

Crystal and Molecular Structure of Thiamine Pyrophosphate Hydrochloride¹

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Abstract: The structure of thiamine pyrophosphate hydrochloride ($C_{12}H_{19}ClN_4O_7P_2S$) has been determined by X-ray single crystal analysis. The crystals are monoclinic (space group $P2_1/c$) with four molecules in the unit cell; $a = 6.815(1)$, $b = 11.171(1)$, $c = 24.541(6)$ Å, $\beta = 94.00(4)^\circ$. The intensity data were obtained using a Picker four-circle diffractometer. The structure was refined by the full matrix least-squares technique to a final R value of 0.037 for the observed reflections. The average estimated standard deviations are 0.002 Å for the bonds involving P, 0.003 Å for those involving S, and 0.004 Å for those involving C and N. The relative conformation of the two planar rings is described and compared for several structures containing the thiamine moiety. A resonance description, consistent with the observed structural features, is presented for both aromatic ring systems. The structure shows that the most acidic proton is on the inner phosphate of the pyrophosphate ester. The normal absence of hydrogen bonding to the bridging oxygen atoms is also a feature of this structure. This property of polyphosphates in the solid state is consistent with the observation that their solvation energies in aqueous solution are less than those of their hydrolysis products.

Thiamine pyrophosphate (TPP) is a coenzyme for a number of different enzyme systems which catalyze the transfer of aldehyde or acyl groups.² Since a detailed structure analysis can be useful in gaining a more complete understanding of the chemical properties of a compound, we have undertaken a study of the structures of a number of thiamine-related compounds. We have previously reported the crystal structure analyses of thiamine pyrophosphate³ and *N*-benzylthiazolium bromide.⁴ In this paper we are reporting the results of a more accurate analysis of TPP. To our knowledge it is the only accurate analysis of a pyrophosphate ester presently on record.

Experimental Section

Single crystals of thiamine pyrophosphate hydrochloride were obtained in sufficiently large size by promoting slow growth over a period of several months. The crystals were grown from a 0.04 *M* solution of the compound in 0.1 *N* HCl which was subsequently diluted with an approximately equal volume of absolute ethanol and then maintained at 0° to -15°. Crystallization was apparent after a week's time, but several months' growth was required before an adequate size was achieved. The crystals grew as thin "boat-shaped" plates with a tendency to twin about the *b* axis through the center of the crystal. It was possible to cleave a single fragment from one of these crystals.

The specimen used in this analysis was a "spear-shaped" plate 0.06 mm thick, 0.55 mm long, and 0.19 mm wide at the base of the spear. It was mounted parallel to the long dimension which was the direction of the *a* axis. The unit cell dimensions were obtained from replicate measurements of the axial reflections on a Picker four-circle automatic diffractometer. All of the pertinent crystal data for this compound are summarized in Table I. All of the unit cell parameters, except α , are within 3σ of the corresponding parameters obtained in the previous analysis. However, since the remeasured axial lengths are all shorter, the unit cell volume is significantly smaller. This, together with a more accurate crystal density, reduces the previous discrepancy between the observed and calculated densities. The present difference is equivalent

Table I. Crystal Data for Thiamine Pyrophosphate Hydrochloride

Diffractometer data	Ni-filtered Cu radiation	Film data ^a
$a = 6.815(0.0013)$ Å		$a = 6.92(0.02)$
$b = 11.171(0.0010)$ Å	$K\alpha_1 = 1.54051$	$b = 11.28(0.05)$
$c = 24.541(0.0060)$ Å	$K\alpha_2 = 1.54433$	$c = 24.82(0.10)$
$\beta = 94.00^\circ(0.04^\circ)$	$K\alpha_1\alpha_2 = 1.54178$	$\beta = 93^\circ 48'(15')$
$V = 1863.7$ Å ³		$V = 1933$ Å ³

Diffractometer data at 22°, ambient temperature

Space group is $P2_1/c$ uniquely determined by systematically absent

reflections $h0l$, $l = 2n + 1$

$0k0$, $k = 2n + 1$

$\rho_m = 1.656(0.004)$ in duplicate by flotation in $CCl_4-CH_2Br_2$

$\rho_x = 1.642$ g cm⁻³, $Z = 4$

MW = 460.77 for $C_{12}N_4O_7P_2SH_{19}Cl$

$F(000) = 956$

$\mu(Cu K\alpha) = 48.82$ cm⁻¹

Coordinates of crystal corners in cm ($\times 10^4$) ^b					
<i>X</i>	<i>Y</i>	<i>Z</i>	<i>X</i>	<i>Y</i>	<i>Z</i>
-94	30	-130	-94	-30	-130
-33	30	273	-33	-30	273
-94	30	-273	-94	-30	-273
94	30	-130	94	-30	-130
33	30	273	33	-30	273
00	30	-273	00	-30	-273

^a Pletcher and Sax.³ ^b With reference to orthogonal axes: *X* along *b**, *Y* along *c**, and *Z* along *a*.

to 0.25 of a molecule of water per asymmetric unit. Since the final difference Fourier gave no indications of a partial water molecule, it must be concluded that if there is any water in the crystal, it is highly disordered. From spatial consideration it is not very likely that the crystal contains any water. Thus, the reported chemical analysis requiring 0.5 a molecule of water per molecule of TPP³ either is in error or it applies to a different crystal modification.

The diffracted intensities were measured^{5a} using a 2θ scan tech-

(5) J. Weijlard, *J. Amer. Chem. Soc.*, **63**, 1160, (1941).

(6) The following computing programs used in this analysis were obtained from the Crystallography Laboratory, University of Pittsburgh: (a) Angle Generation and Data Reduction, R. Shiono; (b) Sign Correlation by the Sayre Equation, P. T. Beurskens, Technical Report (1963); (c) Fourier, A. Zalkin modified by R. Shiono; *E* Value Coefficients, I. L. Karle, H. Hauptman, J. Karle, and A. B. Wing, *Acta Crystallogr.*, **11**, 257, (1958); (d) Least Squares, W. R. Busing, K. O.

(1) Research supported in part by NIH Grants HE-09068 and NS-09178.

(2) A recent review of thiamine catalysis is presented by L. O. Kram-pitz, *Annu. Rev. Biochem.*, **38**, 213 (1969).

(3) J. Pletcher and M. Sax, *Science*, **154**, 1331 (1966).

(4) L. Power, J. Pletcher, and M. Sax, *Acta Crystallogr.*, **B26**, 143 (1970).

nique with a scan range of 2° and a scan rate of $1^\circ/\text{min}$ for all the reflections in one quadrant of the sphere having $\sin \theta \leq 0.903$. The background was measured for 30 sec at each end of the scan range. A total of eight reflections were chosen as standards which were measured after every 20–30 reflections (approximately every 2 hr). Normally no more than two or three of the standard reflections were measured at any given checkpoint. However, all eight standards were measured about once every other day or after any lengthy disruption of the data collection. Over the course of the data collection (about 2 weeks), the intensities of the standard reflections exhibited a slow but continuous decline. The total decrease amounted to about 4%. However, by the end of the third week (during which time some poorly determined reflections were remeasured), the standards had dropped by a total of 10%. This loss of intensity was initially ascribed to a slow deterioration of the crystal specimen. Several months after the data collection was completed, it was discovered that the counting efficiency of the scintillation crystal in the detector system was slowly decreasing. This was undoubtedly the source of the intensity loss during the data collection because there had been no previous indications of any instability of thiamine pyrophosphate crystals. Since the standards had been measured frequently, a correction curve was readily plotted and subsequently applied to the measured intensities. An accurate microscopic determination of the crystal's size and shape permitted a calculation of an absorption correction which was also applied to the intensity data. The structure refinement was carried out using both the corrected and the uncorrected data. The unobserved reflections were given zero weight in the refinement. Reflections were considered unobserved whenever $I \leq 3\sigma(I)$ where $\sigma(I) = [I' + 4(b_1 + b_2)]^{1/2}$, I is the intensity corrected for background, I' is the number of counts accumulated during the scan, and b_1 and b_2 are the number of background counts accumulated at the initial and final setting of the scan range. Of the 3128 independent reflections measured, 359 were considered unobserved by this criterion. An additional 35 observed reflections, which were found later to have been unreliably measured, were also assigned zero weight in the refinement. The intensities were converted to their corresponding structure amplitudes by applying the proper Lorentz and polarization factors.^{6a}

Structure Determination

The structure was initially determined using photographic data by application of the direct method (Beursskens)^{6b} to obtain a set of phases. The complete structure (including hydrogens) was obtained from the E map^{6c} or from subsequent difference Fourier.^{6c} This structure as reported previously³ was refined by full matrix least-squares^{6d} to a final R of 0.12. In the present analysis the refinement was begun utilizing the final positional parameters from the previous determination. The atomic scattering factors employed in the refinement were as follows: Cl^- and S from Dawson;^{7a} O , N , and C from Berghuis, *et al.*;^{7b} P and H from International Tables;^{7c} and the anomalous dispersion corrections for Cl^- , S , and P from International Tables.^{7c} The initial agreement factor was 0.141. Full matrix least-squares refinement^{6d} (anisotropic thermal parameters) of the nonhydrogen atoms lowered the R index to 0.068. The weights assigned to the observational data are those given by $W = 1/\sigma^2$ where $\sigma = \sigma(\text{exp}) + A + BF_o + CF_o^2$, $\sigma(\text{exp})$ is the experimental σ based on counting statistics, F_o is the structure amplitude, and A , B , and C are constants which were

Martin, and H. A. Levy (ORNL-TM-305, 1962), modified by R. Shiono (1965); (e) Absorption Correction, B. M. Craven, Technical Report 45 (1963), using method of W. R. Busing and H. A. Levy, *Acta Crystallogr.*, **10**, 180 (1957); (f) ORTEP, C. K. Johnson (ORNL-3794, 1965); (g) Interatomic Distance and Angle, R. Shiono and S. Chu; (h) Least-Squares Planes, S. Chu using method of V. Schomaker, J. Waser, R. E. Marsh, and G. Bergman, *Acta Crystallogr.*, **12**, 600 (1959); (i) Torsion angles, H. Berman

(7) (a) B. Dawson, *Acta Crystallogr.*, **13**, 403 (1960); (b) J. Berghuis, I. M. Haanappel, M. Potters, B. O. Loopstra, C. H. MacGillivray, and A. L. Veenendaal, *ibid.*, **8**, 478 (1955); (c) International Tables for X-ray Crystallography, Kynoch Press, Birmingham, England, 1965.

empirically assigned the values 0.3, 0.006, and 0.0001, respectively.

The hydrogen positional parameters were refined (fixed thermal parameters were assigned to the hydrogen atoms which were identical with those of the atoms to which the hydrogens were bonded) followed by a further refinement of the nonhydrogen atoms to give a final R factor of 0.057. The difference Fourier calculated at this point was essentially featureless except for an antisymmetric diffraction ripple associated with each of the four heavier atoms for which anomalous dispersion corrections were applied.

Careful scrutiny of the observed and calculated structure factors indicated that certain segments of reflections were appreciably affected by absorption. Even though the crystal is not completely bounded by plane faces, its shape can be closely approximated by eight planes. An absorption correction was calculated based on this approximation to the crystal shape (see Table I) using a version of Busing and Levy's absorption correction program.^{6e} The absorption corrected data were used for two additional cycles of anisotropic refinement on the nonhydrogen atoms followed by one cycle of refinement of the positional parameters for all the atoms. The final agreement factor, $R = \Sigma|F_o - F_c|/\Sigma|F_o|$, over all 3128 reflections was 0.044 and for the 2734 observed reflections R was 0.037. The difference Fourier was essentially unchanged in comparison with the one just prior to the absorption correction. The final atomic coordinates and thermal parameters are listed in Table II. The structure factors are presented in Table III.⁸

Description of the Structure

Thiamine pyrophosphate hydrochloride contains a zwitterion with one negative and two positive charges and a chloride ion with one negative charge. One positive charge is associated with the aromatic thiazolium ring while the other results from protonation of $\text{N}(11)$ in the pyrimidine ring. The single negative charge in the zwitterion is produced by ionization of the acidic hydrogen from the inner phosphate group of the pyrophosphate moiety. In the crystal the net positive charge on the zwitterion is balanced by a chloride ion. A projection of the unit cell contents is shown in Figure 1. Figure 2 contains the interatomic distances and angles^{6g} within the molecule. The intermolecular interactions, which are listed in Table IVA along with Figure 1, describe the three-dimensional packing in the crystal structure. One significant feature of the molecular packing is the short contact distance between the chloride ion and the $\text{C}(2)$ of the thiazolium ring. Since the hydrogen on this carbon atom is acidic and since similar short contacts are observed in other thiamine structures, it is reasonable to describe this interaction as a hydrogen bond. By using the hydrogen bond acceptor radii of Wallwork⁹ we have determined a hydrogen bond donor radius for the CH group. These results, along with some of the values from Wallwork's tabulation, are shown in Table V.

(8) Table III will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to code number JACS-72-3998. Remit check or money order for \$3.00 for photocopy or \$2.00 for microfiche.

(9) S. C. Wallwork, *Acta Crystallogr.*, **15**, 758 (1962).

Table II. Atomic Coordinates

Atom	$x \times 10^4$	$y \times 10^4$	A. Fractional Coordinates ^a			Atom	$x \times 10^3$	$y \times 10^3$	$z \times 10^3$
			$z \times 10^4$						
S(1)	6495 (1)	-827 (.7)	588 (.4)	H(28)	705 (6)	121 (4)	68 (2)		
C(2)	6217 (5)	609 (3)	761 (1)	H(29)	277 (6)	214 (3)	114 (2)		
N(3)	4607 (3)	790 (2)	1021 (1)	H(30) ^b	400 (7)	185 (4)	166 (2)		
C(4)	3503 (4)	-240 (3)	1087 (1)	H (31)	967 (6)	396 (3)	157 (2)		
C(5)	4350 (5)	-1210 (3)	870 (1)	H(32)	734 (6)	268 (4)	180 (2)		
C(6)	4050 (5)	1968 (3)	1259 (1)	H(33)	270 (6)	313 (4)	35 (2)		
C(7)	5501 (4)	2927 (2)	1141 (1)	H(34)	368 (6)	414 (4)	7 (2)		
C(8)	5254 (4)	3700 (2)	678 (1)	H(35)	980 (6)	576 (4)	49 (2)		
N(9)	6627 (3)	4541 (2)	582 (1)	H(36)	1026 (6)	582 (4)	107 (2)		
C(10)	8211 (4)	4615 (2)	914 (1)	H(37)	1103 (6)	504 (4)	77 (2)		
N(11)	8472 (4)	3938 (2)	1366 (1)	H(38)	175 (6)	17 (4)	172 (2)		
C(12)	7122 (5)	3103 (3)	1479 (1)	H(39)	112 (6)	-92 (4)	141 (2)		
N(13)	3729 (4)	3639 (3)	320 (1)	H(40)	61 (6)	39 (4)	119 (2)		
C(14)	9787 (5)	5465 (3)	798 (2)	H(41)	232 (7)	-251 (4)	86 (2)		
C(15)	1615 (5)	-183 (4)	1361 (2)	H(42)	427 (6)	-296 (4)	60 (2)		
C(16)	3708 (5)	-2489 (3)	896 (1)	H(43)	399 (6)	-250 (4)	174 (2)		
C(17)	4425 (5)	-3082 (3)	1428 (1)	H(44)	396 (6)	-391 (4)	145 (2)		
O(18)	6563 (3)	-3103 (2)	1467 (1)	H(45)	865 (6)	102 (3)	253 (2)		
P(10)	7704 (1)	-2859 (.6)	2039 (.3)	H(46)	447 (6)	33 (4)	254 (2)		
O(20)	6655 (4)	-3409 (2)	2483 (1)						
O(21)	9798 (3)	-3154 (2)	1982 (1)						
O(22)	7539 (3)	-1431 (2)	2074 (1)						
P(23)	7160 (1)	-530 (.6)	2554 (.3)						
O(24)	7990 (4)	656 (2)	2351 (1)						
O(25)	4909 (3)	-439 (2)	2527 (1)						
O(26)	8022 (3)	-957 (2)	3082 (1)						
Cl(27)	9878 (1)	2125 (.8)	279 (.3)						

B. Temperature Factor Expression: $\exp -(h^2\beta_{11} + k^2\beta_{22} + l^2\beta_{33} + 2hk\beta_{12} + 2hl\beta_{13} + 2kl\beta_{23})$

Atom ^c	$\beta \times 10^4$						Atom ^c	$\beta \times 10^4$					
	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}		β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
S(1)	193 (2)	64 (1)	19 (.2)	-4 (1)	15 (1)	-3 (.3)	C(14)	152 (8)	76 (3)	14 (1)	-28 (4)	-1 (2)	2 (1)
C(2)	162 (8)	58 (2)	16 (1)	-15 (4)	10 (2)	3 (1)	C(15)	159 (8)	82 (3)	21 (1)	-26 (4)	13 (2)	8 (1)
N(3)	125 (6)	50 (2)	11 (1)	-10 (3)	3 (1)	5 (1)	C(16)	195 (8)	55 (2)	16 (1)	-23 (4)	-14 (2)	3 (1)
C(4)	135 (7)	60 (2)	12 (1)	-21 (3)	-2 (2)	8 (1)	C(17)	164 (8)	60 (3)	16 (1)	-23 (4)	-7 (2)	5 (1)
C(5)	156 (7)	57 (3)	13 (1)	-15 (3)	-5 (2)	4 (1)	O(18)	158 (5)	61 (2)	12 (1)	4 (2)	-1 (1)	-4 (1)
C(6)	169 (8)	52 (2)	13 (1)	-9 (3)	13 (2)	3 (1)	P(19)	144 (2)	43 (1)	11 (.1)	3 (1)	1 (1)	-0 (.2)
C(7)	152 (7)	47 (2)	11 (1)	-3 (3)	7 (1)	2 (1)	O(20)	238 (6)	62 (2)	13 (1)	-37 (3)	7 (1)	0 (1)
C(8)	124 (6)	45 (2)	11 (1)	-3 (3)	4 (1)	2 (1)	O(21)	160 (6)	83 (2)	16 (1)	39 (3)	-1 (1)	5 (1)
N(9)	124 (6)	47 (2)	11 (1)	-11 (2)	1 (1)	2 (1)	O(22)	258 (6)	45 (2)	12 (1)	1 (3)	7 (1)	-2 (1)
C(10)	138 (7)	46 (2)	12 (1)	-2 (3)	2 (1)	-1 (1)	P(23)	126 (2)	42 (1)	12 (.1)	-1 (1)	-5 (1)	-2 (.2)
N(11)	160 (6)	56 (2)	10 (1)	-12 (3)	-7 (1)	2 (1)	O(24)	211 (6)	53 (2)	15 (1)	-26 (3)	-9 (1)	2 (1)
C(12)	188 (8)	55 (2)	11 (1)	-3 (4)	1 (2)	3 (1)	O(25)	140 (5)	52 (2)	28 (1)	6 (2)	-4 (1)	-1 (1)
N(13)	144 (6)	72 (2)	14 (1)	-33 (3)	-4 (1)	12 (1)	O(26)	198 (6)	72 (2)	13 (1)	-2 (3)	-13 (1)	3 (1)
							Cl(27)	156 (2)	91 (1)	18 (.2)	-36 (1)	7 (1)	6 (.3)

^a Standard deviations in parentheses refer to the least significant positions. ^b Not refined after absorption correction. ^c Hydrogens arbitrarily assigned thermal parameters of atoms to which they are bonded.

Table IV. Intermolecular Contacts

Atom	Location of atom c relative to coordinates in Table III	Interatomic dist, Å	abc, deg
a b c		a-c b-c	
A. Hydrogen Bonds			
C(2)-H(28) Cl(27)	x	y z	3.302 2.45 156
N(11)-H(31) O(26)	$2.0 - x$	$0.5 + y$ $0.5 - z$	2.665 1.74 177
N(13)-H(33) Cl(27)	$-1.0 + x$	Y z	3.117 2.22 168
N(13)-H(34) N(9) ^a	$1.0 - x$	$1.0 - y$ $-z$	3.004 2.17 176
O(24)-H(45) O(21)	$2.0 - x$	$0.5 + y$ $0.5 - z$	2.523 1.79 175
O(25)-H(46) O(20)	$1.0 - x$	$0.5 + y$ $0.5 - z$	2.505 1.61 170
B. Pyrimidine Methyl (Nearest Neighbors)			
C(14)-H(36) O(21)	x	$1.0 + y$ q	3.291 2.56 149
C(14)-H(36) O(18)	x	$1.0 + y$ z	3.255 3.02 100
C(14)-H(36) O(26)	$2.0 - x$	$0.5 + y$ $0.5 - z$	3.426 3.05 105
C(14)-H(37) O(26)	$2.0 - x$	$0.5 + y$ $0.5 - z$	3.426 3.05 111
C. Miscellaneous Close Contacts			
C(6)-H(30) O(20)	$1.0 - x$	$0.5 + y$ $0.5 - z$	3.185 2.19 170
C(6)-H(30) O(26)	$1.0 - x$	$0.5 + y$ $0.5 - z$	3.210 2.90
C(5)-O(18)	x	y z	2.929
C(17)-O(20)	x	y z	2.934
C(17)-O(22)	x	y z	3.155

^a This hydrogen bond was overlooked in the initial report of the structure.

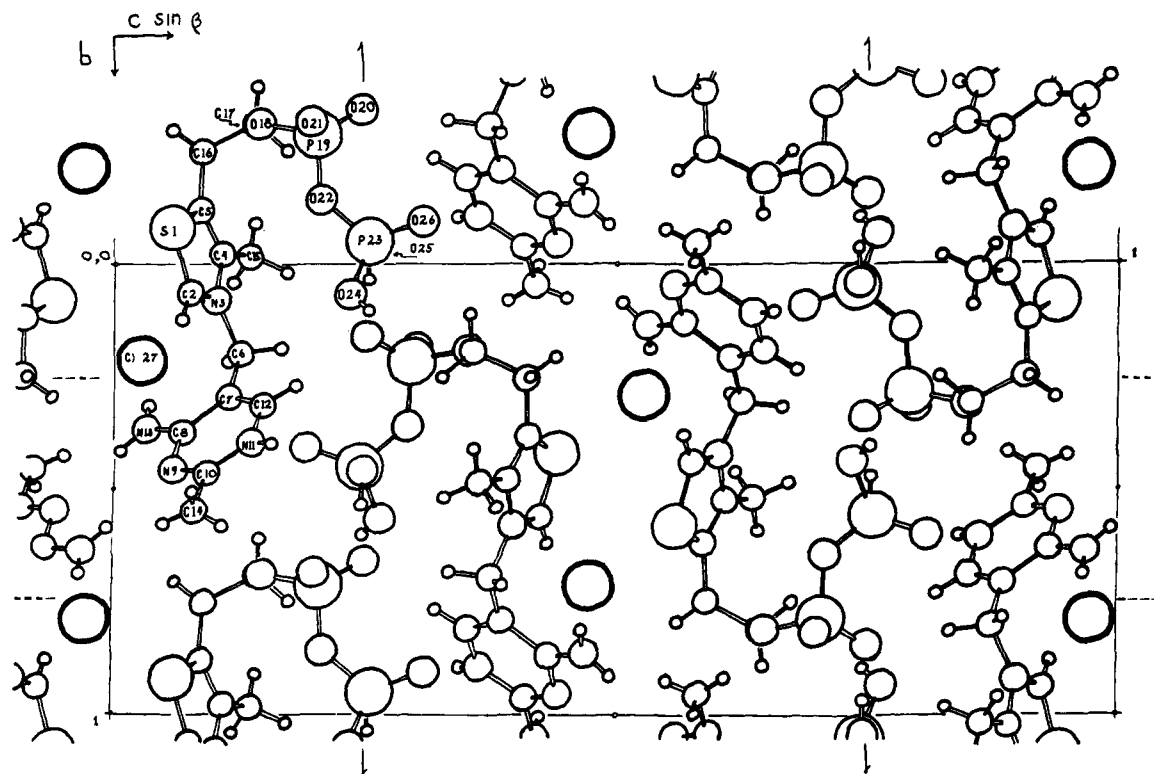


Figure 1. ORTEP^{6f} plot of the *a* axis projection of the contents of a unit cell. The atoms are represented by spheres of arbitrary radii for different atom types.

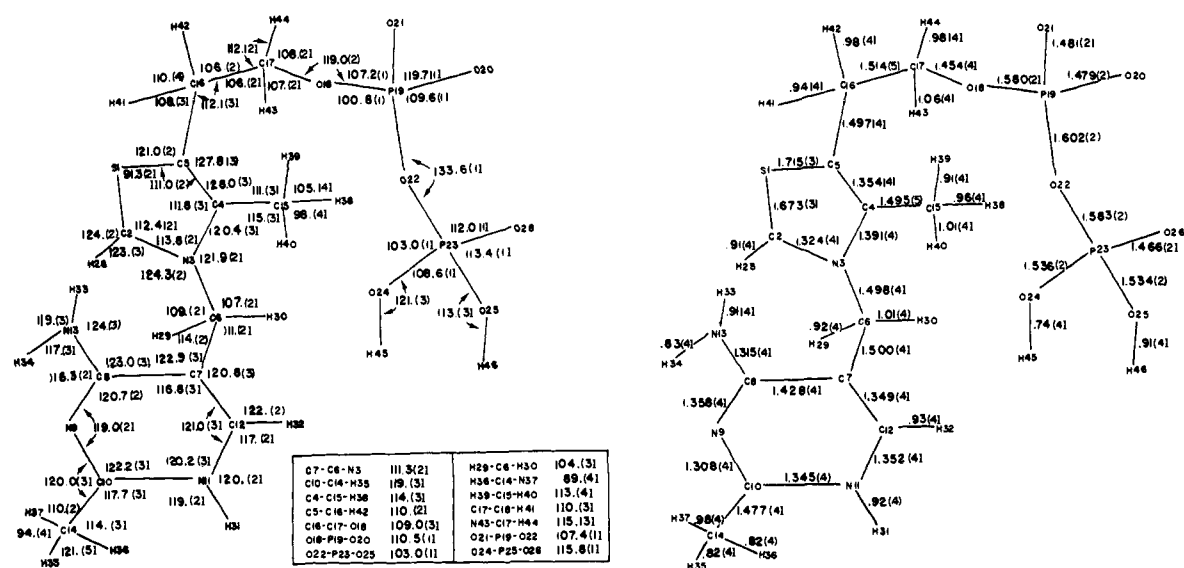


Figure 2. This figure shows the atom numbering scheme along with the interatomic bond distances (ångströms) and the bond angles (degrees). The bond angles which could not be conveniently depicted on the molecular diagram are tabulated between the two drawings. The estimated standard deviations for the bond distances and angles are given in parentheses to the right of each value.

Both of the aromatic rings in this molecule are virtually planar with small but significant deviations from strict planarity. The plane of the pyrimidine ring, referred to the crystal axes in ångströms, is defined by the equation^{6h} $0.51645X - 0.67061Y - 0.56723Z = -1.86280$. The equation defining the plane of the thiazolium ring is $0.45172X - 0.17273Y + 0.84161Z = 3.37153$. The deviations of the atoms from the ring planes are listed in Table VI. The dihedral angle between these two planes is 83.3° . Since the two rings are joined through a methylene bridge carbon, a more

satisfactory description of the relative ring orientation is provided in terms of the torsion angles about the bonds from the methylene carbon to the rings. The angle about the bond from the methylene carbon to the thiazolium ring is specified by ϕ_T . The angle about the bond from the methylene carbon to the pyrimidine ring is defined by ϕ_P . A clockwise rotation of the ring is indicated by a positive angle. The angle $\phi_T = \phi_P = 0^\circ$ is defined by the condition that the normals to the planes of the thiazolium ring, the pyrimidine ring, and the N(3)C(6)C(7) group are all parallel and

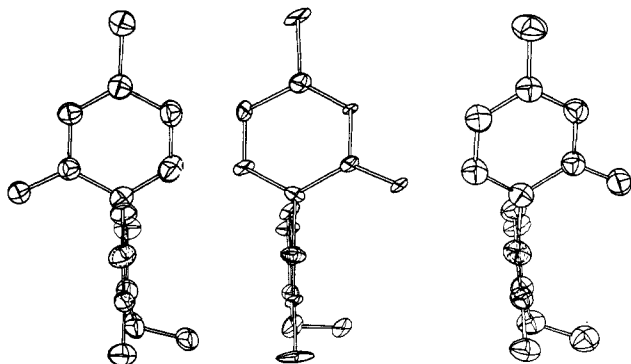


Figure 3. ORTEP^{6f} plots of TPP (left), thiamine monophosphate¹⁰ (center) and thiamine¹¹ (right) as viewed down the bond from the methylene bridge carbon to the thiazolium ring nitrogen. The thiazolium ring is seen edge-on with the bond between the carbons of the dimethylene side chain nearly perpendicular to the ring plane. The pyrimidine ring is seen clearly in projection with the amino nitrogen directed either to the right or left. The atoms are represented by their thermal ellipsoids at the 50% probability level.

oriented in the same direction with the pyrimidine amino group adjacent to the thiazolium C(2). The reference orientation of the thiazolium ring is specified such that the dimethylene side chain, C(16)C(17), is directed to the right when the ring is viewed down the

Table V. Hydrogen Bond Radii

H bond donor radii, Å ^a		H bond acceptor radii, Å ^a	
NH	1.55	N	1.51
NH ⁺	1.41	O	1.40
OH	1.30	O ⁻	1.43
OH ⁺	(1.20)	Cl ⁻	1.79

Compound	Thiazolium C(2) interaction	Distance, Å	Distance - acceptor radius, Å
TPP	CH-Cl	3.30	1.51
Thiamine monophosphate ^b	CH-O	3.05	1.62
Thiamine ^c	CH-Cl	3.48	1.69
Average CH donor radius			1.61

^a See ref 9. ^b See ref 10. ^c See ref 11.

Table VI. Deviations from Least-Squares Planes

Atoms used in calculating plane	Deviations from plane in Å	Atoms not used in calculating plane	Deviations from plane in Å
Pyrimidine Ring			
C(7)	0.018	C(6)	0.062
C(8)	-0.003	N(13)	0.004
N(9)	-0.017	C(14)	0.103
C(10)	0.023	H(31)	0.115
N(11)	-0.007	H(32)	-0.071
C(12)	-0.014	H(33)	-0.012
		H(34)	-0.034
Thiazolium Ring			
S(1)	0.003	C(6)	0.096
C(2)	-0.004	C(15)	-0.028
N(3)	0.004	C(16)	0.101
C(4)	-0.001	H(28)	-0.032
C(5)	-0.001		

(10) I. Karle and K. Britts, *Acta Crystallogr.*, **20**, 118 (1966).

(11) J. Kraut and H. J. Reed, *ibid.*, **15**, 747 (1962).

methylene-nitrogen bond and the methyl substituent is pointing back toward the observer. The conformer whose angles are of the opposite sign is present in the crystal structure too, because of the center of symmetry. Table VII provides a comparison of the relative ring

Table VII. Relative Ring Orientation

Compound	ϕ_T , deg	ϕ_P , deg
TPP	3.6	93.2
Thiamine monophosphate ^a	-19.9	-85.7
Thiamine ^b	-9.4	-73.5

^a See ref 10. ^b See ref 11.

orientation which has been found in three structures containing the thiamine molecule. The striking similarity in the ring orientation in these structures is more clearly demonstrated in Figure 3. The primary difference concerns the structural relationship of the pyrimidine amino group and the dimethylene side chain. In TPP these two substituents are "trans" while in the other two compounds they are "cis." In a triclinic form of TPP the cis configuration has also been observed.¹² In spite of this structural difference it is important to note that in all of these structures the spatial arrangement of the amino group and the "active site" on the thiazolium ring are quite similar. Evidently the relative orientation of the dimethylene side chain is determined by lattice forces. It is, therefore, conceivable that this structural aspect of the coenzyme is specified by the enzyme when it is bound to the enzyme.

The fact that the rings are similarly oriented in all these crystal structures containing thiamine indicates an inherent stability in this geometry. A consequence of this geometry is the close proximity of the amino group to the active site on the thiazolium ring. As previously noted by Kraut and Reed,¹¹ this feature may be of significance both in its conversion to thiochrome and to its catalytic function. It was originally pointed out by Breslow and McNelis¹³ that the enhanced activity of thiamine over other N-substituted thiazolium compounds is much greater than the amount which can be attributed to an inductive stabilization of the carbanion. They further suggested that the amino group on the pyrimidine ring may assist proton removal in one or more steps of the reaction. The active participation of the pyrimidine ring both in the mechanism and in binding to the enzyme has been implicated by several pieces of experimental evidence.^{14,15} Although this stable configuration may be maintained in the enzyme-bound coenzyme, it appears unlikely that this ring orientation is retained after the adduct is formed with the substrate.¹⁶

(12) C. H. Carlisle and D. S. Cook, *ibid.*, **B25**, 1359 (1969).

(13) R. Breslow and E. McNelis, *J. Amer. Chem. Soc.*, **81**, 3080 (1959).

(14) A. Schellenberger, W. Rödel, and H. Rödel, *Hoppe-Seyler's Z. Physiol. Chem.*, **339**, 122 (1964); A. Schellenberger and Kl. Winter, *ibid.*, **344**, 16 (1966); A. Schellenberger, V. Müller, Kl. Winter, and G. Hübner, *ibid.*, **344**, 244 (1966); A. Schellenberger, K. Wendler, P. Creutzburg, and G. Hübner, *ibid.*, **348**, 501 (1967).

(15) A. V. Morey and E. Juni, *J. Biol. Chem.*, **243**, 3009 (1968).

(16) In the crystal structure of 2- α -hydroxyethylthiamine chloride hydrochloride there occurs a substantially different orientation of the two planar rings. Although this structure does not exhibit any intra-

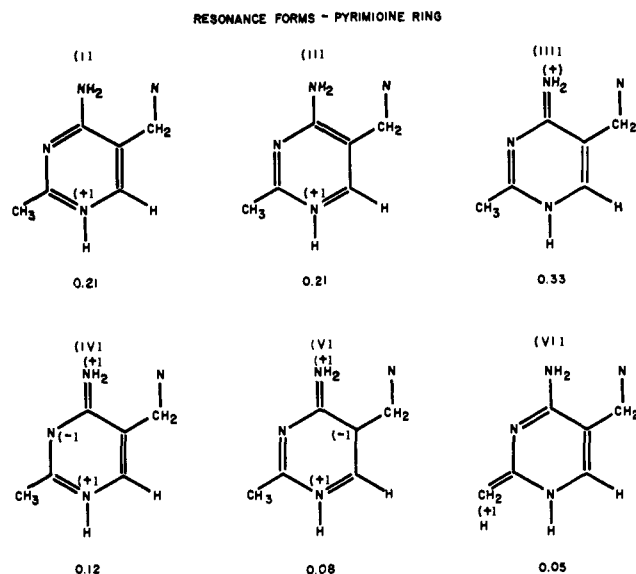


Figure 4. The fractional contributions of the resonance forms for the pyrimidine ring as determined by applying equation 7-3 of L. Pauling, "Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960, p 235.

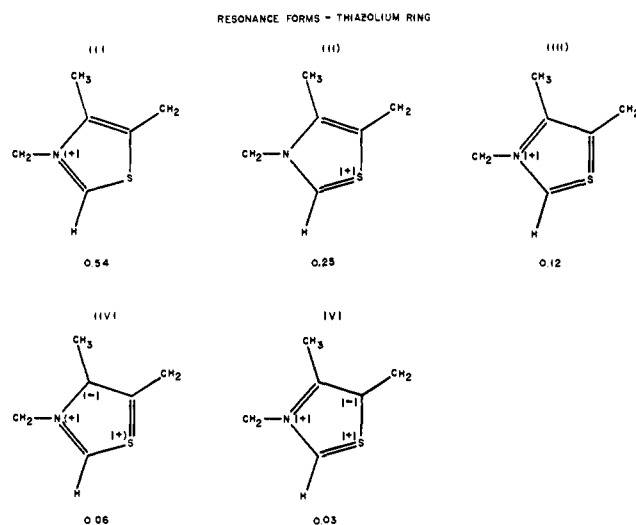


Figure 5. The five resonance forms used to account for the observed bond lengths in the thiazolium ring. The fractional contribution of each canonical form is listed below the corresponding structural diagram.

The observed bond distances for the protonated pyrimidine ring can be accounted for by the six resonance forms shown in Figure 4. The relative contributions of the different forms agree reasonably well with those determined by Kraut and Reed¹¹ with the exception of resonance form VI. The inclusion of resonance form VI was required to account for the bond number of the short C(10)-C(14) bond even though this form would seem very unlikely. Since this bond is significantly shorter in TPP than in other thiamine compounds, it seemed possible that it might be the result of enhanced thermal motion. However, the β 's of Table III indicate that the difference in the thermal motion for

molecular interactions between the pyrimidine amino group and the covalently bound substrate, such interactions may be possible in the presence of the enzyme. M. Sax, P. Pulsinelli, and J. Pletcher, in preparation.

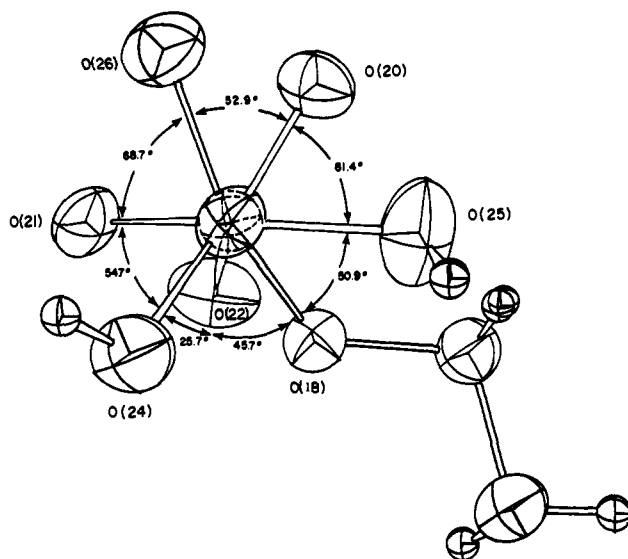


Figure 6. ORTEP^{6f} plot of the pyrophosphate group projected down the P-P vector. The projected angles between the atoms are included in the figure. The atoms are represented by their thermal ellipsoids at the 50% probability level.

C(14) and C(10) is not any larger than for many other pairs of atoms. An investigation of the environment around the C(14) methyl group revealed an interesting structural feature which does contribute to the plausibility of resonance form VI. The closest intermolecular contacts to C(14) are those to O(18), O(21), and O(26) at distances of 3.255, 3.291, and 3.426 Å, respectively (see Table IVB for details). These compare with a predicted van der Waals separation of 3.40 Å. Hence the negative environment of the methyl group could stabilize the hyperconjugation of resonance form VI.

The observed bond distances in the thiazolium ring can be accounted for by the five resonance forms in Figure 5. A comparison of these results with those for the 2- α -hydroxyethyl-substituted thiazolium ring¹⁷ indicates that the various resonance forms, including their relative contributions, are similar except for form III. This difference in the resonance forms places a slightly larger positive charge on carbon atom C(2) as is expected for the unsubstituted thiazolium ring.

Pyrophosphate Group

The structural aspects of the pyrophosphate group are of interest because it contains the so-called "high energy bond." The pyrophosphate group is shown in Figure 6 as viewed down the P-P vector. This view demonstrates the nearly perfect staggered conformation of the oxygen atoms. The staggered conformation is a frequently observed feature of the pyrophosphate ion. In TPP the main distortions from a staggered conformation are those shown by O(18) and O(22). A close examination of the projected angles between the oxygen atoms reveals that there is also a relative twist of about 4° between the two phosphate groups.

The conformation of the pyrophosphate ester can also be described in terms of the torsion angles about the P-O (bridge) bonds common to P(19). These torsion angles were calculated⁶ⁱ according to the right-hand rule of Klyne and Prelog¹⁸ for the atom sequence

(17) M. Sax, J. Pletcher, and P. Pulsinelli (in preparation).

(18) W. Klyne and V. Prelog, *Experientia*, **16**, 521 (1960).

Table VIII. Pyrophosphate Dimensions

Compound	P-OP(R), Å	P-OH, Å	P-O, Å	POP(R), deg	Avg P-O, Å	Ref
Na ₄ P ₂ O ₇ ·10H ₂ O	1.612		1.526 1.510 1.503	130.2	1.538	20
α-Cu ₂ P ₂ O ₇	1.576		1.547 1.519 1.508	157	1.538	21
β-Ca ₂ P ₂ O ₇ 1st molecule	1.617		1.480 1.525 1.531	130.5	1.538	22
	1.637		1.504 1.520 1.538		1.550	
2nd molecule	1.616		1.504 1.527 1.527	137.8	1.544	
	1.590		1.498 1.505 1.562		1.539	
α-Mg ₂ P ₂ O ₇	1.612		1.533 1.507 1.472	144	1.531	23
	1.569		1.539 1.527 1.521		1.539	
TPP	1.602 (1.580) 1.583	1.534 1.536	1.481 1.479 1.466	133.6 (119.0)	1.536 1.530	This paper
Na ₂ H ₂ P ₂ O ₇ ·6H ₂ O	1.598	1.569	1.492 1.495	136.1	1.539	24

P(23)-O(22)-P(19)-O(18)-C(17). The results, which are summarized in Figure 7, can be compared with the tabulation of Shefter, *et al.*,¹⁹ for a large series of phos-

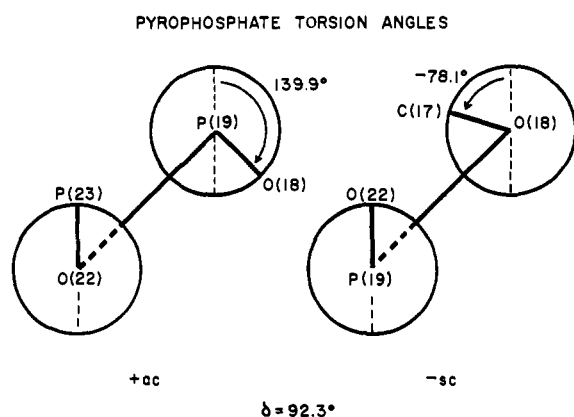


Figure 7. Torsion angles about the O(22)-P(19) and P(19)-O(18) bonds. The dihedral angle, δ , between the planes P(23)-O(22)-P(19) and P(19)-O(18)-C(17) was obtained from the relationship given by Shefter, *et al.*¹⁹

phate diester compounds. It should be noted that the conformation of this diphosphate ester does differ from that of the triphosphate anion (ap, ap) as predicted by Shefter, *et al.* However, as in their dinucleoside phosphate, TPP also exhibits the less favorable anticlinical conformation. It is less clear in TPP that this conformation results from packing constraints although it is possible since the pyrophosphate group does take part in strong hydrogen bond interactions.

(19) E. Shefter, M. Barlow, R. A. Sparks, and K. N. Trueblood, *Acta Crystallogr.*, B25, 895 (1969).

Other structural features which are similar to those observed in the pyrophosphate anion include the P-O-P bond length and the P-O-P bond angle. Table VIII²⁰⁻²⁵ lists a comparison of these bond distances and angles for several representative compounds. Although there appears to be some correlation between a shorter P-OP bond length with a greater P-O-P angle,^{20,25} these variations could be the result of environmental differences. The variations observed in the terminal P-O bond lengths certainly indicate an influence by the number and strength of ligands to the oxygens.

Recently the significance of resonance and electrostatic repulsion as the source of the reactivity of the "high energy" phosphates has been questioned.^{26,27} The studies by George, *et al.*,²⁶ have further demonstrated that the difference between the solvation energy of the reactant and products is large enough to account for the major portion of their thermodynamic reactivity. Structural evidence can be given which suggests the source of at least a part of the observed increase in solvation energy when polyphosphates are hydrolyzed. In the many different structures in which the polyphosphate group has been observed, the P-O-P bridging oxygens are never found to participate as a hydrogen bond acceptor even though such interaction is not sterically prevented. The rather inert character dis-

(20) W. S. McDonald and D. W. J. Cruickshank, *ibid.*, 22, 43 (1967).

(21) B. E. Robertson and C. Calvo, *ibid.*, 22, 665 (1967).

(22) N. C. Webb, *ibid.*, 21, 942 (1966).

(23) C. Calvo, *ibid.*, 23, 289 (1967).

(24) R. L. Collin and M. Willis, *ibid.*, B27, 291 (1971).

(25) D. W. J. Cruickshank, *J. Chem. Soc.*, 5486 (1961).

(26) P. George, R. J. Witonsky, M. Trachtman, C. Wu, W. Dorwart, L. Richman, W. Richman, F. Shurayh, and B. Lentz, *Biochim. Biophys. Acta*, 223, 1 (1970).

(27) J. Pletcher and M. Sax, Abstracts, 160th National Meeting of the American Chemical Society Chicago, Ill., 242, Sept 14-18, 1970, BIOL 242.

played by this bridging position is remarkably demonstrated by the complete isomorphism between the structures of tetrasodium pyrophosphate dodecahydrate and imidodiphosphate.²⁸ A further example is provided by the recent observation²⁴ of the nearly complete isomorphism between disodium dihydrogen phosphate hexahydrate and disodium dihydrogen phosphonate hexahydrate which does not have a bridge oxygen.

(28) M. Larsen, R. Willett, and R. G. Yount, *Science*, **166**, 1510 (1969).

On the other hand, the nonbridging oxygen atoms in both polyphosphate and orthophosphate ions normally exhibit strong interactions (ionic and hydrogen bonding) with the neighboring atoms. Thus, the relative inertness of the bridging oxygens in forming hydrogen bonds in crystals is consistent with the observation that the polyphosphates have lower solvation energies in aqueous solutions than do their hydrolysis products.

Acknowledgment. We wish to acknowledge the helpful comments and suggestions of Dr. L. Pauling.

Crystal and Molecular Structure of the Antihistamine 2-[(2)-Dimethylaminoethyl-2-thenylamino]pyridine Hydrochloride

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Abstract: The crystal structure of the antihistamine 2-[(2)-dimethylaminoethyl-2-thenylamino]pyridine hydrochloride has been determined using three-dimensional diffractometer data. The colorless plates are monoclinic, of space group $P2_1/c$. The unit cell containing eight molecules has dimensions $a = 10.936 \pm 0.003$, $b = 10.417 \pm 0.003$, and $c = 28.256 \pm 0.008$ Å, $\beta = 106.21 \pm 0.02^\circ$. The structure was solved by using a Patterson synthesis and an E map to locate one sulfur and two chlorine atoms. Positions for the remaining atoms were deduced from subsequent electron density syntheses. Thirty-seven of the 40 hydrogen atoms were located and their positions and isotropic temperature factors refined along with the positional and anisotropic thermal parameters for the other atoms. The final residual R was 0.055 for 2983 observed reflections. The two independent molecules assume different geometries. In addition, the thiophene ring in one molecule is physically disordered. Equivalent bond lengths in the two molecules are equal, except for those involving the disordered thiophene group. The nitrogen atom at the center of the molecule is in the sp^2 hybridization state. Both molecules adopt the trans configuration about the carbon-carbon bond of the ethylamine side chain (as was found for histamine itself), thus adding support for the competitive site-occupancy theory of antihistaminic activity.

Although the mechanism of antihistamine activity has not been clearly established, one view is that antihistamines compete with histamine for a receptor site.^{1,2} Nearly all antihistamines are alkylamines and can be classified into seven different groups on the basis of the other substituents.¹ If competition between histamine and antihistamines exists, then we might expect a structural or conformational similarity to exist between these two species. Therefore, structural studies of histamine and several antihistamines were undertaken to provide data on these important molecules. Our results on histamine have been reported,³ as well as a preliminary report⁴ on the antihistamine Histadyl,⁵ 2-[(2)-dimethylaminoethyl-2-thenylamino]pyridine hydrochloride (I).

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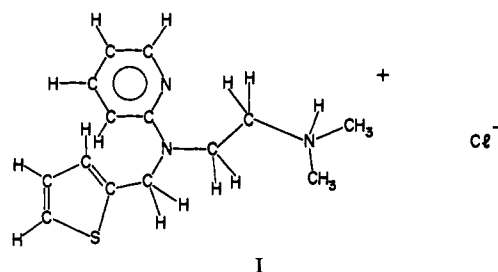
(1) R. E. Wilhelm, *Med. Clin. N. Amer.*, **45**, 887 (1961).

(2) C. Botre⁷, M. Marchetti, C. Del Vecchio, G. Lionetti, and A. Memoli, *J. Med. Chem.*, **12**, 832 (1969).

(3) M. V. Veidis, G. J. Palenik, R. Schaffrin, and J. Trotter, *J. Chem. Soc. A*, 2659 (1969).

(4) G. R. Clark and G. J. Palenik, *J. Amer. Chem. Soc.*, **92**, 1777 (1970).

(5) Histadyl is the registered trade name for the compound 2-[(2)-dimethylaminoethyl-2-thenylamino]pyridine hydrochloride.



Experimental Section

A sample of 2-[(2)-dimethylaminoethyl-2-thenylamino]pyridine hydrochloride, $C_{14}H_{20}N_2S \cdot HCl$, $M = 297.85$, was generously supplied by Eli Lilly and Co. The compound was recrystallized as transparent colorless plates on [001] by cooling from a hot isopropyl alcohol solution. Preliminary investigations by Rose and Williams⁶ had found the monoclinic space group $P2_1/n$, with $a = 27.35$, $b = 10.38$, and $c = 10.96$ Å, $\beta = 96^\circ$, $Z = 8$, $\rho_{\text{obsd}} = 1.273$ g cm^{-3} . For the present study the equivalent space group $P2_1/c$ (no. 14) was chosen and the cell dimensions were redetermined. A small crystal ($0.10 \times 0.05 \times 0.05$ mm) was mounted on the

(6) H. A. Rose and J. G. Williams, *J. Amer. Pharm. Ass.*, **48**, 487 (1959).