100.99(8)°, and Z = 4. Two crystallographically independent phosphoenolpyruvic acid molecules differ in the orientation of the phosphate group relative to the enolpyruvate moiety. The phosphate groups have different tetrahedral distortions, and their P–O(ester) bond-lengths are 1.578(3) and 1.583(3) Å. All OH groups are involved in intermolecular hydrogen bonds.

INTRODUCTION

Phosphoenolpyruvate (PEP) is an important intermediate in carbohydrate metabolism, and in the storage and transfer of energy in biological processes[1]. Of the tribasic acid, monoanion, dianion, and trianion forms of PEP, only the crystal structures of the monoanion as the monopotassium[2], monosodium[3], and monocyclohexylammonium[4] salts have been described. We have been studying the influence of protonation on the geometry of PEP. We have reported[2,4] on the crystal and molecular structures of the PEP moiety and now report on PEP acid.

EXPERIMENTAL

Cyclohexylammonium phosphoenolpyruvate[5] was passed over Dowex 50W(H^+) resin, and slightly hygroscopic, plate-shaped crystals of phosphoenolpyruvic acid were grown by slow concentration of the eluate. The product has m.p. 103 ±1°. The density was measured pycnometrically in CCl_4–$C_2H_4Br_2$. The crystal system was determined from oscillation and Weissenberg photographs. A fragment

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TABLE I

CRYSTAL DATA FOR PHOSPHOENOLPYRUVIC ACID

Molecular formula	$C_3H_5O_6P$
Molecular weight	168.04
Crystal system	Triclinic
Space group	$P\bar{1}$
a (Å)	5.905(5)
b	8.135(8)
c	14.095(15)
α (°)	104.70(8)
β	97.72(8)
γ	100.99(8)
Cell volume (Å^3)	630.9(12)
Z	4
F(000)	344
$\mu(MoK_\alpha)$ (cm^{-1})	4.16
D_c (g.cm^{-3})	1.769(3)
D_m	1.72

(0.55 × 0.50 × 0.15 mm) was cut from a large crystal and sealed in a capillary. A Syntex $P2_1$ diffractometer, MoK_α radiation (λ = 0.71069 Å), and a graphite monochromator were used for measurements of lattice parameters (15 reflections in the range $20 < 2\theta < 27°$) and intensities at T = 297(2) K. An almost full Ewald sphere up to 2θ = 60° was collected, operating in the ω–2θ scan mode. After each group of 50 reflections, two standards were measured; variation ±5%. The intensities were corrected for Lorentz and polarisation factors only. Symmetry-related reflexions among 3944 measurements were averaged to give a final set of 2521 reflexions with $I > 3\sigma(I)$ used for the structure analysis. R_{merg} was 0.0223. The crystal data are listed in Table I.

The structure was solved by the use of direct methods[6]. Refinement was by full-matrix least-squares methods[7], minimising the function $\Sigma w(|F_0| - |F_c|)^2$, with $w = 1/\sigma^2(F_0)$, where $\sigma(F_0)$ was based on counting statistics. The hydrogen atoms were found from difference Fourier synthesis. The positional parameters of H-atoms were refined with constraints d(C–H) = 1.08 Å and d(O–H) = 0.97 Å. The refinement yielded a final R value of 0.0318 and an R_w value of 0.0324. In the final cycle of refinement, no parameter shifted more than 0.1 of its standard deviation. The final difference Fourier synthesis showed maximum and minimum electron densities of 0.45 and −0.36 eÅ^{-3}, respectively. Neutral atomic scattering factors were taken from standard tables[8]. Anomalous dispersion was included for P, O, and C atoms. The atomic positional parameters and equivalent temperature factors are listed in Table II.

DISCUSSION

The molecular structure and the numbering scheme for two crystallographi-

TABLE II

THE FINAL ATOM CO-ORDINATES AND ISOTROPIC THERMAL PARAMETERS AND THEIR ESTIMATED STANDARD DEVIATIONS IN PARENTHESES FOR PHOSPHOENOLPYRUVIC ACID[a]

Atom	*x*	*y*	*z*	U_{iso} or U_{eq}
P-1	0.48325(9)	0.21388(7)	0.35475(4)	0.0238(11)
P-2	−0.07628(9)	0.76982(7)	0.36767(4)	0.0228(11)
O-11	0.73323(27)	0.33041(20)	0.38255(12)	0.0345(37)
O-12	0.31081(28)	0.30972(21)	0.40424(11)	0.0338(37)
O-13	0.48628(26)	0.04177(19)	0.37365(12)	0.0333(37)
O-14	0.40933(27)	0.19401(19)	0.23988(11)	0.0309(33)
O-15	0.41887(32)	−0.08585(25)	0.09312(14)	0.0491(45)
O-16	0.02649(32)	−0.16627(26)	0.05221(15)	0.0520(45)
O-21	0.18794(26)	0.79336(20)	0.40127(12)	0.0305(35)
O-22	−0.11605(29)	0.95414(20)	0.37960(14)	0.0379(44)
O-23	−0.21997(26)	0.66439(19)	0.41943(11)	0.0305(33)
O-24	−0.11917(28)	0.66997(23)	0.25240(11)	0.0389(38)
O-25	−0.10946(32)	0.42199(25)	0.09188(14)	0.0478(44)
O-26	−0.50170(31)	0.34112(24)	0.05021(13)	0.0458(41)
C-11	0.22581(43)	−0.06348(30)	0.10525(16)	0.034(5)
C-12	0.19444(40)	0.08677(30)	0.18342(15)	0.030(5)
C-13	−0.00844(50)	0.12261(40)	0.19618(20)	0.047(7)
C-21	−0.30280(42)	0.43925(29)	0.10631(16)	0.032(5)
C-22	−0.33496(40)	0.57760(29)	0.19224(16)	0.031(5)
C-23	−0.54040(50)	0.60524(40)	0.20710(22)	0.051(7)
H-13	−0.1688(30)	0.0379(34)	0.1487(19)	0.071(11)
H-13′	−0.0167(46)	0.2324(23)	0.2565(14)	0.048(8)
H-23	−0.6960(32)	0.5240(34)	0.1546(18)	0.071(10)
H-23′	−0.5483(50)	0.7045(28)	0.2732(13)	0.061(9)
H-11	0.7408(65)	0.4537(12)	0.3915(29)	0.102(14)
H-12	0.2972(55)	0.3124(45)	0.4724(8)	0.077(11)
H-16	0.0580(64)	−0.2578(35)	0.0001(21)	0.100(14)
H-21	0.2906(56)	0.8966(30)	0.3948(29)	0.106(14)
H-22	−0.2756(26)	0.9691(54)	0.3769(30)	0.107(15)
H-26	−0.4767(57)	0.2593(35)	−0.0085(16)	0.080(11)

[a] $U_{eq} = 1/3\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i\mathbf{a}_j$.

cally independent molecules (**1** and **2**) are illustrated in Fig. 1. The principal interatomic distances and angles are given in Table III*.

The enolpyruvate systems in **1** and **2** are not exactly planar. The angles between the planes formed by C-12, C-13, O-14, and carboxyl in **1** and **2** are 3.6(1) and 3.7(1)°, respectively. With the exception of the monopotassium[2] salt, where the planarity of the enolpyruvate system is stabilized by binding to the K^+ ion, the distortions about the C-11–C-12 and C-21–C-22 bonds in the acid molecules are slightly smaller than in the monoanion forms[3,4].

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*The final anisotropic thermal parameters and observed and calculated structure factors are deposited with, and can be obtained from, Elsevier Science Publishers B.V., BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/420/*Carbohydr. Res.*, 194 (1989) 63–70.

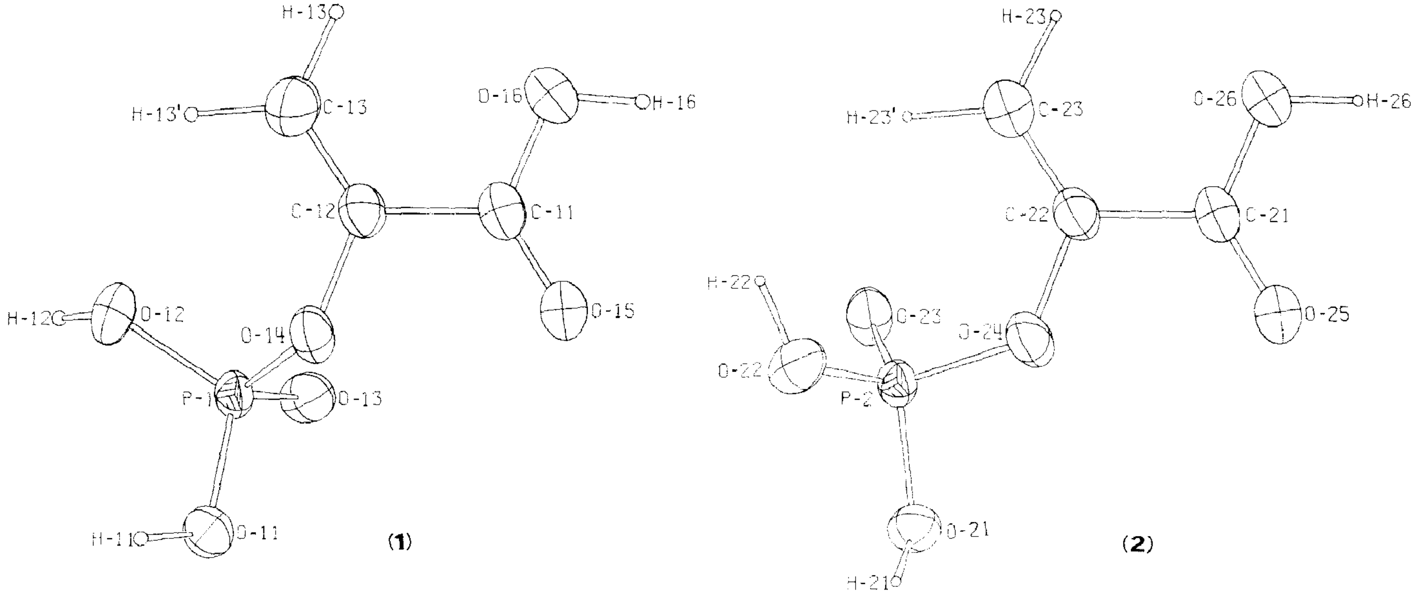

Fig. 1. The molecular structure of two crystallographically independent molecules (**1** and **2**) of phosphoenolpyruvic acid.

TABLE III

PRINCIPAL BOND LENGTHS (Å), BOND ANGLES (DEGREES), AND TORSION ANGLES (DEGREES) IN PHOSPHOENOLPYRUVIC ACID

Bond	*Bond distance*	*Bond*	*Bond distance*
P-1–O-11	1.534(3)	P-2–O-21	1.529(3)
P-1–O-12	1.533(3)	P-2–O-22	1.533(3)
P-1–O-13	1.493(3)	P-2–O-23	1.481(3)
P-1–O-14	1.578(3)	P-2–O-24	1.583(3)
O-14–C-12	1.400(4)	O-24–C-22	1.389(4)
C-11–C-12	1.483(4)	C-21–C-22	1.490(3)
C-12–C-13	1.310(4)	C-22–C-23	1.309(4)
C-11–O-15	1.216(3)	C-21–O-25	1.214(3)
C-11–O-16	1.309(3)	C-21–O-26	1.309(3)

Bonds	*Bond angle*	*Bonds*	*Bond angle*
O-11–P-1–O-12	111.3(2)	O-21–P-2–O-22	106.0(2)
O-11–P-1–O-13	110.0(2)	O-21–P-2–O-23	113.1(2)
O-11–P-1–O-14	103.3(2)	O-21–P-2–O-24	102.8(2)
O-12–P-1–O-13	115.0(2)	O-22–P-2–O-23	115.0(2)
O-12–P-1–O-14	104.6(2)	O-22–P-2–O-24	109.0(2)
O-13–P-1–O-14	111.8(2)	O-23–P-2–O-24	110.2(2)
P-1–O-14–C-12	121.6(2)	P-2–O-24–C-22	125.8(2)
O-14–C-12–C-11	112.2(2)	O-24–C-22–C-21	110.5(3)
O-14–C-12–C-13	122.9(3)	O-24–C-22–C-23	125.9(3)
C-11–C-12–C-13	124.8(3)	C-21–C-22–C-23	123.6(3)
C-12–C-11–O-15	122.3(3)	C-22–C-21–O-25	122.1(3)
C-12–C-11–O-16	113.1(3)	C-22–C-21–O-26	113.2(3)
O-15–C-11–O-16	124.6(3)	O-25–C-21–O-26	124.7(3)
P-1–O-11–H-11	114.6(20)	P-2–O-21–H-21	117.1(20)
P-1–O-12–H-12	119.4(18)	P-2–O-22–H-22	118.2(22)
C-11–O-16–H-16	109.4(20)	C-21–O-26–H-26	111.7(17)

Bonds	*Torsion angle*	*Bonds*	*Torsion angle*
C-13–C-12–C-11–O-15	176.0(5)	C-23–C-22–C-21–O-25	176.5(9)
C-13–C-12–C-11–O-16	−3.9(5)	C-23–C-22–C-21–O-26	−3.8(8)
C-13–C-12–O-14–P-1	68.6(6)	C-23–C-22–O-24–P-2	25.9(9)
C-11–C-12–O-14–P-1	−114.7(6)	C-21–C-22–O-24–P-2	−154.1(8)
C-12–O-14–P-1–O-11	176.0(6)	C-22–O-24–P-2–O-21	162.1(8)
C-12–O-14–P-1–O-12	−67.3(7)	C-22–O-24–P-2–O-22	−85.7(7)
C-12–O-14–P-1–O-13	57.8(6)	C-22–O-24–P-2–O-23	41.3(8)
O-15–C-11–C-12–O-14	−0.7(5)	O-25–C-21–C-22–O-24	−3.5(8)
O-16–C-11–C-12–O-14	179.4(4)	O-26–C-21–C-22–O-24	176.2(7)
O-14–P-1–O-11–H-11	78(3)	O-24–P-2–O-21–H-21	87(4)
O-14–P-1–O-12–H-12	165(3)	O-24–P-2–O-22–H-22	84(3)
C-12–C-11–O-16–H-16	177(3)	C-22–C-21–O-26–H-26	175(3)

The flexibility for rotation about the enolic bond, observed earlier in the monoanion of PEP[2–4], is reflected in the different orientations of the phosphate group relative to the enolpyruvate system. The torsion angles P-1–O-14–C-12–C-11 and P-2–O-24–C-22–C-21 are −114.7(6) and −154.1(8)° in **1** and **2**, respectively.

The phosphate ester bonds in **1** and **2** [1.578(3) and 1.583(3) Å, respectively] are shorter than in the monopotassium [1.622(2) Å][2], the monosodium [1.595(5), 1.610(6) Å][3], both forms of the monocyclohexylammonium [1.604(2), 1.610(2) Å][4], and the dicyclohexylammonium [1.627(3), 1.633(3) Å][9] salts, and belong to the shortest phosphate ester bonds. The enolic bond-lengths in **1** and **2** [1.400(4) and 1.389(4) Å, respectively] are comparable with those observed in PEP monoanions and shorter (except for phenyl dihydrogenphosphate[10]) than the C–O (ester) bond in dihydrogenphosphate esters[11–14]. Such contraction of the C–O (ester) bond is

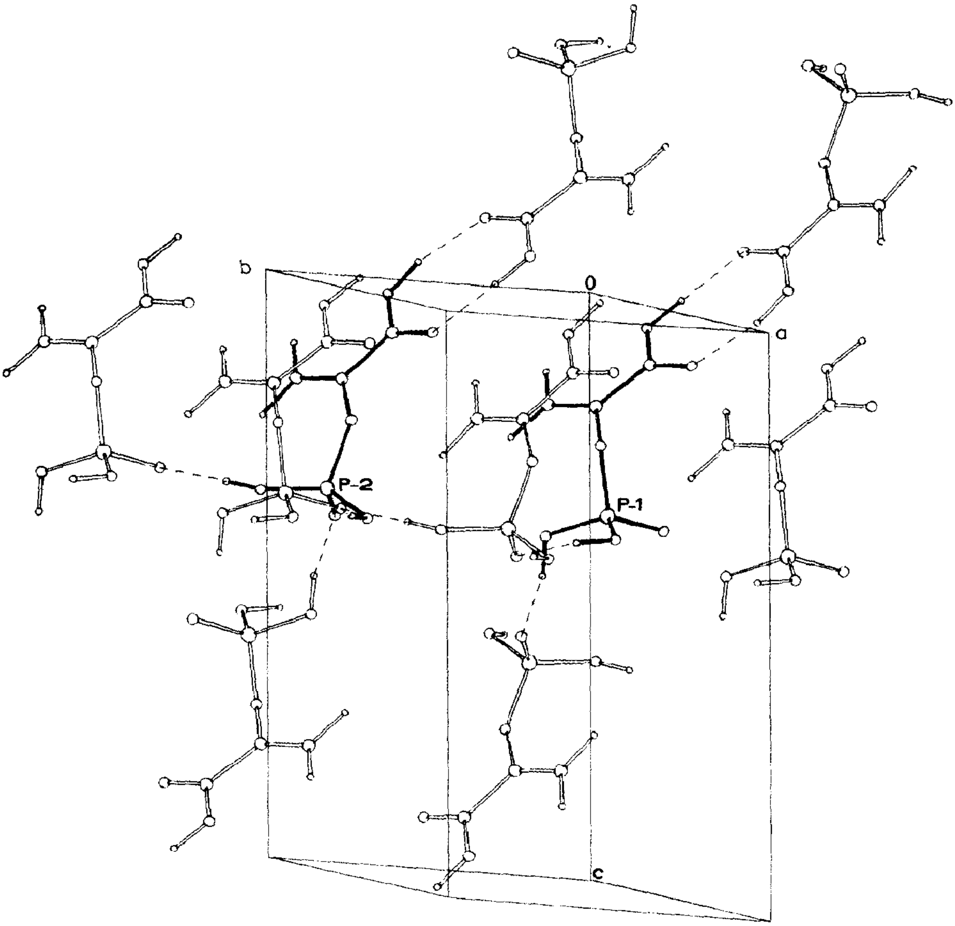

Fig. 2. Molecular packing in the crystal of phosphoenolpyruvic acid.

TABLE IV

HYDROGEN-BOND DISTANCES (Å) AND ANGLES (DEGREES) IN THE CRYSTAL OF PHOSPHOENOLPYRUVIC ACID

O–H · · · O	*O · · · O*	*H · · · O*	*O–H · · · O*
O-11–H-11 · · · O-23[a]	2.586(3)	1.62(2)	172(4)
O-12–H-12 · · · O-23[b]	2.578(3)	1.62(2)	166(3)
O-16–H-16 · · · O-25[c]	2.684(3)	1.71(4)	179(3)
O-21–H-21 · · · O-13[d]	2.562(3)	1.60(3)	169(4)
O-22–H-22 · · · O-13[e]	2.578(3)	1.62(3)	166(4)
O-26–H-26 · · · O-15[c]	2.675(3)	1.72(3)	166(3)

[a]Symmetry codes: $x + 1, y, z$; [b]$-x, 1 - y, 1 - z$; [c]$-x, -y, -z$; [d]$x, 1 + y, z$; [e]$x - 1, 1 + y, z$.

found in phosphate groups attached to C=C or the anomeric carbon in carbohydrates.

Only five structures that involve monoesterified phosphates with two hydrogens on the phosphate group are known, namely, phenyl phosphate[10], DL-serine phosphate[11], *myo*-inositol 2-phosphate[12], uridine 3′-phosphate[13], and 2,2′-anhydro-1-*β*-D-arabinofuranosylcytosine 3′,5′-bisphosphate[14]. In all but one compound[14] (where the phosphate group is involved in the short intramolecular contacts), the unprotonated oxygen atom in the dihydrogenphosphate group is *trans* to C. In contrast, this oxygen atom in **1** and **2** is *gauche* to C. The phosphate groups in **1** and **2** are distorted from tetrahedral, but in different ways [see the O–P–O (ester) bond angles].

The hydroxyl oxygen in the carboxyl group in two crystallographically independent molecules of PEP acid is *trans* to O (ester). The same situation has been observed in other PEP structures, with the exception of the monoclinic form of the cyclohexylammonium salt[4].

Other bond lengths and angles in **1** and **2** do not differ significantly and are normal.

The crystal packing and hydrogen bonding schemes are shown in Fig. 2. The hydrogen-bond distances and angles are listed in Table IV. The enolpyruvate skeletons of **1** and **2** are nearly parallel in the crystal. All OH groups participate in hydrogen bonds forming the three-dimensional network. Each unprotonated oxygen in the phosphate groups in **1** and **2** accepts two hydrogens, whereas the OH groups act as donors only. The carboxyl groups are involved in hydrogen bonds in the way usually observed in carboxylic acid structures, *i.e.*, carboxyl-to-carboxyl.

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