light of the observed[34,35] very strong effect of O_2 on photodecomposition of CH_3Cbl.
(34) G. N. Schrauzer, J. W. Sibert, and R. J. Windgassen, *J. Am. Chem. Soc.*, **90**, 6681 (1968).
(35) H. P. C. Hogenkamp, P. J. Vergamini, and N. A. Matwiyoff, *J. Chem. Soc., Dalton Trans.*, 2628 (1975).
(36) (a) H. C. Heinrich, *Z. Vitam-, Horm.-Fermentforsch.*, **9**, 385 (1958); (b) H. C. Heinrich, *Z. Naturwiss.*, **45**, 269 (1958).
(37) (a) E. L. Lien, L. Ellenbogen, P. Y. Law, and J. M. Wood, *Biochem. Biophys. Res. Commun.*, **55**, 730 (1973); (b) E. L. Lien, L. Ellenbogen, P. Y. Law, and J. M. Wood, *J. Biol. Chem.*, **249**, 890 (1974).
(38) E. L. Lien and J. M. Wood, *Biochim. Biophys. Acta*, **264**, 530 (1972).
(39) (a) E. Hippe, E. Haber, and H. Olesen, *Biochim. Biophys. Acta*, **243**, 75 (1972); (b) E. Hippe and H. Olesen, *ibid.*, **243**, 83 (1972).
(40) G. Kidroni and N. Grossowicz, *Biochim. Biophys. Acta*, **252**, 262 (1971).
(41) R. T. Taylor and M. L. Hanna, *Arch. Biochem. Biophys.*, **141**, 247 (1970).
(42) R. T. Taylor and H. Weissbach, *J. Biol. Chem.*, **242**, 1502 (1967).
(43) H. A. Lee and R. H. Abeles, *J. Biol. Chem.*, **238**, 2367 (1963).
(44) T. Toraya and S. Fukui, *Biochim. Biophys. Acta*, **284**, 536 (1972).
(45) T. Toraya, M. Kondo, Y. Isemura, and S. Fukui, *Biochemistry*, **11**, 2599 (1972).
(46) Z. Schneider, E. G. Larsen, G. Jacobson, B. C. Johnson, and J. Pawelkiewicz, *J. Biol. Chem.*, **245**, 3388 (1970).
(47) (a) J. A. Hamilton, R. L. Blakley, F. D. Looney, and M. E. Winfield, *Biochim. Biophys. Acta*, **177**, 374 (1969); (b) J. A. Hamilton, R. Yamada, R. L. Blakley, H. P. C. Hogenkamp, F. D. Looney, and M. E. Winfield, *Biochemistry*, **10**, 347 (1971); (c) R. Yamada, Y. Tamao, and R. L. Blakley, *ibid.*, **10**, 3959 (1971).
(48) E-I. Ochiai, *J. Inorg. Nucl. Chem.*, **37**, 351 (1975).
(49) S. A. Cockle, H. A. O. Hill, R. J. P. Williams, S. P. Davis, and M. A. Foster, *J. Am. Chem. Soc.*, **94**, 275 (1972).
(50) (a) B. M. Babior, T. H. Moss, and D. C. Gould, *J. Biol. Chem.*, **247**, 4389 (1972); (b) B. M. Babior, T. H. Moss, W. H. Orme-Johnson, and H. Beinert, *ibid.*, **249**, 4537 (1974).
(51) T. H. Finlay, J. Valinsky, A. S. Mildvan, and R. H. Abeles, *J. Biol. Chem.*, **248**, 1285 (1973).
(52) B. M. Babior, *Acc. Chem. Res.*, **8**, 376 (1975).
(53) R. H. Abeles and D. Dolphin, *Acc. Chem. Res.*, **9**, 114 (1976).
(54) D. Dolphin, *Methods Enzymol.*, **18**, 34 (1971).
(55) R. A. Firth, H. A. O. Hill, J. M. Pratt, and R. G. Thorp, *Anal. Biochem.*, **23**, 433 (1968).
(56) C. L. deLigny, P. F. M. Luykx, M. Rehbach, and A. A. Weineke, *Recl. Trav. Chim. Pays-Bas*, **79**, 713 (1960).
(57) H. P. C. Hogenkamp, J. E. Rush, and C. A. Swenson, *J. Biol. Chem.*, **240**, 3641 (1965).
(58) A. L. Van Geet, *Anal. Chem.*, **42**, 679 (1970).
(59) (a) S. Meiboom, *J. Chem. Phys.*, **34**, 375 (1961); (b) L. W. Reeves, *Adv. Phys. Org. Chem.*, **3**, 187 (1965).
(60) T. E. Needham, N. A. Matwiyoff, T. E. Walker, and H. P. C. Hogenkamp, *J. Am. Chem. Soc.*, **95**, 5019 (1973).
(61) J. M. Pratt, "Inorganic Chemistry of Vitamin B_{12}", Academic Press, London, 1972, Table 8.7.
(62) J. D. Brodie and M. Poe, *Biochemistry*, **11**, 2534 (1972).
(63) (a) H. P. C. Hogenkamp, R. D. Tkachuck, M. E. Grant, R. Fuentes, and N. A. Matwiyoff, *Biochemistry*, **14**, 3707 (1975); (b) T. E. Walker, H. P. C. Hogenkamp, T. E. Needham, and N. A. Matwiyoff, *J. Chem. Soc., Chem. Commun.*, 85 (1974).
(64) I. P. Rudakova, T. A. Pospelova, V. A. Borodulina-shvets, B. I. Kurganov, and A. M. Yurkevich, *J. Organomet. Chem.*, **61**, 389 (1973).
(65) A. Allerhand, H. S. Gutowsky, J. Jonas, and R. Meinzer, *J. Am. Chem. Soc.*, **88**, 185 (1966).
(66) A. Abragham, "The Principles of Nuclear Magnetism", Oxford University Press, London, 1961, Chapter 8.
(67) T. C. Farrar and E. D. Becker, "Pulse and Fourier Transform NMR", Academic Press, New York, N.Y., 1971, Chapter 4.
(68) More recently it has been claimed[12c] that alkylcobaloximes, $RCo(dh)_2$, may exist in water as the five-coordinate species. However, the cobalt in $RCo(dh)_2$ is a quite strongly acidic center. In view of the energetics of binding of various bases to $RCo(dh)_2$[13,14,69] it is quite inconceivable that five-coordinate species are present to any extent in aqueous medium. The characteristics of the UV–visible spectra of cobaloximes or cobalamins are affected by many variables; these spectra are not an appropriate technique for detecting a potential five–six-coordination equilibrium.
(69) R. L. Courtright, R. S. Drago, J. A. Nusz and M. S. Nozari, *Inorg. Chem.*, **12**, 2809 (1973).
(70) S. A. Cockle, O. D. Hensens, H. A. O. Hill, and R. J. P. Williams, *J. Chem. Soc., Dalton Trans.*, 2633 (1975).
(71) S. P. Datta and A. K. Grzybowski, *J. Chem. Soc. B*, 136 (1966).
(72) M. T. Davies, P. Mamalis, V. Petrow, and B. Sturgeon, *J. Pharm. Pharmacol.*, **3**, 420 (1951).
(73) P. G. Lenhert, *Proc. R. Soc. London, Ser. A*, **303**, 45 (1968).
(74) R. S. Drago, *Struct. Bonding (Berlin)*, **15**, 73 (1973).

Stereochemistry of Intermediates in Thiamine Catalysis. 2. Crystal Structure of DL-2-(α-Hydroxybenzyl)thiamine Chloride Hydrochloride Trihydrate

James Pletcher,* Martin Sax,* Gary Blank, and Mical Wood

Contribution from the Biocrystallography Lab, VA Hospital, Pittsburgh, Pennsylvania 15240, and Crystallography Department, University of Pittsburgh, Pittsburgh, Pennsylvania 15260.
Received July 6, 1976

Abstract: The structure of 2-(α-hydroxybenzyl)thiamine, an intermediate in a thiamine catalyzed reaction, has been determined by single crystal x-ray diffraction techniques. In this compound the thiamine conformation is similar to that found for the 2-(α-hydroxyethyl) adduct[1] and the minor modifications which do exist account very well for differences that are observed in the NMR spectra[2] of the two compounds. The intramolecular S···O interaction which was first characterized in the previous adduct structures is found in this compound as well. The close intramolecular contact between the overlapped parallel phenyl and pyrimidinium rings is a structural feature that provides additional conformational stability as well as offering some interesting mechanistic implications. The crystal structure was determined using diffractometer data obtained by the θ:2θ scan technique with Cu radiation from a crystal having space group symmetry $C2/c$ and unit cell parameters a = 27.82 (4), b = 7.478 (8), c = 24.11 (3) Å, and β = 110.00 (7)°. The structure was solved by direct methods and refined by least squares to an R = 0.080 for all 3871 independent reflections and an R = 0.065 for the 2340 observed reflections.

Thiamine, in the form of the pyrophosphate ester, is a coenzyme in a number of enzyme systems that catalyze the decarboxylation of α- keto acids and the transfer of aldehyde or acyl groups.[3] Thiamine C(2) adducts are intermediates in the reaction mechanism.[4] Many of these intermediates are sufficiently stable under mildly acidic conditions to be isolated. It has previously been shown with 2-(α-hydroxyethyl)thiamine, HET, that when substitution occurs on C(2) the molecular conformation with respect to the C(3,5′) bridge carbon atom undergoes a substantial change from that which characterizes the free thiamine molecule.[1] Besides having a different molecular conformation, the adduct compounds display an apparent conformational stability that is imparted through an intramolecular S···O interaction with the O(2α1) oxygen.

Although this apparent attractive force between sulfur and oxygen manifests itself through the structural parameters, it has mechanistic implications as well. In order to examine these structural features further, the crystal structures of other C(2) intermediates are being studied. The 2-(α-hydroxybenzyl)thiamine, HBT, was of particular interest in that C(2α) phenyl substituent provides a much bulkier group to accommodate and its NMR spectrum[2] shows that there are likely structural changes in comparison with HET.

Experimental Section

The compound was kindly supplied by Dr. Henry Sable of Case Western Reserve University and was recrystallized from water by the addition of two volumes of acetone at −20 °C. Colorless crystals grew with a bladed morphology and appeared stable to atmospheric conditions at room temperature. They are monoclinic and are elongated in the direction of the *b* axis. Although they had a marked tendency to grow as multiple crystals, one was found that appeared suitable for data collection. The Wissenberg photographs indicated reflections for planes with *hkl* indices when $h + k = 2n$, with *h*0*l* indices when $l = 2n$ ($h = 2n$), and with 0*k*0 indices when $k = 2n$. This pattern is consistent with space group symmetry *Cc* or *C*2/*c*. Although both space groups will contain the two enantiomers present in the solution of the racemic mixture which is formed in the synthesis of the compound, the centrosymmetric space group, *C*2/*c*, was arbitrarily selected. This choice was confirmed by the subsequent solution and refinement.[5a] The crystals exhibited a marked anisotropic mosaic character. The one selected for data collection had a mosaic spread ~1.5° about an axis parallel to the [0,1,0] zone axis, while the mosaic distribution about axes perpendicular to this direction was <0.5°. The cell parameters were determined from a least-squares fit of the centered setting angles for 12 pair (average values for ±2θ settings) of reflections measured on a Picker FACS-1 diffractometer using graphite monochromated Cu Kα radiation.[6] The crystal data are summarized in Table I. The intensity data were collected to a sin θ limit of 0.8989 using the θ:2θ scan technique at a scan rate of 2°/min over a range in 2θ of 3° plus an increment to accommodate spectral dispersion. Background counts were accumulated for 20 sec at each end of the scan range. Three standard reflections were monitored during the data collection. The intensity of an 0*k*0 reflection exhibited less than ±2.5% fluctuation but the two *h*0*l* reflections showed greater variations which were inconsistent and could not be improved by recentering.[5b] No correction was applied to the intensity data because of the disparity between the standard reflections and the constancy of the 0*k*0 standard. Of the 3871 independent reflections measured, 1497 were considered unobserved based on the criterion that $|F| \leq 6\sigma(F)$.[7a] The structure amplitudes were converted to *E*'s[7b] and the structure was solved using MULTAN.[8] The structure was refined by full-matrix anisotropic least squares using the CRYLSQ program of the x-ray system[9] in which the quantity minimized is $\Sigma w(|F_o| - k|F_c|)^2$ where *k* is a single scale factor and $w = 1/\sigma^2$. In the final cycles of refinement, the positions and isotropic thermal parameters of all the hydrogen atoms, except H(5γ) which could not be located, were also refined with the total list of parameters separated into five blocks. The blocks were constituted as follows: (1) the thiazolium ring, the C(2) substituent minus the phenyl ring and the chloride ions; (2) the N(3) substituent minus the pyrimidine ring and the C(4) and C(5) substituents; (3) the pyrimidine ring; (4) the phenyl ring and; (5) the water molecules. The atomic scattering factors for C, N, O, S, and Cl^- were those of Cromer and Mann[10] and the H scattering factor was from Steward, Davidson, and Simpson.[11] The real and imaginary anomalous dispersion corrections for S and Cl were taken from international tables.[12] The final weights assigned to the observations were determined from scheme No. 3 of WTLSSQ[9] with coefficients $A = 0.0$, $B = 0.36$, and $C = 50$. The unobserved reflections and an additional 34 that appeared to be effected by extinction were assigned zero weight. The refinement converged with $R = 0.080$ for all 3871 reflections and $R = 0.065$ for the 2340 observed reflections; $[\Sigma w|F_o| - |F_c|)^2/(\text{no. reflections} - \text{no. variables})]^{1/2} = 0.598$. The final difference Fourier was relatively clean ($\sigma(\rho) \approx 0.05$ electron/Å^3) with most of the residual density associated with the bonds between atoms. The largest peaks (max = 0.55 electron/Å^3) and holes (max = −0.48 electron/Å^3) are residual ripples associated with the S and Cl atoms. The inability to definitively locate the H(5γ) hydrogen probably stems from the close proximity of O(5γ) to both S(1) and Cl(2) which places the possible hydrogen positions in the region of the diffraction ripple for these two heavy atoms. Although several small peaks (0.2 to 0.35 electron/Å) on the final difference map are within reasonable bonding distance of O(5γ), none is clearly preferable. A probable position for H(5γ) is best selected by considering the hydrogen bonding scheme. The final coordinates and thermal parameters are listed in Table II. The structure factor table is available as supplementary material (see paragraph at end of paper for details).

Table I. Crystal Data for HBT·Cl·HCl·3H_2O[a]

$C_{19}H_{24}Cl_2N_4O_2S \cdot 3H_2O$ fw = 497.44
(Cu $K\alpha_1\alpha_2$) = 1.54178 Å
a = 27.82 (4) Å
b = 7.478 (8)
c = 24.11 (3)
β = 110.00 (7)°
V = 4713.3 Å^3
Space group = $C2/c$, $Z = 8$
ρ_o = 1.398 by flotation in benzene–CCl_4
ρ_c = 1.402 g/cm^3
μ(Cu Kα) = 36.34 cm^{-1}, $F(000)$ = 2112
mp = 175–183 °C with decomposition; loss of water begins at ~45 °C (uncorrected temperatures determined with thermoline melting point apparatus)
Crystal dimensions:
0.08 mm along *a*
0.48 mm along *b*
0.02 mm along *c*

[a] Data measured at ambient temperature, ~22 °C.

Description of Crystal and Molecular Structures

The structure of HBT, although of interest in its own right, provides an interesting subject for comparison with the 2-(α-hydroxyethyl) derivative of thiamine, HET. The bond distances and valency angles for HBT are given in Figure 1. In general they agree very well with the values for HET.[1] The largest differences seen are for the intracyclic bonds of the thiazolium ring and for the C(2α)–O(2α1) bond. These differences possibly reflect changes in the electronic structure of the molecule.

In HBT, the thiamine conformation is that of one of the S forms having torsion angles $\phi_T = +92.7$, $\phi_P = -167.3$ (see ref 13 for definition of torsion angles and description of conformations). Although this is the same basic conformation as that found in HET ($\phi_T = -100.3$, $\phi_P = -145.6$), the change in the relative sign of ϕ_T results in subtle but perhaps significant differences in its properties (influence on its NMR spectra in discussion). These differences are clearly seen by comparing the molecular structure in Figure 2 with that in Figure 4 of Sax, Pulsinelli, and Pletcher.[1] The most important difference is found in the environment of the C(2α) substituent. In both of these structures there is a significant intramolecular interaction between the O(2α1) oxygen and the thiazolium S(1) which aligns the C(2α)–O(2α1) synplanar to the C(2)–S(1) bond, thereby "fixing" the conformation of the C(2) substituent. (This aspect will be discussed in greater detail later.) Although the configuration of the C(2α) substituents is the same, the environment with respect to the pyrimidine is different. In HET the C(2β) methyl is directed nearly normal to the thiazolium ring and pointed in the opposite direction of the pyrimidine while the H(2α) hydrogen is directed under the pyrimidine ring. On the other hand in HBT, the C(2β) phenyl ring is oriented parallel to the pyrimidine ring forming a close intramolecular stacked pair rather than pointing away from the pyrimidine ring. The nearly parallel stacking and the close intramolecular contacts are readily apparent from the least-squares planes and dihedral angles which are listed in Table III and from the table of contact distances in Table IV. The formation of the stacked rings which favors this alternate S

Table II. Fractional Coordinates and Temperature Factors for HBT·Cl·HCl·3H_2O

(a) Nonhydrogen Atoms[a]

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
S(1)	705 (0.5)	3788 (2)	5431 (0.6)	44 (0.8)	17 (0.6)	37 (0.8)	−2 (0.6)	16 (0.6)	1 (0.6)
C(2)	564 (2)	4657 (7)	4756 (3)	34 (3)	18 (3)	45 (3)	3 (2)	21 (3)	−1 (2)
N(3)	704 (2)	6380 (6)	4772 (2)	36 (2)	17 (2)	34 (2)	4 (2)	17 (2)	7 (2)
C(4)	940 (2)	7035 (7)	5344 (3)	37 (3)	18 (3)	44 (3)	3 (2)	23 (3)	3 (2)
C(5)	977 (2)	5770 (7)	5765 (3)	34 (3)	16 (3)	40 (3)	−1 (2)	16 (2)	2 (2)
C(2α)	313 (2)	3591 (8)	4202 (3)	32 (3)	26 (3)	34 (3)	1 (2)	8 (2)	4 (2)
O(2α1)	175 (2)	1927 (6)	4414 (2)	49 (3)	26 (2)	40 (2)	−13 (2)	12 (2)	−2 (2)
C(2β1)	665 (2)	3316 (7)	3851 (2)	36 (3)	21 (3)	35 (3)	−5 (2)	11 (2)	−6 (2)
C(2β2)	506 (3)	3838 (9)	3271 (3)	41 (3)	35 (3)	44 (3)	−1 (3)	12 (3)	1 (3)
C(2β3)	835 (3)	3646 (10)	2948 (3)	53 (4)	49 (4)	39 (4)	2 (3)	19 (3)	1 (3)
C(2β4)	1317 (3)	2927 (9)	3216 (3)	48 (4)	42 (4)	63 (4)	−6 (3)	30 (4)	−12 (3)
C(2β5)	1476 (2)	2404 (8)	3793 (3)	35 (3)	32 (3)	59 (4)	1 (3)	16 (3)	−11 (3)
C(2β6)	1153 (2)	2583 (7)	4112 (3)	40 (3)	21 (3)	42 (4)	0 (2)	10 (3)	−3 (2)
C(3,5′)	627 (2)	7492 (8)	4242 (3)	39 (3)	28 (3)	43 (3)	5 (3)	17 (3)	13 (3)
C(5′)	1081 (2)	7483 (7)	4040 (2)	34 (3)	17 (2)	39 (3)	−3 (2)	11 (2)	3 (2)
C(6′)	1539 (2)	6752 (7)	4362 (3)	38 (3)	19 (3)	42 (3)	2 (2)	13 (3)	3 (2)
N(1′)	1926 (2)	6735 (7)	4148 (2)	36 (3)	22 (2)	45 (3)	3 (2)	13 (2)	0 (2)
C(2′)	1864 (2)	7354 (7)	3609 (3)	43 (3)	20 (3)	42 (3)	−6 (2)	16 (3)	−2 (2)
C(2′α)	2300 (3)	7170 (11)	3395 (4)	46 (4)	58 (5)	60 (5)	9 (4)	27 (4)	10 (4)
N(3′)	1432 (2)	8064 (7)	3268 (2)	42 (3)	27 (2)	45 (3)	1 (2)	18 (2)	4 (2)
C(4′)	1036 (2)	8179 (7)	3472 (2)	37 (3)	16 (2)	39 (3)	−1 (2)	13 (2)	2 (2)
N(4′α)	615 (2)	8923 (7)	3123 (3)	39 (3)	38 (3)	40 (3)	11 (2)	13 (2)	19 (2)
C(4α)	1114 (3)	8931 (8)	5460 (3)	65 (5)	23 (3)	56 (4)	−17 (3)	30 (4)	−5 (3)
C(5α)	1226 (3)	5929 (8)	6416 (3)	47 (4)	28 (3)	38 (3)	−2 (3)	10 (3)	−5 (3)
C(5β)	1193 (3)	4252 (9)	6752 (3)	54 (4)	35 (3)	29 (3)	7 (3)	8 (3)	6 (3)
O(5γ)	671 (2)	3908 (6)	6662 (2)	45 (2)	39 (2)	50 (2)	0 (2)	15 (2)	14 (2)
Cl(1)	2094 (0.7)	3943 (3)	5636 (0.8)	58 (1)	60 (1)	55 (1)	17 (1)	20 (0.8)	8 (0.8)
Cl(2)	485 (0.6)	−107 (2)	6732 (0.7)	48 (1)	30 (0.8)	49 (1)	−3 (0.7)	14 (0.7)	−7 (0.6)
O(W1)	1849 (2)	−99 (8)	7131 (3)	63 (3)	52 (3)	83 (4)	5 (3)	29 (3)	12 (3)
O(W2)	2236 (2)	127 (7)	5143 (3)	55 (3)	41 (3)	61 (4)	5 (2)	16 (3)	12 (3)
O(W3)	2501 (3)	2290 (9)	3007 (3)	74 (4)	82 (4)	62 (4)	−14 (4)	22 (3)	1 (3)

(b) Hydrogen Atoms[b]

Atom	x	y	z	U	Atom	x	y	z	U
H(2α)	−1 (3)	408 (9)	393 (3)	40 (17)	H(4′α2)	32 (3)	900 (10)	320 (3)	48 (19)
H(2α1)	−4 (2)	145 (9)	406 (3)	32 (16)	H(4α1)	133 (3)	915 (9)	528 (3)	38 (17)
H(2β2)	17 (3)	435 (11)	311 (3)	61 (22)	H(4α2)	125 (2)	917 (9)	587 (3)	39 (17)
H(2β3)	71 (3)	392 (13)	251 (4)	81 (26)	H(4α3)	75 (6)	957 (23)	537 (7)	157 (56)
H(2β4)	150 (3)	299 (10)	299 (3)	54 (21)	H(5α1)	157 (2)	634 (8)	653 (2)	22 (14)
H(2β5)	184 (3)	188 (11)	396 (3)	59 (21)	H(5α2)	106 (4)	692 (15)	657 (4)	93 (31)
H(2β6)	124 (2)	236 (8)	453 (3)	21 (13)	H(5β1)	139 (2)	429 (8)	714 (3)	29 (15)
H(3,5′1)	34 (2)	713 (8)	398 (2)	27 (14)	H(5β2)	132 (3)	332 (13)	657 (4)	71 (25)
H(3,5′2)	56 (3)	874 (10)	437 (3)	49 (18)	H(5γ)				
H(6′)	158 (2)	649 (8)	474 (3)	23 (14)	H(W11)	207 (3)	−71 (13)	745 (4)	60 (25)
H(1′)	218 (3)	620 (10)	431 (3)	39 (19)	H(W12)	159 (5)	17 (16)	730 (5)	106 (36)
H(2′α1)	258 (4)	729 (14)	368 (4)	77 (29)	H(W21)	231 (3)	44 (13)	485 (4)	70 (29)
H(2′α2)	220 (4)	733 (17)	296 (6)	115 (38)	H(W22)	227 (5)	85 (17)	540 (6)	91 (43)
H(2′α3)	240 (5)	575 (21)	334 (6)	135 (45)	H(W31)	277 (5)	263 (16)	301 (5)	94 (38)
H(4′α1)	60 (2)	923 (9)	279 (3)	33 (17)	H(W32)	260 (3)	222 (11)	342 (4)	60 (24)

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

conformation could be an important property of the pyrimidine ring of thiamine. This is even more likely in view of the recently completed structure of the picrolonate salt of thiamine in which the crystal structure is dominated by stacking interactions between the pyrimidine ring and the picrolonate anion.[14] Another consequence of this pyrimidine–phenyl interaction is that the H(2α) proton is now directed away from the pyrimidine ring, thus permitting greater access for interaction with neighboring groups. In HBT this hydrogen forms a hydrogen bond to O(5γ) of a neighboring molecule (Table V). This is reminescent of the C(2)–H···X hydrogen bond which is characteristic of the unsubstituted thiamine structures. The studies of Mieyal, Bantle, Votaw, Rosner, and Sable[2] involving the NMR spectra of various thiamine C(2) adducts show that the H(2α) is acidic like the H(2) hydrogen in unsubstituted thiamine. In addition they have shown that H(2α) is more acidic in HBT than in HET. Whether this property has any bearing on the formation of a hydrogen bond in the HBT structure while it is absent in HET is uncertain. It could be related simply to the different accessibility of a hydrogen bond acceptor in the two crystal structures.

As mentioned previously, the conformation of the C(2) adduct in HBT is similar to that observed in HET and in 2-(α-hydroxyethyl)-3,4-dimethylthiazolium bromide.[1] In all three of these structures the C(2α)–O(2α1) bond is syn-planar with the C(2)–S(1) bond and a close intramolecular contact

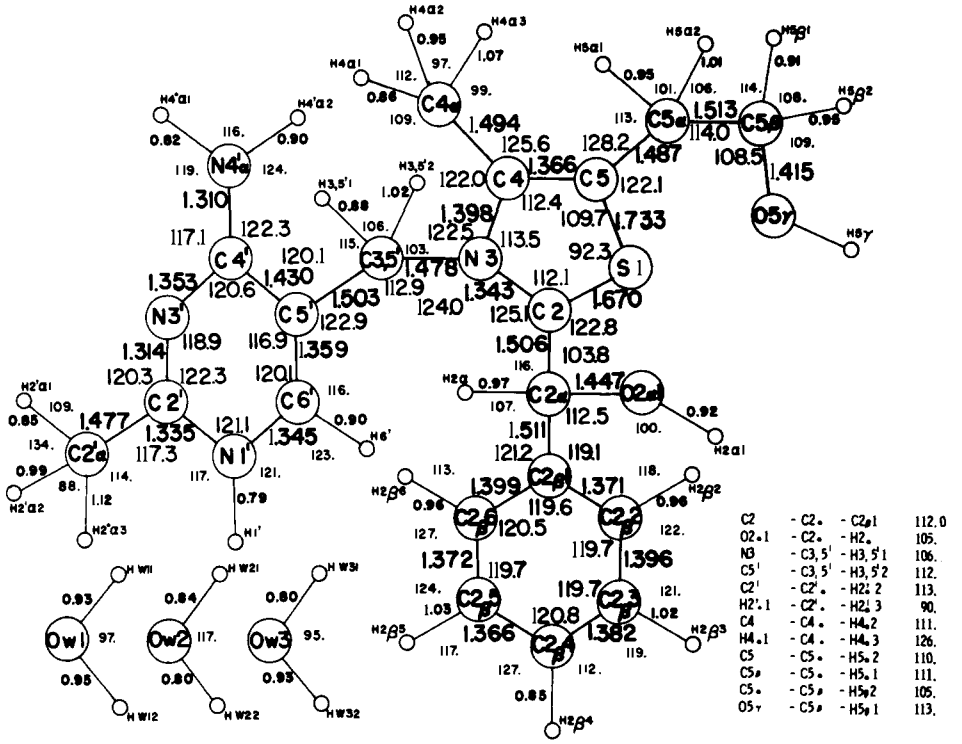

Figure 1. Schematic representation of the molecule showing the atomic numbering scheme, the bond distances (Å), and valence angles (deg). For the nonhydrogen atoms the standard deviations in the bonds range from 0.005 to 0.012 and average 0.008 and in the angles they range from 0.3 to 0.8 and average 0.5. For values involving hydrogen atoms the standard deviations in the bonds vary from 0.05 to 0.17 and average 0.09 while those for the angles cover the range from 3.0 to 12.0 and average 6.0.

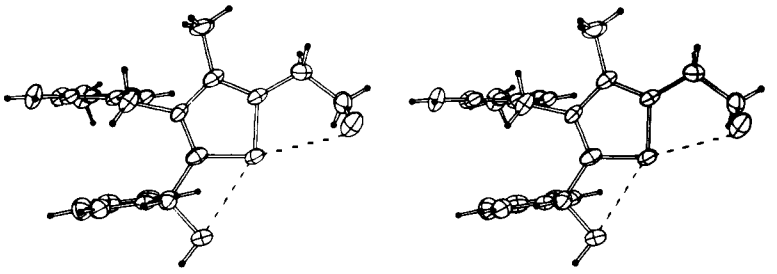

Figure 2. Stereoscopic view[7c] of HBT looking along normal to thiazolium ring. The dashed lines indicate the close contacts of S(1) with both O(2α1) and O(5γ). The molecule shown is centrically related to the one for which coordinates are listed in Table II to permit a direct comparison with HET in Figure 4 of Sax, Pulsinelli, and Pletcher.[1] In comparison with HET, it is apparent that the conformations of the C(2) substituents are similar but the pyrimidine rings are oriented in the opposite direction.

exists between O(2α1) and S(1). In HBT there is even greater coplanarity as evidenced by the smaller displacement of O(2α1) from the plane of the thiazolium ring (Table III) and from the torsion angle about the C(2α)-C(2) bond (Figure 3). On the basis of structural evidence it was previously proposed that an electrostatic attraction existed between S(1) and O(2α1) and that this stabilized the observed conformation. There is further substantiation of this idea in the HBT structure because along with the smaller torsion angle (8.4° compared with 18.4 and 20.6) the S(1)···O(2α1) distance (Table V) is shorter (2.764 Å compared with 2.852 and 2.901) while the S(1)-C(2)-C(2α) and C(2)-C(2α)-O(2α1) angles are the same or smaller on the average. That the C(2α)-O(2α1) bond is 0.025 Å longer, (3σ) may be a chemically significant consequence of the closer S···O distance. Although the C(2) adduct conformation is similar to that in the two previous adduct structures, the disposition of H(2α1) does not reflect the nearly perpendicular orientation previously observed for the O(2α1)-H(2α1) bond with respect to the thiazolium ring (Figure 3). It is of interest that the observed H(2α1) position in the current structure infers that the sp^2 lone pair orbital of O(2α1) is favorably aligned for an electrostatic interaction with S(1) whereas in the two previous adduct structures it was the pπ orbital that was favorably aligned. Additional studies will be required to determine if there is a conformational preference for either or both of these two orientations. The relative stability of these two orientations of the O(2α1)-H(2α1) bond may be influenced by crystal packing. It can be seen in the present structure that the crystal packing places a centrosymmetrically related thiazolium ring parallel to the ring at a distance (3.6 Å) that precludes the perpendicular positioning of both a hydrogen bond acceptor and the hydrogen atom itself. In fact, the actual chloride ion acceptor is nearly in the plane of the thiazolium ring (Table III).

The C(5) side chain vividly demonstrates the influence of packing forces on the conformation of "flexible" structural features. In the first several structures of thiamine containing compounds to have been reported,[15] the C(5α)-C(5β) bond was oriented approximately perpendicular to the thiazolium ring (torsion angle $\phi_{5\alpha}$ = S(1)-C(5)-C(5α)-C(5β) ≈ ± 90°)

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

Plane	A	B	C	D	σ	Displacements
Thiazolium	−958	286	354	3556	10	**S(1) 7, C(2) −7, N(3) 4, C(4) 3, C(5) 7,** C(2α) −38, O(2α1) 156, H(2α1) 324, H(2α) 686, C(2β1) −1334, C(3,5′) −5, C(4α) 43, C(5α) −83, C(5β) −66, O(5γ) 1173, Cl(1) −3486, Cl(2) 871
Pyrimidinium	187	897	312	8624	16	**N(1′) 17, C(2′) −6, N(3′) −10, C(4′) 14, C(5′) −3, C(6′) −13,** H(1′) −92, C(2′α) −63, N(4′α) 31, H(4′α1) −27, H(4′α2) −11, C(3,5′) −81, H(6′) 119, Cl(1) −650
Phenyl	287	915	168	4355	3	**C(2β1) 3, C(2β2) −1, C(2β3) 0, C(2β4) 0, C(2β5) 2, C(2β6) −4,** C(2α) 52, H(2α) 23, O(2α1) −1111, C(2) 1206, H(2β2) 19, H(2β3) −93, H(2β4) 96, H(2β5) 1, H(2β6) 77

Dihedral Angles, deg
Thiazolium–pyrimidinium, 84.9; thiazolium–phenyl, 90.3; pyrimidinium–phenyl, 8.9

[a] Coefficients × 10^3 in $Ax + By + Cz = D$ are referred to crystallographic axes in Å. Displacements of the atoms from the plane are in Å × 10^3. Boldface type designates atoms used to define the planes. Standard deviation in least-squares planes in Å × 10^3.

Table IV. Separation between Pyrimidinium and Phenyl Rings

(a) Distance of Atoms from Least-Squares Planes in Å

Atom	Pyrimidinium plane	Atom	Phenyl plane
C(2β1)	−3.157	N(1′)	3.470
C(2β2)	−3.325	C(2′)	3.625
C(2β3)	−3.525	C(2′α)	3.761
C(2β4)	−3.556	N(3′)	3.629
C(2β5)	−3.390	C(4′)	3.474
C(2β6)	−3.198	N(4′α)	3.506
		C(5′)	3.263
		C(6′)	3.258

(b) Intramolecular Separation between Pairs of Atoms in Å

Atom pair		Distance	Atom pair		Distance
C(2β1)	C(3,5′)	3.274 (8)	C(2β4)	N(3′)	3.853 (9)
	C(5′)	3.301 (7)		C(2′)	3.631 (10)
	C(6′)	3.461 (8)		N(1′)	3.664 (9)
C(2β2)	C(5′)	3.375 (8)	C(2β5)	N(1′)	3.472 (8)
	C(4′)	3.530 (8)		C(6′)	3.510 (9)
C(2β3)	C(4′)	3.593 (9)	C(2β6)	C(6′)	3.284 (8)
	N(3′)	3.660 (9)		C(5′)	3.671 (8)

(c) Intermolecular Separation between Pairs of Atoms in Å

C(2β5)	C(4′)[a]	3.381 (8)

[a] $x, y - 1, z$.

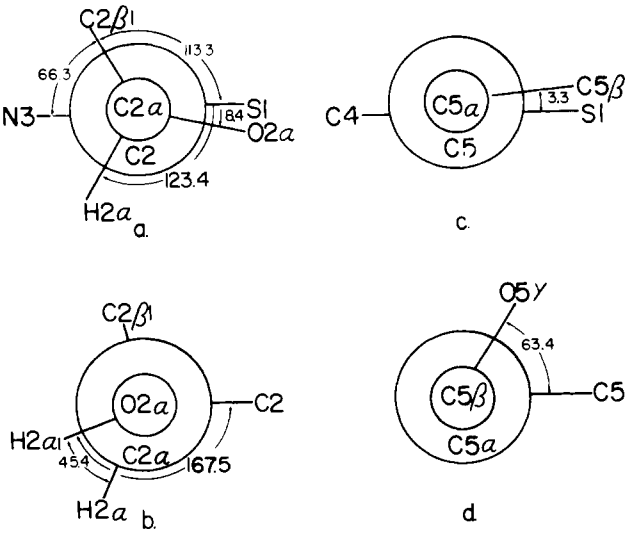

Figure 3. Torsion angles of thiamine substituents presented in Newman projections of a molecule which is centrosymmetrically related to one for which coordinates are listed in Table II in order to allow direct comparison with Figures 6 and 7 in Sax, Pulsinelli, and Pletcher (ref 1). (a) The projection down the C(2α)–C(2) bond; (b) the projection down the O(2α1)–C(2α) bond; (c) the projection down the C(5α)–C(5) bond; and (d) the projection down the C(5β)–C(5α).

$\phi_{5\beta}$ = C(5)–C(5α)–C(5β)–O(5γ)) showed greater variability in order to accommodate different hydrogen bonding interactions. In none of these structures was there any evidence of close contact between S(1) and O(5γ) of the type which is characteristically seen between S(1) and O(2α1) because a close approach was not possible when $\phi_{5\alpha} \approx \pm 90°$. The structure of thiochrome,[16] which is the planar tricyclic oxidation product of thiamine, provided the first example of a close contact between S(1) and O(5γ) which was permitted because $\phi_{5\alpha}$ was substantially less than 90°, a conformation favored by the packing and hydrogen bonding scheme. HBT is one of several thiamine structures[17] which have now shown a close S(1)···O(5γ) contact (3.003 Å) which is made possible by the conformation of the C(5) side chain that is determined by packing and hydrogen bonding considerations. In fact HBT is an extreme example with $\phi_{5\alpha} = 3.3°$ and $\phi_{5\beta} = 63.4°$ (the angles calculated with the same molecule that was used for ϕ_T and ϕ_P). It is thus apparent that structurally flexible moieties in thiamine assume conformations that are largely determined by the packing and intermolecular hydrogen bonding forces in the crystal, while those entities that are stabilized by intramolecular bonding forces and/or are limited by intramolecular repulsion normally vary but little in conformation.

The packing of the molecule and the hydrogen bonding network are shown in Figure 4. The intramolecular stacking between the phenyl and pyrimidinium rings actually forms a continuous column extending through the crystal. The closest intermolecular ring contact occurs between C(4′) and C(2β5) with a separation of 3.381 (8) Å. The intra- and intermolecular ring stacking is clearly shown in Figure 5. Although the intermolecular overlap is not as extensive as the intramolecular contact, it is typical of dipole-induced dipole interactions of pyrimidine rings as described by Bugg, Thomas, Sundaralingam, and Rao.[18] An extensive hydrogen bonding network exists (Table V) in the crystal structure. Cl(2) accepts four hydrogen bonds: two of these are from the N(4′α) hydrogens on molecules related by the twofold axis; the other two are from hydrogens on O(2α1) and O(5γ) from molecules related by a center of symmetry. N(1′) donates a hydrogen bond to O(W2). O(W2) in turn donates hydrogen bonds to two Cl(1) ions that are related by a center of symmetry. O(W3) donates a third hydrogen bond to Cl(1). Its second hydrogen participates as a donor to O(W1). O(W1) donates a hydrogen bond to O(W3). These water molecules and Cl(1) form a hydrogen

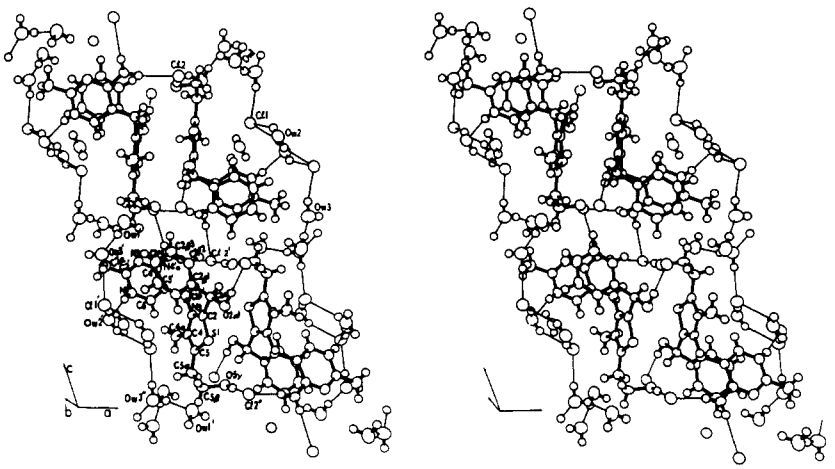

Figure 4. Stereo packing diagram[7c] for DL-2-(α-hydroxybenzyl) thiamine chloride hydrochloride trihydrate. The contents shown are contained in the volume which extends in the direction of the *a* axis from $-1/4$ to $1/4$, in the direction of the *b* axis from 0 to 1, and in the direction of the *c* axis from $-1/4$ to $3/4$. The labeled thiamine derivative is related by the twofold axis at $x = 0, z = 1/4$ to the molecule whose coordinates are listed in Table II. However, the unprimed chloride ions and water molecules do correspond to those whose coordinates are in Table II while the primed labels designate atoms related by various symmetry operations. Covalent bonds are depicted by broad dark lines; hydrogen bonds are shown as single thin lines.

bonded sheet that separates the HBT molecules that are stacked along the direction of the *b* axis.

Discussion

It has been noted that in the crystal structure of HET[1] the molecular conformation is consistent with the NMR spectrum report by Mieyal et al.[2] The NMR spectra, which were also presented for HBT and 2-(α-hydroxy-α-cyclohexylmethyl)thiamine (HCMT), showed that the HBT spectrum contains several significant differences from those of the nonaromatic adducts, HET and HCMT, which were attributed to the increased electronegativity of the phenyl ring and to steric effects which altered the shielding of affected hydrogens.[2] The crystal structure illustrates the structural features that can account for the observed spectral changes. The largest shifts are associated with the H(2α) and H(6′) hydrogens. As discussed above, the H(2α) which was over the pyrimidinium ring in HET is directed away from the ring in HBT. This hydrogen is also in the plane of the phenyl ring (displacement = 0.023 Å (Table III)). In this position the hydrogen experiences deshielding which is consistent with the downfield shift of the resonance absorption. The H(6′) proton resonance exhibits an upfield shift which is consistent with the increased shielding that would result from the hydrogen being positioned almost directly over N(3). In addition that hydrogen could experience shielding from the phenyl ring which is stacked parallel to the pyrimidinium ring. The phenyl ring probably provides the slightly increased shielding for C(3,5′) and C(2′α) hydrogens which would account for their small upfield shifts. These spectral correlations support the idea that conformation seen in the crystal structure is also the predominant one in solution.

This structure analysis provides additional support for the idea that the S···O interaction helps to stabilize the positioning of the C(2) adduct with respect to rotation about the C(2)–C(2α) bond and that it may assist in the catalytic mechanism. The observation of the remarkable similarity in conformation of the C(2α)–O(2α1) bond with respect to C(2)–S(1) in the three adduct structures seen to date is significant only in relation to the extent of its possible conformational flexibility. A recent calculation by Jordan[19] on the rotational barriers in 2-(α-hydroxyethyl)-3-methylthiazolium ion indicates that there is a nearly flat energy minimum when the C(2) side chain is rotated over a range of ~130° which includes the conformation observed in the crystal structure (see Figure 6 in ref 1 and Figures 8 and 9 in ref 19). Since this range extends from ~30 ccw to ~100° cw with respect to the structure observed (Figure 6, ref 1), it is apparent that the C(2α)–O(2α1) bond could be positioned as much as 120° with respect to C(2)–S(1). In fact even lower rotational barriers may have been obtained in the theoretical calculations[19] if the C(2β) methyl and N(3) methyl groups had been allowed to rotate as the C(2) substituent was incremented. Zero-order approximations of rotational barriers can be obtained using CPK space filling models of

Table V. Selected Intermolecular and Intramolecular Contacts

(a) Hydrogen Bonds

Atom a	Atom b	Atom c	Distance, Å a–c	Distance, Å b–c	Angle, deg a–b–c
O(2α1)–H(2α1)···Cl(2)[i]			3.065 (4)	2.14 (6)	174 (6)
O(5γ)–H(5γ)···Cl(2)			3.062 (5)		
N(4′α)–H(4′α1)···Cl(2)[ii]			3.365 (7)	2.54(8)	177 (6)
N(4′α)–H(4′α2)···Cl(2)[iii]			3.315 (7)	2.44 (9)	162 (6)
N(1′)–H(1′)···O(W2)[iv]			2.752 (7)	1.98 (6)	168 (8)
C(2α)–H(2α)···O(5γ)[iii]			3.381 (8)	2.43 (6)	163 (4)
O(W1)–H(W11)···O(W3)			2.794 (9)	1.88 (8)	169 (9)
O(W2)–H(W21)···Cl(1)[iv]			3.143 (8)	2.37 (11)	153 (7)
O(W2)–H(W22)···Cl(1)			3.167 (6)	2.47 (13)	146 (11)
O(W3)–H(W32)···Cl(1)[iv]			3.210 (7)	2.32 (9)	160 (7)
O(W3)–H(W31)···O(W1)[iv]			2.863 (10)	2.21 (12)	140 (11)

(b) Close Contacts around S(1)

C(2)–S(1)···O(2α1)			2.764 (4)	57.0
C(5)–S(1)···O(2α1)				149.1
C(2)–S(1)···O(5γ)			3.003 (5)	151.1
C(5)–S(1)···O(5γ)				70.8

(c) Nearest Neighbors around C(2′α) Methyl

C(2′α)–H(2′α1)	Cl(1)[vi]	3.749 (9)	3.2 (1)	122
C(2′α)–H(2′α1)	O(W2)[iv]	3.74 (1)	3.2 (1)	119
C(2′α)–H(2′α2)	O(W1)[ii]	3.61 (1)	2.8 (1)	138
C(2′α)–H(2′α2)	O(W3)[vii]	3.61 (1)	2.7 (1)	147
C(2′α)–H(2′α3)	O(W3)	3.86 (1)	2.8 (1)	169

(d) Symmetry Code

	x, y, z		
i	$-x, -y, 1-z$	v	$x, -y, 1/2+z$
ii	$x, 1-y, -1/2+z$	vi	$1/2-x, 3/2-y, 1-z$
iii	$-x, 1-y, 1-z$	vii	$1/2-x, 1/2+y, 1/2-z$
iv	$1/2-x, 1/2-y, 1-z$		

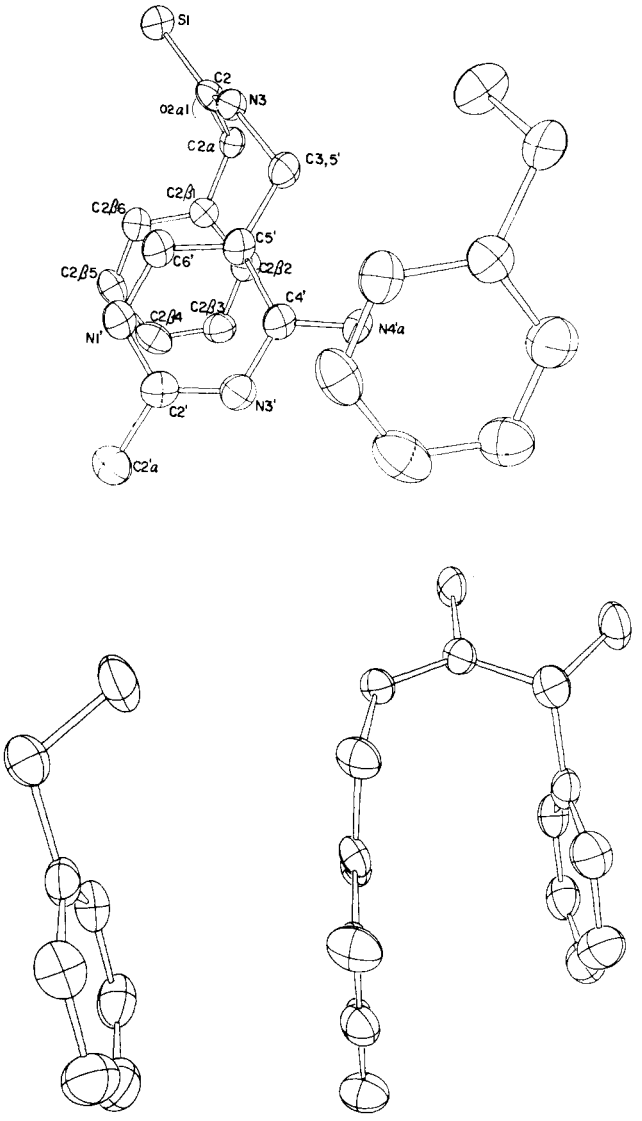

Figure 5. Intra- and intermolecular ring stacking between the overlapped rings in HBT. The top figure, which is viewed along the normal to the pyrimidine ring, shows the extensive overlap between the phenyl and pyrimidine rings within the molecule. The ring which is offset to the right is the C(2) substituent of the molecule translated one unit along *b*. The bottom figure illustrates the same three rings when the pyrimidine ring is viewed edge on. The nonparallel appearance of the two phenyl rings on either side of the figure is a result of the perspective in the ORTEP[7c] drawing.

2-(α-hydroxyethyl)-3-methylthiazolium ion, HET and HBT. In none of the three models are significant barriers encountered over a range of at least 270° when molecular flexibility is permitted for any single bonded substituent. Surprisingly in HBT, complete 360° rotation can be achieved although the C(3,5′) methylene, C(2β) phenyl contacts did distort the S(1)-C(2)-C(2α) bond angle through ~80° of the rotational range. Since it appears that there should be substantial flexibility in the C(2) adduct conformation, the close S···O interaction in the three adduct structures does indicate that there is a significant attractive force between them which is responsible for the highly restricted conformational range actually observed.

Not only would the S···O interaction restrict the conformation but, as previously mentioned,[1,20] it has possible mechanistic implications as well. For example, in the final release of the product from the coenzyme as outlined in Breslow's mechanism[4] a proton must be released from O(2α1). The low pK_a which has been found for this hydrogen[21] is consistent with this electrostatic S···O. Thus the thiazolium S is better suited sterically and electronically to facilitate this deprotonation than the frequently mentioned N(4′α) amino group. This interaction would also provide the specific electrostatic substrate-coenzyme interaction would also provide the specific electrostatic substrate-coenzyme interaction that was suggested by Crosby and Lienhard[22] as a significant binding force in the nonpolar environment which has been found to characterize the active site of the enzyme.[23,24]

It has been suggested that the $C(2)^{\delta-}$-$S(1)^{\delta+}$ dipole in the thiazolium "ylide" could assist in aligning the substrate $>C^{\delta+}=O^{\delta-}$ in the initial adduct formation.[19,20] The HBT structure indicates that the pyrimidine ring may also assist in substrate-coenzyme alignment. As mentioned above in the description of the structure, the phenyl ring stacks over the pyrimidine ring with significantly close contacts between them. In order to maintain both the synplanar C(2)-S(1), C(2α)-O(2α1) conformation and the most effective ring stacking, the HBT structure is limited to the one described as S(+−). (See ref 13b defining symbols.) Interestingly, it can be seen with CPK models that the S(−+) and S(−−) conformations can accommodate either the ring stacking interaction or the S···O contact but not both simultaneously. In both the S(++) and S(−−) conformations the ring stacking appears to be less effective in that there is a smaller extent of ring overlap. It, therefore, seems possible that both of these factors are operative in stabilizing the observed conformation. The ring stacking tendency which is a characteristic of pyrimidines[18] may be an important structural determinant both in the association between the coenzyme and apoenzyme and between the coenzyme and substrate. In regard to the latter, it is of interest that a CPK model of the lactate adduct of thiamine (intermediate following pyruvate addition) can assume the S(+−) conformation in which the carboxyl group stacks over the pyrimidine ring while maintaining the S···O contact with the α hydroxyl group. Spectral studies with enzymes,[25] NMR studies with thiamine and indoles,[26] and the crystal structure of thiamine picrolonate[14] all support the plausibility of an association between the pyrimidine ring and a planar molecular moiety in the enzyme. However, additional studies are needed before this enzyme-coenzyme association can be explicitly defined.

Acknowledgment. We thank the University of Pittsburgh Computer Center for providing the computing time and facilities used in this research. This research was supported in part by NIH Grant AM 18898.

Supplementary Material Available: Structure factor table (7 pages). Ordering information is given on any current masthead page.

References and Notes

(1) M. Sax, P. Pulsinelli, and J. Pletcher, *J. Am. Chem. Soc.*, **96**, 155 (1974).
(2) J. J. Mieyal, G. Bantle, R. G. Votaw, I. A. Rosner, and H. Z. Sable, *J. Biol. Chem.*, **246**, 5213 (1971).
(3) For a general review of thiamine catalysis see L. O. Krampitz, *Annu. Rev. Biochem.*, **38**, 213 (1969). A detailed review of the structural and mechanistic aspects of thiamine catalysis has recently been prepared by A. A. Gallo, J. J. Mieyal, and H. Z. Sable, *Bioorg. Chem.*, in press.
(4) R. Breslow, *J. Am. Chem. Soc.*, **80**, 3719 (1958).
(5) (a) The remote possibility that the centrosymmetrically related molecules in *C2/c* are only related by pseudosymmetry and that the space group is truely *Cc* with two independent molecules was not tested at the end of the refinement because of the amount of computing time that would be required and the limited accuracy of the data set. (b) The intensity of an *h*00 reflection showed gradual fluctuations from −4 to +17% while the intensity of an 00*l* reflection gradually varied between +1 and −18%. The crystal centering and orientation showed no significant changes throughout the data collection even as determined using the standard reflections. Also the cell parameters did not show any significant variations. The final structure amplitudes do not give any evidence of systematic differences that are consistent with the behavior of the two *h*0*l* standard reflections; i.e., F_o is not systematically smaller than F_c for the *h*0*l* data when *h* is small and *l* is large, nor is F_o systematically larger than F_c when *h* is large and *l* is small.
(6) The DOS software system for diffractometer control, data collection, and data reduction was obtained with the instrument from Picker Corp. Some

routines in the program in current use have been modified locally.
(7) (a) This criterion is equivalent to $I \leq 3\sigma(I)$; the terms are defined the same as those in M. K. Wood, M. Sax, and J. Pletcher, *Acta Crystallogr., Sect. B*, **31**, 76 (1975). (b) Programs written or modified by R. Shiono are contained in various Technical Reports from the Department of Crystallography, University of Pittsburgh. (c) The ORTEP program is by C. K. Johnson, ORNL-3794, 1965.
(8) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. A*, **27**, 368 (1971).
(9) J. M. Stewart, G. J. Kruger, H. L. Ammon, C. Dickinson, and S. R. Hall, "The X-ray System," Technical Report TR-192, University of Maryland; implemented on DECSYSTEM 10 by S. Ernst and B. Poppleton.
(10) D. T. Cromer and J. B. Mann, *Acta Crystallogr., Sect. A*, **24**, 321 (1968).
(11) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).
(12) "International Tables for X-ray Crystallography," Vol. III, Kynoch Press, Birmingham, England, 1968, p 214.
(13) (a) The conformation of thiamine with respect to the methylene bridge carbon joining the thiazolium and pyrimidine rings was orignally defined in terms of the dihedral angles ϕ_T and ϕ_P (J. Pletcher and M. Sax, *J. Am. Chem. Soc.*, **94**, 3998 (1972)). ϕ_T was the angle between the normals to the thiazolium ring and the bridge plane (the bridge plane contains the methylene bridge atom and the atom in each ring system to which it is bonded). ϕ_P was the angle between the normals to the pyrimidine ring and the bridge plane. The reference conformation ($\phi_T = \phi_P = 0°$) was defined in terms of the idealized structure presented in thiochrome, the planar tricyclic oxidation product of thiamine, in which the pyrimidine amino group is bonded to the thiazolium C(2). The sense of rotation (right handed) was specified as positive when the ring was rotated clockwise as viewed down the bond from the bridge carbon to the ring. Since thiamine frequently is present in crystal structures as centrosymmetrically related pairs (true of all thus far done), the torsion angles for the centrosymmetrically related molecules will have the same magnitudes but opposite signs. If the structural relationship between the 5β hydroxyethyl side chain (site of pyrophosphate esterification for primary binding to enzyme) and the pyrimidine amino group is of importance, then this structural information can be easily conveyed through the sense of rotation of the torsion angles provided that a reference orientation is also specified. In order to incorporate this feature into the definition of the angles ϕ_T and ϕ_P, the orientation was arbitrarily specified in terms of the thiazolium ring. The molecule selected for specifying the angles was the one in which the C(5) side chain was directed to the right when the thiazolium ring was viewed edge on down the bond from the methylene bridge to N(3) with C(2) positioned to the north and C(4) positioned to the south. (It is important to note that thiamine is a disymmetric molecule and that the two centrosymmetrically related conformers seen in the crystal structures are readily interconverted in solution. However the C(2) adduct structures, such as HET and HBT, contain an asymmetric center and hence the centrosymmetrically related molecules are stereoisomers. The reference orientation thus selects one of the stereoisomers.) Thus with $\phi_T \approx 0°$ for both TPP·HCl and thiamine·Cl·H_2O their ϕ_P values of $+93$ and $-76°$, respectively, convey the anti and syn relationship between N(4′α) and O(5γ) in these two structures. In order to simplify the calculation of the magnitudes for the conformation angles, they were redefined in terms of the torsion angles as ϕ_T = C(5′)–C(3,5′)–N(3)–C(2) and ϕ_P = N(3)–C(3,5′)–C(5′)–C(4′) (see ref 1). The reference angles, the sense of rotation, and reference orientation were maintained as originally defined. (b) Since thiamine in over a dozen different structures has been observed to assume only two basic conformations, it is convenient to specify the conformation by means of a simplified notation (G. Blank, M. Ridrigues, J. Pletcher, and M. Sax, *Acta Crystallogr., Sect.B*, **32**, 2970 (1976)). **F** is used to designate the conformation which is characteristic of thiamine when it is free of substituents on C(2) ($\phi_T \approx 0°$, $\phi_P \approx \pm 90°$). **S** is used to designate the conformation which is characteristic of thiamine when it is substituted on C(2) ($\phi_T \approx \pm 100°$, $\phi_P \approx \pm 150°$). The specific form can be designated by appending the signs for ϕ_T and ϕ_P in that order in parentheses after the letter. Thus **S**(+−) specifies $\phi_T \approx 100°$, $\phi_P \approx -150°$. The **V** conformation, which was first described qualitatively by A. Schellenberger, *Angew. Chem., Int. Ed. Engl.*, **6**, 1024 (1967), and further characterized by J. E. Biaglow, J. J. Mieyal, J. Suchy, and H. Z. Sable, *J. Biol. Chem.* **244**, 4054 (1969), is defined in terms of the torsion angles by $\phi_T \approx \pm 90°$, $\phi_P \approx \mp 90°$).
(14) W. Shin, J. Pletcher, G. Blank, and M. Sax, Pittsburgh Diffraction Conf., Abstract B4, Mellon Institute, Pittsburgh, Pa., Nov. 5–7, 1975. Accepted for publication in *J. Am. Chem. Soc.*
(15) These early structures which showed this feature were thiamine·Cl·HCl, J. Kraut and H. J. Reed, *Acta Crystallogr.*, **15**, 747 (1962); thiamine·PO_4H·H_2PO_4, I. Karle and K. Britts, *ibid.*, **20**, 118 (1966); TPP·HCl, J. Pletcher and M. Sax, *Science*, **154**, 1331 (1966); *J. Am. Chem. Soc.*, **94**, 3998 (1972).
(16) J. Pletcher, M. Sax, C. S. Yoo, J. Chu, and L. Power, *Acta Crystallogr., Sect. B*, **30**, 496 (1974).
(17) Other thiamine structures which have a close S(1)···O(5γ) contact include two polymorphs of the neutral zwitterion of TPP, G. Blank, M. Wood, J. Pletcher, and M. Sax, American Crystallographic Association Meeting, Abstract H5, Charlottesville, Va., Mar. 9–13, 1975; thiamine picrolonate, ref 14; thiamine·I·HI, W. E. Lee and M. F. Richardson, personal communication, submitted to *Can. J. Chem.*
(18) C. E. Bugg, J. M. Thomas, M. Sundaralingam and S. T. Rao, *Biopolymers*, **10**, 175 (1971).
(19) F. Jordan, *J. Am. Chem. Soc.*, **98**, 808 (1976).
(20) P. D. Pulsinelli, Ph.D. thesis, University of Pittsburgh, Pittsburgh, Pa., 1970, pp 69–79.
(21) J. Crosby, R. Stone, and G. E. Lienhard, *J. Am. Chem. Soc.*, **92**, 2891 (1970).
(22) J. Crosby and G. E. Lienhard, *J. Am. Chem. Soc.*, **92**, 5707 (1970).
(23) J. Ullrich, *Angew. Chem., Int. Ed. Engl.*, **8**, 82 (1969).
(24) J. H. Wittorf and C. J. Gubler, *Eur. J. Biochem.*, **14**, 53 (1970).
(25) G. A. Kochetov and R. A. Usmanov, *Biochem. Biophys. Res. Commun.*, **41**, 1134 (1970); C. P. Heinrich, K. Noack, and O. Wiss, *ibid.*, **44**, 275 (1971); G. A. Kochetov, R. A. Usmanov, and A. T. Meukh, *ibid.*, **54**, 1619 (1973).
(26) J. E. Biaglow, J. J. Mieyal, J. Suchy, and H. Z. Sable, *J. Biol. Chem.*, **244**, 4054 (1969); J. J. Mieyal, J. Suchy, J. E. Biaglow, and H. Z. Sable, *ibid.*, **244**, 4063 (1969); J. Suchy, J. J. Mieyal, G. Bantle, and H. Z. Sable, *Ibid.*, **247**, 5905 (1972).