Computations were carried out on a HITAC 8700 computer at the Computer Centre of Tokyo Institute of Technology.

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Thiamine Pyrophosphate Tetrahydrate: A Structure with the Pyrophosphate Ester in an Extended Conformation

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(*Received* 17 *January* 1977; *accepted* 18 *April* 1977)

Thiamine pyrophosphate, cocarboxylase, is a coenzyme in a number of enzyme systems for which its pyrophosphate group is believed to provide the principal mode of binding to the enzyme. A previous structure determination of thiamine pyrophosphate (TPP) as the hydrochloride, in which the pyrophosphate ester is mono-ionized, indicated that the pyrophosphate was folded back over the thiazolium ring of the thiamine. In the current structure, TPP is a neutral zwitterion with its pyrophosphate doubly ionized and its pyrimidine ring still protonated. In contrast to the hydrochloride structure, the pyrophosphate ester in the neutral zwitterion is extended away from the thiamine moiety. This structure also presents a model which differs substantially from that previously proposed [Carlisle & Cook, *Acta Cryst.* (1969), B**25**, 1359–1367] for the TPP neutral zwitterion, which is shown here to have some unacceptably close intermolecular contacts. For the current structure there is good agreement between the structural features in the two independent molecules, not only with respect to the pyrophosphate ester conformation but also in terms of the bond distances and angles. In addition, both thiamine molecules exhibit the characteristic F conformation with respect to the C(3,5′) methylene bridge atom. The crystal structure was determined using diffractometer data obtained by the $\theta:2\theta$ scan technique with Cu radiation from a crystal having space-group symmetry $P\bar{1}$ ($P1$) and unit-cell parameters a = 13·418 (35), b = 12·296 (9), c = 15·572 (13) Å, α = 56·62 (4), β 96·27 (8), γ = 92·67 (1)°. The structure was solved by direct methods and refined by full-matrix least squares to R 0·045 for 6416 observed reflections and R = 0·048 for all 6864 reflections.

Introduction

Thiamine pyrophosphate, which is also known as cocarboxylase, is a coenzyme in enzyme systems which catalyze the decarboxylation of α-keto acids and the transfer of aldehyde or acyl groups. The conformation of the coenzyme is important for several aspects of the catalytic mechanism (Sax, Pulsinelli & Pletcher, 1974; Gallo, Mieyal & Sable, 1977; Pletcher, Sax, Blank & Wood, 1977). The pyrophosphate ester of the C(5) dimethylene side chain is generally considered to provide the primary site for attachment to the various enzymes and thus an understanding of its conformational properties will be helpful in later studies of the enzyme–coenzyme complexes. An examination of the currently known thiamine structures reveals that there is a marked variability in the $\varphi_{5\alpha}$ torsion angle but that the $\varphi_{5\beta}$ angle is normally found at $\sim\pm60°$ (Shin, Pletcher, Blank & Sax, 1977). In the thiamine pyrophosphate hydrochloride (TPP.HCl) structure, the mono-ionized pyrophosphate group was observed to be folded back over the positively charged thiazolium ring (Pletcher & Sax, 1966, 1972). It was suggested that the minimization of separation of unlike charges was largely responsible for the stabilization of the folded conformation of the pyrophosphate side chain. This appeared to be supported by the NMR studies of Gallo & Sable (1975) and Gallo, Hansen, Sable & Swift (1972) who reported that thiamine pyrophosphate (TPP) existed in solution in both the folded and extended conformations in the presence of metal ions. They suggested that coordination of the metal ion to the pyrophosphate reduced the effective negative charge which decreased its attraction to the positively charged rings in thiamine. In order to examine further the influence of the charge distribution in TPP, we initiated a study of TPP in various states of ionization.

Experimental

A 0·1 *M* aqueous solution of thiamine pyrophosphate hydrochloride (Sigma) was titrated with one equivalent of 0·1 *M* NaOH (final *p*H = 4·0). The entire sample was recovered as an amorphous precipitate following the addition of 1·35 volumes of acetone and storage at −20°C. The precipitate was collected by suction filtration, washed with aqueous acetone and acetone, and then air dried at room temperature. The compound was recrystallized from a 0·07 *M* aqueous solution by the dropwise addition of acetone (~0·5 volumes) to trace permanent turbidity. After one week in a closed container at room temperature the resulting precipitate was collected by suction filtration, washed with aqueous acetone and acetone and then air dried at room temperature. The precipitate consisted largely of white amorphous or microcrystalline material, although there were a number of larger clear crystals with either a tabular or prismatic morphology. With many of the larger crystals the two forms appeared to grow epitaxially although they could be fairly easily cleaved. The diffraction patterns of the crystals showed that the two morphological forms were different polymorphs of TPP. This paper deals with the polymorph having the prismatic morphology (the other will be reported later).

Weissenberg photographs indicated that the crystals were triclinic. On the basis of previously determined thiamine structures, the centrosymmetric space group $P\bar{1}$ was assumed. A single crystal, which was mounted along the prism axis (*b*), varied in length from 0·46 to 0·58 mm because of the obliquely sloping faces at either end of the prism. The nearly square cross-section measured 0·08 × 0·10 mm. The cell parameters were determined from a least-squares fit of setting angles (2θ values ranged from 40 to 74°) for 12 centered reflections on a Picker FACS-1 diffractometer system using monochromated Cu *Kα* radiation (Picker FACS-1 Disk Operating System, 1972). The cell parameters and other crystal data are summarized in Table 1.* The similarity of the cell parameters to those of Carlisle & Cook (1969) for TPP.$4H_2O$ (hereafter referred to as C & C) indicated that our crystals may have a similar, or perhaps even the same, structure. In either event, it was decided to pursue the analysis since the refinement of C & C was terminated at 28% and more highly refined structural parameters of the neutral zwitterion were desirable.

The present crystals had good diffraction characteristics and appeared to be stable to X-rays during preliminary photographic examination. The intensity data were collected to a 2θ limit of 127° using the θ:2θ scan technique over a scan range of 1·75° (plus an increment to allow for spectral dispersion) at a scan

Table 1. *Crystal data for thiamine pyrophosphate tetrahydrate*

$C_{12}H_{18}N_4O_7P_2S.4H_2O$, M_r = 496·4, space group $P\bar{1}$ ($P1$); ρ_o = 1·542 (by flotation in CCl_4–$CHCl_3$), ρ_c = 1·548 g cm^{-3}, Z = 4, μ(Cu *Kα*) = 33·4 cm^{-1}, $F(000)$ = 1040, m.p. 207°C (begins to lose water of hydration at ~45°C; Thermolyne melting-point apparatus, uncalibrated).

Comparative cell parameters

	Initial*	Final*	Mean†	C & C‡	Reduced mean	Reduced C & C
a(Å)	13·452 (2)	13·383 (2)	13·418 (35)	13·42 (3)	12·296	12·24
b(Å)	12·290 (2)	12·303 (2)	12·296 (9)	12·24 (3)	13·418	13·42
c(Å)	15·563 (3)	15·581 (2)	15·572 (13)	15·57 (3)	13·528	13·45
α(°)	56·65 (1)	56·60 (1)	56·62 (4)	56·33 (33)	94·78	96·30
β(°)	96·21 (1)	96·33 (1)	96·27 (8)	95·77 (33)	106·00	105·56
γ(°)	92·68 (1)	92·67 (1)	92·67 (1)	90·42 (33)	92·67	90·42
V(Å^3)	2136·4	2128·4	2132·3	2117 (23)	2132·2	2114

* At ~24°C.
† Used in analysis.
‡ Carlisle & Cook (1969).

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* During the data collection the cell parameters were calculated from three centered reflections whenever automatic recentering was requested. Because of the obvious drift in cell parameters, they were redetermined from a least-squares fit of the 12 centered reflections at the end of the data collection. Since the unit cell changed gradually during the data collection, the mean values of the initial and final determinations were used for the unit-cell parameters in the crystal structure analysis.

Table 2. *Positional parameters*

Positional parameters for S and P are $\times 10^5$, for C, N and O $\times 10^4$, and for H $\times 10^3$. Estimated standard deviations in parentheses are for the least significant figures.

(*a*) The non-hydrogen atoms

Molecule *A*	*x*	*y*	*z*	Molecule *B*	*x*	*y*	*z*
S(1)	3886 (5)	55208 (6)	14529 (5)	S(1)	55823 (5)	46694 (6)	38250 (5)
C(2)	947 (2)	6972 (2)	847 (2)	C(2)	6053 (2)	3152 (2)	4474 (2)
N(3)	1868 (1)	6924 (2)	1257 (2)	N(3)	6781 (2)	3031 (2)	4030 (2)
C(4)	2172 (2)	5689 (2)	2102 (2)	C(4)	7002 (2)	4187 (2)	3113 (2)
C(5)	1438 (2)	4798 (2)	2317 (2)	C(5)	6395 (2)	5186 (3)	2886 (2)
C(3,5′)	2561 (2)	8055 (2)	846 (2)	C(3,5′)	7379 (2)	1808 (3)	4477 (2)
N(1′)	1655 (2)	10742 (2)	−1821 (2)	N(1′)	6504 (2)	−821 (2)	7001 (1)
C(2′)	1130 (2)	11507 (3)	−1661 (2)	C(2′)	6006 (2)	−1604 (3)	6749 (2)
C(2′α)	638 (3)	12702 (3)	−2588 (2)	C(2′α)	5538 (3)	−2782 (3)	7590 (3)
N(3′)	1039 (2)	11238 (2)	−731 (2)	N(3′)	5932 (2)	−1377 (2)	5816 (2)
C(4′)	1478 (2)	10123 (2)	104 (2)	C(4′)	6358 (2)	−282 (2)	5055 (2)
N(4′α)	1324 (2)	9871 (2)	1016 (2)	N(4′α)	6258 (2)	−117 (2)	4139 (2)
C(5′)	2048 (2)	9283 (2)	−31 (2)	C(5′)	6878 (2)	607 (2)	5266 (2)
C(6′)	2122 (2)	9650 (2)	−1013 (2)	C(6′)	6944 (2)	261 (2)	6264 (2)
C(4α)	3203 (2)	5471 (3)	2622 (2)	C(4α)	7825 (3)	4237 (3)	2526 (2)
C(5α)	1479 (2)	3382 (3)	3157 (2)	C(5α)	6368 (2)	6558 (3)	1949 (2)
C(5β)	1302 (2)	2501 (3)	2763 (2)	C(5β)	6205 (2)	7589 (3)	2166 (2)
O(5γ)	287 (1)	2705 (2)	2301 (2)	O(5γ)	5199 (1)	7467 (2)	2440 (1)
P(1)	−1718 (5)	18218 (6)	19066 (5)	P(1)	48441 (5)	82421 (6)	29057 (5)
O(1–1)	−44 (1)	377 (2)	2932 (1)	O(1–1)	5128 (1)	9721 (2)	2097 (1)
O(1–2)	−1240 (1)	2213 (2)	1562 (2)	O(1–2)	3747 (2)	8090 (2)	2918 (2)
O(1–3)	461 (1)	1827 (2)	1181 (1)	O(1–3)	5449 (2)	7852 (2)	3895 (1)
P(2)	−6427 (5)	−4525 (6)	39365 (5)	P(2)	45898 (6)	108326 (7)	9847 (5)
O(2–1)	−1097 (1)	545 (2)	4104 (2)	O(2–1)	4307 (2)	10204 (2)	387 (2)
O(2–2)	−1454 (1)	−1141 (2)	3695 (1)	O(2–2)	3706 (1)	11358 (2)	1149 (1)
O(2–3)	130 (1)	−1306 (2)	4812 (1)	O(2–3)	5439 (2)	11808 (2)	530 (2)
Water							
O(*W*1)*A*	2131 (2)	3208 (2)	147 (2)	O(*W*1)*B*	−2672 (2)	6762 (2)	4740 (2)
O(*W*2)*A*	−3098 (2)	1808 (3)	2244 (2)	O(*W*2)*B*	1846 (2)	7694 (2)	3082 (2)
O(*W*3)*A*	−2378 (2)	4489 (3)	−78 (2)	O(*W*3)*B*	1322 (2)	5183 (3)	4530 (2)
O(*W*4)*A*	−426 (2)	3957 (3)	4401 (2)	O(*W*4)*B*	4435 (3)	5784 (4)	80 (4)

(*b*) Hydrogen atoms

Molecule *A*				Molecule *B*			
H(2)	63 (2)	769 (3)	25 (2)	H(2)	581 (2)	249 (3)	509 (2)
H(3,5′1)	280 (2)	811 (3)	142 (2)	H(3,5′1)	755 (2)	174 (3)	394 (2)
H(3,5′2)	314 (2)	784 (3)	60 (2)	H(3,5′2)	803 (2)	190 (3)	481 (2)
H(1′)	163 (2)	1089 (3)	−241 (2)	H(1′)	651 (2)	−98 (3)	768 (3)
H(6′)	251 (2)	917 (3)	−118 (2)	H(6′)	730 (2)	79 (3)	649 (2)
H(2′α1)	86 (3)	1280 (4)	−309 (3)	H(2′α1)	597 (4)	−329 (5)	820 (4)
H(2′α2)	−2 (4)	1287 (5)	−258 (4)	H(2′α2)	494 (3)	−266 (5)	766 (3)
H(2′α3)	86 (4)	1367 (6)	−262 (4)	H(2′α3)	518 (4)	−324 (5)	737 (4)
H(4′α1)	161 (2)	918 (3)	162 (2)	H(4′α1)	602 (3)	−80 (3)	410 (3)
H(4′α2)	105 (2)	1037 (3)	105 (2)	H(4′α2)	664 (2)	34 (3)	373 (2)
H(4α1)	364 (3)	556 (4)	214 (3)	H(4α1)	778 (3)	518 (4)	187 (3)
H(4α2)	336 (3)	620 (4)	283 (3)	H(4α2)	845 (3)	375 (4)	299 (4)
H(4α3)	332 (3)	458 (4)	317 (3)	H(4α3)	771 (4)	369 (5)	233 (4)
H(5α1)	216 (2)	320 (3)	353 (2)	H(5α1)	701 (2)	676 (3)	159 (2)
H(5α2)	102 (2)	314 (3)	360 (3)	H(5α2)	581 (2)	662 (3)	146 (2)
H(5β1)	135 (2)	161 (3)	338 (3)	H(5β1)	628 (2)	846 (3)	152 (2)
H(5β2)	170 (2)	273 (3)	224 (2)	H(5β2)	670 (2)	741 (3)	277 (2)
H(2–1)	−64 (3)	90 (4)	456 (3)	H(2–1)	500 (0)	1000 (0)	0 (0)
Water							
H(*W*11)*A*	158 (4)	280 (5)	44 (4)	H(*W*11)*B*	−221 (3)	754 (4)	438 (3)
H(*W*12)*A*	262 (4)	268 (5)	45 (4)	H(*W*12)*B*	−326 (3)	700 (4)	454 (3)
H(*W*21)*A*	−247 (3)	191 (3)	212 (3)	H(*W*21)*B*	209 (4)	637 (6)	410 (5)
H(*W*22)*A*	−342 (4)	208 (5)	177 (4)	H(*W*22)*B*	247 (2)	777 (3)	309 (2)
H(*W*31)*A*	−195 (4)	393 (6)	41 (5)	H(*W*31)*B*	178 (3)	451 (4)	494 (3)
H(*W*32)*A*	−230 (4)	517 (6)	−8 (4)	H(*W*32)*B*	52 (4)	457 (5)	441 (3)
H(*W*41)*A*	−32 (3)	319 (4)	475 (3)	H(*W*41)*B*	413 (6)	570 (7)	9 (5)
H(*W*42)*A*	−111 (6)	402 (8)	483 (6)				

rate of 1° min^{-1} with graphite-monochromated ($2\theta_M = 26{\cdot}14°$) Cu $K\alpha$ radiation. Background counts were accumulated for 20 s at either end of the scan range. Three standard reflections were monitored after every 50 reflections. During the data collection the intensity of the standards fluctuated and showed an overall decrease of ~15% either as a result of radiation damage or a loss of water of crystallization. Not only was there a loss in intensity but there was also a gradual change in cell parameters for which periodic corrections were made during the data collection.[*]

The intensity data were corrected for the intensity fluctuations and for the Lorentz–polarization factor, but no correction for absorption was applied. Of the 6864 independent reflections measured, 448 were considered unobserved according to the criterion $|F| \le 6\sigma(F)$, where $\sigma(F)$ is the same as previously given in Wood, Sax & Pletcher (1975).

Structure determination and refinement

The fact that our crystals were not completely stable under the conditions of the diffraction experiment indicated a possible source of difficulty in the refinement by C & C. An attempt was made to refine the coordinates of C & C with our data. The lack of success in this endeavor indicated that the structures were different and an independent solution was therefore initiated.

The structure was solved by direct methods using a single quartet relationship (Hauptman, 1974) and *MULTAN* (Germain, Main & Woolfson, 1971) in a manner similar to that described by Blank, Rodrigues, Pletcher & Sax (1976). The structure was refined by full-matrix least squares (Shiono, 1971) minimizing the function $\Sigma w(|F_o| - k|F_c|)^2$, where k is a single scale factor and w, the weight, is assigned in a manner similar to that described by Wood, Sax & Pletcher (1975). The nonhydrogen atoms were refined with anisotropic temperature factors while the hydrogen atoms, which were located from difference Fourier syntheses, were refined with isotropic thermal parameters. As a result of the large number of parameters involved in the refinement, only about one quarter of them could be refined in a single cycle. The atomic scattering factors for C, N, O, P and S were those of Cromer & Waber (1965) while that for H was from Stewart, Davidson & Simpson (1965). The real and imaginary anomalous-dispersion corrections for P and S were from *International Tables for X-ray Crystallography* (1968). The refinement converged at $R = 0{\cdot}045$ for 6416 observed reflections and $R = 0{\cdot}048$ for all 6864 reflections.[†]

The final structure indicated a minor disorder in that the second hydrogen on $(W4)B$ could not be found and there was residual density on the center of symmetry between two symmetry-related O atoms, O(2–1)B, in the terminal phosphate of molecule B. From this analysis it was not possible to distinguish between the two different models in which the phosphate disordered H atom was either located midway between the two O atoms, maintaining centrosymmetric symmetry, or alternately distributed between the two, requiring noncentrosymmetric space-group symmetry. In an attempt to resolve this problem a sample of the crystals[*] was submitted for analysis by optical second harmonic generation (Dougherty & Kurtz, 1975). Their analysis produced a weak signal indicating the disordered model with noncentrosymmetric space-group symmetry. However, no effort was made to refine the disordered model in $P1$ and in the list of final coordinates (Table 2) the hydrogen H(2–1)B is given for the position midway between the 'symmetry-related' O atoms.

Because the molecular structure of this TPP.$4H_2O$ neutral zwitterion was observed to differ substantially from that found by C & C, an extensive comparative analysis of the two was carried out. In the course of this analysis it was discovered that published coordinates of C & C produced a number of unacceptably close inter- and intramolecular contacts (intermolecular contact distances short by as much as 48σ). This finding indicates that the C & C structural model is in error and probably accounts for the convergence of that refinement at $R \simeq 28\%$. Therefore the C & C structure will not be considered further.[†]

Description of molecular and crystal structures

The bond distances and angles for the two independent molecules in this structure are presented in Fig. 1(a)

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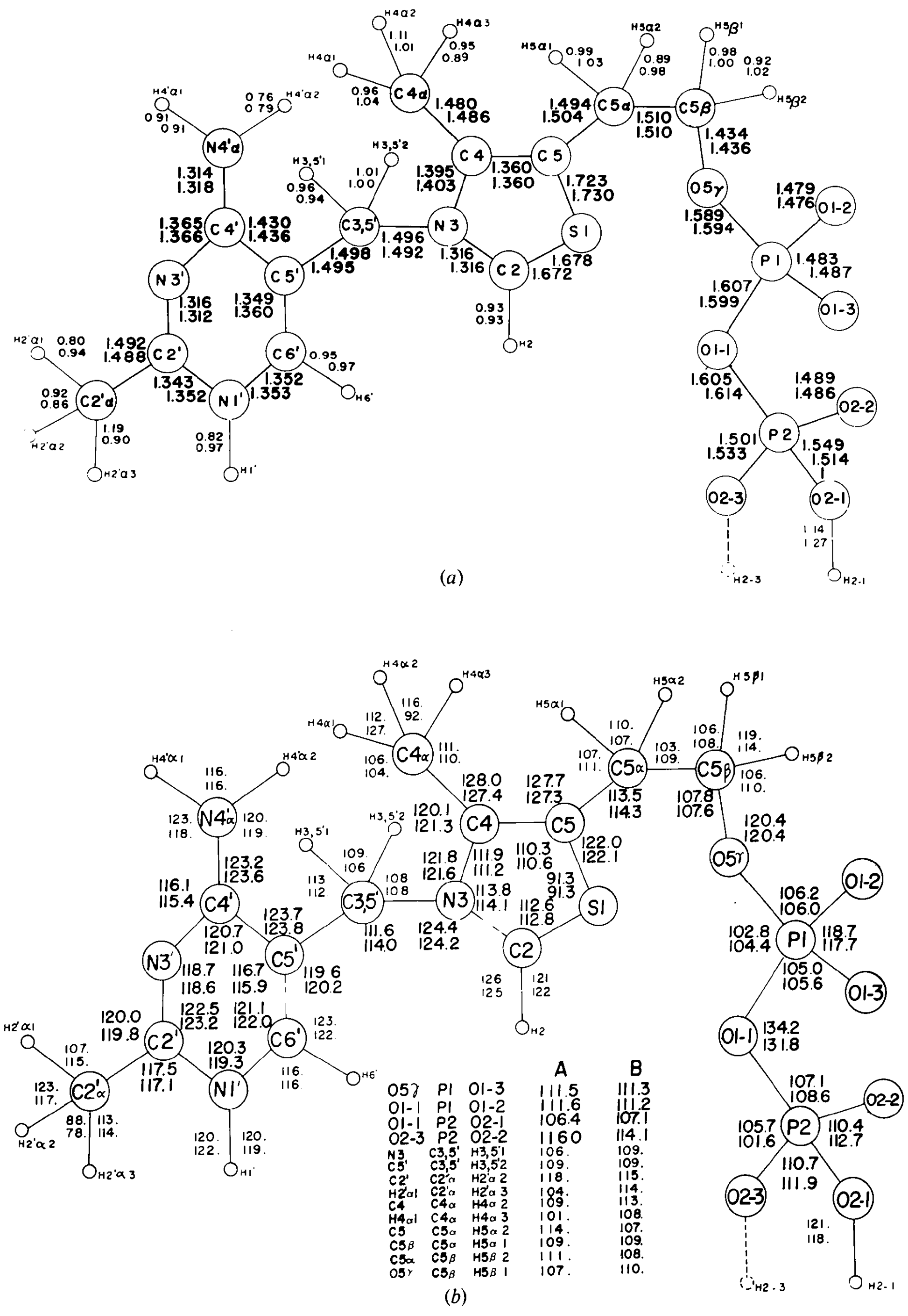
[*] See footnote on p. 3350.

[†] Lists of structure factors and thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32686 (10 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

[*] Since there were not a sufficient number of the prismatic crystals to run the analysis, the test was actually run on a sample of the microcrystalline material which very likely contained both polymorphs.

[†] Although the proposed model of C & C is not correct, it remains undetermined whether the crystal used by C & C has the same structure as the one presented here or whether it is a third polymorph of the neutral zwitterion with a currently unknown structure. It is of interest that the following close intermolecular contacts suggest the possibility that the pyrophosphate groups should be interchanged, resulting in an extended instead of a folded conformation: C(36)···C(10) [C(5β)B···C(5β)A], 2·08 Å; O(35)···C(10) [O(5γ)B···C(5β)A], 2·30 Å; C(36)···O(8) [C(5β)B···O(1–2/3)A], 2·45 Å; C(37)···O(7) [C(5α)B···O(1–3/2)A], 2·59 Å. (The notation in square brackets is the atom designation, used in this paper, which corresponds to the labeling of C & C.) However, other equally serious contacts, such as S(39)···C(17), 2·25 Å; C(40)···C(17), 2·24 Å; C(43)···O(35), 1·99 Å; C(52)···C(20), 2·52 Å and N(49)···C(22), 2·44 Å, indicate that other aspects of the model also require change. A comparison of the two data sets reveals little correlation except for the $h00$ reflections. Our efforts to achieve an independent solution have thus far been unsuccessful.



			A	B
O5γ	P1	O1-3	111.5	111.3
O1-1	P1	O1-2	111.6	111.2
O1-1	P2	O2-1	106.4	107.1
O2-3	P2	O2-2	116.0	114.1
N3	C3,5'	H3,5'1	106.	109.
C5'	C3,5'	H3,5'2	109.	109.
C2'	C2'α	H2'α 2	118.	115.
H2'α1	C2'α	H2'α 3	104.	114.
C4	C4α	H4α 2	109.	113.
H4α1	C4α	H4α 3	101.	108.
C5	C5α	H5α 2	114.	107.
C5β	C5α	H5α 1	109.	109.
C5α	C5β	H5β 2	111.	108.
O5γ	C5β	H5β 1	107.	110.

Fig. 1. (*a*) Bond distances (Å) and atomic-numbering scheme for both molecules of thiamine pyrophosphate. In each pair, the upper and lower values are for molecules *A* and *B* respectively. The estimated standard deviations for the P–O and S–C bonds range from 0·002 to 0·003 Å. The e.s.d.'s for the C–C, C–N and C–O bonds are all 0·004 Å, except C(2′)–C(2′α) which is 0·005 Å. The bonds involving H have e.s.d.'s in the range from 0·03 to 0·06 Å with an average value of 0·04 Å. The O(2–1)*B*–H(2–1)*B* distance is for the disordered H position on the center of symmetry. The corresponding disordered H on O(2–3)*B* was not located. (*b*) Valence angles (°) for both molecules of thiamine pyrophosphate. In each pair, the upper and lower values are for molecules *A* and *B* respectively. The estimated standard deviations for C–S–C and the angles involving only P and O atoms are 0·1°, except for O(2–1)*B*–P(2)*B*–O(2–3)*B* which is 0·2°. The e.s.d.'s for the remaining angles involving C, N, O and P range from 0·2 to 0·3° with an average of 0·2°. The e.s.d.'s for angles involving H range from 2·0 to 5·0° with an average of 3·0°.

and (*b*). In general the values agree very well showing differences less than 3σ with most being less than 1σ (the greater variation in the pyrophosphate parameters will be discussed later). This level of agreement indicates that the crystal instability during the data collection has not seriously affected the thiamine parameters and, therefore, they can be reliably compared with those of other thiamine structures. (An error in the cell parameters would introduce a systematic error into the bond lengths. However, an examination of the structure using the initial and final cell parameters indicates that corresponding bonds in the two molecules are not similarly influenced by changes in the cell parameters.)

When the current structure is compared with TPP.HCl (Pletcher & Sax, 1972), the bonds for the thiamine moiety agree to within better than 3σ, with a few notable exceptions. The largest difference seen (in the thiamine bonds of this structure) is that for C(5β)–O(5γ) which is 5σ shorter in both molecules *A* and *B*. Although there is no definite explanation for this difference, it is very likely related to the bonding in the pyrophosphate because the O(5γ)–P(1) is from 4 to 7σ longer. The reason for the lengthening of the S(1)–C(5) bond by 3 to 5σ is also unclear but it does appear to be significant. The lengthening of the C(2′)–C(2′α) bond by 3 to 4σ very likely reflects the different crystalline environment of the methyl group in the two structures. The electronegative groups that were clustered around the C(2′α) methyl as nearest neighbors are not observed in the present structure. The variation in this bond length with the environment of the methyl group has been noted in a number of thiamine structures (Pletcher & Sax, 1972; Blank *et al.*, 1976; Pletcher *et al.*, 1977; Shin *et al.*, 1977). This property of the pyrimidine methyl is consistent with the observed exchange of the 2′α H atoms in acid media (Hutchinson, 1971).

From the present structure it is evident that the second ionization in TPP occurs at the terminal phosphate producing a quadruply charged neutral zwitterion with two negative charges on the pyrophosphate ester and a positive charge on both the aromatic thiazolium and protonated pyrimidine rings. This can be seen from the bond lengths which are characteristic of the pyrimidinium ring in contrast to those of unprotonated thiamine (Pletcher, Sax, Sengupta, Chu & Yoo, 1972; Shin *et al.*, 1977). The location and refinement of the proton on N(1′) provides further substantiation that the pyrimidine is fully protonated. The ionization state of the pyrophosphate group is also evident from the bond lengths and the location of the H atoms. It is particularly clear in molecule *A* that a P(OH) has ionized on both the inner and terminal phosphates. Although this same condition applies to molecule *B*, it is less clear because the remaining proton is disordered between O(2–1) and O(2–3). The length of P(2)–O(2–3) would tend to indicate a greater extent of protonation but only one of the disordered protons was located; namely, H(2–1), which lies midway between two O(2–1)*B* atoms at the unrealistically long OH distance of 1·27 Å.

The parameters of the pyrophosphate group appear to exhibit greater variation than is found in the thiamine moiety. Of course the differences in ionization between TPP.HCl and the present structure would be expected to influence the structural parameters. In addition, however, Baur (1974) has shown that the bond lengths in phosphate tetrahedra vary with the sum of the bond strengths received by the O atoms, including hydrogen bonds. From a knowledge of the O coordination and a set of empirical relationships, he was able to predict the geometrical parameters of the phosphate tetrahedra to a reasonable degree of accuracy. The results of a similar set of calculations for this structure are recorded in Table 3. In general the observations and Baur's predictions are in good agreement, although they are not as good as those obtained by Baur (1974) for TPP.HCl. This is not at all surprising in light of the disorder in this structure. It is interesting, though, that the disordered model for molecule *B* gives better agreement with the observed values.

The conformation of the pyrophosphate is nearly the same for the two molecules. This can be seen from a comparison of the torsion angles and pseudo torsion angles in Fig. 2. The conformation does not exhibit any unusual features but assumes the expected preferred values (Shefter, Barlow, Sparks & Trueblood, 1969). This differs from TPP.HCl which had one of the 'less preferred' conformations. The most significant conformational differences between these two ionization states is found with respect to the two bridge bonds, O(5γ)–P(1) and P(1)–O(1–1). In the current structure there is a rotation of O(1–1) $\simeq$ 135° about the former bridge bond and a rotation of P(2) $\simeq -60°$ about the latter. These two changes are primarily responsible for the change from the folded to the extended form.

In spite of the above-mentioned differences between the two pyrophosphate structures, there are some interesting similarities between them which are revealed in the pseudo torsion angles about the P(1)–P(2) vector (compare Fig. 2 with Fig. 6 of Pletcher & Sax, 1972). The staggered arrangement of the O atoms shows a remarkable similarity even though there are differences in the individual torsion angles. This feature very likely reflects the importance of nonbonded interactions in the pyrophosphate group (Wood *et al.*, 1975; Sundaralingam, 1969). It was also noted in TPP.HCl that the negative charge was predominantly on the same side of the pyrophosphate, opposite to the side esterified to the thiamine molecule. That same condition exists in the current structure.

The conformation of thiamine with respect to C(3,5′) is conveniently specified in terms of the torsion angles about the bonds to the thiazolium and pyrimidine rings, designated by φ_T and φ_P respectively. [The definition of

Table 3. *Comparison of observed phosphate distances* (Å) *and angles* (°) *with predicted values*

Predicted values are those obtained by the method of Baur (1974).

(*a*) Distances. 'Coordination' summarizes the number and types of atoms bonded to individual O atoms; H specifies a bonded hydrogen and *A*H designates a hydrogen-bond acceptor. The observed distance minus the predicted × 10^3 is listed under Δ_1. The values in parentheses under P(2)*B* are for the model with hydrogen not disordered.

		(*A*)			(*B*)	
	Coordination	Predicted	Δ_1	Coordination	Predicted	Δ_1
P(1) oxygen atoms						
O(5γ)	P,C	1·574	15	P,C	1·579	15
O(1–1)	2P	1·607	0	2P	1·611	−12
O(1–2)	P,2*A*H	1·488	−9	P,*A*H	1·471	5
O(1–3)	P,2*A*H	1·488	−5	P,2*A*H	1·492	−5
Mean |Δ|			7			9
Average P—O		1·539	1		1·538	1
P(2) oxygen atoms						
O(1–1)	2P	1·611	−6	2P (2P)	1·611 (1·615)	3 (−1)
O(2–1)	P,H	1·557	−8	P,$\frac{1}{2}$H (P,*A*H)	1·514 (1·475)	0 (39)
O(2–2)	P,2*A*H	1·492	−3	P,2*A*H (P,2*A*H)	1·492 (1·496)	−6 (−10)
O(2–3)	P,2*A*H	1·492	9	P,$\frac{1}{2}$H (P,H)	1·514 (1·561)	19 (−28)
Mean |Δ|			7			7 (20)
Average P—O		1·538	−2		1·533 (1·537)	4 0

(*b*) Angles. The observed bond angle minus the predicted value is listed under Δ_2. The Δ_{2c} column contains the difference after the predicted values have been corrected for the effect on non-bonded interactions (Wood, Sax & Pletcher, 1975; Yoo, Abola, Wood, Sax & Pletcher, 1975).

		(*A*)			(*B*)	
	Predicted	Δ_2	Δ_{2c}	Predicted	Δ_2	Δ_{2c}
P(1) oxygen atoms						
O(5γ), O(1–1)	102·0	0·8	−2·0	101·4	3·0	0·2
O(5γ), O(1–2)	110·4	−4·2	−0·1	111·3	−5·3	−1·2
O(5γ), O(1–3)	110·4	1·1	−0·3	109·6	1·7	0·3
O(1–1), O(1–2)	108·0	3·6	2·2	108·8	2·4	1·0
O(1–1), O(1–3)	108·0	−3·0	1·1	107·2	−1·6	2·5
O(1–2), O(1–3)	117·7	1·0	1·0	118·8	−1·1	−1·1
Mean |Δ|		2·3	1·1		2·5	1·0
P(2) oxygen atoms						
O(1–1), O(2–1)	102·7	3·7	2·3	104·9	2·2	0·8
O(1–1), O(2–2)	107·2	−0·1	−0·1	106·4	2·2	0·8
O(1–1), O(2–3)	107·2	−1·5	2·6	104·9	−3·3	0·8
O(2–1), O(2–2)	111·3	−0·9	−0·9	114·0	−1·3	−1·3
O(2–1), O(2–3)	111·3	−0·6	−0·6	112·2	−0·3	−0·3
O(2–2), O(2–3)	116·8	−0·8	−0·8	114·0	0·1	0·1
Mean |Δ|		1·3	1·2		1·6	0·7

these angles and other pertinent conformational data are summarized in Pletcher *et al.* (1977).] Both molecules have the **F** conformation which is characteristic of thiamine when it is free of C(2) substituents with φ_T and φ_P values of 5·4 and 85·8° for (*A*) and 20·5 and 100·5° for (*B*). The conformation of the C(5) side chain of thiamine has been noted to vary readily in different crystal structures in order to accommodate differences in crystal packing. However, a recent analysis of this structural feature has shown that its variability is not completely random (Shin *et al.*, 1977). For example, the torsion angle $\varphi_{5\alpha}$, S(1)—C(5)—C(5α)—C(5β), varies from 3 to 105° although two-thirds of the structures have angles with values between

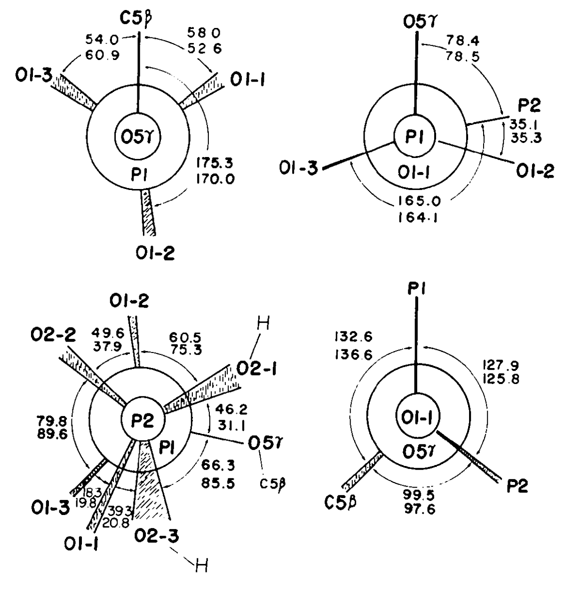


Fig. 2. Newman projections for the pyrophosphate group in both molecules of TPP. The upper and lower values are for molecules *A* and *B* respectively. The top diagrams illustrate the torsion angles (°) about the P–O bridge bonds at P(1). The bottom diagrams show the pseudo torsion angles about the P(2)–P(1) and the O(1–1)–O(5γ) vectors.

60 and 90°. For the $\varphi_{5\beta}$ angle, C(5)–C(5α)–C(5β)–O(5γ), there is a pronounced limitation with practically all of the observed values lying within 10° of ±60°. The values of these two torsion angles determine whether or not a close intramolecular S···O interaction can form with O(5γ) in a manner similar to that of the structurally significant S(1)···O(2α1) in the active intermediates of thiamine (Sax *et al.*, 1974; Pletcher *et al.*, 1977). In order for O(5γ) to come close enough to S(1), $\varphi_{5\alpha}$ must be less than $\simeq 70°$ and $\varphi_{5\beta}$ must assume its negative value. Both of these conditions are met with $\varphi_{5\alpha}$ and $\varphi_{5\beta}$ having values of 58·5 and −66·1° for (*A*) and 40·5 and −68·6° for (*B*). As expected from the smaller value of $\varphi_{5\alpha}$ for molecule *B*, its S···O(5γ) contact distance is significantly shorter than that for molecule *A*. These and other significant contact distances are recorded in Table 4.

The planarity of the rings in both molecules is typical of that observed in other thiamine structures in that the rings are essentially planar, although there are small deviations from strict planarity. As commonly found, the deviations from the least-squares planes (Table 5) are greater for the pyrimidinium rings than for the thiazolium rings. The closer proximity of O(5γ) to the thiazolium plane as indicated above for molecule *B* is shown quite clearly by a comparison of the deviations

Table 4. *Selected intermolecular and intramolecular contacts*

(*a*) Hydrogen bonds

a *b* *c*	*a–c* (Å)	*b–c* (Å)	*abc* (°)	*a* *b* *c*	*a–c* (Å)	*b–c* (Å)	*abc* (°)
N(4′α)*A*–H(4′α2)···O(1–3)*A*$^{\rm i}$	2·863 (3)	2·10 (3)	174 (3)	N(4′α)*B*–H(4′α1)···O(1–3)*B*$^{\rm ii}$	2·874 (4)	1·96 (4)	175 (4)
N(4′α)*A*–H(4′α1)···O(*W*2)*B*	2·866 (4)	1·98 (4)	163 (3)	N(4′α)*B*–H(4′α2)···*W*(2)*A*$^{\rm iii}$	2·782 (4)	2·07 (4)	150 (4)
N(1′)*A*–H(1′)···O(2–2)*A*$^{\rm iv}$	2·663 (3)	1·84 (3)	174 (3)	N(1′)*B*–H(1′)···O(2–2)*B*$^{\rm v}$	2·624 (3)	1·67 (4)	168 (4)
O(2–1)*A*–H(2–1)···O(2–3)*A*$^{\rm vi}$	2·560 (3)	1·42 (4)	176 (4)	O(2–1)*B*–H(2–1)···O(2–1)*B*$^{\rm vii}$	2·531 (4)		
O(*W*1)*A*–H(*W*11)···O(1–3)*A*	2·815 (4)	1·93 (6)	176 (6)	O(*W*1)*B*–H(*W*12)···O(1–3)*B*$^{\rm ix}$	2·745 (4)	1·94 (5)	170 (5)
O(*W*1)*A*–H(*W*12)···O(2–2)*B*$^{\rm ii}$	2·810 (4)	1·97 (6)	176 (6)	O(*W*1)*B*–H(*W*11)···O(2–2)*A*$^{\rm i}$	2·745 (4)	1·73 (5)	176 (4)
O(*W*2)*A*–H(*W*21)···O(1–2)*A*	2·733 (4)	1·88 (4)	167 (4)	O(*W*2)*B*–H(*W*22)···O(1–2)*B*	2·588 (4)	1·76 (4)	172 (4)
				O(*W*2)*B*–H(*W*21)···O(*W*3)*B*	2·756 (4)	1·61 (7)	123 (4)
O(*W*3)*A*–H(*W*31)···O(1–2)*A*	2·893 (4)	2·05 (7)	163 (6)				
O(*W*3)*A*–H(*W*32)···O(*W*1)*A*$^{\rm iv}$	2·780 (4)	1·94 (6)	178 (6)	O(*W*3)*B*–H(*W*31)···O(*W*1)*B*$^{\rm viii}$	2·694 (4)	1·83 (5)	157 (5)
				O(*W*3)*B*–H(*W*32)···O(*W*4)*A*	2·778 (5)	1·45 (5)	169 (4)
O(*W*4)*A*–H(*W*42)···O(*W*3)*B*$^{\rm viii}$	2·798 (5)	1·79 (9)	136 (7)	O(*W*4)*B*–H(*W*4–)···O(*W*3)*A*$^{\rm iv}$	2·764 (6)		
O(*W*4)*A*–H(*W*41)···O(2–3)*A*$^{\rm vi}$	2·811 (4)	2·04 (4)	162 (4)	O(*W*4)*B*–H(*W*4–)···O(2–3)*B*$^{\rm vii}$	2·567 (6)		
				O(*W*4)*B*–H(*W*4–)···O(*W*4)*B*$^{\rm x}$	2·638 (8)		
C(2)*A*–H(2)···O(1–3)*A*$^{\rm iv}$	3·090 (4)	2·32 (3)	140 (3)	C(2)*B*–H(2)···O(1–3)*B*$^{\rm v}$	3·077 (4)	2·31 (3)	139 (3)

(*b*) Close contacts around S(1)

C(2)*A*–S(1)···O(5γ)*A*	2·952 (2)	153·3 (1)	C(2)*B*–S(1)···O(5γ)*B*	2·917 (2)	166·2 (1)
C(5)*A*–S(1)···O(5γ)*A*		73·0 (1)	C(5)*B*–S(1)···O(5γ)*B*		75·4 (1)
C(2)*A*–S(1)···O(*W*1)*A*$^{\rm iv}$	3·802	95·1	C(2)*B*–S(1)···O(*W*1)*B*$^{\rm viii}$	4·501	91·6
C(5)*A*–S(1)···O(*W*1)*A*$^{\rm iv}$		172·1	C(5)*B*–S(1)···O(*W*1)*B*$^{\rm viii}$		156·3

(*c*) Symmetry code

None	*x*,	*y*,	*z*	(iv)	−*x*,	1 − *y*,	−*z*	(viii)	−*x*,	1 − *y*,	1 − *z*
(i)	*x*,	1 + *y*,	*z*	(v)	1 − *x*,	1 − *y*,	1 − *z*	(ix)	−1 + *x*,	*y*,	*z*
(ii)	*x*,	−1 + *y*,	*z*	(vi)	−*x*,	−*y*,	1 − *z*	(x)	1 − *x*,	1 − *y*,	−*z*
(iii)	1 + *x*,	*y*,	*z*	(vii)	1 − *x*,	2 − *y*,	−*z*				

from the thiazolium ring plane. Although it is not readily apparent in the packing diagram (Fig. 3), the pyrimidinium rings show stacking interactions between centrosymmetrically related molecules. The overlap, though similar for both molecules *A* and *B*, is somewhat more extensive for (*B*) as can be seen from the data in Table 5.

The hydrogen-bonding network that extends throughout the crystal is quite similar for the two molecules, including the distribution and bonding of the water molecules. This can be seen in the stereo packing diagram (Fig. 3) and from the list of hydrogen-bonding interactions in Table 4. The main differences involve the terminal phosphate group and the (*W*4) water molecule. In molecule *A* the terminal phosphate forms a cyclic dimer around a center of symmetry with O(2–1)H donating to O(2–3). The terminal phosphate is also linked to another centrosymmetrically related molecule through a chain of water molecules in which (*W*4)*A* and (*W*1)*B* donate to O(2–3) and O(2–2) respectively. These two water molecules are in turn bonded to two centrosymmetrically related (*W*3)*B* molecules; one (*W*3)*B* donates to both (*W*4)*A* and (*W*1)*B* while (*W*4)*A* donates to the other (*W*3)*B*. The centrosymmetrically related water molecules extend this chain to the symmetry-related phosphate. A different bonding pattern exists for molecule *B* in that O(2–1)H bonds directly across a center of symmetry resulting in a disordering of the H. A diffuse H position was found on the center but the disordered hydrogen on O(2–3) was not located. This disorder extends through the chain of water molecules which presents a slightly different pattern from that described above. In a manner similar to that above, (*W*1)*A* donates to O(2–2) and receives a hydrogen bond from (*W*3)*A*; (*W*3)*A* also accepts a hydrogen bond from (*W*4)*B*, but (*W*3)*A* does not donate one to (*W*4)*B*. Instead, (*W*4)*B* is bonded directly across a center of symmetry and thus extends the disordered chain through the crystal as ···O(2–3)–P(2)–O(2–1)···O(2–1)–P(2)–O(2–3)···(*W*4)*B*···(*W*4)*B*···O(2–3)–. The remainder of the hydrogen-bonding scheme is essentially the same for both molecules. The N(1′)H···O(2–2) bond linking the extreme ends of centrosymmetrically related molecules to form a cyclic dimer is similar to a scheme observed in only one previous thiamine structure (Shin *et al.*, 1977).

An interesting aspect of the molecular packing is found in the absence of any direct hydrogen bonding between TPP molecules *A* and *B*, although both (*A*) and (*B*) are bonded to their own symmetry-related molecules. The only linkages between (*A*) and (*B*) molecules occur through hydrogen-bonded water molecules. The water molecules lie in two channels that extend through the crystal approximately parallel to the *a* axis. One channel is at $(\frac{1}{2},\frac{1}{2})$, the other is at $(\frac{1}{2},0)$. The water molecules in the channel at $(\frac{1}{2},\frac{1}{2})$ (1*B*, 2*B*, 3*B* and 4*A*) form clusters in which they are bonded to one another and to both thiamine molecules. There is no direct link between the water clusters in this channel but they are joined only through hydrogen bonds to phosphates. The water molecules in the channel at $(\frac{1}{2},0)$ (1*A*, 2*A*, 3*A* and 4*B*) form similar clusters although three of them lie nearly in the plane at $z = 0$. Again these clusters are not directly linked but are bonded together only through phosphate groups. With this

Table 5. *Least-squares planes*

Coefficients $\times 10^4$ in $Ax + By + Cz = D$ are referred to crystallographic axes in Å. Standard deviations in least-squares planes and the displacements of atoms from the planes are in Å $\times 10^3$. Boldface type designates atoms used to define planes. Displacements of atoms in parallel pyrimidinium rings are given as pairs of numbers representing the perpendicular distance from the plane and the projected distance in the plane to the center of the ring.

	Molecule *A*					Molecule *B*				
	A	*B*	*C*	*D*	σ	*A*	*B*	*C*	*D*	σ
Thiazolium	−4439	6734	9145	64123	4	5924	5861	7036	119885	6
Pyrimidinium	8056	5532	2050	85229	12	8529	−5130	−1695	61146	15

Molecule *A*	Molecule *B*
S(1) −3, C(2) 3, N(3) −1, C(4) −2, C(5) 3, C(3,5′) −64, C(4α) −56, C(5α) 3, C(5β) −1182, O(5γ) −1067, H(2) −60	**S(1) 5, C(2) −3, N(3) 0, C(4) 4, C(5) −6,** C(3,5′) 84, C(4α) 53, C(5α) −65, C(5β) 786, O(5γ) 198, H(2) −2
N(1′) −8, C(2′) −4, N(3′) 11, C(4′) −6, C(5′) −5, C(6′) 12, C(2′α) −18, N(4′α) −52, C(3,5′) −5, H(1′) −119, H(6′) 52, H(4′α1) −25, H(4′α2) 8	**N(1′) −1, C(2′) −11, N(3′) 8, C(4′) 5, C(5′) −16, C(6′) 14,** C(2′α) −24, N(4′α) 29, C(3,5′) 8, H(1′) −70, H(6′) 36, H(4′α1) 191, H(4′α2) 283
[Molecule at −*x*, 2 − *y*, −*z*]	[Molecule at 1 − *x*, −*y*, 1 − *z*]
N(1′) (−3435, 4826), C(2′) (−3438, 3805), N(3′) (−3453, 2526), C(4′) (−3436, 2603), C(5′) (−3438, 4012), C(6′) (−3454, 4905), C(2′α) (−3424, 4520), N(4′α) (−3388, 1907), H(4′α1) (−3420, 2589), H(4′α2) (−3442, 1184), H(1′) (−3321, 5579), H(6′) (−3494, 5833)	N(1′) (−3423, 4092), C(2′) (−3414, 3143), N(3′) (−3432, 1834), C(4′) (−3430, 1804), C(5′) (−3408, 3228), C(6′) (−3439, 4106), C(2′α) (−3399, 4035), N(4′α) (−3453, 1307), H(4′α1) (−3622, 709), H(4′α2) (−3710, 2007), H(1′) (−3351, 5004), H(6′) (−3453, 5057)

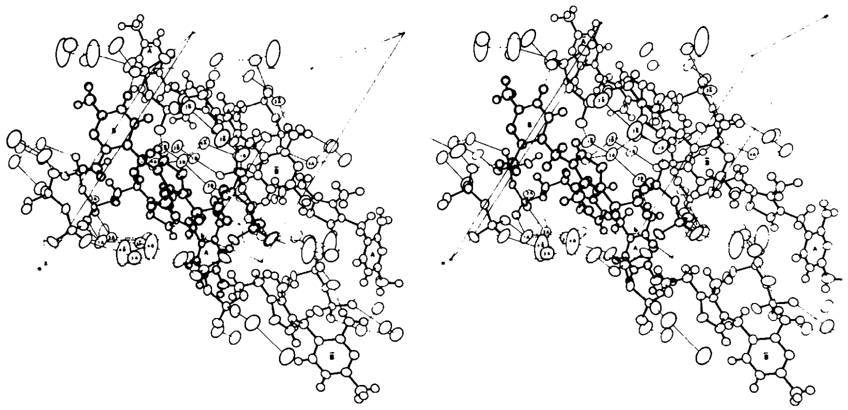


Fig. 3. Stereoscopic packing diagram of the crystal structure. The atoms of the thiamine molecules whose coordinates are listed in Table 2 are shaded and the pyrimidinium rings are labeled *A* and *B* to distinguish the two independent molecules. The primed molecules are related to those in Table 2 by translation only. The barred molecules are centrosymmetrically related to the ones in the table. The water molecules, for which all the H atoms have been omitted for improved clarity, are not shaded but are only labeled. The hydrogen bonding is shown by the thin lines between atoms.

extensive hydrogen bonding it is difficult to understand the loss of water and the shift in cell parameters during the data collection.

Discussion

The NMR studies of solutions of metal ion complexes (Ni^{2+} and Mn^{2+}) with TPP by Gallo & Sable (1975) and Gallo, Hansen, Sable & Swift (1972) show that the conformation of the C(5) pyrophosphate ester side chain is sensitive to specific metal ion ligands, temperature and *p*H. Their studies further indicated that the pyrophosphate side chain can coexist in solution in a folded and in an extended conformation and that the extended conformation is favored at neutral *p*H. The population of the folded form appears to be related to the coordination affinity of the metal ion pyrophosphate towards the hydrated N(1′) of the pyrimidine ring (Gallo & Sable, 1975). The crystal-structure results support the existence of the two pyrophosphate conformations, with the extended one being favored under less acidic conditions. In fact, the results of the present analysis indicate that the extended form is favored following the ionization of the second proton from TPP. Thus the ionization of the terminal phosphate seems to be an important factor which favors the extended conformation in TPP, as was also indicated by Gallo & Sable (1975) for the TPP metal ion complexes.

The reason for the stability of the extended conformation in TPP remains unclear. It had been suggested that the folded conformation of TPP.HCl formed in order to minimize the charge separation between the two positively charged thiamine rings and the negatively charged pyrophosphate (Pletcher & Sax, 1966, 1972). Clearly, the present structure does not support this idea since both rings remain positively charged and the pyrophosphate is now doubly negatively charged.*

This structure also clearly shows that the terminal phosphate ionizes before the N(1′) proton on the pyrimidine ring. It is indeed significant that the refined thermal parameters for the H atoms give no indication that the H(2–1) disordering in molecule *B* involves the H(1′)*B* which is hydrogen-bonded to O(2–2)*B*.

It is again noteworthy that although there is extensive hydrogen bonding of the pyrophosphate groups, the O(1–1) bridging O atoms do not serve as hydrogen-bond acceptors. This characteristic feature of polyphosphates, which has been noted previously, provided an explanation for the observed difference in solvation energy between polyphosphates and their hydrolysis products (Pletcher & Sax, 1972; George, Witonsky, Trachtman, Wu, Dorwart, Richman, Richman, Shurayh & Lentz, 1970).

The predominance of the **F** conformation of thiamine (Shin *et al*., 1977) is also supported by this structure. The deviation of φ_T from 0° for molecule *B* is the largest observed to date for those having the **F** conformation. However, it is of interest that this deviation moves C(2)H away from the N(4′α) amino, *i.e.* away from the proposed **V** conformation (Schellenberger, 1967; Biaglow, Mieyal, Suchy & Sable, 1969). In this same vein it is noteworthy that in 11 of the 13 thiamine molecules with the **F** conformation, the deviations of φ_T from 0° are such as to move C(2)H away from N(4′α). This is the case whenever φ_T and φ_P

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* It has been pointed out by the referee that this condition is satisfied by the molecular packing in the crystal. We agree that packing considerations may account for the extended conformation. However, in light of the latest solution studies (Gallo & Sable, 1975) we believe that the extended form is preferred following ionization on the terminal phosphate. The presence of divalent metal ions tends to stabilize the folded form through coordination with the pyrimidine ring although the extended form still predominates for this state of ionization.

have the same sign. These data for all of the known thiamine structures have been summarized in Table 5 of Shin, Pletcher, Blank & Sax (1977).

We wish to acknowledge help from the University of Pittsburgh Computing Center which has provided computing time and facilities. This work was supported in part by NIH grant AM 18898.

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