

radii (vanadium, 0.74 Å) total about 0.10 Å more than those for the Fe–O separation yet the observed mean value for the V–O separation is 1.981 Å. This value suggests that the V–O bond is more covalent than the corresponding Fe–O bond. (Values for V–O separation in vanadyl(IV) bisacetylacetonate and bisbenzoylacetonate have been determined to be near 1.97 Å by Pfluger (1968); no other trivalent V–O separations are known to the authors.)

For two of the three chelate rings, the vanadium ion in the α form lies in the least-squares plane formed by the five atoms of the 2,4-pentanedione ring. Only the ring atoms are included in calculation of the least-squares plane because steric effects from crystal packing appear systematically to affect methyl groups in isomorphous sets of 2,4-pentanedione compounds (Morosin, 1967). A comparison of the corresponding values found in $\text{Fe}(\text{Acac})_3$ and $\alpha\text{-V}(\text{Acac})_3$ is given in Table 5. In both of these, it is clear that the metal ion lies off the least-squares plane in only one of the rings. For $\beta\text{-V}(\text{Acac})_3$ the metal ion lies off the plane for all three chelate rings. Since the structures of the Ga, In or Sc analogs have not been reported, one is not able to invoke packing effects for the β form.

Fig. 3 illustrates the anisotropic thermal parameters for the two forms of $\text{V}(\text{Acac})_3$. In general the root mean square amplitudes are greater along directions perpendicular to the planes formed by the chelate rings than along chemical bonds. However, short contact separations between C(3x) and C(3x) on different molecules in $\alpha\text{-V}(\text{Acac})_3$ and between C(2x) and C(12) as well as between C(2x) and C(3x) on different molecules

in $\beta\text{-V}(\text{Acac})_3$ account for part of the departures from the expected directions of the thermal ellipsoids.

Table 5. *Distance of metal from ideal ligand plane*

	Metal	A*	B*	σ^*
$\alpha\text{-V}(\text{Acac})_3^*$	0.003	0.010	0.015	0.016
	0.227	0.045	0.151	0.029
	0.004	0.111	0.134	0.026
$\text{Fe}(\text{Acac})_3$	0.001	0.027	0.052	0.006
	0.266	0.088	0.208	0.016
	0.081	0.070	0.139	0.025
$\beta\text{-V}(\text{Acac})_3$	0.089	0.001	0.049	0.012
	0.173	0.032	0.072	0.006
	0.075	0.038	0.063	0.004

* $\text{Acac} = \text{C}_5\text{H}_7\text{O}_2$; A is the methyl carbon closer to least-squares plane; B other methyl-carbon; and σ is the standard deviation of the atoms (those defining the plane) from the least-squares plane.

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The Crystal Structure of Thiamine Pyrophosphate

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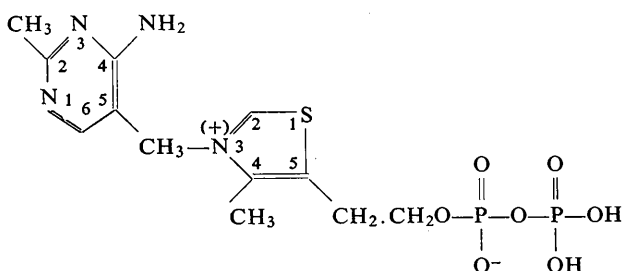
An X-ray crystal structure analysis of triclinic thiamine pyrophosphate is reported. The space group is $P\bar{1}$ with $a = 13.42$, $b = 12.24$, $c = 15.57$ Å, $\alpha = 56^\circ 20'$, $\beta = 95^\circ 46'$, $\gamma = 90^\circ 25'$ and with two molecules in the asymmetric unit. The structure and molecular conformation differ from those of monoclinic thiamine pyrophosphate hydrochloride. The parameters of both the thiamine and pyrophosphate portions of the molecule are in general agreement with parameters of related crystal structures. The structure possesses layer-type characteristics and there is some evidence for end-to-end hydrogen bonding of the pyrophosphate groups, the oxygen atoms of which are in the staggered conformation.

The structure was solved by use of the three-dimensional Patterson function and successive partial Fourier syntheses of electron density, and refined to an R value of 27.9% on 4920 non-zero observed reflexions.

Introduction

Thiamine, or vitamin B_1 , is an essential dietary factor for all animals. In man its deficiency is the cause of

the disease beriberi. Thiamine, however, functions in its coenzyme form, cocarboxylase or thiamine pyrophosphate, (TPP). The structural formula and conventional thiamine ring numbering is shown below.



The vitamin is transported in the host as either thiamine or its monophosphate; the metabolically reactive form, TPP, is synthesized at the site of catalysis. TPP was first isolated from yeast by Lohmann & Schuster (1937) soon after the final structural assignment of thiamine was published by Williams (1936), but it may be more readily produced synthetically (Weijlard & Tauber, 1938).

TPP is a coenzyme for numerous enzymic decarboxylations of α -keto acids. The mode of action of TPP-catalysed reactions has been explained by Breslow (1958) and Breslow & McNelis (1959). Review articles on thiamine and TPP have been published by Jansen (1949) and Pullman & Spanjaard (1961).

The purpose of this structure analysis was to determine the configuration of the terminal pyrophosphate group with respect to the thiamine portion of the molecule and to test whether the thiamine moiety remains unchanged in the crystalline form of the coenzyme. During the course of this work, the structures of both monoclinic TPP hydrochloride (Pletcher & Sax, 1966) and triclinic thiamine monophosphate (Karl & Britts, 1966) were reported. TPP hydrochloride differs from the present TPP in its molecular conformation, space group ($P2_1/c$ compared with $P\bar{1}$), and in the presence of ionic chlorine. Thiamine monophosphate was originally believed to be TPP hydrochloride but subsequent direct method analysis proved the substance to be hydrolysed TPP, *i.e.* thiamine monophosphate possessing free orthophosphate ions but no chlorine. Relevant, also, is the earlier reported structure of monoclinic thiamine hydrochloride (Kraut & Reed, 1962).

Experimental

Crystals were prepared by slow evaporation of TPP chloride from a water and acetone mixture. Colourless, brittle crystals of tabular habit, showing the predominant form $\{010\}$, resulted. Both the initial and final collection of crystal intensity data were obtained from oscillation and Weissenberg photographs with $\text{Cu } K\alpha$ radiation. The density of the crystals was determined by flotation using carbon tetrachloride and methylene chloride.

Crystal data

Empirical formula: $\text{C}_{12}\text{H}_{18}\text{N}_4\text{S}_2\text{P}_2\text{O}_7$. Molecular weight = 424.1.

Crystal system: triclinic.

Space group: $P\bar{1}$

$$a = 13.42 \pm 0.03 \text{ \AA}$$

$$\alpha = 56^\circ 20' \pm 20'$$

$$b = 12.24 \pm 0.03 \text{ \AA}$$

$$\beta = 95^\circ 46' \pm 20'$$

$$c = 15.57 \pm 0.03 \text{ \AA}$$

$$\gamma = 90^\circ 25' \pm 20'$$

$$V = 2117 \pm 23 \text{ \AA}^3$$

$$D_M = 1.56 \text{ g.cm}^{-3}$$

$$D_c = 1.56 \text{ g.cm}^{-3}, \text{ assuming 4 TPP molecules and 16 water molecules in the unit cell.}$$

Linear absorption coefficient ($\text{Cu } K\alpha$) = 30.8 cm^{-1} .

Crystal sizes used for data collection:

$$0.25 \times 0.30 \times 0.30 \text{ mm (c axis vertical).}$$

$$0.32 \times 0.28 \times 0.34 \text{ mm (a axis vertical).}$$

The intensities were visually estimated and corrected for Lorentz and polarization effects but not for absorption. The bulk of the data were collected from two crystals, one mounted with the c axis vertical and a second with the a axis vertical. Of the 9660 possible reflexions in the half sphere of reflexion, a total of 7108 were observed, of which 4920 had measurable intensities greater than zero.

The X-ray intensities were initially scaled, layer by layer, and put on an approximately absolute scale by the method of Wilson (1942). An average isotropic temperature factor of 3.2 \AA^2 was deduced at this stage. The estimated relative standard deviations of the intensities averaged 20% for reflexions of moderate intensity, increasing to 25% for the strong reflexions and 30% for the weak reflexions.

It was recognized at an early stage of the analysis that the large number of low intensity reflexions would probably impose restrictions on the accuracy of the structure determination. This has indeed proved to be the case. Hence, no accuracy is claimed for the work put forward, but the results may be considered reliable owing to the fact that the two molecules in the asymmetric unit have approximately the same conformation.

Structure determination

The structure analysis commenced with the location of the phosphorus-phosphorus vectors of the pyrophosphate groups from the three-dimensional Patterson function. The terms were sharpened and modified by a function given by

$$F^2(\text{sharpened}) = \frac{F_o^2}{\sum f^2} \exp \left[\frac{4.5 \sin^2 \theta}{\lambda^2} \right].$$

Reflexions too weak to be observed from the photographs were approximately allowed for by allotting to those reflections for which $0.3 < \sin \theta < 0.7$, weights of half the threshold value.

The Patterson function revealed strong pseudo-monoclinic features, with the majority of vectors falling

on the sections near $U=0$ and $U=\frac{1}{2}$, suggesting a layer-type structure. Many of the peaks were related by approximate $\frac{1}{2}a, \frac{1}{2}b$ and to a lesser extent, $\frac{1}{2}c$ translations. A pronounced peak at a distance of 2.9 Å from the origin was observed, indicative of a possible phos-

phorus-phosphorus vector of a pyrophosphate group. Further vectors of similar length throughout the Patterson function showed the pyrophosphate groups to be lying in approximately the same orientation in the bc plane and at heights of $x \approx \frac{1}{4}$ and $\frac{3}{4}$. The following

Table 1(a). Positional and thermal parameters for thiamine pyrophosphate molecules 1 and 2

Molecule 1				Molecule 2					
	$X \times 10^3$	$Y \times 10^3$	$Z \times 10^3$	$B (\text{Å}^2)$		$X \times 10^3$	$Y \times 10^3$	$Z \times 10^3$	$B (\text{Å}^2)$
O(1)	307	472	701	2.6	O(27)	160	637	412	3.0
O(2)	131	547	667	4.8	O(28)	325	660	467	3.3
O(3)	190	453	574	3.6	O(29)	183	737	507	3.7
P(4)	208	447	674	3.4	P(30)	227	729	422	2.8
O(5)	193	293	750	2.6	O(31)	187	875	326	3.6
P(6)	241	189	866	2.7	P(32)	215	987	215	3.1
O(7)	327	120	847	4.7	O(33)	177	100	213	4.0
O(8)	187	068	945	3.5	O(34)	150	937	160	5.3
O(9)	253	253	920	2.7	O(35)	323	981	189	2.4
C(10)	277	173	030	2.0	C(36)	313	975	097	2.0
C(11)	330	270	048	2.0	C(37)	402	967	040	3.0
C(12)	347	402	965	3.8	C(38)	417	833	093	5.5
S(13)	321	517	993	3.5	S(39)	423	715	065	3.7
C(14)	323	637	871	4.9	C(40)	410	593	193	4.3
N(15)	333	581	821	4.5	N(41)	398	640	250	4.5
C(16)	377	463	871	4.1	C(42)	400	780	190	3.7
C(17)	430	390	841	2.9	C(43)	401	808	269	3.2
C(18)	353	677	719	2.8	C(44)	367	553	363	2.6
C(19)	370	798	707	3.3	C(45)	323	417	413	3.2
C(20)	293	867	732	3.0	C(46)	223	396	399	3.0
N(21)	317	975	732	3.0	N(47)	190	271	442	3.8
C(22)	400	009	703	2.2	C(48)	243	167	500	2.9
N(23)	473	949	680	3.4	N(49)	340	180	523	2.8
C(24)	457	837	683	3.8	C(50)	377	303	477	2.8
N(25)	203	830	758	2.7	N(51)	161	492	328	4.0
C(26)	432	133	697	4.2	C(52)	200	037	557	4.0

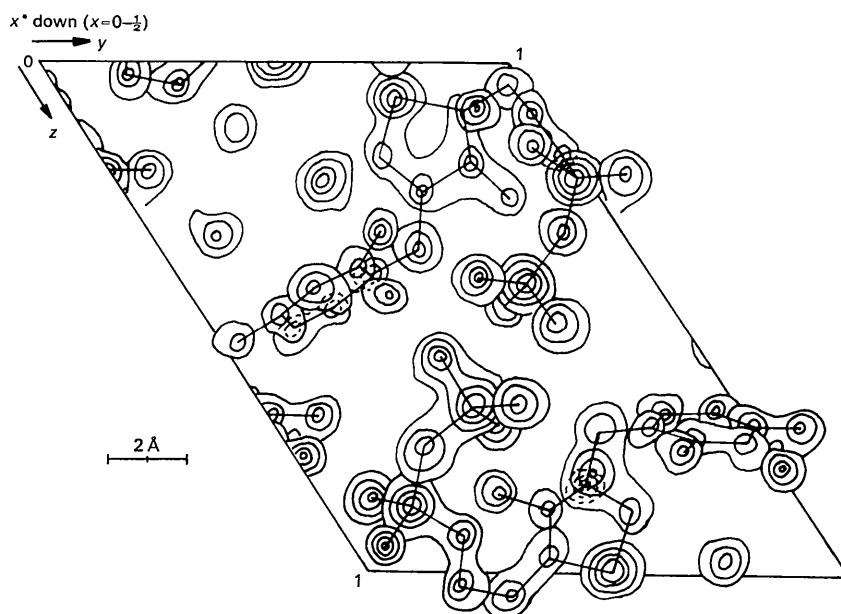


Fig. 1. A composite electron density map of the final distribution, showing sections superposed down the a axis. (Several contours for the phosphorus and sulphur atoms have been omitted for clarity).

approximate coordinates for the four phosphorus atoms of the asymmetric unit were deduced:

(0.21, 0.45, 0.68); (0.24, 0.19, 0.86); (0.22, 0.72, 0.43); (0.22, 0.98, 0.22).

Positions for the sulphur atoms and chlorine ions believed at this stage to be present could not be assigned. Chlorine was later, in fact, shown to be absent.

A partial Fourier synthesis with terms phased on the phosphorus positions revealed approximate locations of most of the atoms in molecule 1, but only partial evidence was present for molecule 2. Refinement of the structure was slow and the resolution of molecule 2, especially the thiazole ring, was initially poor for many of the early electron density maps. Successive Fourier syntheses, with increasing terms, finally led to the conclusion that chlorine was absent, its effective scattering weight being allowed for by the inclusion of two more water molecules of crystallization per TPP molecule. Later, electron density maps together with a final ($F_o - F_c$) difference map enabled the 52 atoms of the two TPP molecules together with the most likely positions of the eight waters of crystallization in the asymmetric unit to be located. No attempt was made to locate hydrogen atoms. Fig. 1 shows the final composite electron density map phased on all the non-zero observed reflexions.

Refinement

Some refinement of atomic positions had already proceeded in parallel with the successive partial Fourier syntheses. This, together with improved, inter-zonal $F_o \sim F_c$ scaling and correlation of data in the later stages, reduced the R value from 49% to 35%. Least-squares refinement of the three-dimensional data of positional parameters of all the atoms, individual iso-

tropic temperature factors and an overall scale factor were then applied. The final R value after 5 cycles fell to 27.9%, calculated on the 4920 non-zero observed reflexions. The final scale and average temperature factors were found to be 1.5 (relative to the original Wilson plots) and 3.6 \AA^2 respectively. The final shifts in the positional and thermal parameters were small, averaging 0.001 \AA and 0.2 \AA^2 respectively. Anisotropic refinement was not attempted as the large number of parameters involved would have necessitated a prohibitive amount of computing. The final calculated and observed structure factors may be obtained on request from the authors.

Results and discussion

General

The atoms of molecules 1 and 2 of the asymmetric unit are numbered 1–26 and 27–52 respectively see Fig. 2, and the most probable sites for the eight waters of crystallization numbered 53–60. Primed symbols are reserved for centrosymmetrically related atoms. The numbering here differs from the conventional chemical thiamine-ring numbering system given earlier. Tables 1(a) and (b) list the final positional and isotropic thermal parameters respectively of the 60 atoms in the asymmetric unit. Fig. 2 illustrates the bond distances and angles and Tables 2(a) and (b) tabulate respectively the same bond distances and angles for the two molecules. These collectively have average standard deviations of 0.04 \AA and 3° respectively.

The TPP molecule

Fig. 3. gives comparative sketches of a TPP molecule of the present analysis and related thiamine molecules, namely thiamine hydrochloride (Kraut & Reed, 1962), thiamine monophosphate (Karle & Britts, 1966) and TPP hydrochloride (Pletcher & Sax, 1966). The

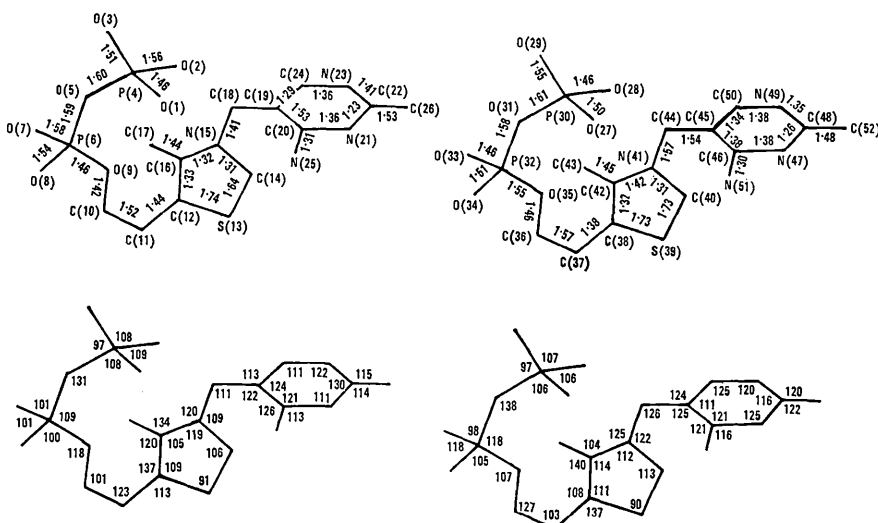


Fig. 2. Bond distances and angles for TPP.

Table 1(b) *Most probable positional and thermal parameters for water molecules of crystallization*

	$X \times 10^3$	$Y \times 10^3$	$Z \times 10^3$	$B (\text{\AA})^2$
O(53)	157	767	965	3.8
O(54)	093	027	768	3.5
O(55)	117	601	807	4.0
O(56)	253	140	343	4.1
O(57)	263	433	234	3.9
O(58)	157	340	130	4.0
O(59)	010	427	450	3.5
O(60)	003	866	090	3.6

TPP molecule can best be described as being an irregular S-shape with the terminal pyrophosphate group bent back over, and above, the organic thiamine portion containing the thiazole and pyrimidine rings, which lie approximately at right angles to each other. As in TPP hydrochloride (Pletcher & Sax, 1966), it would appear that the zwitterionic charge distribution within the molecule is largely minimized by the bending back of the negatively charged pyrophosphate group towards the positively charged thiazole ring.

The present approximate analysis, as expected, gives slight discrepancies between the parameters of the two molecules in the asymmetric unit. However, the general level of agreement is good, and both molecules compare favourably with the published parameters of related molecules containing the thiamine moiety. Slight conformational differences in the dimethylene side chain linking the thiazole ring to the pyrophosphate group for the two molecules is found. The conformations of both TPP molecules of the asymmetric

Table 2(a). *Bond lengths for thiamine pyrophosphate*

The average standard deviation of bond lengths is 0.04 Å.

Molecule 1		Molecule 2	
Bond	Distance (Å)	Bond	Distance (Å)
O(1)—P(4)	1.46	O(27)—P(30)	1.50
O(2)—P(4)	1.56	O(28)—P(30)	1.46
O(3)—P(4)	1.51	O(29)—P(30)	1.55
O(5)—P(4)	1.60	O(31)—P(30)	1.61
O(5)—P(6)	1.59	O(31)—P(32)	1.58
O(7)—P(6)	1.58	O(33)—P(32)	1.46
O(8)—P(6)	1.54	O(34)—P(32)	1.51
O(9)—P(6)	1.46	O(35)—P(32)	1.55
O(9)—C(10)	1.42	O(35)—C(36)	1.46
C(10)—C(11)	1.52	C(36)—C(37)	1.47
C(11)—C(12)	1.44	C(37)—C(38)	1.38
C(12)—S(13)	1.74	C(38)—S(39)	1.73
S(13)—C(14)	1.64	S(39)—C(40)	1.73
C(14)—N(15)	1.31	C(40)—N(41)	1.31
N(15)—C(16)	1.32	N(41)—C(42)	1.42
C(16)—C(17)	1.44	C(42)—C(43)	1.45
C(16)—C(12)	1.33	C(42)—C(38)	1.38
N(15)—C(18)	1.41	N(41)—C(44)	1.57
C(18)—C(19)	1.41	C(44)—C(45)	1.54
C(19)—C(20)	1.53	C(45)—C(46)	1.38
C(20)—N(21)	1.36	C(46)—N(47)	1.38
C(20)—N(25)	1.31	C(46)—N(51)	1.30
N(21)—C(22)	1.23	N(47)—C(48)	1.26
C(22)—C(26)	1.53	C(48)—C(52)	1.48
C(22)—N(23)	1.41	C(48)—N(49)	1.35
N(23)—C(24)	1.36	N(49)—C(50)	1.38
C(24)—C(19)	1.29	C(50)—C(45)	1.34

unit, however, approximate to those found in thiamine hydrochloride (Kraut & Reed, 1962) and thiamine monophosphate (Karle & Britts, 1966), but differ from TPP hydrochloride (Pletcher & Sax, 1966) in that the latter's side chain extends to the opposite side of the thiazole ring. The difference between the TPP molecules of the present analysis and those of TPP hydrochloride is not so striking as would first appear from Fig. 3, if their enantiomorphs are also considered. Flexibility in the orientations of the thiazole and pyrimidine rings and the disposition of the terminal portion of the pyrophosphate group with respect to the thiazole ring make the TPP and enantiomorph TPP hydrochloride (or *vice versa*) molecule basically similar.

The thiamine parameters

The thiamine parameters of TPP are in general agreement with those of earlier reported thiamine structures. Flexibility in the orientations of the planes of the thiazole and pyrimidine rings, however, is apparent. Equations for least-squares planes were derived for the thiazole and pyrimidine rings, together with their ring attached atoms, by use of a program written by Dr J. Milledge of University College, London. Table 3 gives the results of the analysis, from which it is seen that the most significant deviations from planarity occur for the three atoms of each molecule attached to the thiazole rings [*i.e.* C(11), C(17), C(18) and C(37), C(43), C(44)]. The resulting dihedral angles *i.e.* the angles between the normals to the planes of the two rings are 70° and 85° for molecules 1 and 2 respectively. Corresponding angles reported for related structures are: 76° for thiamine hydrochloride (Kraut & Reed, 1962); 90° for thiamine monophosphate (Karle & Britts, 1966); and 84° for TPP hydrochloride (Pletcher & Sax, 1966). Hence, the general ring conformations are basically similar in all structures, conversions from one to another being possible by rotation of ±10° about the central methylene carbon. Such a conformation is likely to be preserved in the catalytically active molecule.

The pyrophosphate parameters

A general survey of organic monophosphates (Karle & Britts, 1966) gives the following average bond lengths P—OR 1.60 Å; P—OH 1.57 Å; P=O 1.51 Å; and P—O⁻ 1.50 Å, with an unprotonated O—P—O angle in many cases several degrees larger than the tetrahedral value. Exceptions to these general values include the consistently short values of dibenzyl phosphoric acid (Dunitz & Rollett, 1956) for all bond types by as much as 0.5 Å and the short P=O value of 1.46 Å and the partially double-bonded P—OH value of 1.48 Å in pyridoxal phosphate oxime (Barrett, 1967).

The bond distances and angles of the pyrophosphate group in TPP, of that in TPP hydrochloride (Pletcher & Sax, 1966 — to date the only other detailed organic pyrophosphate to be reported), and in the inorganic pyrophosphates (sodium pyrophosphate decahydrate,

MacArthur & Beevers, 1957; magnesium pyrophosphate, Calvo, 1965; and calcium pyrophosphate, Webb, 1966) are in general agreement with the above values. The central P—O—P bond angle in TPP, however, is much larger, approximately 135°, and the central P—O bond length is also large at 1.59 Å. The staggered conformation is observed for both pyrophosphate groups in the two TPP molecules when viewing the oxygen atoms along a line joining the phosphorus atoms. Previous pyrophosphates studies also report this conformation, except for that of Webb (1966), who reports an eclipsed conformation.

The TPP crystal structure

Fig. 4 shows molecules 1 and 2 of the asymmetric unit, outlined in solid lines, and their centrosymmetrically related molecules, outlined in dotted lines, projected down the *a* axis. Fig. 5 shows a photographic

view of a model of the unit cell. A noticeable feature is the layering of the structure, with water molecules holding pairs of layers together. Molecules 1 and 2 of the asymmetric unit are discretely contained within one half of the unit cell and are of the same enantiomorphic form, with an approximate twofold axis lying perpendicular to (100), relating them.

Hydrogen atoms were not located, and the choice of possible sites for the eight water molecules was aided by consideration of likely distances between hydrogen donors and acceptors. Table 4 lists the observed short, and possibly hydrogen-bonded, intermolecular contacts. The arrangement of the four molecules in the cell is such that direct intermolecular hydrogen bonding is only possible between both ends of the pyrophosphate groups of molecules within the same layer, and there is evidence, Table 4(c), for such bonding in the present analysis. Further indirect hydrogen bonding appears to

Table 2(b). Bond angles for thiamine pyrophosphate

Average standard deviation 3°.

Molecule 1		Molecule 2	
Bond	Angle (degrees)	Bond	Angle (degrees)
*O(1)—P(4)—O(3)	112	O(27)—P(30)—O(39)	103
*O(2)—P(4)—O(5)	119	O(28)—P(30)—O(31)	131
O(1)—P(4)—O(2)	109	O(27)—P(30)—O(28)	106
O(2)—P(4)—O(3)	108	O(28)—P(30)—O(29)	107
O(3)—P(4)—O(5)	97	O(29)—P(30)—O(31)	97
O(1)—P(4)—O(5)	108	O(27)—P(30)—O(29)	106
P(4)—O(5)—P(6)	131	P(30)—O(31)—P(32)	139
*O(7)—P(6)—O(9)	126	O(33)—P(32)—O(35)	121
*O(8)—P(6)—O(5)	123	O(34)—P(32)—O(31)	98
O(5)—P(6)—O(7)	101	O(31)—P(32)—O(33)	98
O(7)—P(6)—O(8)	101	O(33)—P(32)—O(34)	118
O(8)—P(6)—O(9)	100	O(34)—P(32)—O(35)	105
O(9)—P(6)—O(5)	109	O(35)—P(32)—O(31)	118
P(6)—O(9)—C(10)	118	P(32)—O(35)—C(36)	107
O(9)—C(10)—C(11)	101	O(35)—C(36)—C(37)	127
C(10)—C(11)—C(12)	123	C(36)—C(37)—C(38)	103
C(16)—C(12)—S(13)	109	C(42)—C(38)—S(39)	111
C(11)—C(12)—S(13)	113	C(37)—C(38)—S(39)	137
C(11)—C(12)—C(16)	137	C(37)—C(38)—C(42)	108
C(12)—S(13)—C(14)	91	C(38)—C(39)—C(40)	90
S(13)—C(14)—N(15)	106	S(39)—C(40)—N(41)	113
C(14)—N(15)—C(16)	119	C(40)—N(41)—C(42)	112
C(14)—N(15)—C(18)	109	C(40)—N(41)—C(44)	123
C(16)—N(15)—C(18)	120	C(42)—N(41)—C(44)	125
C(12)—C(16)—N(15)	105	C(38)—C(42)—C(43)	114
C(12)—C(16)—C(17)	120	C(38)—C(42)—C(43)	140
C(17)—C(16)—N(15)	134	C(43)—C(42)—N(41)	104
N(15)—C(18)—C(19)	111	N(41)—C(44)—C(45)	126
C(18)—C(19)—C(20)	122	C(44)—C(45)—C(46)	125
C(18)—C(19)—C(24)	113	C(44)—C(45)—C(50)	124
C(20)—C(19)—C(24)	124	C(46)—C(45)—C(50)	111
C(19)—C(20)—N(25)	127	C(45)—C(46)—N(51)	121
C(19)—C(20)—N(21)	121	C(45)—C(46)—N(47)	122
N(25)—C(20)—N(21)	113	N(51)—C(46)—N(47)	116
C(20)—N(21)—C(22)	111	C(46)—N(47)—C(48)	125
N(21)—C(22)—C(26)	114	N(47)—C(48)—C(52)	122
N(21)—C(22)—N(23)	130	N(47)—C(48)—N(49)	116
N(23)—C(22)—C(26)	115	N(47)—C(48)—C(52)	120
C(22)—N(23)—C(24)	122	C(48)—N(49)—C(50)	120
N(23)—C(25)—C(19)	111	N(49)—C(48)—C(52)	126

* Bond angles not shown in Fig. 3.

occur through both ends of the pyrophosphate groups *via* water molecules O(59), O(59'), O(60), O(60') to the centrosymmetrically related molecules, Table 4(a) and (b). Of the ten pyrophosphate oxygen atoms of the asymmetric unit, all except O(7) give evidence for hydrogen bonding.

All the remaining possible hydrogen-bonded intermolecular contacts [see Table 4(d)] are *via* water molecules of crystallization. The atoms C(14), N(25) and correspondingly C(40), N(51) of the thiamine portions are all possible sources of hydrogen bonding. Such bonding might sensibly contribute to the stability of the thiamine ends of the molecules and would perhaps limit the observed freedom of rotation of the thiamine and pyrimidine rings about the central methylene carbons C(18) and C(44). No evidence for hydrogen bonding to any of the nitrogen atoms in the pyrimidine rings was observed.

No water molecules of crystallization were found near the region $x \approx \frac{1}{2}$. This is partly to be expected as both molecules in a layer are of the same enantio-

Table 4. *Short intermolecular contacts some of which are due to hydrogen bonds*

(a) Water to phosphate (Å)		(c) Phosphate to phosphate (Å)	
O(54)-O(8)	3.22	O(3)-O(29)	3.02
O(55)-O(2)	2.62	O(3)-O(28)	2.89
O(56)-O(33)	2.37	O(3)-O(27)	2.36
O(58)-O(33)	2.47	O(2)-O(29)	2.45
O(59)-O(2)	2.39	O(1)-O(29)	3.26
O(59)-O(3)	3.16	O(1)-O(28)	3.08
O(59)-O(3)	2.84	O(8)-O(34)	2.90
O(59)-O(27)	3.17		
O(59)-O(27)	2.98		
O(59)-O(29)	3.05		
O(60)-O(34)	2.56		
O(60)-O(8)	2.55		
(b) Water to water (Å)		(d) Others (Å)	
O(53)-O(54)	3.01	O(53)-N(25)	3.03
O(56)-O(57)	3.49	O(54)-N(25)	2.91
O(56)-O(58)	3.28	O(55)-C(14)	2.96
O(57)-O(58)	2.73	O(55)-N(25)	2.76
O(59)-O(59)	2.95	O(56)-N(49)	3.36
O(60)-O(60)	2.70	O(57)-C(40)	2.64
		O(57)-N(51)	2.49
		O(59)-N(51)	2.83

Table 3. *Planarity of rings in thiamine pyrophosphate*

Molecule 1 Thiazole ring Atom deviation (Å)		Molecule 1 Pyrimidine ring Atom deviation (Å)		Molecule 2 Thiazole ring Atom deviation (Å)		Molecule 2 Pyrimidine ring Atom deviation (Å)	
C(11)*	-0.25	C(18)*	0.06	C(37)*	-0.19	C(44)*	0.03
C(12)	-0.06	C(19)	-0.04	C(38)	0.06	C(45)	0.02
S(13)	0.15	C(20)	-0.02	S(39)	0.02	C(46)	0.08
C(14)	0.03	N(21)	0.05	C(40)	0.04	N(47)	0.00
N(15)	-0.20	C(22)	-0.01	N(41)	0.05	C(48)	-0.07
C(16)	0.09	N(23)	-0.01	C(42)	0.01	N(49)	-0.03
C(17)*	0.25	C(24)	-0.02	C(43)*	0.18	C(50)	-0.03
C(18)*	-0.15	N(25)*	-0.03	C(44)*	-0.20	N(51) *	-0.06
		C(26)*	0.02			C(52)*	0.07
r.m.s. deviation		r.m.s. deviation		r.m.s. deviation		r.m.s. deviation	
	0.08 Å		0.02 Å		0.07 Å		0.03 Å

* Ring attached atom.

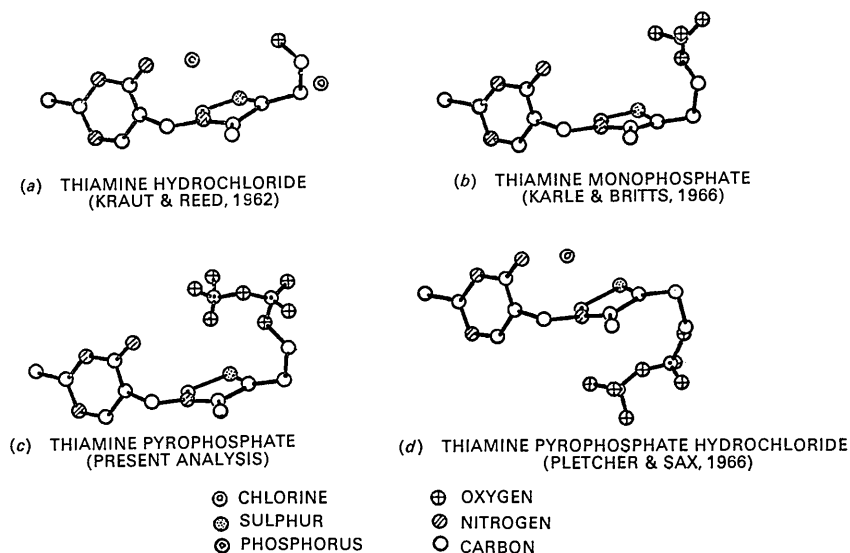


Fig. 3. Sketches of related 'thiamine' molecules.

morphic form, with the majority of possible hydrogen bonding sites tending to point away from the region near $x = \frac{1}{2}$. Further, it is difficult to postulate likely sites in this region at which water could simultaneously be hydrogen bonded to the molecules and be significant in contributing to the cohesion of the structure. Hence it seems likely that the intermolecular contacts between the thiamine portions of molecules of different enantiomorphic form in the separate layers are weakly bonded, possibly by van der Waals forces only.

Comparison of TPP and TPP hydrochloride

TPP hydrochloride (Pletcher & Sax, 1966) contains a chlorine ion counter-balanced in charge by protonation of the pyrimidine ring nitrogen atom opposite the attached amino group, in addition to the pyrophosphate and thiazole charges of the present TPP analysis. The presence of these two extra charges would appear to determine, to a large extent, the packing of molecules in the unit cell with alternating layers of positive and negative charge running through the structure. Intermolecular hydrogen bonding, *via* the pyrophosphate groups and pyrimidine ring nitrogen atoms, between all the monoclinically related molecules is observed. Waters of crystallization (1.5 per molecule) were not found, despite location of the hydrogen atoms, and appear insignificant for the structure cohesion.

The present TPP analysis, with no chlorine ions present, is less dependent upon ionic charge considerations. It is feasible that, with the zwitterionic charges of each molecule already more or less satisfied (by proximity of thiazole ring and pyrophosphate group),

the structure should be of a more open, less rigid, layer-type. The association of TPP molecules 1 and 2 of the asymmetric unit in half the unit cell, so defining a layer, is reasonable with the pseudo-monoclinic nature of the analysis. The relatively large number of waters of crystallization appear to play a more significant part in the structure cohesion. The values of unit-cell volume and density for TPP correspond more closely to thiamine monophosphate (Karle & Britts, 1966), having almost the same contents per unit volume, than to TPP hydrochloride. In conclusion, the present analysis has shown a triclinic form of TPP to exist, with a structure different from the independently determined monoclinic structure of TPP hydrochloride.

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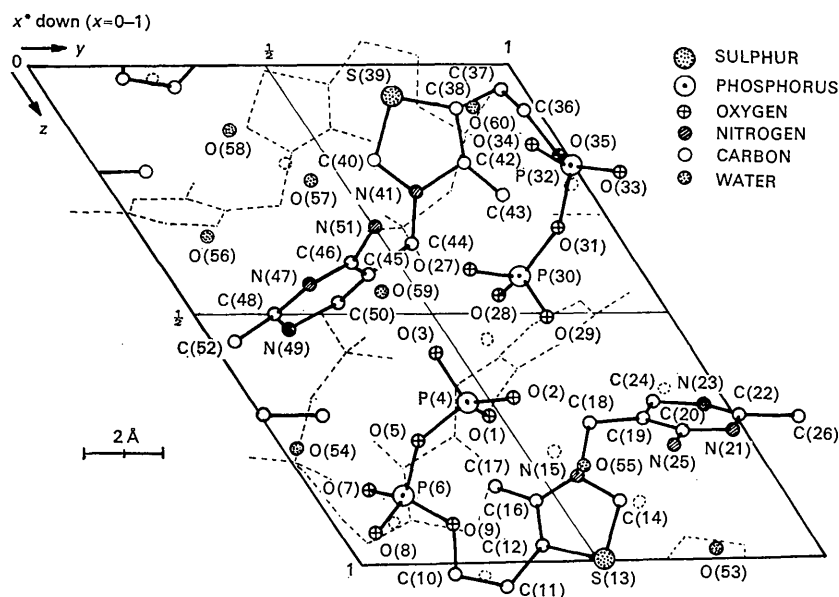


Fig. 4. The TPP unit cell contents projected down the *a* axis. The 'spotted' sulphur atoms in this Figure should not be confused with the 'spotted' phosphorus atoms of Fig. 5.)

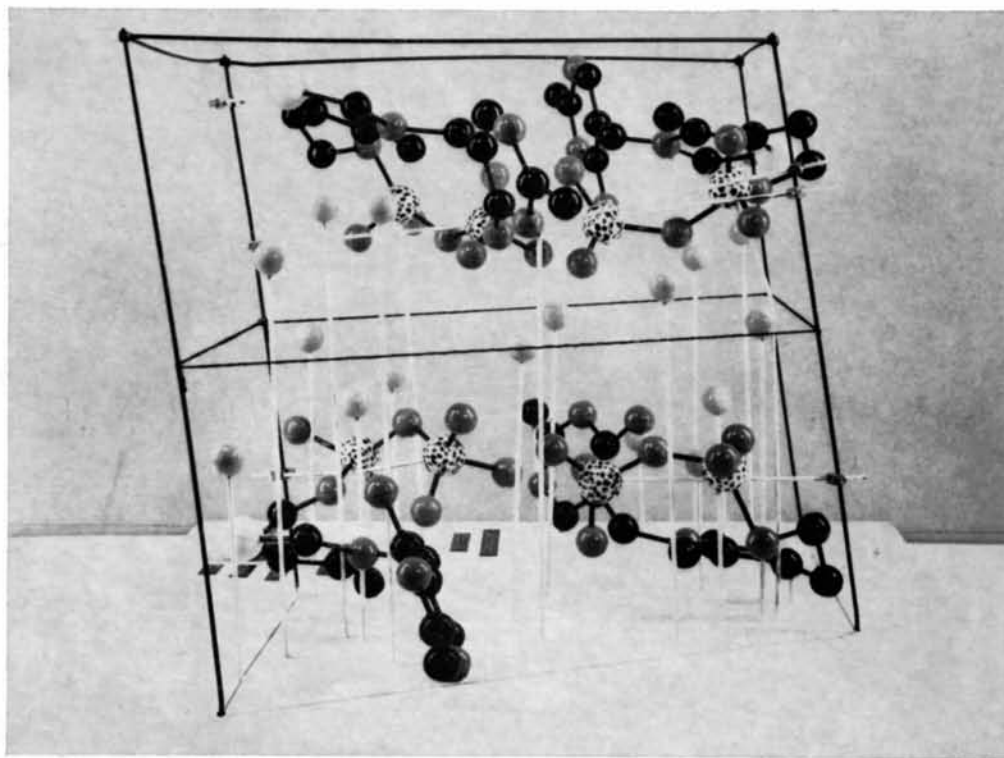


Fig. 5. Photograph of a model showing the TPP unit cell structure viewed approximately along $[0\bar{2}1]$. (Note the end to end juxtaposition of the pyrophosphate groups of the two molecules in the lower half of the photograph).

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The Crystal Structure of L-Alanyl-L-alanine Hydrochloride

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The crystal structure of L-alanyl-L-alanine hydrochloride, $^+H_3NCH(CH_3)CONHCH(CH_3)COOH \cdot Cl^-$, has been determined. The crystal is orthorhombic with space group $P2_12_12$; the unit-cell dimensions are: $a=9.51$, $b=19.72$, $c=5.38$ Å. Final refinement was made by block-diagonal least-squares method to an R of 0.079 and this gave a standard deviation of about 0.012 Å in bond lengths among the non-hydrogen atoms. The bond lengths and angles agree with those found so far in amino acids and peptides. Both the carboxyl and the peptide group are planar within the limits of experimental error, and the dihedral angle between the planes is 27° . The internal rotation angles in the peptide backbone are similar to those in the antiparallel pleated sheet configuration of polypeptide.

Introduction

Accurate structure analyses by X-ray of various kinds of amino acids and peptides have been carried out at many places mainly with biochemical interest. Most of these peptides, however, contain at least one glycyl residue. The only example containing an alanyl residue is glycyl-L-alanine hydrochloride (Tranter, 1956), whereas alanine is one of the most frequently appearing components in proteins. The present work on L-alanyl-L-alanine hydrochloride has been undertaken in order to provide some fundamental knowledge on the conformations of the alanyl residues in proteins, in particular, this knowledge is of essential importance to the structure of tussah silk fibroin.

Experimental

L-Alanyl-L-alanine hydrochloride was obtained in the form of needle-like crystals elongated along the c axis by dissolving the peptide in a slight excess of 2*N* HCl and evaporating to dryness *in vacuo* at room temperature. Since the crystals are hygroscopic, the specimen was coated with a thin-film of collodion during the X-ray experiment.

Unit-cell dimensions were determined by the least-squares calculation, using 17 $0kl$ and 21 $hk0$ reflexions whose Bragg angles were measured on zero-layer Weissenberg photographs taken with Cu $K\alpha$ radiation and calibrated with aluminum powder lines. The density was measured by flotation in a benzene-carbon tetrachloride mixture.

Crystal data :

$$\begin{aligned} a &= 9.51 \pm 0.01 \text{ \AA} , & \rho_{\text{obs}} &= 1.29_0 \text{ g.cm.}^{-3} \\ b &= 19.72 \pm 0.01 \text{ \AA} , & \rho_{\text{cal}} &= 1.29_5 \text{ g.cm.}^{-3} \\ c &= 5.38 \pm 0.01 \text{ \AA} . & Z &= 4 . \end{aligned}$$

The systematic absence of the odd orders of $h00$ and $0k0$ reflexions were observed, hence the space group was found to be $P2_12_12$.

The three-dimensional intensity data for nickel-filtered Cu $K\alpha$ radiation were collected from multiple-film, equi-inclination Weissenberg photographs of the layer line 0-7 about the a axis and 0-4 about the c axis. The intensities were visually estimated by comparison with a standard scale. They were corrected for Lorentz and polarization factors; no absorption and extinction corrections were applied. Corrections for variation in spot-size on higher-layer photographs were made by the method presented by Phillips (1954). Since the intensity data from the c axis photographs seemed to be less reliable than those from the a axis, the former were used only for reflexions outside the region of the a axis photographs. Thus 1273 intensity data were obtained of which 183 were too weak to be observed.

Determination of the structure

The coordinates of the chloride ion were deduced from the Patterson function. The electron density distribution and the minimum function were synthesized on the basis of the coordinates of the chloride ion. All the non-hydrogen atoms were identified, though