

Molecular Structures of Amino Acids and Peptides.

II. A Redetermination of the Crystal Structure of L-O-Serine Phosphate. A Very Short Phosphate-Carboxyl Hydrogen Bond

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L-O-Serine phosphate crystallized from aqueous solution in the orthorhombic space group $P2_12_12_1$, with cell dimensions $a = 7.737 \pm 0.0003$, $b = 10.167 \pm 0.0003$ and $c = 9.136 \pm 0.0004$ Å. The calculated and observed densities, assuming $Z = 4$, are 1.718 and 1.739 g.cm⁻³ respectively. The structure was solved by the heavy-atom technique and refined by full-matrix least squares to an R index of 0.027, using 713 reflections collected on a Picker automatic diffractometer. The estimated standard deviations in the bond distances and bond angles are P-O, 0.003; C-C, C-N, C-O, 0.005; C-H, N-H, O-H, 0.04 Å; O-P-O, 0.12°; P-O-C, 0.20°; the remaining bond angles are 0.25°. The structure includes a very short

$$\begin{array}{c} \text{O} \\ || \\ \text{P}=\text{O} \cdots \text{H}-\text{O}-\text{C} \end{array}$$

hydrogen bond, P=O···H-OC- 2.492 Å, between a phosphate oxygen atom and the carboxyl group. The shortest hydrogen bond, P-O···H-O-P, between phosphate groups is 2.558 Å. Three more hydrogen bonds involve the ammonium group which is protonated by one of the phosphate protons to

$$\begin{array}{c} + \\ \text{NH}_3\text{CH}(\text{COOH})\text{CH}_2\text{OPO}_3\text{H} \end{array}$$

yield a zwitterion, NH₃CH(COOH)CH₂OPO₃H. The N-H···O-P hydrogen bond is 2.797 Å. The carbonyl oxygen atom and the phosphate ester oxygen atom do not participate in hydrogen bonding; the latter feature is characteristic of all known phosphate esters. Variations in bond distances and bond angles in the phosphates is attributed to conformational and hydrogen bonding differences. The molecular structures and conformations of the accurately analyzed amino acids are reviewed. It is seen that the C(1) (*sp*²)-C(2) (*sp*³) bond distance of amino acids is similar to that of the C(2) (*sp*³)-C(3) (*sp*³) bond. Also, the ammonium nitrogen atom, as a general rule, is not coplanar with the carboxyl group. The P-O bond distances and the O-P-O valence angles in the known monophosphate monoanions are tabulated.

Introduction

The recent solution of the structure of DL-serine phosphate monohydrate in our laboratory (Putkey & Sundaralingam, 1970) showed the presence of two very short hydrogen bonds involving the phosphates. A previous communication on the structure of L-serine phosphate by McCallum, Robertson & Sim (1959) showed the presence of a similar short hydrogen bond, in this case between a phosphate group and a carboxyl group. These workers did not publish the atomic coordinates. The present redetermination of the structure of L-serine phosphate was undertaken to obtain more accurate information relating to this short hydrogen bond, bond distances and bond angles.

Experimental

Colorless crystals were grown from an aqueous solution of L-serine phosphate obtained from Cyclo Chemical Corporation. All X-ray data were taken using a

crystal of dimensions 0.2 × 0.2 × 0.4 mm, which was cut from a large rectangular prismatic crystal. Weissenberg and precession photographs showed the space group extinctions $h = 2n + 1$ for $h00$, $k = 2n + 1$ for $0k0$, and $l = 2n + 1$ for $00l$ reflections, confirming the space group $P2_12_12_1$ obtained in the earlier study (McCallum *et al.*, 1959). Precise cell constants were calculated from a least-squares refinement of the angular settings 2θ , χ , φ of twenty reflections measured on the Picker automated diffractometer. The density was measured by the flotation method using a Westphal balance and a mixture of chloroform and bromoform. A summary of the crystal data is given in Table 1.

Cu $K\alpha$ intensity data up to $2\theta = 134^\circ$ were measured on the Picker four-angle diffractometer using a scan rate of two degrees per minute. Individual background measurements were not taken. Instead, a table of background as a function of 2θ was constructed by scanning at appropriate regions of the reciprocal sphere for the entire 2θ range for which data were collected. Background for each reflection was interpolated from the above table and subtracted from the total scan to obtain the net peak count. The criterion for rejecting weak reflections was essentially that of Klug & Alexander (1954). A reflection was considered observed if $I_{\text{corr}} = n\sigma(I)$, where $\sigma(I) = (I_{\text{scan}} + I_{\text{bkg}})^{1/2}$, $I_{\text{corr}} = I_{\text{scan}}$

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$-I_{\text{bkg}}$ and $n=1.5$. Altogether 745 reflections were scanned, and 719 were significantly above background and were utilized in the structure analysis.

The determination and refinement of crystal structure

The phosphorus atom was located using the conventional heavy-atom method on Harker sections of a sharpened three-dimensional Patterson synthesis. The sharpened coefficients were obtained by multiplying each F^2 by $(\sin \theta/\lambda)^2$ (Jacobsen, Wunderlich & Lipscomb, 1961). The remainder of the non-hydrogen atoms were located by means of a three-dimensional electron density synthesis phased upon the phosphorus atom position. A structure factor calculation based on the non-hydrogen atom coordinates and a uniform isotropic temperature factor of 3.0 \AA^2 gave an R value of 0.344, which dropped to 0.078 in three cycles of isotropic least-squares refinement. The Oak Ridge National Laboratory least-squares program *ORFLS* (Busing, Martin & Levy, 1962), modified for the UNIVAC 1108 machine by Dr S. T. Rao of our laboratory, was used in the refinement. A difference

electron density map computed at this stage showed unequivocally the positions of five hydrogen atoms. Location of the remaining hydrogen atoms was hindered by anisotropic effects of the heavier atoms. Two more isotropic least-squares cycles, including these five hydrogen atoms, lowered the R value to 0.066. At this stage, two anisotropic least-squares cycles on the non-hydrogen atoms reduced the R value to 0.053. Another difference map, including the contribution of the non-hydrogen atoms and the above five hydrogen atoms in the F_o , revealed the remaining three hydrogen atoms (Fig. 1). Until this point, the refinement was executed with unit weights. In subsequent least-squares cycles, a modified Hughes (1941) weighting scheme was employed. Two additional anisotropic least-squares cycles refining the positional and anisotropic temperature factors of the non-hydrogen atoms and only the positions of the hydrogen atoms gave an R value of 0.032. The hydrogen atoms were given the anisotropic thermal parameters of the atom to which they were bonded, and these were not refined. Eliminating six reflections suffering from secondary extinction effects made the final $R=0.027$, and the weighted $R=0.038$.

The atomic scattering factors used in this work were taken from *International Tables for X-ray Crystallography* (1962). The hydrogen atom scattering factors were from Stewart, Davidson & Simpson (1965). Table 2 is a listing of the observed and calculated structure factors. The final atomic parameters and their estimated standard deviations are given in Table 3. The principal axes of the thermal ellipsoids are presented in Table 4.

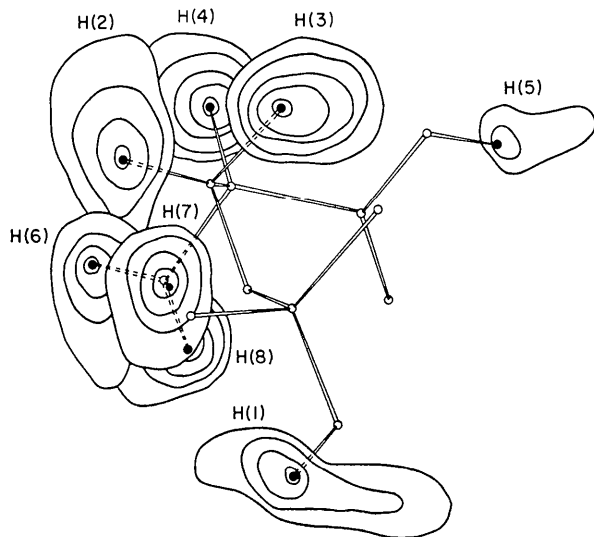


Fig. 1. Difference electron density map showing the hydrogen atoms contoured at intervals of $0.1 \text{ e.}\text{\AA}^{-3}$, beginning at $0.1 \text{ e.}\text{\AA}^{-3}$.

Discussion of the crystal structure

The bond distances and angles in L-serine phosphate are shown in Figs. 2 and 3 respectively. The standard deviations in the bond lengths and angles are: P-O, 0.003; C-C, C-N, C-O, 0.005; C-H, N-H, O-H, 0.04 \AA ; O-P-O, 0.12; P-O-C, 0.20; C-C-C, C-C-O, 0.25°; and for angles involving hydrogen atoms, 1.8°. The bond distances involving the non-hydrogen atoms from the earlier work (the standard deviations were not published) are compared with the present results in Table 5. The agreement in the two analyses is generally

Table 1. *Crystal data for L-serine phosphate*

	$(\lambda_{\text{Cu } K\alpha} = 1.5418 \text{ \AA})$	
	This work	McCallum <i>et al.</i> (1959)
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
a	$7.737 \pm 0.0003 \text{ \AA}$	$7.79 \pm 0.01 \text{ \AA}$
b	$10.167 \pm 0.0003 \text{ \AA}$	$10.24 \pm 0.04 \text{ \AA}$
c	$9.136 \pm 0.0004 \text{ \AA}$	$9.09 \pm 0.02 \text{ \AA}$
V	718.5 \AA^3	725.1 \AA^3
Z	4	4
D_m	1.739 g.cm^{-3}	1.707 g.cm^{-3}
D_x	1.718 g.cm^{-3}	1.692 g.cm^{-3}
Crystal dimensions	$0.2 \times 0.2 \times 0.4 \text{ mm}$	

Table 4. Description of the thermal ellipsoids in L-serine phosphate

u_i is the root-mean-square displacement corresponding to the i th axis of the ellipsoid. C_{ia} , C_{ib} and C_{ic} are the direction cosines of the i th axis with respect to the crystal axes a, b, c .

	Axis i	u_i	C_{ia}	C_{ib}	C_{ic}
P	1	0.144	0.3018	-0.9435	0.1366
	2	0.154	0.2551	-0.0581	-0.9652
	3	0.159	-0.9186	-0.3261	-0.2232
O(1)	1	0.164	-0.9946	-0.0910	-0.0488
	2	0.175	0.0591	-0.1140	-0.9917
	3	0.236	0.0847	-0.9893	0.1188
O(2)	1	0.143	0.2268	-0.9739	-0.0050
	2	0.174	-0.9643	-0.2239	-0.1415
	3	0.232	0.1368	0.0365	-0.9899
O(3)	1	0.155	-0.1397	-0.8992	-0.4146
	2	0.178	0.0171	0.4164	-0.9089
	3	0.220	-0.9900	0.1341	0.0428
O(4)	1	0.160	-0.2504	-0.0356	0.9675
	2	0.174	-0.4284	0.9002	-0.0778
	3	0.273	0.8682	0.4340	0.2407
O(5)	1	0.153	0.1538	-0.9823	0.1074
	2	0.197	-0.3243	-0.1529	-0.9335
	3	0.262	-0.9334	-0.1087	0.3420
O(6)	1	0.172	-0.3340	0.8922	-0.3039
	2	0.198	0.5568	0.4469	0.7002
	3	0.298	-0.7605	-0.0646	0.6461
C(1)	1	0.157	-0.5487	0.8350	0.0416
	2	0.178	0.8178	0.5464	-0.1806
	3	0.199	0.1735	0.0651	0.9827
C(2)	1	0.156	0.6682	0.6928	-0.2711
	2	0.170	-0.6657	0.7195	0.1978
	3	0.205	0.3321	0.0483	0.9420
C(3)	1	0.163	-0.6190	0.6389	-0.4566
	2	0.177	0.7849	0.4824	-0.3890
	3	0.217	0.0283	0.5992	0.8001
N	1	0.153	0.3210	-0.9462	0.0393
	2	0.173	-0.9466	-0.3219	-0.0168
	3	0.226	0.0285	-0.0318	-0.9991

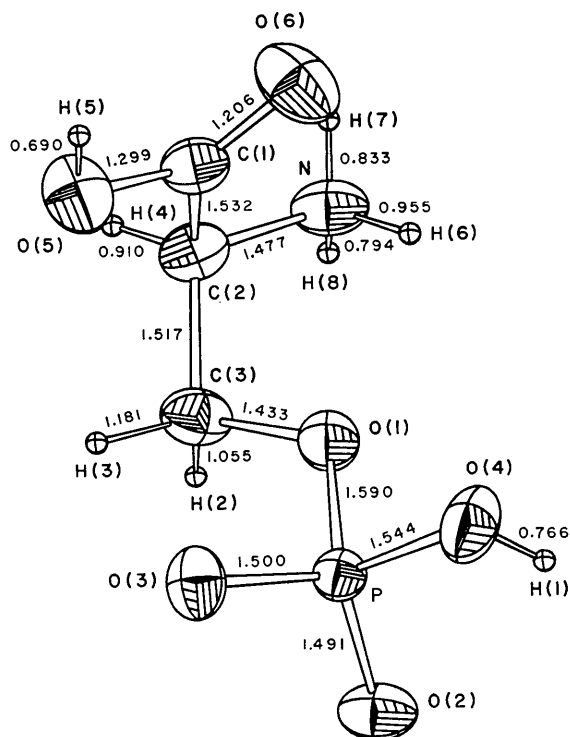


Fig. 2. Bond distances in L-serine phosphate.

good, except for the bond distances C(1)-O(5), C(3)-O(1) and P-O(2).

Table 5. Bond distances involving non-hydrogen atoms in L-serine phosphate

	This work	McCallum <i>et al.</i> (1959)
P—O(1)	1.590 (3)	1.608
P—O(2)	1.491 (2)	1.517
P—O(3)	1.500 (2)	1.497
P—O(4)	1.544 (3)	1.560
O(1)—C(3)	1.433 (5)	1.466
C(3)—C(2)	1.517 (5)	1.526
C(2)—N	1.477 (5)	1.468
C(2)—C(1)	1.532 (5)	1.541
C(1)—O(5)	1.299 (4)	1.321
C(1)—O(6)	1.206 (5)	1.201

The carboxyl C(1)-C(2) bond distance of 1.532 Å is significantly longer (about 6σ) than the value of 1.50 Å normally associated with a Csp^2-Csp^3 single bond and is slightly longer than the C(2)-C(3) bond distance. The remaining bond distances in L-serine phosphate are close to the normal ones. Data for the C(1)-C(2) and C(2)-C(3) bond distances from the more accurately analyzed ($\sigma < 0.015$ Å) amino acid structures are presented in Table 6. In general the C(1)-C(2) (sp^2-sp^3) bond distance is comparable with the

Table 6. Stereochemical features of some amino acids with standard deviations in bond distances less than 0.015 Å

	σ	C(1)-C(2)* (sp^2-sp^2)	C(2)-C(3)* (sp^3-sp^3)	ψ_1	ψ_2	N...O(2)	C(3)...O(1)	C(3)...O(2)	Reference
L-Alanine	0.004	1.525	1.525	161.5	340.7	2.682	3.079	3.326	Simpson & Marsh (1966)
L-Arginine. 2H ₂ O†	0.01	1.547	1.542	167.9	349.3	2.708	2.987	3.384	Karle & Karle (1964)
L-Aspartic acid‡	0.004	1.543	1.518	144.8	322.2	2.737	3.191	3.095	Derissen, Endeman & Peerdeman (1968)
DL- α -n-Butyric acid	A 0.011 B 0.009	1.512 1.509	1.564 1.564	162.5 150.9	339.4 328.8	2.667 2.726	3.108 3.240	3.285 3.226	Ichikawa & Iitaka (1968)
L-Cysteine	0.012	1.51	1.51	176.4	357.0	2.654	2.940	3.424	Harding & Long (1968)
L-Cystine	0.015	1.543	1.509	168.3	342.4	2.623	2.999	3.419	Oughton & Harrison (1959)
Glycine	0.005	1.523	—	198.3	340.9	2.690	—	—	Marsh (1958)
β -Glycine	0.015	1.521	—	156.3	332.7	2.700	—	—	Iitaka (1960)
γ -Glycine	0.011	1.527	—	168.3	345.0	2.687	—	—	Iitaka (1961)
L-Histidine. HCl. 2H ₂ O	0.011	1.530	1.527	179.5	0.4	2.668	2.919	3.527	Donohue & Caron (1964)
Hydroxy-L-proline†	0.009	1.516	1.532	178.0	356.9	2.665	3.005	3.444	Donohue & Trueblood (1952)
L-Lysine. HCl. 2H ₂ O	0.004	1.529	1.524	162.1	340.1	2.661	3.043	3.301	Wright & Marsh (1962)
L-Ornithine. HCl	0.006	1.541	1.529	144.2	321.9	2.774	3.254	3.119	Chiba, <i>et al.</i> (1967)
L-Phenylalanine. HCl*	0.015	1.505	1.550	177.5	358.9	2.655	2.915	3.551	Vainshtein & Gurskaya (1964)
DL-Serine	0.009	1.528	1.513	181.3	3.8	2.630	2.896	3.990	Gurskaya (1968)
DL-O-Serine phosphate monohydrate	0.004	1.522	1.518	164.7	344.8	2.742	2.939	3.335	Shoemaker, Barreau, Donohue & Lu (1953)
L-O-Serine phosphate	0.005	1.532	1.518	178.8	357.4	2.631	2.880	3.413	Putkey & Sundaralingam (1970)
L-Threonine	0.007	1.517	1.542	156.1	333.9	2.672	3.170	3.276	This work
L-O-Tyrosine sulfate-K. 2H ₂ O	0.005	1.528	1.538	164.6	341.5	2.685	3.028	3.335	Shoemaker, Donohue, Shoemaker & Corey (1950)
L-Valine. HCl‡	0.009	1.509	1.557	170.2	348.8	2.640	3.042	3.456	Fries & Sundaralingam (1969a)
L-Valine	0.007	1.527	1.541	156.0	335.8	2.671	3.167	3.326	Ando <i>et al.</i> (1967)

* The weighted average bond distances are: C(1)-C(2), 1.527 ± 0.003 Å and C(2)-C(3), 1.526 ± 0.004 Å.

† Published coordinates are of D-configuration.

‡ Carboxyl groups are not ionized.

Table 7. Bond distances and angles

	P=O (Å)	P-O ⁻ (Å)	P-OH (Å)	P-OR (Å)	∑(P-O) (Å)	P-OC (Å)	σ* (Å)
Adenosine 3'-phosphate	1.477	1.486	1.579	1.612	6.154	1.440	0.005
Adenosine 5'-phosphate	1.495	1.503	1.557	1.591	6.146	1.475	0.011
2-Aminoethanol phosphate	1.493	1.504	1.551	1.591	6.144	1.429	0.005
Cytidine 3'-phosphate (orthorhombic)	1.483	1.501	1.553	1.611	6.149	1.431	0.003
Cytidine 3'-phosphate (monoclinic)	1.480	1.498	1.588	1.610	6.196	1.435	0.009
Galactosamine 1-phosphate	1.499	1.510	1.553	1.606	6.168	1.434	0.003
L-Serine phosphate	1.491	1.500	1.544	1.590	6.125	1.433	0.003
Weighted average (<i>l</i>)	1.490	1.502	1.554	1.602	6.149	1.434	
σ _m †	0.003	0.003	0.007	0.006	0.009	0.006	

* Reported estimated standard deviation.

† Standard deviations in the mean, $\sigma_m = [\sum(I-l)^2/n(n-1)]^{1/2}$.

O-P-O valence angle in the C-O-P-O-H system is similar to that observed for the C-O-P-O-C system of phosphodiester (Sundaralingam, 1969). The weighted average bond distances and bond angles involving the monoanionic monophosphates are shown in Fig. 5. The small variations in the individual P-O bond distances and the O-P-O valence angles may be attributed to the number and strengths of the hydrogen bonds to the phosphate group and the conformation of the atom sequence H-O-P-O-C of the phosphate. Conformational changes usually produce changes in nonbonded interactions and therefore affect the molecular dimensions.

As in DL-serine phosphate, the C(2)-C(3)-O(1) bond angle of 105.3° in L-serine phosphate is considerably less than the tetrahedral value.

Molecular conformation

The conformation of the molecule is shown in Fig. 2 and compared with those of DL-serine and L_s-threonine in Fig. 6 and Table 8. Although the molecular conformation is similar to that of DL-serine phosphate, nonetheless there are significant differences of 2 to 20° in the torsion angles of the two compounds. These differences in the torsion angles are attributable to hydrogen bonding and molecular packing forces. Similar and even larger variations in the torsion angles of the serine (Table 8) and the serine phosphate residues of membrane proteins and lipids might conceivably occur during ion transport across biological membranes (Sundaralingam, 1968).

The ammonium group is almost perfectly staggered to the substituent on C(2). The ammonium nitrogen atom is only slightly displaced from the plane of the carboxyl group; the torsion angle N-C(2)-C(1)-O(6) is -2.6°. The torsion angles (Lakshminarayanan, Sasi-sekharan & Ramachandran, 1967)

$$\psi_1[\text{N}-\text{C}(2)-\text{C}(1)-\text{O}(1)]$$

and

$$\psi_2[\text{N}-\text{C}(2)-\text{C}(1)-\text{O}(2)]$$

for the accurately analyzed amino acid structures are

shown in Table 6. It is seen that as a general rule the ammonium nitrogen atom is not coplanar with the carboxyl group [the α-hydroxy acids show a similar feature (Sundaralingam & Putkey, 1969)]; the torsion angle ψ_2 assumes only small (0-4°) positive values and a large range (-1.1 to -38.1°) of negative values. Thus, the deviation from coplanarity is such that the pref-

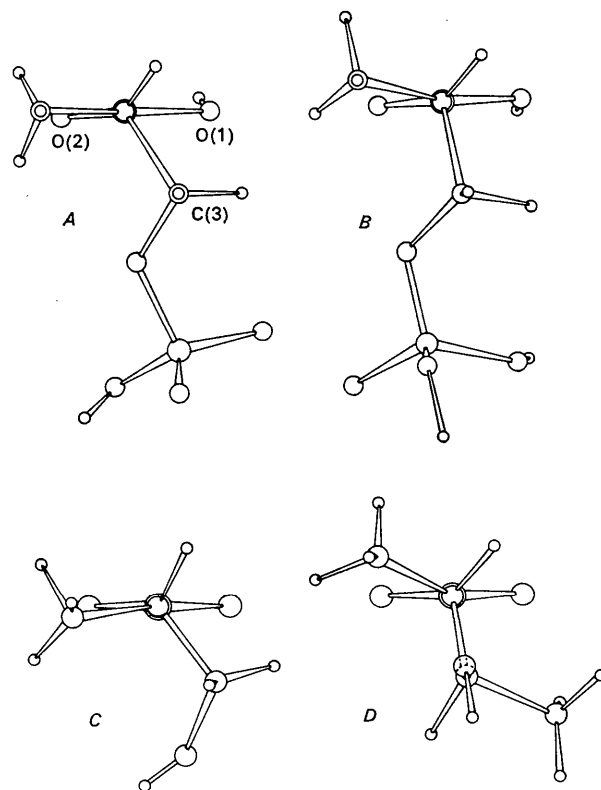


Fig. 6. Conformations of (a) DL-serine phosphate, (b) L-serine phosphate, (c) DL-serine, and (d) L_s-threonine, viewed down the C_α-C' bond. Note that the hydroxy group is *anti* to the ammonium groups in (a) and (b), and the ammonium nitrogen atom is displaced on the opposite side of the carboxyl group in (c).

in monoanionic monophosphates

O=P-O ⁻ (°)	HO-P-O ⁻ (°)	O=P-OH (°)	O=P-OR (°)	-O-P-OR (°)	HO-P-OR (°)	P-O-C (°)	σ (°)	Reference
117.2	112.3	107.1	110.7	103.4	105.5	119.1	0.3	Sundaralingam (1966)
118.2	106.9	110.2	108.7	108.7	106.9	114.7	0.4	Kraut & Jensen (1963)
117.0	109.3	109.8	103.9	109.6	106.2	118.7	0.2	Kraut (1961)
116.0	106.1	113.6	110.1	108.5	101.5	121.2	0.2	Sundaralingam & Jensen (1965a)
117.5	105.4	112.6	105.5	110.9	104.3	118.5	1.0	Bugg & Marsh (1967)
117.2	110.8	109.6	109.4	107.3	101.3	122.1	0.15	Fries & Sundaralingam (1966b)
114.8	109.6	112.2	108.8	109.0	101.5	121.5	0.2	Putkey & Sundaralingam (1970)
116.6	109.4	110.7	108.5	108.0	102.9	120.6		
0.43	1.04	0.84	0.96	0.90	1.00	1.07		

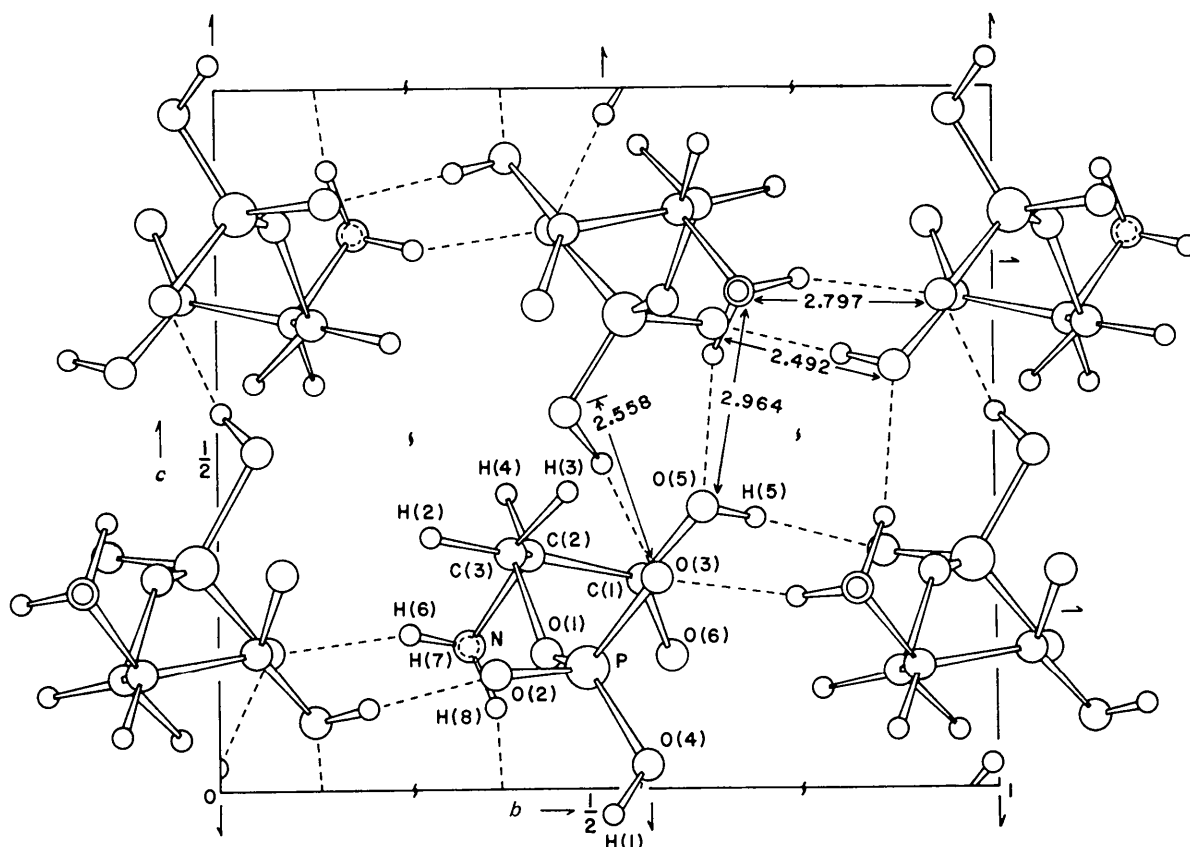


Fig. 7. View of the unit cell down the a axis showing the hydrogen bonding pattern and the alternating regions of hydrogen bonded and hydrophobic bonded channels; see text.

erential twist of the carboxyl group with respect to the ammonium group is counterclockwise (Fig. 6) (Lakshminarayanan *et al.*, 1967), irrespective of whether the carboxyl group is ionized or unionized. There is probably a simple reason for this preferential twist of the carboxyl group; the intramolecular $C_{\beta} \cdots O(1)$ distance increases for a counterclockwise twist of the carboxyl group from the value at $\psi_2 = 0^\circ$, but decreases for a

clockwise twist of the carboxyl group. In *DL*-serine, ψ_2 is 3.8° , and the $C(3) \cdots O(1)$ van der Waals contact is only 2.896 \AA ; consequently, any appreciable increase in the positive ψ_2 values will produce severe nonbonded $C(3) \cdots O(1)$ interactions. In *L*-ornithine hydrochloride, ψ_2 is -38.1° , and the $C(3) \cdots O(1)$ distance (3.254 \AA) is beyond the van der Waals contact distance, while the $C(3) \cdots O(2)$ distance (3.119 \AA)

is now approaching the van der Waals distance; thus any further appreciable increase in the negative ψ_2 value will produce unfavorable $C(3)\cdots O(2)$ contacts. Therefore, the preferred value of ψ_2 is centered at about -15.0° for the amino acids (Table 6).

Hydrogen bonding

There are five hydrogen bonds in this crystal structure; four of these are shown in Fig. 7 and the fifth is shown in Fig. 8. The only two oxygen atoms not participating in hydrogen bonds are the carbonyl oxygen atom of the carboxyl group and the ester oxygen atom of the phosphate group. DL-Serine phosphate exhibited a similar property. To date there is no known example of an intermolecular hydrogen bond involving the phosphate ester oxygen atom. However, in the amino

acid structures with a carboxylate group, both the carboxyl oxygen atoms are usually involved in hydrogen bonding, while in the amino acid derivatives with the un-ionized carboxyl group, e.g. L-serine phosphate, DL-serine phosphate, L-valine hydrochloride, L-phenylalanine hydrochloride, the carbonyl group may not necessarily be involved in hydrogen bonding as demonstrated by the former two examples. The very

short hydrogen bond $P=O\cdots H-O-C$ of 2.492 Å occurs between the phosphate oxygen atom O(4) and the carboxyl hydroxyl oxygen atom O(5) of the $0k0$ screw-related molecule. The shortest phosphate-phosphate hydrogen bond, $P-O\cdots H-O-P$, 2.558 Å, involves the phosphates related by the $00l$ screw axis.

Table 8. Torsion angles in L-serine phosphate, DL-serine and L_s-threonine

Bond sequence	L-serine phosphate	DL-serine	L _s -threonine
O(2)-P — O(1)-C(3)	80.1°		
O(3)-P — O(1)-C(3)	-45.8		
O(4)-P — O(1)-C(3)	-161.4		
P — O(1)-C(3)-C(2)	153.2		
O(5)-C(1)-C(2)-C(3)	-60.9	-55.8	-82.6
O(5)-C(1)-C(2)-N	178.7	181.3	156.1
O(6)-C(1)-C(2)-C(3)	117.7	126.7	95.1
O(6)-C(1)-C(2)-N	-2.6	3.8	-26.1
C(1)-C(2)-C(3)-O(1)	-57.1	-53.1	-177.4
N — C(2)-C(3)-O(1)	61.3	69.2	-54.8
O(1)-P — O(4)-H(1)	-97.0		

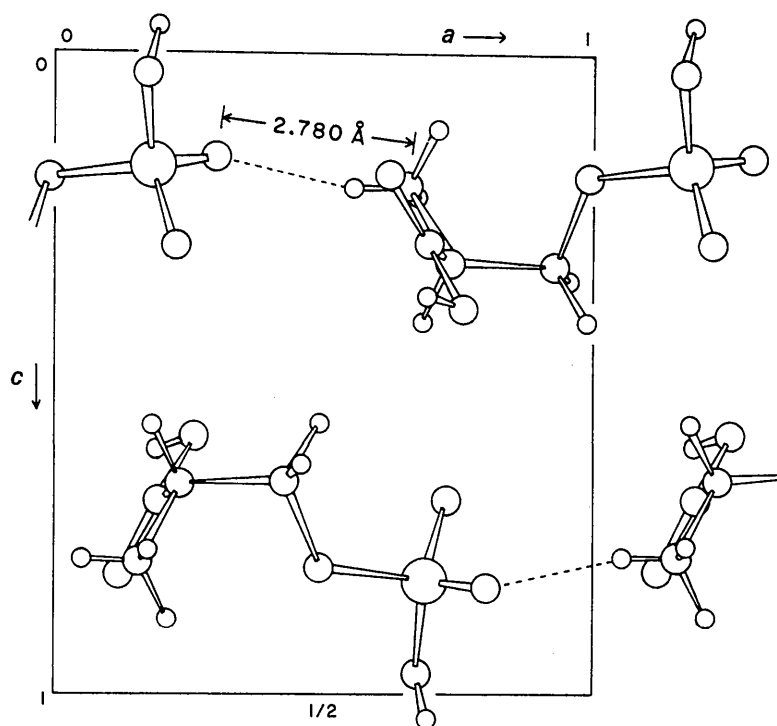


Fig. 8. View down the b axis of the unit cell showing the hydrogen bond not depicted in Fig. 7.

Table 9. Hydrogen bonds in L-serine phosphate

Reference molecule	Symmetry molecule	Key to bonds			Distances	Angles
		x	y	z		
(1)	A_1	$-\frac{1}{2}+y$	$-\frac{1}{2}+y$	$\frac{1}{2}-z$	$A_1A_2A_3$	$H_4A_3A_4$
(2)	A_3	$-\frac{1}{2}+y$	$-\frac{1}{2}+y$	$\frac{1}{2}-z$	$A_2A_3^*$	$A_2A_3A_4$
(3)	$O(3)$	$-\frac{1}{2}+y$	$-\frac{1}{2}+y$	$\frac{1}{2}-z$	A_2H^*	$H_4A_3A_4$
(4)	$O(2)$	$-\frac{1}{2}+y$	$-\frac{1}{2}+y$	$\frac{1}{2}-z$	A_3H^*	$H_4A_3A_4$
(5)	$O(3)$	$-\frac{1}{2}+y$	$-\frac{1}{2}+y$	$\frac{1}{2}-z$	A_2H	$H_4A_3A_4$
(6)	$O(5)$	$-\frac{1}{2}+y$	$-\frac{1}{2}+y$	$\frac{1}{2}-z$	A_3H	$H_4A_3A_4$
	$C(1)$	$-\frac{1}{2}+y$	$-\frac{1}{2}+y$	$\frac{1}{2}-z$	A_2H	$H_4A_3A_4$
	$C(2)$	$-\frac{1}{2}+y$	$-\frac{1}{2}+y$	$\frac{1}{2}-z$	A_3H	$H_4A_3A_4$
	$C(2)$	$-\frac{1}{2}+y$	$-\frac{1}{2}+y$	$\frac{1}{2}-z$	A_2H	$H_4A_3A_4$

* Standard deviations are given in parentheses.

There was little question here that the proton was attached to phosphate oxygen atom O(4), although the electron density of the proton was slightly diffuse. These two short hydrogen bonds run approximately normal to each other. The remaining three hydrogen bonds involve the protonated amino group; the two shorter hydrogen bonds, N-H...O(2), 2.780 Å, and N-H...C(3), 2.797 Å, are between the amino group and two different phosphate groups, and the third hydrogen bond, N-H...O(5), 2.964 Å, is between the amino group and the carboxyl hydroxyl group. It may be noted that the -NH₃⁺ group of amino acids is always involved in three hydrogen bonds. L-Serine phosphate occurs as a zwitterion with the amino group protonated by a phosphate proton. Terminal amino groups of proteins may be hydrogen bonded to phosphates in a similar manner.

The phosphate group is involved in five hydrogen bonds (Fig. 9); O(4) is involved in a donor hydrogen bond, while O(2) and O(3) are each involved in two acceptor hydrogen bonds. All of the hydrogen bonds in this structure are nonlinear (Table 9), *i.e.* the proton does not lie on the line joining the donor and acceptor. The $A_2-H \cdots A_3$ angles range from 153 to 168°, while the $H-A_2 \cdots A_3$ angles vary from 8 to 20°. The largest $H-A_2 \cdots A_3$ angle of 20° occurs in the case of the shortest hydrogen bond.

Viewed down the a axis, the crystal structure may be described as composed of hydrogen bonded and hydrophobic 'bonded' channels running down alternate $h00$ screw axes (Fig. 7). The hydrophobic channel is composed of the methylene protons, the carbonyl oxygen atom (which is not involved in hydrogen bonding) and the 'back end' of the phosphate hydroxyl group.

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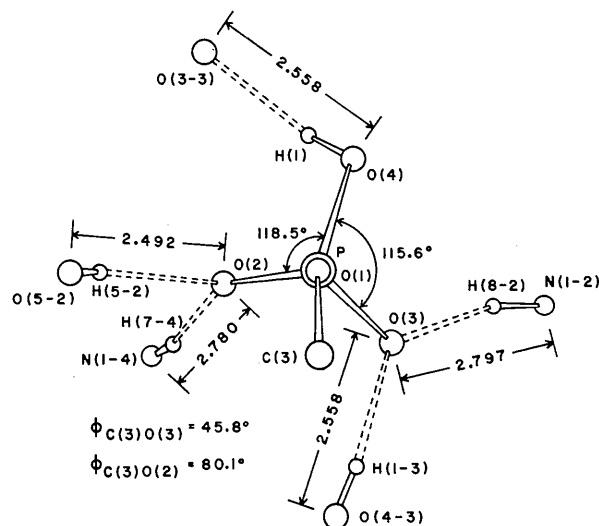


Fig. 9. The hydrogen-bonded surroundings of the phosphate group. Numbers appearing after hyphens are the symmetry numbers of Table 9.

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The Refinement of the Crystal Structure of the Perylene-Tetracyanoethylene Complex

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Crystals of the 1:1 complex of perylene and tetracyanoethylene (TCNE) are monoclinic, space group $P2_1/a$ with $a = 15.763$, $b = 8.234$, $c = 7.346$ Å, $\beta = 96.4^\circ$, $Z = 2$. The structure was refined by the block-diagonal least-squares method, on the basis of three-dimensional photographic data. Perylene and TCNE molecules are stacked alternately along the b -axis direction, making their molecular planes almost parallel to each other. The mean separation of the molecular planes is 3.186 Å.

Introduction

There is a common feature in the π -molecular complex; the donor and acceptor molecules are alternately

stacked, plane-to-plane, along some crystal axis with relatively small separation between molecular planes. The relative orientations of donor and acceptor molecules in the crystal are various (Prout & Wright, 1968).