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Ring Stacking Interactions between Thiamin and Planar Molecules as Seen in the Crystal Structure of a Thiamin Picrolonate Dihydrate Complex

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Abstract: Thiamin has been crystallized as the salt of picrolonic acid, 3-methyl-4-nitro-1-(*p*-nitrophenyl)-2-pyrazolin-5-one. The structure, which was determined by x-ray diffraction techniques, shows that the picrolonate anion forms two different types of stacking interactions with the neutral pyrimidine ring of thiamin but does not exhibit any planar overlap with the positively charged thiazolium ring. Even though the stacking interactions dominate the crystal packing, thiamin maintains the characteristic F conformation. The analysis of this structure supports the inherent stability of the F conformation. The crystal structure was determined using diffractometer data obtained by the θ–2θ scan technique with Cu radiation from a crystal with space group symmetry $P\bar{1}$ and unit cell parameters a = 10.730 (3), b = 11.161 (2), c = 12.719 (3) Å; α = 108.50 (7), β = 100.62 (10), and γ = 107.88 (7)°. The structure was solved by direct methods and refined by full-matrix least squares to a final R = 0.064 for all 4182 independent reflections and R = 0.045 for the 3142 observed reflections.

Thiamin is a precursor of thiamin pyrophosphate (TPP) which is a coenzyme for enzyme systems catalyzing the transfer of aldehyde or acyl groups such as pyruvate decarboxylase and transketolase. Neither the mode of coenzyme binding nor the nature of the enzyme catalytic site is completely known, although a number of investigators have probed various aspects of this problem. The current understanding of the coenzyme binding has emerged from studies of pyruvate decarboxylase or transketolase using TPP analogues and inhibitors,[2-5] from the UV and CD spectra of the TPP–transketolase complex,[6-8] and from studies of the interaction between thiamin and indoles using NMR and UV techniques.[9,10] Although lacking in detail, the binding of the coenzyme to the enzyme is generally considered to involve an ionic interaction with the pyrosphosphate group, a hydrophobic interaction with the 2′- and 4-methyl substituents, and a charge-transfer interaction between the positively charged thiamin and tryptophan residues in the enzyme.

In the studies which originally suggested the charge-transfer interaction, Biaglow et al.[9] concluded that both the pyrimidine and the thiazolium rings are involved in π–π interactions with indoles which serve to delocalize the positive charge on thiamin. In order to account for the details of the interaction of the indole with both rings of thiamin in solution, thiamin was pictured as assuming a V conformation ($\phi_T \approx \pm 90°$; $\phi_P \approx \mp 90°$)[11] which, on the average, formed a close contact between the six-membered ring of indole and the methylene bridge carbon at the apex of the V. The V conformation, with the 4′-amino group adjacent to the active C(2) on the thiazolium ring, is the form first proposed by Schellenberger[2,12] as the active form of the enzyme bound coenzyme. Results from more than a dozen crystal structures present a different picture for the preferred conformation of thiamin with respect to its C(3,5′) methylene bridge. Basically two conformations have been observed. The S form ($\phi_T \approx \pm 100°$, $\phi_P \approx \pm 150°$) is characteristic of thiamin when C(2) is substituted; the F form ($\phi_T \approx 0°$; $\phi_P \approx \pm 90°$) is characteristic of thiamin when C(2) is free of substituents. These results are also at variance with theoretical calculations which indicate a relatively large apparent freedom of rotation about the two bonds to the methylene bridge carbon, especially when C(2) is unsubstituted.[13]

In an effort to help resolve the conflicting viewpoints about the thiamin conformation and to investigate the parameters controlling its conformation, we have been attempting to crystallize thiamin with molecular fragments that could possibly associate with TPP in the enzyme binding site and to observe how they interact and how such molecules might influence the conformation of thiamin. Tryptophan and other indoles are compounds of obvious interest. Although suitable crystalline complexes have not yet been obtained with any of the indoles, thiamin does crystallize readily with picrolonic acid, 3-methyl-4-nitro-1-(*p*-nitrophenyl)-2-pyrazolin-5-one, in the form of its anion. Picrolonate has been used as a reagent for the identification and assay of thiamin[14] and it has the apparent structural characteristics[15] that enable it to form a molecular complex with thiamin. This structure does provide the opportunity to examine the influence that would be exerted on the thiamin conformation by incorporating a large organic anion into the crystal structure and to examine the mode of association between thiamin and a planar, heterocyclic ring system.

Experimental Section

Pale yellow, bladed crystals grew from an approximately equimolar mixture of thiamin chloride and sodium picrolonate in aqueous solution when it was allowed to stand open to the atmosphere at room temperature. This procedure was adapted from a method for thiamin analysis described by Alexandrova and Alexandrov.[14] The thiamin chloride was prepared by dissolving thiamin chloride hydrochloride (Nutritional Biochemicals) in water, titrating with 1 equiv of sodium hydroxide, and precipitating with acetone at 0 °C. The precipitate was collected by suction filtration, washed with aqueous acetone and acetone, and then air-dried at room temperature. The sodium picrolonate was prepared by dissolving picrolonic acid (Sigma) in water and ti-

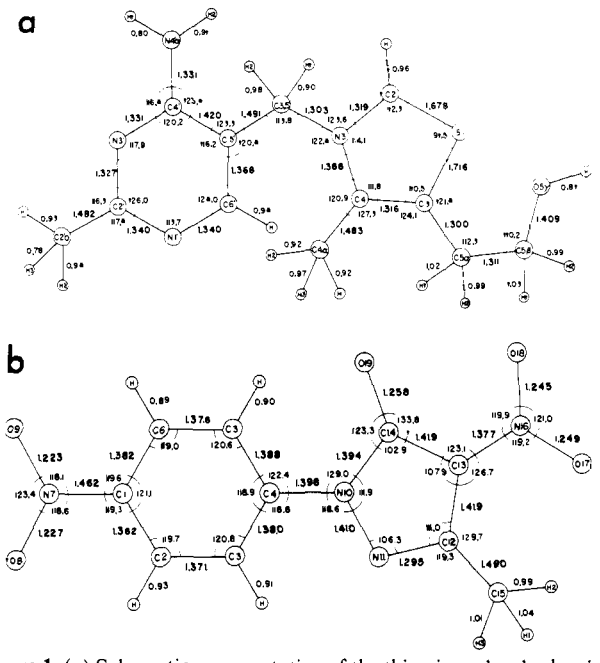

Figure 1. (a) Schematic representation of the thiamin molecule showing the atomic numbering scheme, the bond distances (Å), and valence angles (deg). The esd's for bonds between nonhydrogen atoms range from 0.003 to 0.005 and average 0.004, while those involving hydrogen vary from 0.03 to 0.04 with an average value of 0.03. The esd's for the angles between nonhydrogen atoms span the range from 0.1 to 0.3 and average 0.2. Hydrogen atoms in the text are assigned the same numerical designation as the atom to which they are bonded. When more than one hydrogen is bonded to the same atom, the hydrogen sequence number shown in the figure is appended to the numerical designation. Hence, the amino hydrogens are designated as H(4′α_1) and H(4′α_2). (b) Schematic representation of the picrolonate molecule showing the atomic numbering scheme, the bond distances (Å), and valence angles (deg). The esd's are the same as stated for the thiamin molecule. All references in the text to picrolonate atoms have the suffix P appended to the atom designation in the figure. Hydrogen numbering follows the same scheme as described for the thiamin molecule.

trating with 1 equiv of sodium hydroxide. No attempt was made to isolate the sodium picrolonate but thiamin chloride was added to this solution.

The resulting crystals were triclinic as determined from oscillation and Weissenberg photographs. The space group which was initially assumed to be $P\bar{1}$ was confirmed later in the crystal structure determination and refinement. The unit cell parameters were determined by a least-squares fit of the orientation and 2θ angles for 12 reflections measured with Cu Kα radiation on a Picker FACS-1 diffractometer.[16] The values used for each reflection were the average of four separate measurements taken at ($\pm 2\theta$, χ) and ($\pm 2\theta$, 180° + χ). All of the pertinent crystal data are summarized in Table I. The intensity data were collected with graphite monochromated Cu Kα radiation on a Picker FACS-1 x-ray diffractometer from a crystal which was 0.8 mm along a, 0.096 mm along (0, 1, $\bar{1}$), and 0.016 mm along (0, 1, 1). The crystal was mounted with the a axis approximately parallel to the φ axis and the reflection data were collected by θ–2θ scan technique over a scan range of 1.7° at a scan rate of 1°/min and a 20-s background count at each end of the scan range.

Three standard reflections were monitored after each 50 data reflections. The intensities of the standard reflections showed gradual fluctuations of less than ±3% throughout the entire data collection. At the completion of the data collection those reflections showing a background ratio greater than 3.0 were remeasured with a scan range of 2.0° plus a variable amount to accommodate the wavelength dispersion. The intensity data were converted to relative structure factor amplitudes after correction for Lorentz and polarization effects appropriate for graphite-monochromated ($2\theta_m$ = 26.16°) radiation as the intensity data were collected.[16] The $|F|$'s were scaled to correct for the gradual fluctuation of the standards.[17a] Of the 4182 independent reflections measured within the range of $\theta \leq 63.5°$, 1040

Table I. Crystal Data for Thiamin Picrolonate Dihydrate

$[C_{12}H_{17}N_4OS]^+[C_{10}H_7N_4O_5]^-\cdot 2H_2O$; mol wt 564.56
a = 10.730 (3), b = 11.161 (2), c = 12.719 (3) Å
α = 108.50 (7), β = 100.62 (10), γ = 107.88 (7)°
V = 1306.0 Å^3
D_m = 1.438 (2) g cm^{-3} by flotation in CCl_4-CH_2Cl_2
D_c = 1.435 (3) g cm^{-3}
Z = 2; $F(000)$ = 592
μ(Cu Kα) = 16.6 cm^{-1}; space group $P\bar{1}$
mp 120 °C dec (loss of water of crystallization apparent at ≈102 °C)

[a] Cell parameters at 23 °C.

(25%) were considered unobserved, as defined by $|F| \leq 6\,\sigma(F)$.[17b] No correction for the absorption and the extinction effects was made.

Structure Determination and Refinement. The structure was solved with the program MULTAN[18] assuming that the space group is $P\bar{1}$. From the initial E map it was possible to identify the positions of all 37 nonhydrogen atoms excluding the oxygen atoms of the two water molecules. Three cycles of isotropic full-matrix least-squares refinement (ORFLS) reduced the conventional R value from 0.377 to 0.243 and a subsequent difference Fourier map gave the positions of the remaining two oxygen atoms. After three further cycles of refinement, which lowered the R value to 0.110, 24 of the hydrogen atoms were located in a difference Fourier map. The function minimized in the refinement was $\Sigma\omega(|F_o| - k|F_c|)^2$ where k is a single scale factor and $\omega = 1/\sigma^2(|F_o|)$ where $\sigma(|F_o|)$ is the standard deviation in $|F_o|$ based on counting statistics. During the final phase of the refinement the thermal parameters of the nonhydrogen atoms were refined anisotropically, the anomalous dispersion correction was applied for the S atom, the four remaining H atoms were located and included in the analysis, and ω was defined by $1/(A + B|F_o| + C|F_o|^2)$ where A = 5.0, B = 1.0, and C = 0.18357 were empirically adjusted. The H thermal parameters were not refined but were assigned as the isotropic equivalents of the atoms to which they were bonded. The atomic scattering factors for S, O, N, and C are from Cromer and Waber[19] and that for H is from Stewart et al.[20] The $\Delta f'$ and $\Delta f''$ values for S are from the International Tables.[21] The final R value was 0.045 for the 3142 observed reflections and 0.064 over all 4182 reflections. The weighted R was 0.061.

In the final cycle of refinement only nine of the 436 parameters refined had shifted by more than one esd with the largest being 1.44 esd for C(14P). The final difference Fourier was relatively clean. The most prominent feature was a characteristically observed series of peaks and holes clustered around the S atom position. The largest ones had densities of ≈0.35 e/Å^3 and ≈−0.78 e/Å^3. The largest peak not associated with the sulfur position was ≈0.14 e/Å^3. The final atomic parameters are listed in Table II. The structure factor table is available as supplementary material (see paragraph at end of paper regarding supplementary material). The atomic numbering scheme for both molecules is given in Figure 1.

Description of the Structure

Thiamin Molecule. Although thiamin exists in this complex as the unprotonated base, there are no distinctive differences in its structural features when compared with the protonated thiamins having the F conformation. The molecular dimensions of the thiamin molecule in this structure (Figure 1, a), for which the pyrimidine N(1′) is deprotonated, are in good agreement with those of thiamin chloride monohydrate (TCM),[22] the only other thiamin structure in this state of ionization. The N(1′)–C(2′) and C(2′)–N(3′) bond distances in the pyrimidine ring show a difference of 0.01 Å from those of TCM. These might be induced by the different environment around N(1′), in that N(1′) of this structure forms a hydrogen bond with O(5γ) whereas N(1′) of TCM does not form any hydrogen bond. The two methyl groups, C(2′α) and C(4α), have bond distances of 1.482 and 1.483 Å, respectively. Although these bonds are shorter than expected for an sp^2–sp^3 C–C single bond,[23,24] no critical analysis can be made because the bond length values have not been corrected for thermal

Table II. Fractional Coordinates and Temperature Factors for Thiamin Picrolonate Dihydrate

A. Nonhydrogen Atoms[a]

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
Thiamin									
S(1)	−301 (0.7)	7850 (0.7)	203 (0.5)	49 (0.3)	53 (0.4)	51 (0.3)	13 (0.3)	17 (0.3)	12 (0.3)
C(2)	299 (3)	6803 (3)	−621 (2)	46 (1)	44 (1)	39 (1)	8 (1)	8 (1)	11 (1)
N(3)	1649 (2)	7362 (2)	−375 (2)	42 (1)	36 (1)	31 (1)	9 (1)	7 (1)	11 (1)
C(4)	2271 (2)	8667 (2)	515 (2)	47 (1)	34 (1)	35 (1)	8 (1)	9 (1)	14 (1)
C(4α)	3792 (3)	9407 (3)	946 (3)	51 (2)	43 (2)	54 (2)	7 (1)	9 (1)	10 (1)
C(5)	1330 (3)	9101 (2)	917 (2)	55 (1)	41 (1)	40 (1)	12 (1)	17 (1)	17 (1)
C(5α)	1585 (4)	10445 (3)	1861 (3)	70 (2)	39 (1)	64 (2)	13 (1)	30 (2)	16 (1)
C(5β)	1257 (3)	10281 (3)	2925 (2)	65 (2)	42 (1)	49 (2)	19 (1)	16 (1)	9 (1)
O(5γ)	−174 (2)	9586 (2)	2662 (2)	66 (1)	47 (1)	77 (1)	29 (1)	33 (1)	32 (1)
C(3,5′)	2452 (3)	6675 (3)	−1014 (2)	44 (1)	42 (1)	41 (1)	15 (1)	3 (1)	12 (1)
N(1′)	688 (2)	2905 (2)	−2852 (2)	60 (1)	38 (1)	47 (1)	21 (1)	19 (1)	19 (1)
C(2′)	160 (2)	2943 (2)	−3875 (2)	48 (1)	37 (1)	40 (1)	18 (1)	18 (1)	12 (1)
C(2′α)	−606 (4)	1614 (3)	−4895 (3)	83 (2)	40 (1)	48 (2)	27 (1)	16 (1)	10 (1)
N(3′)	258 (2)	4066 (2)	−4064 (2)	51 (1)	33 (1)	37 (1)	18 (1)	12 (1)	12 (1)
C(4′)	953 (2)	5294 (2)	−3152 (2)	40 (1)	34 (1)	38 (1)	16 (1)	13 (1)	14 (1)
N(4′α)	1001 (3)	6399 (2)	−3364 (2)	70 (1)	35 (1)	39 (1)	17 (1)	4 (1)	12 (1)
C(5′)	1573 (2)	5359 (2)	−2037 (2)	40 (1)	38 (1)	39 (1)	14 (1)	11 (1)	14 (1)
C(6′)	1409 (3)	4128 (3)	−1960 (2)	52 (1)	43 (1)	38 (1)	18 (1)	11 (1)	18 (1)
Picrolonate									
C(1P)	6882 (3)	3278 (3)	6111 (2)	49 (1)	74 (2)	61 (2)	32 (1)	23 (1)	38 (1)
C(2P)	7344 (3)	4666 (3)	6457 (3)	53 (2)	70 (2)	54 (2)	18 (1)	2 (1)	25 (1)
C(3P)	7054 (3)	5208 (3)	5660 (2)	54 (2)	53 (2)	54 (2)	12 (1)	4 (1)	20 (1)
C(4P)	6299 (2)	4364 (2)	4510 (2)	42 (1)	51 (1)	50 (1)	18 (1)	16 (1)	21 (1)
C(5P)	5838 (3)	2951 (3)	4170 (3)	80 (2)	55 (2)	53 (2)	20 (1)	12 (1)	19 (1)
C(6P)	6113 (3)	2401 (3)	4969 (3)	79 (2)	58 (2)	76 (2)	28 (2)	24 (2)	32 (2)
N(7P)	7201 (3)	2709 (3)	6967 (3)	66 (2)	88 (2)	82 (2)	42 (1)	32 (1)	52 (2)
O(8P)	8011 (3)	3497 (3)	7941 (2)	87 (2)	124 (2)	77 (2)	43 (1)	14 (1)	59 (2)
O(9P)	6649 (3)	1465 (3)	6669 (2)	106 (2)	96 (2)	109 (2)	54 (2)	38 (2)	67 (2)
N(10P)	6032 (2)	4963 (2)	3728 (2)	47 (1)	47 (1)	49 (1)	11 (1)	9 (1)	19 (1)
N(11P)	6672 (2)	6402 (2)	4112 (2)	45 (1)	43 (1)	62 (1)	8 (1)	6 (1)	20 (1)
C(12P)	6166 (2)	6700 (3)	3270 (2)	38 (1)	48 (1)	66 (2)	11 (1)	13 (1)	24 (1)
C(13P)	5178 (2)	5500 (3)	2315 (2)	43 (1)	56 (1)	48 (1)	16 (1)	12 (1)	24 (1)
C(14P)	5071 (3)	4361 (3)	2618 (2)	50 (1)	48 (1)	46 (1)	11 (1)	13 (1)	16 (1)
C(15P)	6652 (4)	8160 (3)	3408 (4)	64 (2)	52 (2)	96 (3)	7 (1)	−2 (2)	32 (2)
N(16P)	4354 (2)	5445 (2)	1319 (2)	61 (1)	62 (1)	60 (1)	23 (1)	12 (1)	27 (1)
O(17P)	4563 (2)	6517 (2)	1140 (2)	79 (1)	85 (1)	96 (2)	26 (1)	10 (1)	59 (1)
O(18P)	3394 (3)	4342 (2)	625 (2)	94 (2)	69 (1)	68 (1)	17 (1)	−16 (1)	18 (1)
O(19P)	4358 (2)	3114 (2)	2114 (2)	99 (2)	45 (1)	55 (1)	6 (1)	3 (1)	15 (1)
Water									
O(W1)	3767 (3)	1711 (2)	−237 (2)	107 (2)	55 (1)	69 (1)	21 (1)	17 (1)	15 (1)
O(W2)	8092 (3)	775 (2)	1866 (2)	120 (2)	44 (1)	72 (1)	34 (1)	−18 (1)	10 (1)

B. Hydrogen Atoms[b]

Atom	x	y	z	U	Atom	x	y	z	U
Thiamin					Thiamin				
H(2)	−25 (3)	588 (3)	−117 (2)	49	H(4′α$_2$)	141 (3)	725 (3)	−279 (3)	54
H(4α$_1$)	422 (3)	893 (3)	125 (3)	58	H(6′)	184 (3)	410 (3)	−126 (2)	48
H(4α$_2$)	414 (3)	939 (3)	34 (3)	58	Picrolonate				
H(4α$_3$)	410 (3)	1035 (3)	151 (3)	58	H(2P)	783 (3)	524 (3)	726 (3)	68
H(5α$_1$)	99 (3)	1087 (3)	152 (3)	61	H(3P)	734 (3)	614 (3)	592 (3)	63
H(5α$_2$)	255 (3)	1109 (3)	208 (3)	61	H(5P)	533 (3)	240 (3)	343 (3)	69
H(5β$_1$)	178 (3)	975 (3)	320 (2)	57	H(6P)	581 (3)	150 (3)	477 (3)	76
H(5β$_2$)	156 (3)	1121 (3)	354 (3)	57	H(15P1)	705 (4)	827 (3)	274 (3)	91
H(5γ)	−34 (3)	880 (3)	271 (3)	66	H(15P2)	597 (4)	857 (3)	331 (3)	91
H(2′α$_1$)	−152 (3)	150 (3)	−518 (3)	62	H(15P3)	724 (4)	881 (4)	424 (3)	91
H(2′α$_2$)	−64 (3)	81 (3)	−479 (3)	62	Water				
H(2′α$_3$)	−20 (3)	142 (3)	−532 (3)	62	H(W11)	382 (4)	237 (4)	45 (3)	85
H(3,5′1)	300 (3)	652 (3)	−49 (2)	47	H(W12)	398 (4)	239 (4)	−51 (3)	85
H(3,5′2)	301 (3)	735 (3)	−126 (2)	47	H(W21)	750 (4)	5 (4)	126 (4)	87
H(4′α$_1$)	67 (3)	627 (3)	−403 (3)	54	H(W22)	857 (4)	56 (4)	217 (4)	87

[a] Estimated standard deviation in parentheses is for the least significant figure. Positional parameters × 10^4; thermal parameters, which are coefficients of the expression $\exp[-2\pi^2(h^2a^{*2}U_{11} + \ldots 2hka^*b^*U_{12} + \ldots)]$, × 10^3. [b] Positional parameters × 10^3; thermal parameter, which is the coefficient for the expression $\exp[-(8\pi^2U)\sin^2\theta/\lambda^2]$, × 10^3.

motion. However, it is of interest that these values fall between the even shorter C(2′)–C(2′α) in thiamin pyrophosphate[25] and Mg thiamin[26] where there are close contacts with neighboring electronegative atoms and the longer bond in TCM[22] where the methyl group is not in a polar environment. In this structure both methyl groups are surrounded by electronegative atoms

Table III. Selected Intermolecular and Intramolecular Contacts

Atom a	Atom b	Atom c	Distance, Å a-c	Distance, Å b-c	Angle, deg, a-b-c
			A. Hydrogen Bonds		
N(4′α)-H(4′α$_1$)···N(3′)[a]			3.107 (3)	2.30 (3)	179 (3)
N(4′α)-H(4′α$_2$)···O(W2)[b]			2.854 (4)	1.98 (4)	162 (3)
O(5γ)-H(5γ)···N(1′)[c]			2.762 (3)	1.90 (4)	179 (3)
O(W2)-H(W21)···O(W1)			2.720 (4)	1.87 (4)	166 (4)
O(W2)-H(W22)···O(5γ)[c]			2.814 (4)	2.10 (5)	167 (5)
O(W1)-H(W11)···O(19P)			2.742 (4)	1.91 (4)	148 (4)
O(W1)-H(W11)···O(18P)			2.971 (4)	2.33 (4)	126 (3)
O(W1)-H(W12)···O(17P)			2.911 (4)	2.12 (4)	144 (4)
C(2)-H(2)···O(8P)[d]			3.389 (4)	2.47 (3)	161 (3)
			B. Close Contacts around S(1)		
C(2)-S(1)···O(5γ)				3.064 (2)	147.4 (2)
C(5)-S(1)···O(5γ)					71.0 (2)
C(2)-S(1)···O(18P)[c]				3.194 (3)	96.4 (2)
C(5)-S(1)···O(18P)[c]					168.7 (2)
			C. Miscellaneous Contacts		
C(3,5′)-H(3,5′1)···O(17P)			3.306 (4)	2.42 (3)	170 (3)
C(5P)-H(5P)···O(19P)			2.887 (4)	2.28 (3)	125 (3)
C(2′α)-H( )···C(15P)[e]			3.686 (6)		

[a] x, y, z transformations: $-x, 1-y, -1-z$; [b] $x, 1+y, z$; [c] $-x, 1-y, -z$; [d] $-1+x, y, -1+z$; [e] $-1+x, -1+y, -1+z$.

Table IV. Least-Squares Planes and Dihedral Angles[a]

Plane		A	B	C	D	σ	Displacements
1	Thiazolium	−1238	8066	−11154	6145	11	**S(1) −3, C(2) −2, N(3) 8, C(4) −10, C(5) 8,** C(3,5′) 66, C(4α) −82, C(5α) 7, H(2) −63, O(5γ) −1361, O(18P)[b] −464
2	Pyrimidine	10339	−1824	−5575	1785	10	**N(1′) −14, C(2′) 5, N(3′) 6, C(4′) −8, C(5′) −1, C(6′) 12,** C(2′α) 23, N(4′α) −42, C(3,5′) 98, H(6′) 78, H(4′α$_1$) 13, H(4′α$_2$) −93
3	Pyrazolonate	9841	−2641	−6922	2035	11	**N(10P) 10, N(11P) −6, C(12P) 1, C(13P) 5, C(14P) −9,** C(4P) −110, C(15P) −3, N(16P) −101, O(17P) −55, O(18P) − 274, O(19P) −32
4	Phenyl	10422	−2184	−5246	3247	5	**C(1P) 4, C(2P) 0, C(3P) −2, C(4P) −1, C(5P) 5,** C(6P) −7, N(7P) 12, O(8P) 172, O(9P) −136, N(10P) O, H(2P) −40, H(3P) −48, H(5P) −17, H(6P) −17
5	Nitro (N7P)	10181	−3126	−5900	2376	2	**C(1P) 1, N(7P) −2, O(8P) 1, O(9P) 1**
6	Nitro (N16P)	9306	−3191	−7631	1299	10	**C(13P) −3, N(16P) 9, O(17P) −3, O(18P) −3**

Planes	Dihedral angle, deg	Planes	Dihedral angle, deg	Planes	Dihedral angle, deg
1 and 2	77.5	2 and 3	10.13	3 and 4	10.31
		2 and 4	2.01	3 and 6	6.73
		2 and 5	8.28	4 and 5	7.51
				5 and 6	10.24

Intermolecular Distances × 10^3 (Å) of Atoms from Least-Squares Planes

Atom	Plane 2	Atom	Plane 2	Atom	Plane 4	Atom	Plane 3
C(1P)	3438	C(4P)	3527	N(10P)	3502	C(1P)	3434
C(2P)	3407	C(5P)	3558	N(11P)	3653	C(2P)	3282
N(7P)	3482	N(10P)	3478	C(12P)	3503	C(3P)	3406
O(8P)	3332	N(11P)	3292	C(13P)	3235		
		C(12P)	3400	C(14P)	3214		
		C(13P)	3671	N(16P)	2912		
		C(14P)	3743	O(17P)	2988		
		C(15P)	3241				
		O(19P)	3971				

[a] The coefficients × 10^3 are given for the planes which are expressed by the equation $Ax + By + Cz = D$ where x, y, and z are in fractional unit cell coordinates. The displacements of the atoms from the plane are in Å × 10^3. Atoms used to define the plane are in boldface type. σ is the root mean square displacement of atoms defining the plane. [b] $-x, 1-y, -z$.

Table V. Comparison of Torsion Angles in Thiamin Structures

Compound	ϕ_T	ϕ_P	$\phi_{5\alpha}$	$\phi_{5\beta}$	Ref
F forms					
Thiamin picrolonate dihydrate	6.0	82.5	65.9	−68.2	This paper
Thiamin Cl·HCl·H_2O	−9.0	−76.1	103.4	−53.8	28
Thiamin Br·HBr·0.5H_2O	−2	−77	86	64	29
Thiamin I·HI	−6	81	62	64	30
Thiamin Cl·H_2O	−2.6	−76.8	66.8	64.6	22
Thiamin·$CuCl_4$	−14.1	−82.6	97.2	−60.6	31
Thiamin Cl·0.5[UO_2Cl_4]	5.4	−83.7	79.8	57.1	32
Thiamin Cl·HCl·0.5[$Mg(H_2O)_6$]Cl·2H_2O	−2.0	−77.4	105.1	−53.5	26
Thiamin-PO_3H·H_2PO_4·3H_2O	−6.6	−85.4	76.4	66.1	33
Thiamin-$P_2O_6H_2$·HCl	2.7	93.1	92.2	−62.9	25
Thiamin-P_2O_6H·4H_2O (A)	5.4	85.8	58.5	−66.1	27
Thiamin-P_2O_6H·4H_2O (B)	20.5	100.5	40.5	−68.6	27
Thiamin-P_2O_6H·4.5H_2O	8.6	92.4	53.7	−65.3	27
S forms					
Thiamin·$CdCl_4$·H_2O	110.4	137.3	83.0	67.8	34
2-(α-Hydroxyethyl)thiamin Cl·HCl	−100.3	−145.6	81.8	30.1 (−66.7)[a]	35
2-(α-Hydroxybenzyl)thiamin Cl·HCl·3H_2O	92.7	−167.3	3.3	63.4	36
V forms					
Oxythiamin Cl·HCl·H_2O (A)	105.5	−62.8	61.9	−66.8	37
Oxythiamin Cl·HCl·H_2O (B)	101.5	−64.2	68.7	−66.3	37
Thiochrome·2H_2O	−0.7	−1.9	32.2	−69.3	38
Av abs value F conf (rms)	7.0 (5.3)	84.2 (7.4)	76.0 (20.2)	62.7 (5.0)	
Av abs value S conf (rms)	101.1 (8.9)	150.1 (15.5)	56.0 (45.7)	57.0 (18.0)	
Av abs value all compds (rms)			69.4 (25.4)	62.3 (8.8)	

[a] Value for disordered oxygen position.

but the distances are not less than van der Waals contacts (Table III).

All the bond distances in the thiazolium ring are in good agreement with those of other thiamin structures. Least-squares planes of the thiazolium and pyrimidine rings are given in Table IV. The planarities are good within the range of the experimental error. The torsion angles, ϕ_T and ϕ_P, are 6.0 and 82.5°, respectively, in comparison with the respective values of −2.6 and −76.8° for TCM. The conformation of the 5-(β-hydroxyethyl) side chain is quite different from that of TCM. One aspect of this difference is apparent from the opposite signs for ϕ_P which indicates that in this structure O(5γ) and N(4′α) are "anti" whereas in TCM they are "syn" related.[11b] Prior to this structure, all thiamins with the F conformation were "syn" except for the three thiamin pyrophosphate structures[25,27] which were all "anti". There are no indications at present whether or not this structural feature has any biological or mechanistic significance. However, the disposition of the C(5) side chain does appear to be readily influenced by the hydrogen-bonding interactions of O(5γ). This variability is reflected in the torsion angles $\phi_{5\alpha}$ = [S(1)-C(5)-C(5α)-C(5β)] and $\phi_{5\beta}$ = [C(5)-C(5α)-C(5β)-O(5γ)] as seen in different thiamin structures listed in Table V.

Picrolonate Molecule. The structure of picrolonic acid has not previously been determined. Structural features of the picrolonate anion therefore must be compared with compounds that are similar to picrolonic acid. Those which have the greatest structural similarity are the antipyrines, 1-phenyl-2,3-dimethyl-5-pyrazolones,[39-41] and several other pyrazolones[42] and pyrazoles.[43,44] The molecular dimensions of picrolonate are shown in Figure 1, b. Although it is less meaningful to calculate bond orders and the contributions of canonical resonance forms on the basis of bond distances that are not corrected for thermal vibration, the bond orders based on uncorrected distances still show some characteristic features of the molecule. For example, the negative charge that results from the ionization of the proton on N(11P) is distributed over the N(16P) nitro and O(19P) keto groups, through the C(13P)-C(14P) segment of the ring. The C(13P)-N(16P) bond to the nitro group at 1.377 Å shows considerable double bond character and contrasts markedly with the C(1P)-N(7P) bond (1.462 Å) of the phenyl nitro group which is typical of the single bond length usually observed. The C(14P)-O(19P) bond (1.238 Å) is significantly shorter than that seen in most of the pyrazolone structures which indicates an increased double bond character. This bond length is typical of that in the peptide carbonyl. While the C(13P)-C(14P) bond is somewhat longer than that in most of the pyrazolones, it is not unusual. However, what is unusual is the equivalent lengths for the C(12P)-C(13P) and C(13P)-C(14P) bonds. The N(10P)-N(11P) bond at 1.410 Å and N(11P)-C(12P) bond at 1.295, which are more nearly a single and double bond, respectively, indicate a greater electron localization than occurs in 1-phenyl-3-methyl-5-pyrazolone,[42a] the most closely related of the pyrazolones. The bond to the C(15P)-methyl substituent (1.490) is slightly shorter than expected for an sp^2-sp^3 single bond.[23,24] Some of the shortening undoubtedly results from thermal motion but the crystalline environment may also have an influence as was discussed above for the methyl substituents on thiamin. Although the C(4P)-N(10P) bond (1.398 Å) is slightly shorter than in the other phenylpyrazolones, it does not represent a significant electron delocalization between the two rings.

The least-squares planes for the picrolonate molecule are given in Table IV. The tabulated atomic deviations indicate that the pyrazolonate ring as well as the phenyl ring is essentially planar. It is not clear whether the higher degree of planarity observed in this pyrazolonate ring is a consequence of the specific ring substitution and ionization or whether this is influenced by the molecular packing. Clearly, the packing has

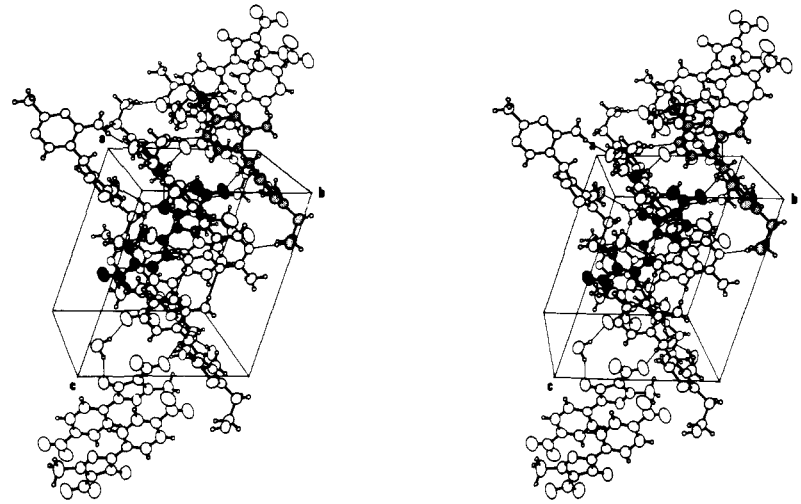

Figure 2. Stereoscopic packing diagram of thiamin picrolonate structure as viewed approximately down the columns of the overlapped rings. Hydrogen bonds are shown as single solid lines. The shaded atoms correspond to those for which coordinates are listed in Table II, A. Picrolonate and O(W1) are designated by the heavy shading; thiamin and O(W2) are designated by light shading.

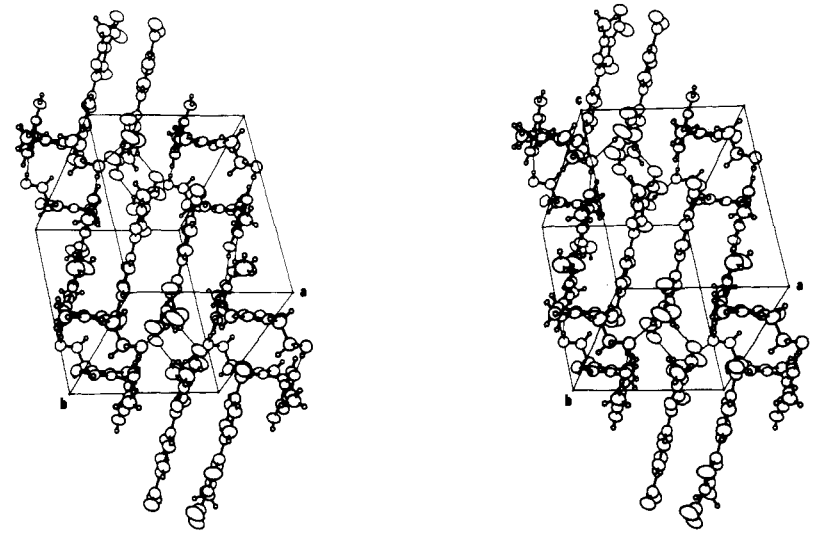

Figure 3. Stereoscopic packing of the structure as viewed perpendicular to the columns of the overlapped rings. In this view the thiazolium rings, which are also seen edge on, are observed to be a part of a sheet that separates columns of stacked rings.

influenced the dihedral angle between the planes. The phenyl–pyrazolonate dihedral angle, which is 10.31°, is substantially smaller than the usually observed values which have been reported in the range from 19.6[42] up to 73.10°.[40] There is not sufficient double bond character in the C(4P)–N(10P) bond to provide any significant barrier to rotation. The planar stacking (discussed in greater detail below) seen in picrolonate is an uncommon feature in the phenylpyrazolone structures. In a recent antipyrine complex,[40] there is a partial overlap between half molecules where coplanarity of the rings is not required. An interesting consequence of the near coplanarity of the rings in the present structure is the close intramolecular contact (2.887 Å) between the O(19P) oxygen atom and C(5P) of the phenyl ring. The O(19P)···H(5P) contact is also short at 2.28 Å with C(5P)–H(5P)···O(19P) forming an angle of 125°.

Packing and Hydrogen Bonding. Figures 2 and 3 show the stereoscopic ORTEP[45] packing drawings of the structure. A feature of significance in this structure is the planar stacking which exhibits three types of ring interactions as it extends through the crystal in a column as –A–B–B′–A–B–B′···. The thiamin and picrolonate molecules each form individual molecular layers and these layers overlap in a direction approximately parallel to the *a* axis. The minimal repeating unit can be considered as the two picrolonate molecules related by the center of symmetry at (½, ½, ½) and the two thiamin molecules related by the center of summetry at (0, ½, ½). The two centrosymmetrically related pyrimidine rings, connected by a pair of N(4′α)···N(3′) hydrogen bonds, lie on top of one picrolonate molecule which results in two different types of stacking interaction. One type is seen in the pyrimidine–nitrophenyl overlap (Figure 4, a) and the other is seen in the pyrimidine–pyrazolonate overlap (Figure 4, b). This picrolonate molecule is in turn stacked with its centrosymmetrically related picrolonate molecule in a fashion shown in Figure 4, c. If just half of the picrolonate molecule is considered at one time, then the stacking pattern described above becomes more readily apparent. Table IV shows the distance of each atom from the least-squares planes of the overlapped rings. The intermolecular distances between individual atoms in the stacked planes are included in Figure 4. The dihedral angles between the least-squares planes are shown in Table IV. In this structure the staggered or partial overlap seen in Figures 4, a, and 4, c, is typical of the dipole-induced dipole interactions described by Bugg et al.[15] for this type of compound. The interaction seen in Figure 4, b, is much less frequently observed in ring stacking but resembles charge-transfer type structures. However, the contact distances are not as short as expected for charge-

transfer interactions.[46] In fact, the self-association between picrolonate anions appears closer than either of the pyrimidine–picrolonate contacts. The C(2P) atom of the benzene ring is significantly close to the pyrazolonate ring (3.282 Å) and its interatomic distances with C(13P) and C(14P) of the pyrazolonate ring are 3.350 and 3.363 Å, respectively. This close contact and the shortening of the bonds to the C(2P) atom may be related phenomena. All other contacts are the normal van der Waals contacts. It is interesting that only the formally neutral pyrimidine ring of the thiamin molecule takes part in the plane stacking and that the positively charged thiazolium ring does not stack with the picrolonate anion.

The view of the structure as seen in Figure 3 shows two parallel columns of stacked rings running along the direction of the *a* axis. The columns are separated by a plane, nearly parallel to the *xy* plane, which contains the thiazolium rings, water molecules, and the nitro groups that connect all the molecules by hydrogen bonding in the directions along the *a* and *b* axes. There are seven unique hydrogen bonding schemes in the structure which are tabulated in Table III. In each thiamin layer all the thiamin molecules are connected by three different types of cyclic dimers through centrosymmetrically related hydrogen bonds; N(4′α) → N(3′) related by (0, ½, ½), O(5γ) → N(1′) related by (0, ½, 0), and N(4′α) → O(W2) → O(5γ) related by (0, 0, 0). In each picrolonate layer two picrolonate molecules are linked together by a pair of O(17P) ← O(W1) → O(19P) hydrogen bonds related by (½, ½, 0). Hence, this hydrogen bonding network links the stacked pairs of picrolonate anions along the direction of the *c* axis. Along the *b* direction there are only van der Waals contacts between picrolonates. Two water molecules which are involved in the hydrogen bonds with thiamin and picrolonate molecules additionally form O(W2) → O(W1) hydrogen bonding so that there is a three-dimensional hydrogen bonding network. The two water molecules form a distorted trigonal configuration. One hydrogen atom, H(W11), of O(W1) may form a bifurcated hydrogen bond although the bonding angle with O(18P) is poor.

As a consequence of the partial positive charge on the thiazolium S(1), there is a characteristic tendency for negative ions or electronegative atoms to form close contacts with sulfur in the plane of the ring along the directions of the C(2)–S(1) and C(5)–S(1) bonds.[35,47] This property is of particular importance in the C(2) adduct structures. Although the intramolecular association with O(5γ) was not present in the earlier thiamin structures, it has been observed in a number of more recent structures[36] and attests to the significance of this attractive interaction. Two such S···O interactions are present in this crystal structure: the S(1)···O(5γ) with a separation of 3.060 Å and the S(1)···O(18P) with a value of 3.194 Å.

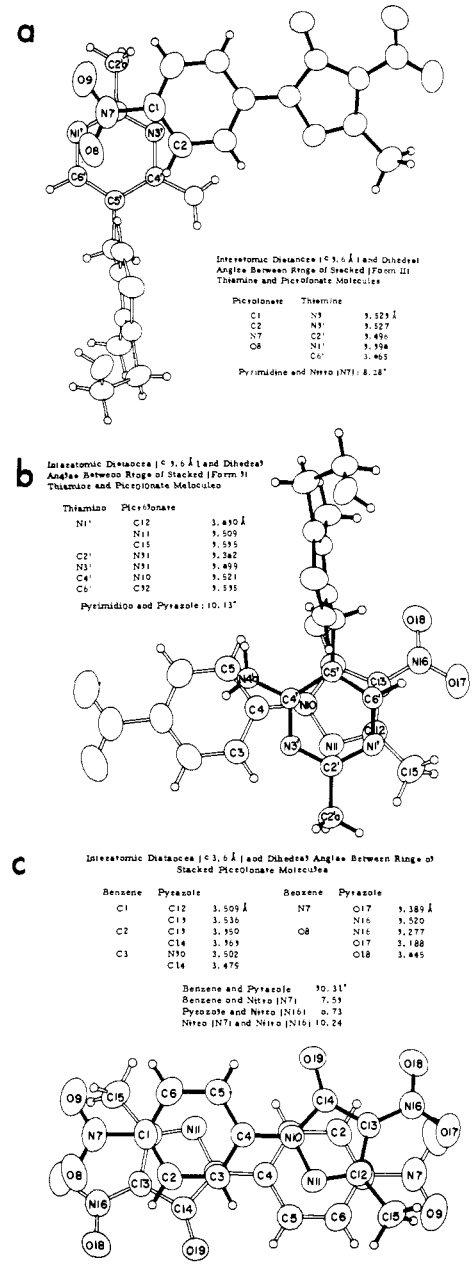

Figure 4. The three modes of ring stacking which are found in the crystal structure. Each pair is projected down the normal to the plane of the ring which is on top. The upper molecule is designated by the solid bonds. View a shows overlap between the pyrimidine and the nitrophenyl rings. View b shows the stacking of the pyrimidine and pyrazolonate rings. View c shows the overlap of the picrolonate molecules.

Discussion

In order to obtain a complete description of thiamin catalysis, it is necessary to understand the structural aspects of the molecular system. An important structural parameter in this system is the conformation of thiamin with respect to its C(3,5′) bridge atom. On the basis of molecular orbital calculations[13,48] or from an examination of molecular models[49] there is an apparent continuum of conformations having nearly equal stability. Even in the C(2) adducts of thiamin, where there is less freedom of rotation about the C(3,5′) bridge bonds, there is a considerable range of conformations that are apparently stable. With this apparent range of nearly equally probable conformations, it would be reasonable to expect that in different crystal structures containing thiamin a representative distribution of these conformations would be found. However, a quite contrary picture is seen from the many thiamin structures that have been determined. In these structures there are basically two conformations that have been observed which are designated as the S and F forms.[11b] The S form is characteristic of the thiamin when it is substituted at C(2) and is consistent with the interpretation of the NMR spectra of these compounds.[35,36,50] When thiamin is free of substituents on C(2), it has been found in the F form in over a dozen cases but only once in the S conformation.[34] None have yet been found in any other conformation including the proposed V form.[2,9,12,51]

In order to account for the discrepancy between the crystal structure results and the conformations suggested from other data, it has been proposed that packing or lattice forces have stabilized the observed solid-state conformation.[2,13,34] With the wide variation in molecular environment represented in crystal structures, it is highly unlikely that crystal packing forces are responsible for stabilizing the F form unless the

accessibility to the groups participating in intermolecular associations is a function of the variations in the torsion angles ϕ_T and ϕ_P. The predominant interactions seen in these structures involve hydrogen bonding with N(1′), N(3′), N(4′α), and O(5γ) [and its phosphate esters when O(5γ) is esterified]. Of secondary importance are the hydrogen bonding by C(2) and the electrostatic interaction with S(1). From an examination of CPK molecular models, it is clear that the accessibility to N(1′), N(3′), O(5γ), and S(1) is totally independent of the torsion angles. Access to C(2) and one of the N(4′α) hydrogens is restricted over a small range of both torsion angles but the other amino hydrogen remains unrestricted. Even when there is restricted access, the restricted groups still form hydrogen bonds.[52] Since there is nearly unlimited accessibility to the intermolecular bonding groups in thiamin, it is difficult to envision how the crystal packing forces could impose any limit on the range of torsion angles.

The present structure provides substantial additional support for the idea that the F conformation is the predominant form of free thiamin because the F conformation is retained in spite of the ring stacking interaction between the large picrolonate anion and thiamin. This stacking interaction is a dominating feature of the crystal structure. It is significant that in the packing of the thiamin picrolonate complex into the crystal structure, the rings of the picrolonate molecule are forced into a nearly coplanar alignment, a condition not previously observed in other phenylpyrazalone structures. This coplanar alignment results in a significantly close contact between O(19P) and C–H(5P) of the phenyl ring, indicating an energetically less favorable conformation for the picrolonate anion.

By considering all of the available structural data, both for this structure and other thiamin structures, the evidence strongly indicates that the conformation of thiamin with respect to the C(3,5′) methylene bridge is largely influenced by its intramolecular properties and is much less susceptible to the influence of intermolecular interactions. The conformation of the C(5) side chain provides a marked contrast in that the structural data show that it will readily adjust to accommodate intermolecular interactions with its neighboring environment in various different structures. However, the data in Table V show that even the C(5) side chain assumes a limited range of conformations. The largest variation is seen in $\phi_{5\alpha}$ which shows values from 3 to 105° (the values are all positive as a consequence of the molecular selection procedure used in the definition of ϕ_T and ϕ_P) although a majority fall in the range from 60 to 90°. The values for $\phi_{5\beta}$ show a bimodal distribution with practically all of them falling within 10° of ±60°. There is a slightly greater population of values around −60° (60%) which moves O(5γ) toward S(1) and results in a close S···O contact when $\phi_{5\alpha}$ is also less than ≈70°. Perhaps the S···O interaction also influences the conformation of the C(5) side chain, albeit to a lesser extent, as has been found for the C(2) side chain of the intermediates of thiamin catalysis.[35,36]

Thiamin has been observed to form association complexes with aromatic compounds.[7,9,10] The mode of association in these complexes has been attributed to charge-transfer complex formation which has also served as the basis for identifying by UV and CD spectral changes, a thiamin–tryptophan complex in transketolase.[6,8,53] The thiamin picrolonate structure provides a detailed example of the association between thiamin and planar ring systems. There are several aspects of this complex which may be relevant to the functional properties of thiamin. One of these is the preference for association with the pyrimidine ring. This was an unexpected result because (1) previous data suggested that the charged thiazolium ring was essential for complex formation,[9] (2) both the thiazolium and pyrimidine rings were reported to form a donor–acceptor complex,[9] and (3) the negatively charged pyrazolone ring appeared to be ideally suited for forming a planar complex with the positively charged thiazolium ring. The observed association of the neutral pyrimidine ring with both the neutral nitrophenyl and the pyrazolone anion illustrates the greater likelihood for the pyrimidine ring of thiamin to form a planar complex than for the thiazolium ring to be involved in such a complex.

A further point of interest in this structure concerns the type of interaction between the two components. Although previous data have been interpreted in terms of a charge-transfer interaction, it is unlikely that thiamin forms this type of association. It is much more likely that the association with thiamin involves dipole-induced dipole interactions as is characteristic of purines and pyrimidines.[15] It is indeed pertinent that the observed weaker association between thiamin and tryptophol in a less polar solvent[54] is consistent with expectations for dipole-induced dipole interactions[15] but is contrary to expectations for a charge-transfer complex. It is also of interest that in their study of purine and pyrimidine compounds, Bugg et al.[15] found stacking interactions to be quite specific and a major stabilizing force in the solid state and probably in solution as well. It is reasonable to expect that the stacking interaction with the pyrimidine ring of thiamin could be important in its binding to the enzyme. Although the influence that such an interaction could exert on the conformation of the coenzyme cannot be ascertained from the presently available data, it does seem rather unlikely that ring stacking interactions stabilize alternate thiamin conformations such as the proposed V form.[2,9,12] The pyrimidine stacking interaction could also play a directive role with the substrate both before and after addition to the coenzyme.[36]

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Supplementary Material Available: structure factor table for the thiamin picrolonate dihydrate complex (4 pages). Ordering information is given on any current masthead page.

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