

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



hydrogen bond, $\text{P}=\text{O} \cdots \text{H}-\text{OC}$ 2.492 Å, between a phosphate oxygen atom and the carboxyl group. The shortest hydrogen bond, $\text{P}-\text{O} \cdots \text{H}-\text{O}-\text{P}$, 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



yield a zwitterion, $\text{NH}_3^+\text{CH}(\text{COOH})\text{CH}_2\text{PO}_3^-$. The $\text{N}-\text{H} \cdots \text{O}-\text{P}$ 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 $\text{C}(1) (\text{sp}^2)-\text{C}(2) (\text{sp}^3)$ bond distance of amino acids is similar to that of the $\text{C}(2) (\text{sp}^3)-\text{C}(3) (\text{sp}^3)$ 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 $0.2 \times 0.2 \times 0.4$ 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.

$\text{Cu K}\alpha$ 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_c , 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.

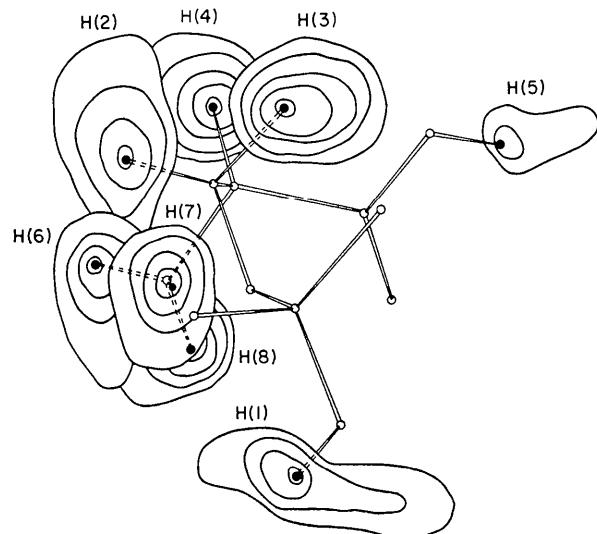


Fig. 1. Difference electron density map showing the hydrogen atoms contoured at intervals of $0.1 \text{ e.}\text{\AA}^{-3}$, beginning at $0.1 \text{ e.}\text{\AA}^{-3}$.

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*

($\lambda_{\text{Cu K}\alpha} = 1.5418 \text{ \AA}$)

Crystal system Space group	This work $P2_12_12_1$	McCallum <i>et al.</i> (1959)
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 2. Calculated and observed structure amplitudes $\times 10$

The following six reflections were suspected of suffering from large secondary extinction and therefore were not used in the final refinement cycles. 200 2602 2830; 020 2646 2829; 130 1628 1741; 211 1427 1523; 002 2545 3018; 102 1883 2025

0 < x <		U(0,6)				1 < x <				2 < x < 2				3 < x < 4				4 < x < 5				5 < x < 6				6 < x < 7									
4	1075	1061	0	1019	1019	0	198	208	0	530	527	0	80	69	0	169	203	2	55	75	0	426	425	0	166	166	0	280	289	2	60	55			
5	321	314	1	485	485	1	275	275	1	507	511	0	49	49	0	296	290	1	164	168	0	237	241	0	784	782	3	214	212						
6	268	261	3	324	321	3	591	589	3	334	341	0	305	305	0	196	185	3	254	254	0	94	82	0	217	217	0	217	217	0	217	217			
7	167	161	1	137	132	4	530	523	4	305	301	0	88	85	0	258	262	3	683	689	0	138	132	0	198	202	5	138	132	0	198	202			
8	0	0	0	103	103	0	121	126	0	675	676	0	7	151	163	0	116	100	0	292	292	0	177	180	0	67	67	0	101	99	0	101	99		
9	841	501	7	77	76	7	977	966	7	673	678	3	133	134	0	215	219	7	641	640	0	130	130	8	105	103	0	7	7	0	7	7	0	7	7
10	157	1077	0	123	115	0	101	119	0	526	525	9	205	209	0	250	255	0	133	130	0	648	648	0	216	216	0	806	802	0	190	194			
11	55	72	0	109	109	10	286	274	10	270	266	11	184	193	1	630	610	1	162	165	0	244	244	0	176	176	0	217	217	0	190	194			
12	363	402	1	155	149	1	16	16	1	1056	1051	3	122	123	0	244	244	0	176	176	0	266	266	0	197	197	0	217	217	0	217	217			
13	86	75	3	81	76	3	122	120	3	174	177	0	506	526	0	184	186	2	204	200	2	150	152	0	247	245	0	197	197	0	217	217			
14	509	567	5	205	185	1	557	571	0	592	612	5	87	87	0	225	224	1	256	259	9	144	123	3	511	513	5	53	53	0	174	159			
15	368	360	6	200	180	2	244	236	1	736	742	6	519	523	0	230	216	0	194	194	0	498	485	0	255	250	0	174	159						
16	136	136	0	10	9	0	477	575	2	240	230	0	300	300	0	3	3	0	406	421	0	315	312	0	217	217	0	174	159						
17	0	0	0	5	5	0	386	390	4	284	289	0	9	221	226	0	265	279	1	216	213	8	191	193	0	141	144	0	141	144					
18	0	0	0	556	545	0	142	145	5	253	259	10	467	467	1	277	267	7	70	64	2	323	322	0	196	197	0	217	217	0	217	217			
19	599	628	2	171	159	8	64	63	7	580	578	3	117	117	0	57	63	0	201	202	0	269	292	0	181	176	0	217	217	0	217	217			
20	349	818	3	268	278	9	92	89	8	156	141	3	141	141	0	208	199	0	192	192	0	154	154	0	231	231	0	197	197	0	217	217			
21	351	301	4	31	20	0	1	1	1	243	240	0	835	845	0	240	236	0	194	194	0	217	217	0	117	117	0	217	217	0	217	217			
22	0	0	0	1	1	1	0	0	0	403	393	11	138	130	1	595	607	7	140	126	1	152	146	8	56	50	0	331	321	0	133	132			
23	125	125	1	1	1	0	0	0	0	380	377	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
24	124	124	1	1	1	0	0	0	0	376	373	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
25	123	123	1	1	1	0	0	0	0	375	373	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
26	122	122	1	1	1	0	0	0	0	374	373	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
27	121	121	1	1	1	0	0	0	0	373	373	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
28	120	120	1	1	1	0	0	0	0	372	372	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
29	119	119	1	1	1	0	0	0	0	371	371	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
30	118	118	1	1	1	0	0	0	0	370	370	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
31	117	117	1	1	1	0	0	0	0	369	369	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
32	116	116	1	1	1	0	0	0	0	368	368	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
33	115	115	1	1	1	0	0	0	0	367	367	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
34	114	114	1	1	1	0	0	0	0	366	366	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
35	113	113	1	1	1	0	0	0	0	365	365	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
36	112	112	1	1	1	0	0	0	0	364	364	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
37	111	111	1	1	1	0	0	0	0	363	363	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
38	110	110	1	1	1	0	0	0	0	362	362	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
39	109	109	1	1	1	0	0	0	0	361	361	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
40	108	108	1	1	1	0	0	0	0	360	360	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
41	107	107	1	1	1	0	0	0	0	359	359	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
42	106	106	1	1	1	0	0	0	0	358	358	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
43	105	105	1	1	1	0	0	0	0	357	357	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
44	104	104	1	1	1	0	0	0	0	356	356	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
45	103	103	1	1	1	0	0	0	0	355	355	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
46	102	102	1	1	1	0	0	0	0	354	354	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
47	101	101	1	1	1	0	0	0	0	353	353	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
48	100	100	1	1	1	0	0	0	0	352	352	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
49	99	99	1	1	1	0	0	0	0	351	351	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
50	98	98	1	1	1	0	0	0	0	350	350	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
51	97	97	1	1	1	0	0	0	0	349	349	5	256	260	0	101	100	0	247	247	0	136	149	0	217	217	0	217	217	0	217	217			
52	96	96	1	1	1	0	0	0	0	348	348	5	256	260	0	101	100	0	247	247	0														

Table 3. Final positional coordinates and anisotropic temperature factors for L-serine phosphate*

Standard deviations are shown in parentheses. All non-hydrogen parameters are $\times 10^5$; all hydrogen parameters are $\times 10^4$.

x/a	y/b	z/c	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
P	18373 (10)	47330 (7)	17671 (9)	816 (13)	405 (7)	564 (9)	33 (9)	5 (10)
O(1)	-931 (30)	42231 (26)	19560 (26)	898 (40)	1050 (29)	734 (30)	-60 (29)	20 (31)
O(2)	30099 (31)	35744 (21)	16406 (29)	998 (39)	401 (20)	126 (35)	58 (25)	-89 (37)
O(3)	22563 (33)	56539 (21)	29991 (25)	1584 (49)	490 (21)	716 (29)	-81 (27)	-32 (32)
O(4)	17093 (42)	55131 (26)	3206 (27)	2087 (58)	735 (27)	671 (30)	-418 (39)	290 (39)
O(5)	-24151 (42)	62348 (26)	39977 (30)	2125 (65)	464 (22)	994 (33)	134 (33)	-274 (41)
O(6)	-37993 (41)	58137 (25)	19025 (35)	2201 (61)	609 (24)	1395 (43)	132 (33)	-703 (50)
C(1)	-30383 (45)	54688 (32)	29889 (39)	987 (54)	514 (30)	929 (44)	84 (37)	41 (47)
C(2)	-26658 (44)	40147 (31)	32952 (48)	935 (58)	511 (31)	954 (46)	-48 (33)	-138 (52)
C(3)	-7301 (45)	37788 (37)	33433 (48)	972 (57)	668 (34)	953 (46)	52 (44)	-26 (57)
N	-34642 (43)	32453 (31)	21021 (41)	965 (52)	458 (26)	1202 (47)	50 (32)	-19 (46)
H(1)	2005 (54)	5073 (38)	-312 (24)					
H(2)	-466 (59)	2779 (43)	3554 (47)					
H(3)	-125 (53)	4534 (39)	4181 (41)					
H(4)	-3186 (61)	3768 (40)	4145 (46)					
H(5)	-2869 (60)	6806 (39)	3864 (41)					
H(6)	-2901 (52)	3584 (44)	1252 (42)					
H(7)	-4539 (58)	3299 (38)	2092 (45)					
H(8)	-3271 (50)	2493 (41)	2271 (39)					

* Anisotropic thermal parameters are in the form $\exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2hk\beta_{12} + 2hl\beta_{13} + 2kl\beta_{23})]$.

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

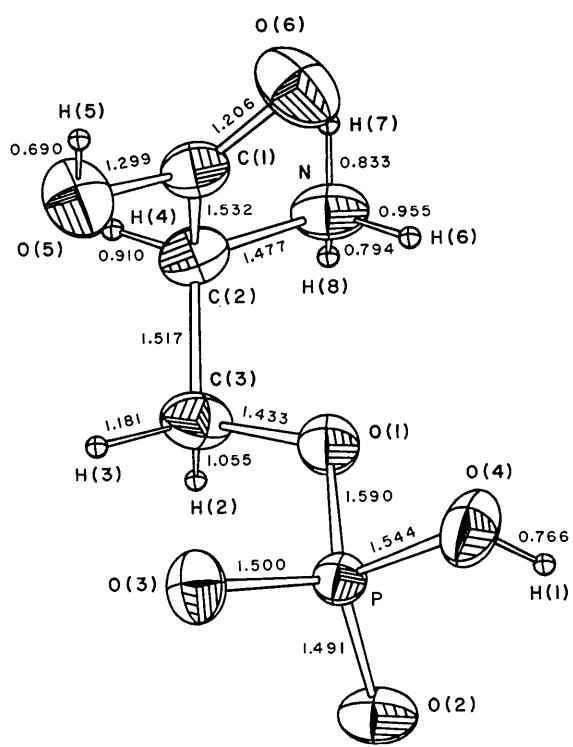


Fig. 2. Bond distances in L-serine phosphate.

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 et al. (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 ³)	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 & Peerde- man (1968)
DL- α -n-Butyric acid	A 0.011	1.512	1.564	162.5	339.4	2.667	3.108	3.285	Ichikawa & Itaka (1968)
B 0.009	1.509	1.564	150.9	328.8	2.726	2.654	2.940	3.226	Harding & Long (1968)
0.012	1.51	1.51	176.4	357.0	2.623	2.999	3.424	3.419	Oughton & Harrison (1959)
L-Cystine	0.015	1.543	1.509	168.3	342.4	2.690	—	—	Marsh (1958)
Glycine	0.005	1.523	—	198.3	340.9	332.7	2.700	—	Itaka (1960)
β -Glycine	0.015	1.521	—	156.3	345.0	2.687	—	—	Itaka (1961)
γ -Glycine	0.011	1.527	—	168.3	345.0	2.668	2.919	3.527	Donohue & Caron (1964)
L-Histidine·HCl·2H ₂ O	0.011	1.530	1.527	179.5	0.4	2.665	3.005	3.444	Donohue & Trueblood (1952)
Hydroxy-L-proline†	0.009	1.516	1.532	178.0	356.9	2.661	3.043	3.301	Wright & Marsh (1962)
L-Lysine·HCl·2H ₂ O	0.004	1.529	1.524	162.1	340.1	321.9	2.774	3.254	Chiba, <i>et al.</i> (1967)
L-Ornithine·HCl	0.006	1.541	1.529	144.2	321.9	2.714	2.915	3.119	Vainshtein & Gurskaya (1964)
L-Phenylalanine·HCl*	0.015	1.505	1.550	177.5	358.9	2.655	—	3.551	Gurskaya (1968)
DL-Serine	0.009	1.528	1.513	181.3	3.8	2.630	2.896	3.990	Shoemaker, Barieau, Donohue & Lu (1953)
DL-O-Serine phosphate	0.004	1.522	1.518	164.7	344.8	2.742	2.939	3.335	Putkey & Sundaralingam (1970)
monohydrate									This work
L-O-Serine phosphate	0.005	1.532	1.518	178.8	357.4	2.631	2.880	3.413	Shoemaker, Donohue, Scho- maker & Corey (1950)
L-Threonine	0.007	1.517	1.542	156.1	333.9	2.672	3.170	3.276	Fries & Sundaralingam (1969a)
L-O-Tyrosine	0.005	1.528	1.538	164.6	341.5	2.685	3.028	3.335	Ando <i>et al.</i> (1967)
sulfate-K·2H ₂ O	0.009	1.509	1.557	170.2	348.8	2.640	3.042	3.456	Mallikarjunan & Rao (1969)
L-Valine·HCl†	0.007	1.527	1.541	156.0	335.8	2.671	3.167	3.326	
L-Valine									

* 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.

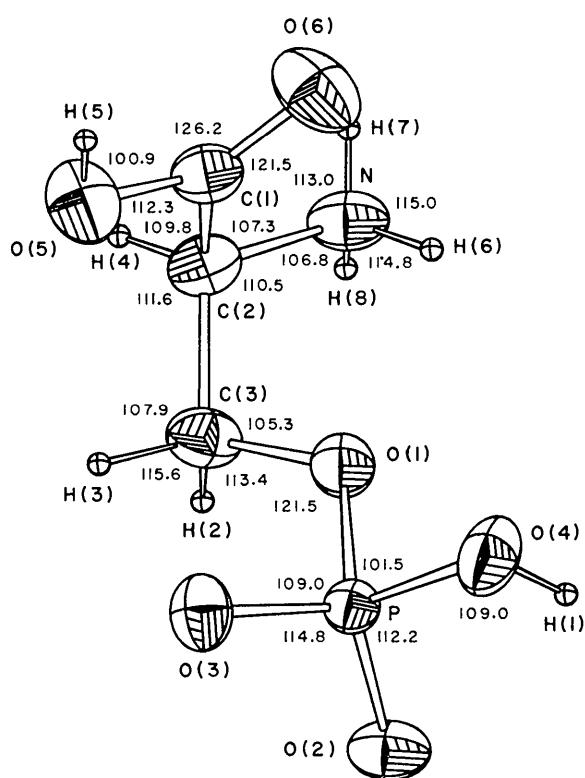


Fig. 3. Bond angles in L-serine phosphate. The bond angles not shown in the Figure are: O(1)PO(2), 108·8; O(3)PO(4), 109·6; C(1)C(2)C(3), 110·1; H(4)C(2)N, 111·6; C(2)C(3)H(2), 110·4; H(3)C(3)O(1), 103·4; C(2)NH(6), 107·4; and H(7)NH(8), 115·6°.

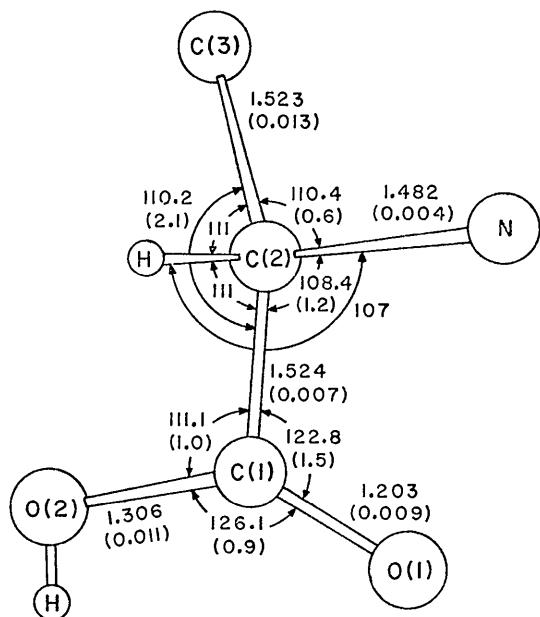


Fig.4. Weighted average bond distances and angles in the amino acid residues with an un-ionized carboxyl group.

C(2)–C(3) (sp^3 – sp^3) bond distance. We attribute the lengthening of the C(1)–C(2) bond distance from the normal sp^2 – sp^3 value to the presence of the α -amino substituent, which is *syn* to the carbonyl group, thus providing nonbonded interactions between the carbonyl group and the ammonium group (see below). A similar lengthening of the carboxyl C–C bond is not observed in the normal carboxylic acids.

The weighted average bond distances and angles for the amino acid residues with an un-ionized carboxyl group are shown in Fig. 4. These values may be compared with those of the ionized amino acids reported recently by Marsh & Donohue (1967). Apart from the differences in the P-O bond distances, the agreement between the bond distances in L-serine phosphate and DL-serine phosphate is quite good. In the monoanionic monophosphates, the negative charge on the phosphate group is mainly delocalized among the phosphate bonds, P-O(2) (1.491 Å) and P-O(3) (1.500 Å); however, there is a tendency for the P-O⁻ bond to be slightly longer than the P=O bond, as seen in all of the known phosphates (Table 7). The P-O bond length order in monoanionic phosphates is

$$\text{P-OC} > \text{P-OH} > \text{P-O}^{\ddagger-} \quad (\text{P=O-P}\leftrightarrow\text{O}^-),$$

with a difference of about 0.05 Å between P-OC and P-OH on one hand and P-OH and P-O⁴⁻ on the other (Cruickshank, 1961; Sundaralingam & Jensen, 1965b). Hence, a knowledge of the P-O bond distances is sufficient to indicate the presence or absence of a hydrogen substituent on the phosphate oxygen atoms.

The O-P-O valence angles in L-serine phosphate range from 101.5° to 114.8°. The smallest angle involves the substituted oxygen atoms O(1) and O(4), while the largest angle involves the unsubstituted atoms O(2) and O(3). The latter angle is about 2° smaller than the other known monoanionic phosphates (Table 7). The

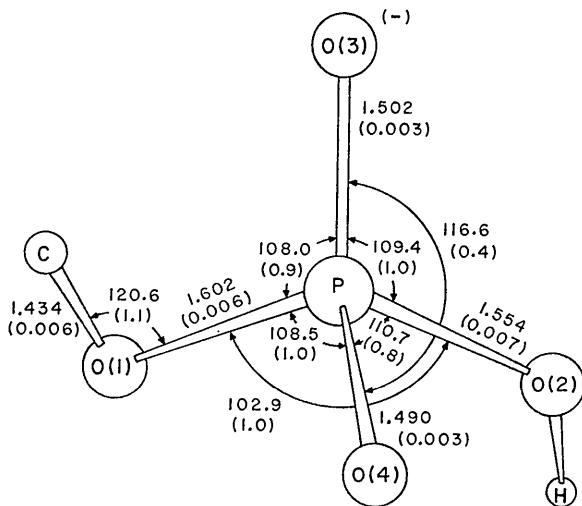


Fig. 5. Weighted average bond distances and angles in the monoanionic monophosphate.

Table 7. Bond distances and angles

	P=O (Å)	P-O ⁻ (Å)	P-OH (Å)	P-OR (Å)	$\sum(P-O)$ (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 (\bar{I})	1.490	1.502	1.554	1.602	6.149	1.434	
$\sigma_m \dagger$	0.003	0.003	0.007	0.006	0.009	0.006	

* Reported estimated standard deviation.

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

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 phosphodiesters (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-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)

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

and

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

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-

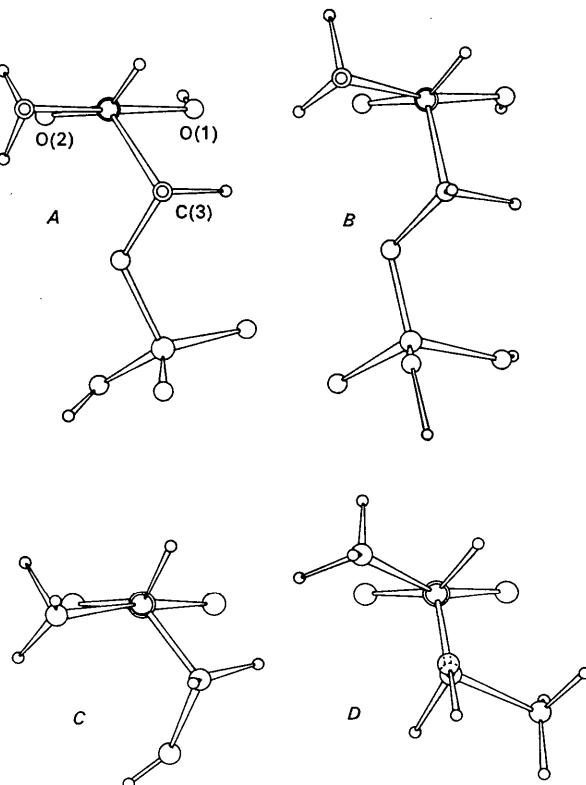


Fig. 6. Conformations of (a) DL-serine phosphate, (b) L-serine phosphate, (c) DL-serine, and (d) L-threonine, viewed down the $C_\alpha-C'$ bond. Note that the hydroxy group is *anti* to the ammonium groups in (a) and (b), and the ammonium nitrogen atom is displaced on the opposite side of the carboxyl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

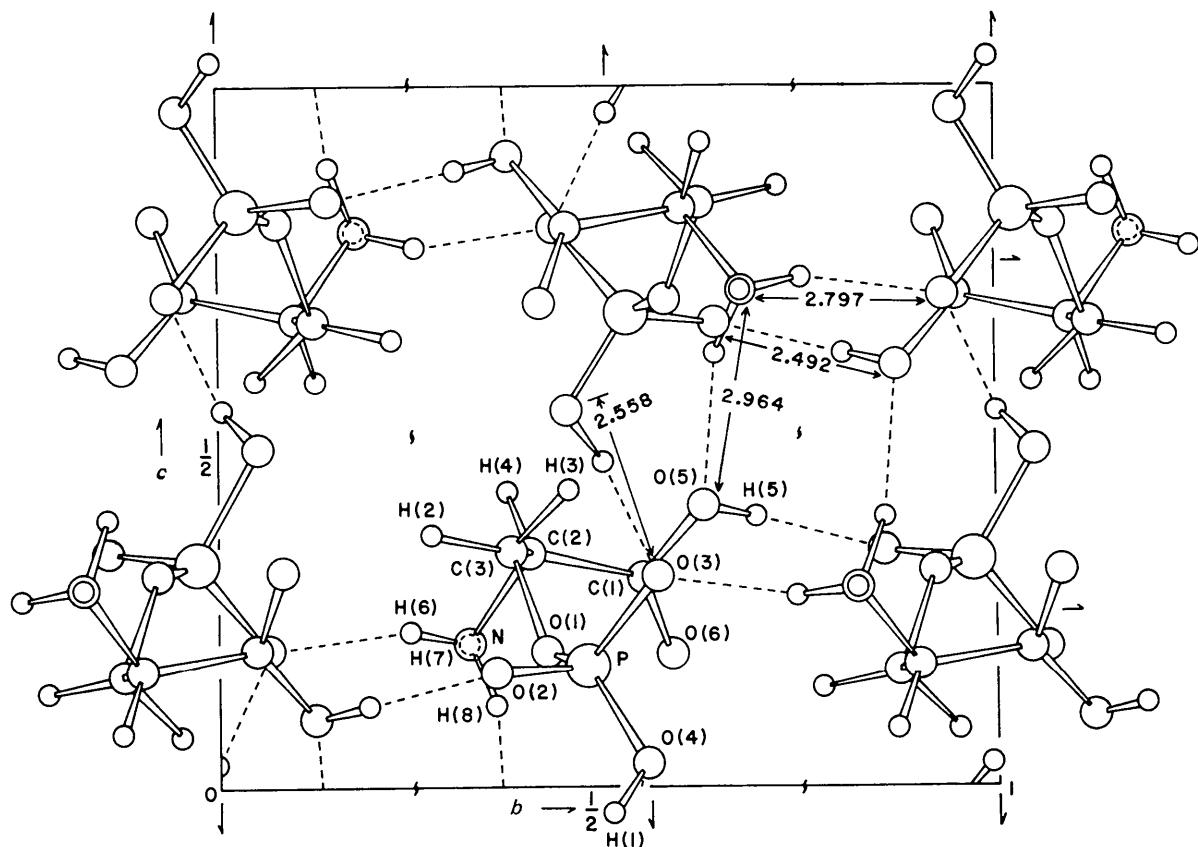


Fig. 7. View of the unit cell down the a axis showing the hydrogen bonding pattern and the alternating regions of hydrogen bonded and hydrophobic bonded channels; see text.

ential 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 non-bonded $C(3)\cdots O(1)$ 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)…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 $\text{P}=\text{O}\cdots\text{H}-\text{O}-\text{C}-$ of 2.492 \AA 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, $\text{P}-\text{O}\cdots\text{H}-\text{O}-\text{P}$, 2.558 \AA , 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		

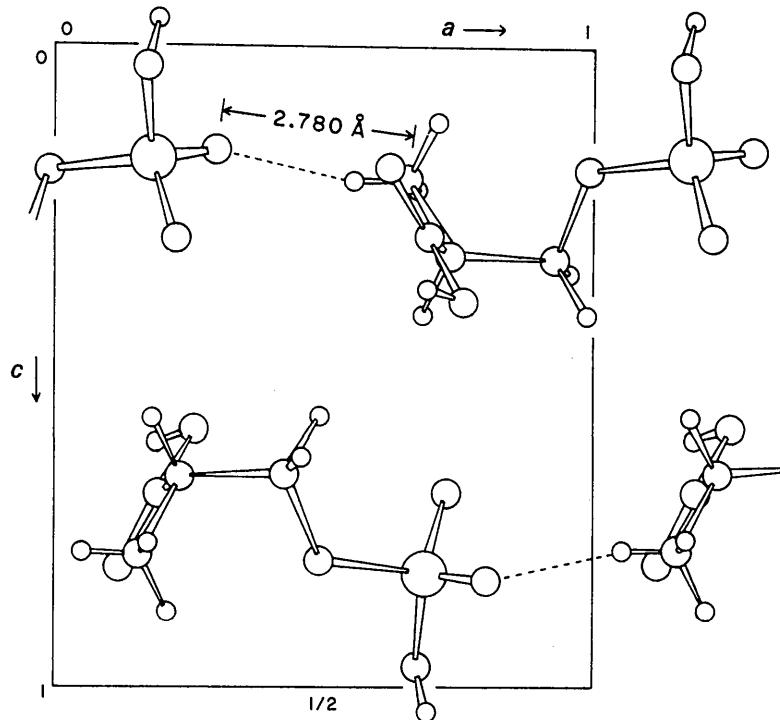


Fig. 8. View down the b axis of the unit cell showing the hydrogen bond not depicted in Fig. 7.

Table 9. Hydrogen bonds in L-serine phosphate

Reference molecule	Symmetry-related molecules				Key to bonds
	A_1	A_2	A_3	A_4	
(1)	x	$-\frac{1}{2}y$	$\frac{1}{2}z$		
(2)	$-x$	$-\frac{1}{2}+y$	$\frac{1}{2}-z$		
(3)	$-x$	$\frac{1}{2}+y$	$\frac{1}{2}-z$		
(4)	$\frac{1}{2}-x$	$1-y$	$-\frac{1}{2}+z$		
(5)	$-\frac{1}{2}-x$	$1-y$	$-\frac{1}{2}+z$		
(6)	$x-1$	y	z		

Reference molecule	Distances				Angles
	A_2H	A_3H^*	A_2A_3 *	$A_1A_2A_3$	
	0.794 Å	2.043 (0.04) Å	2.797 (0.004) Å	112.57°	$A_2H_A_3$
	P	P	P	109.75	$H_A_3A_4$
2	O(3)	O(2)	O(2)	152.62	$A_2A_3A_4$
3	P	P	P	138.29	
4	P	P	P	131.29	
5	C(1)	O(5)	O(5)	20.97	
6	P	O(2)	O(2)	117.04	
				113.58	
				168.36	
				117.04	
				142.94	
				150.53	
				114.40	
				132.85	
				133.22	
				132.85	
				167.57	
				117.28	
				8.73	

* 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 $-\text{NH}_3^+$ 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 $A_2\text{-H}\cdots A_3$ angles range from 153 to 168°, while the $\text{H}\cdots A_2\cdots A_3$ angles vary from 8 to 20°. The largest $\text{H}\cdots A_2\cdots A_3$ 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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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 $a=15.763$, $b=8.234$, $c=7.346 \text{ \AA}$, $\beta=96.4^\circ$, $Z=2$. 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 \AA .

Introduction

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

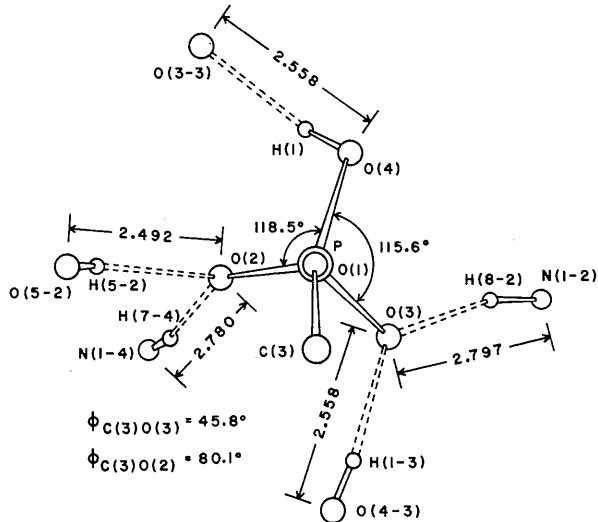


Fig. 9. The hydrogen-bonded surroundings of the phosphate group. Numbers appearing after hyphens are the symmetry numbers of Table 9.

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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).