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Stereochemistry of Intermediates in Thiamin Catalysis. 3. Crystal Structure of DL-2-(α-Hydroxybenzyl)oxythiamin Chloride Hydrochloride Trihydrate, an Inhibitor Adduct

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Abstract: The C(2) adduct of the oxythiamin inhibitor assumes a conformation that is nearly identical with that of the same derivative of thiamin, even though the unsubstituted oxythiamin was observed with a unique conformation not seen in any thiamin structure. The parameters of the oxopyrimidine ring agree well with those in oxythiamin. The crystal structure was determined using diffractometer data obtained by the θ:2θ scan technique with Mo radiation from a crystal having $P2_1/c$ space group symmetry and unit cell parameters a = 13.956 (5), b = 7.407 (3), c = 25.102 (8) Å, and β = 115.48 (2)°. The structure was solved by direct methods and refined by full-matrix least squares to an R = 0.098 for all 4520 reflections and R = 0.049 for the 2653 observed reflections.

2-(α-Hydroxybenzyl)oxythiamin, HBOT, is a C(2) adduct of the thiamin antagonist, oxythiamin. Although oxythiamin is an inhibitor of thiamin catalysis, it can react with substrate to form an intermediate in the holoenzyme system containing oxythiamin instead of thiamin as a cofactor, so that the inhibitory function appears at the step of product release.[2] Many of the intermediates of thiamin catalysis (C(2) adducts) are sufficiently stable under mildly acidic conditions to be isolated. From the crystal-structure analyses of 2-(α-hydroxyethyl)thiamin, HET,[3] and 2-(α-hydroxybenzyl)thiamin, HBT,[4] which are such intermediates, it has been shown that when there is a substituent on C(2) the molecular conformation with respect to the C(3,5′) bridge carbon atom is substantially different from that which characterizes the free thiamin molecule. Besides the change in the preferred conformation with respect to the bridge carbon, the adduct compounds display an apparent conformational stability that is imparted through an intramolecular S···O interaction with the O(2α1) oxygen and through the intramolecular ring stacking interaction between the pyrimidine and the phenyl rings in HBT. These structural features are thought to have important mechanistic properties in thiamin catalysis. The determination of the structural parameters for HBOT is of special interest, since it is known that oxythiamin assumes a conformation that differs from that of free thiamin.[5] From NMR studies, Gallo[6] finds a ring stacking interaction in HBOT similar to that which occurs in HBT, but suggests that the interaction in HBOT is weaker.

Experimental Section

Colorless tabular crystals from a sample of DL-2-(α-hydroxybenzyl)oxythiamin chloride hydrochloride trihydrate, which was kindly given to us by Dr. H. Sable at Case Western Reserve University, were grown from aqueous acetonitrile (1:6) by the addition of an equal volume of acetone at ~5 °C. The crystals are monoclinic with space group symmetry $P2_1/c$ as determined from Weissenberg photographs which indicate systematically absent reflections for $0k0$ when k is odd and $h0l$ when l is odd. The crystal used in the analysis was mounted with b approximately parallel to the φ axis and was thinly coated with epoxy as a precaution against changes in the crystal during the analysis. The cell parameters were determined from a least-squares fit of the setting angles for 12 centered reflections[7] (setting angles for each reflection were obtained from four separate measurements taken at $\pm 2\theta$, χ and $\pm 2\theta$, $180 + \chi$). The crystal data are summarized in Table I.

The intensity data were measured on a Picker FACS-I diffractometer with graphite-monochromated Mo Kα radiation ($2\theta_M$ = 11.97°) using a θ:2θ scan technique at a scan rate of 2°/min over a scan range of at least 2.4° to a sin θ/λ limit of 0.650 Å^{-1} (the scan range was adequate to accommodate the split diffraction profile which gave a maximum separation of 0.5° in ω along the C* axis). Background counts were accumulated for 20 s at the end of the scan range. Three standard reflections, which were monitored after every 50 reflections, showed gradual fluctuations throughout the analysis of less than ±3%. The data were corrected for this slow variation. Among the 6374 measured reflections, 4520 independent reflections, whose net intensities were above zero, were used for subsequent structure determination. Among 4520 reflections, 1867 reflections were treated as unobserved by the criterion of $F \leq 6\sigma(F)$.[8] Neither absorption nor extinction corrections were applied.

The structure amplitudes were converted to Es[9] and the structure was solved using MULTAN.[10] Coordinates of all 28 nonhydrogen atoms excluding the oxygen atoms of the three water molecules were determined from the E map using 306 E values ($E \geq 1.76$) with their calculated phases as coefficients. Conventional application of full-matrix least-squares refinement[9] (minimizing $\Sigma\omega(|F_o| - k|F_c|)^2$, where k is a single scale factor and $\omega = 1/\sigma^2(|F_o|)$), and difference Fourier syntheses located the remaining three oxygen atoms and all of the hydrogen atoms. In the last three cycles of refinement, parameters were separated into two blocks which consisted of (1) the thiazolium ring, the C(2), C(4), and C(5) substituents, and the two

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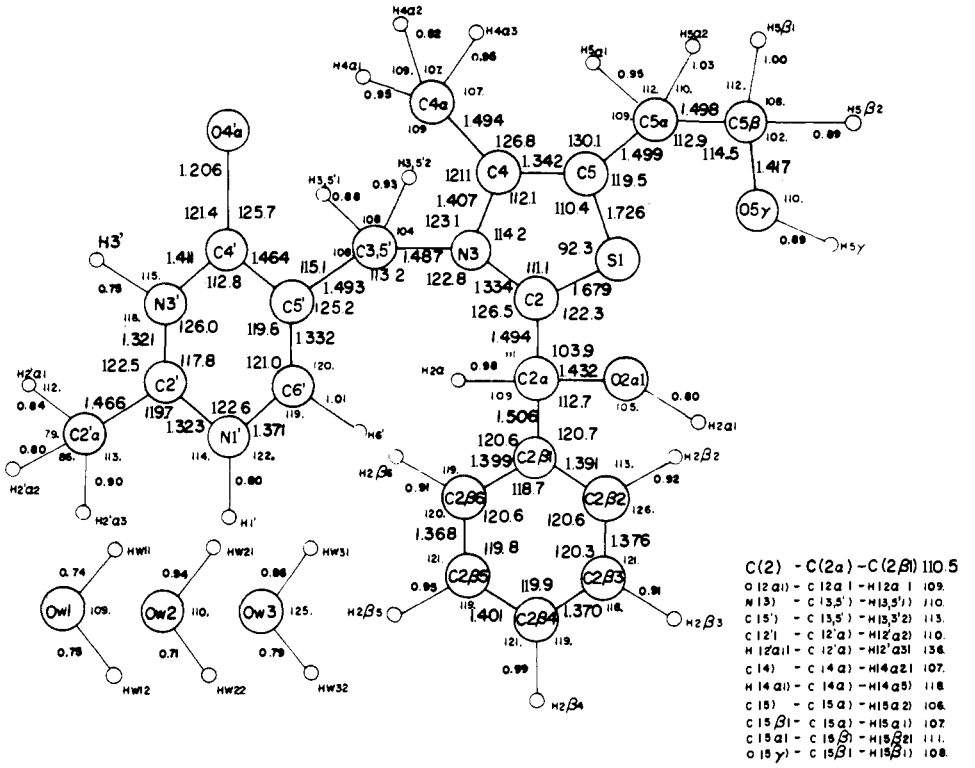

Figure 1. Schematic representation of the HBOT molecule showing the atomic numbering scheme, the bond distances (angstroms), and the bond angles (degrees). The esd's for bonds between nonhydrogen atoms range from 0.003 to 0.005 and average 0.004. The esd's involving hydrogen vary from 0.03 to 0.04 with an average value of 0.03. The esd's for the bond angles between nonhydrogen atoms range from 0.1 to 0.4 and average 0.3, while those involving hydrogen atoms range from 2.0 to 4.0 and average 2.5.

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

$C_{19}H_{22}N_3O_3S$·Cl·HCl·3H_2O	fw = 498.4
λ (Mo Kα) = 0.7107 Å	
a = 13.956 (5) Å	
b = 7.407 (3) Å	
c = 25.102 (8) Å	
β = 115.48 (2)°	
V = 2342.5 Å^3	
space group, $P2_1/c$	Z = 4
ρ_o = 1.414 (1) g/cm^3 by flotation in benzene-CCl_4	
ρ_c = 1.413 g/cm^3	
μ (Mo Kα) = 4.06 cm^{-1}	$F(000)$ = 1056
mp 159–161 °C (uncorrected values determined with thermolyne melting point apparatus)	
maximum crystal dimensions	bounding faces
0.42 mm along a	main faces (0,0,1), (0,0,$\bar{1}$)
0.32 mm along b	edge faces (0,$\bar{1}$,0), (0,1,0),
0.10 mm along c*	(4,1,0), (4,$\bar{1}$,0), (4,$\bar{1}$,0), ($\bar{3}$,1,0)

[a] Data measured at ambient temperature, ~25 °C.

chlorine atoms; and (2) the pyrimidinium ring, including all of its substituents, the two chlorines, S(1), and the three water molecules. Anisotropic thermal parameters were refined for the nonhydrogen atoms, but a B value of 4.0 was arbitrarily assigned to all of the hydrogen atoms. The atomic scattering factors for Cl^-, S, O, N, and C are from Cromer and Waber[11] and that for H is from Stewart et al.[12] The $\Delta f'$ and $\Delta f''$ values for S and Cl are from the International Tables.[13] The final R values were 0.049 for the 2653 observed reflections and 0.098 for all 4520 reflections. The weighted R was 0.032 and the value $[\Sigma(|F_o| - |F_c|)^2/(\text{no. of reflections} - \text{no. of variables})]^{1/2}$ was 1.2758. The final positional parameters are in Table II. The table of anisotropic thermal parameters and the structure factor table are available as supplementary material (see paragraph at end of paper for details).

Description of the Crystal and Molecular Structure

The schematic representation of the molecule in Figure 1 contains the bond distance and angle data as well as the atomic labeling. The structure of HBOT provides an interesting subject for comparison with both oxythiamin and the other thiazolium C(2) adducts. The bond distances in the oxopyrimidine ring, which is protonated in both HBOT and oxythiamin,[5] are in reasonable agreement with nearly all, agreeing to better than 3σ considering the larger standard deviations of the latter. The C(4′)-O(4′α) and C(5′)-C(6′) bonds show almost pure double bond character while N(3′)-C(4′) and C(4′)-C(5′) exhibit only minor multiple bond character. Hence, the formal positive charge that results from the protonation of the pyrimidine ring is not widely distributed over the oxopyrimidine ring. The tentative calculation of bond order shows that the positive charge is confined mostly through the N(1′)-C(2′)-N(3′) bonds and slightly on the N(1′)-C(6′) bond. This enhanced localization presents a marked contrast to the pyrimidine ring of thiamin where more extensive resonance occurs.[14a]

The shortening of the C(2′)-C(2′α) bond by 5σ compared to the normal sp^2-sp^3 C-C bond length[15] is common in thiamin structures when the neighboring atoms of the C(2′α) methyl group are electronegative. Such an environment increases the plausibility of the hyperconjugational resonance forms needed to account for the short bond.[14a] In this structure there are six water molecules or chloride ions around the methyl group, two of which are closer than the van der Waals contact distance (Table III).

Comparison of the thiazolium ring of HBOT with that of the other C(2) adduct structures provides a reasonably consistent picture of bond distances and angles. However, some of the bonds appear to exhibit somewhat greater variation than might be expected from the individual estimated standard deviations. It is uncertain whether or not some of these corre-

Table II. Atomic Coordinates for DL-2-(α-Hydroxybenzyl)oxythiamin Chloride Hydrochloride Trihydrate[a]

	x	y	z		x	y	z
			Nonhydrogen Atoms[b]				
Cl(1)	5547.3 (0.7)	1616 (1)	927.5 (0.4)	C(5α)	7102 (3)	3122 (5)	2502 (2)
Cl(2)	8195.3 (0.6)	6151 (1)	−1549.8 (0.4)	C(5β)	7701 (3)	3761 (5)	3125 (2)
S(1)	8437.5 (0.6)	1140 (1)	2156.3 (0.3)	O(5γ)	8697 (2)	2911 (3)	3440 (1)
C(2)	8921 (2)	2072 (4)	1712 (1)	C(3,5′)	8904 (3)	4936 (5)	1199 (1)
N(3)	8612 (2)	3788 (3)	1592(1)	N(1′)	6436 (2)	4219 (4)	−206 (1)
C(4)	7954 (2)	4391 (4)	1851 (1)	C(2′)	6716 (2)	4800 (4)	−617 (1)
C(5)	7768 (2)	3089 (4)	2166 (1)	N(3′)	7678 (2)	5484 (4)	−444 (1)
C(2α)	9551 (2)	1016 (4)	1467 (1)	C(4′)	8448 (2)	5651 (4)	146 (1)
O(2α1)	9783 (2)	−638 (3)	1794 (1)	C(5′)	8093 (2)	4911 (4)	571 (1)
C(2β1)	8919 (2)	732 (4)	813 (1)	C(6′)	7114 (2)	4248 (4)	383 (1)
C(2β2)	9314 (2)	1290 (5)	417 (1)	C(2′α)	5955 (3)	4706 (7)	−1240 (2)
C(2β3)	8729 (3)	1056 (5)	−180 (2)	O(4′α)	9313 (2)	6279 (3)	263 (1)
C(2β4)	7742 (3)	283 (5)	−394 (2)	O(W1)	4061 (2)	2247 (4)	2430 (1)
C(2β5)	7331 (3)	−291 (5)	−2 (2)	O(W2)	4612 (2)	2403 (4)	−425 (1)
C(2β6)	7914 (3)	−67 (4)	592 (2)	O(W3)	4717 (3)	−144 (4)	1812 (1)
C(4α)	7550 (3)	6286 (5)	1768 (2)				
			Hydrogen Atoms[c]				
H(2α)	1022 (2)	163 (4)	154 (1)	H(3,5′1)	951 (2)	455 (4)	121 (1)
H(2α1)	1022 (3)	−113 (5)	172 (1)	H(3,5′2)	903 (2)	607 (4)	138 (1)
H(2β2)	1000 (2)	172 (4)	60 (1)	H(1′)	587 (2)	372 (5)	−33 (1)
H(2β3)	899 (2)	137 (4)	−44 (1)	H(3′)	787 (3)	568 (5)	−68 (1)
H(2β4)	733 (2)	12 (4)	−83 (1)	H(6′)	687 (2)	370 (4)	67 (1)
H(2β5)	667 (2)	−88 (4)	−16 (1)	H(2′α1)	609 (3)	547 (5)	−144 (2)
H(2β6)	763 (2)	−39 (4)	85 (1)	H(2′α2)	618 (3)	408 (5)	−142 (2)
H(4α1)	719 (2)	653 (5)	136 (1)	H(2′α3)	546 (3)	384 (5)	−131 (1)
H(4α2)	808 (3)	695 (5)	192 (1)	H(W11)	354 (3)	262 (6)	222 (2)
H(4α3)	716 (2)	643 (4)	200 (1)	H(W12)	436 (3)	296 (5)	266 (2)
H(5α1)	686 (2)	193 (5)	252 (1)	H(W21)	484 (2)	215 (5)	−2 (1)
H(5α2)	648 (2)	399 (4)	228 (1)	H(W22)	458 (3)	158 (5)	−58 (2)
H(5β1)	728 (2)	362 (4)	336 (1)	H(W31)	493 (3)	24 (5)	156 (1)
H(5β2)	788 (3)	492 (5)	313 (1)	H(W32)	462 (3)	47 (5)	204 (1)
H(5γ)	862 (3)	202 (5)	349 (2)				

[a] Estimated standard deviation in parentheses is for the least significant figure. Positions are designated in fractional coordinates of unit-cell axes. [b] Positional parameters × 10^4. [c] Positional parameters × 10^3.

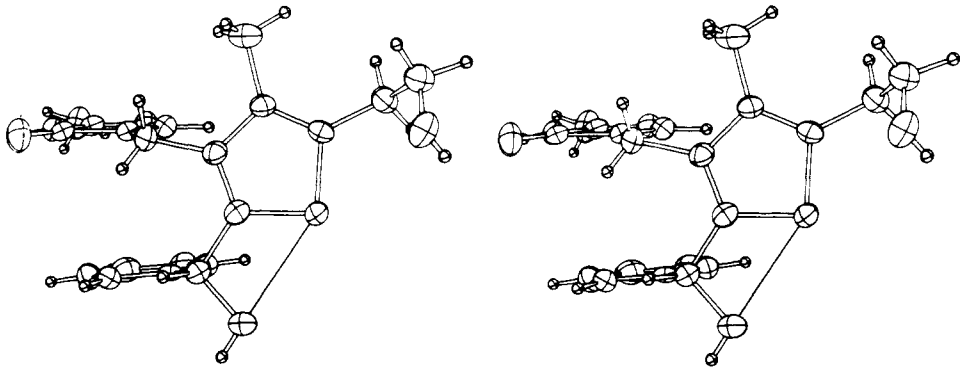

Figure 2. Stereoscopic view of HBOT looking along normal to thiazolium ring. The single line indicates the close contact of S(1) with O(2α1). The molecule shown is centrically related to the one for which coordinates are listed in Table II to permit a direct comparison with Figure 2 of HBT.[4]

late with the nearest neighbor environment as has been observed for the bond to the C(2′α) methyl. For example, C(4)-C(5) is shorter by 3σ in HBOT in comparison with the values in the other adduct structures. It was further noted that the chloride ion, Cl(1), is positioned over the thiazolium ring at a distance from C(5) which is less than the van der Waals contact distance by more than 3σ (Table III). However, any possible significance to this correlation is reduced by the finding of a similarly positioned chloride ion in HBT at a distance (3.506) slightly greater than the van der Waals distance. The C(4)-C(5) bond in HBT is the longest of the four structures. Since that chloride ion in both structures is extensively hydrogen bonded, including C(6′) in HBOT, it is likely that its close approach to the thiazolium ring is merely a consequence of other packing constraints.

It is of interest to examine how the adduct at C(2) influences the parameters of the thiazolium ring. On the basis of the initial adduct structures, it was observed that adduct formation introduced small but possibly significant changes in the thiazolium ring, namely, the S(1)-C(2) bond, which increased ~0.02 Å, and the S(1)-C(2)-N(3) angle, which decreased ~1°.[3] A comparison of the average values obtained from currently available structures with esd's ≤ 0.005 Å yields a similar conclusion, even though the statistical significance is not much improved. The values in Table IV further indicate that C(2)-N(3) is also lengthened by an amount similar to the

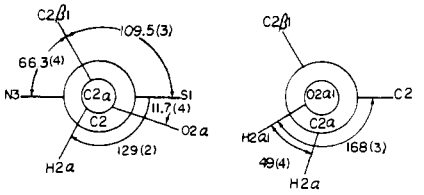

Figure 3. Torsion angles around C(2α) and O(2α1) presented as Newman projection of molecule, which is centrosymmetrically related to the one for which coordinates are listed in Table II.

S(1)-C(2) bond.

The planarities of the rings (Table V) are typical of those for other thiamin structures. The small deviations from strict planarity occasionally appear to be systematically correlated with certain structural features, but the effects are usually too small to be accepted with any confidence. For example, in this structure the N(1′) and C(4′) atoms are displaced to one side of the ring plane, while the remaining atoms are in the plane or displaced to the opposite side of the plane. This same condition exists in HBT and thiamin-picrolonate complex, both of which also exhibit significant ring stacking interactions with the pyrimidine ring.

A very surprising feature of this structure analysis is the striking similarity between HBOT and HBT. For example, in HBT the conformation of the thiamin rings with respect to the methylene bridge displays an **S** conformation having torsion angles $\varphi_T = +92.9\ (4)°$, $\varphi_P = -165.9\ (3)°$.[21a] In HBOT those values are +92.7 and −167.3°, respectively. Furthermore, the C(2) substituent in HBOT has the same conformation as that in HBT, so that again there is a close intramolecular stacking between the pyrimidine and phenyl rings. The similarities are clearly seen by comparing the molecular structure in Figure 2 with that of HBT. The torsion angles are given in Figure 3. The nearly parallel disposition of the phenyl and pyrimidinium rings is indicated by the small value of the dihedral angle in Table V. In comparison with HBT, the rings are more nearly parallel in HBOT by 1.6°. The major difference in the conformations of HBOT and HBT is that of the C(5) side chain, which can be seen in Figure 2 of both structures. The conformation of the C(5) side chain, which exhibits structural flexibility in thiamin, appears to be readily influenced by crystal packing forces.[4] A comparison of torsion angles of $\varphi_{5\alpha}$ and $\varphi_{5\alpha}$[21b] for all known thiamin structures is given in Table V of

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					
O(2α1)-H(2α1)···O(5γ)[i]			2.660 (4)	1.86 (4)	175 (4)
O(5γ)-H(5γ)···Cl(2)[ii]			3.091 (3)	2.41 (4)	169 (4)
N(1′)-H(1′)···O(W2)			2.719 (4)	1.93 (4)	166 (3)
N(3′)-H(3′)···Cl(2)			3.194 (3)	2.45 (4)	170 (3)
C(2α)-H(2α)···Cl(2)[vi]			3.712 (3)	2.75 (3)	168 (3)
C(6′)-H(6′)···Cl(1)			3.608 (3)	2.69 (3)	152 (2)
O(W1)-H(W11)···Cl(2)[iv]			3.197 (3)	2.45 (4)	179 (4)
O(W1)-H(W12)···O(W3)[v]			2.734 (5)	1.99 (4)	172 (4)
O(W2)-H(W21)···Cl(1)			3.123 (3)	2.19 (4)	174 (3)
O(W2)-H(W22)···Cl(1)[iii]			3.203 (3)	2.50 (4)	167 (4)
O(W3)-H(W31)···Cl(1)			3.191 (3)	2.33 (4)	173 (3)
O(W3)-H(W32)···O(W1)			2.758 (4)	1.99 (4)	165 (4)
(b) Close Contacts around S(1)					
C(2)-S(1)···O(2α1)				2.749 (3)	56.7 (1)
C(5)-S(1)···O(2α1)					149.0 (1)
(c) Nearest Neighbors around C(2′α) Methyl and Other Close Contacts					
C(2′α)	O(W1)[ii]		3.554 (6)		
C(2′α)	O(W3)[iv]		3.633 (6)		
C(2′α)-H(2′α1)···Cl(2)			3.690 (5)	3.11 (4)	128 (5)
C(2′α)	Cl(1)[iv]		3.722 (5)		
C(2′α)	O(W2)		3.730 (6)		
C(2′α)	O(W1)		3.736 (6)		
Cl(1)	C(5)		3.489 (3)		

Symmetry Code

none	x	y	z				
i	$2-x$,	$-\frac{1}{2}+y$,	$\frac{1}{2}-z$	iv	$1-x$,	$1-y$,	$-z$
ii	x,	$\frac{1}{2}-y$,	$\frac{1}{2}+z$	v	$1-x$,	$\frac{1}{2}+y$,	$\frac{1}{2}-z$
iii	$1-x$,	$-y$,	$-z$	vi	$2-x$,	$1-y$,	$-z$

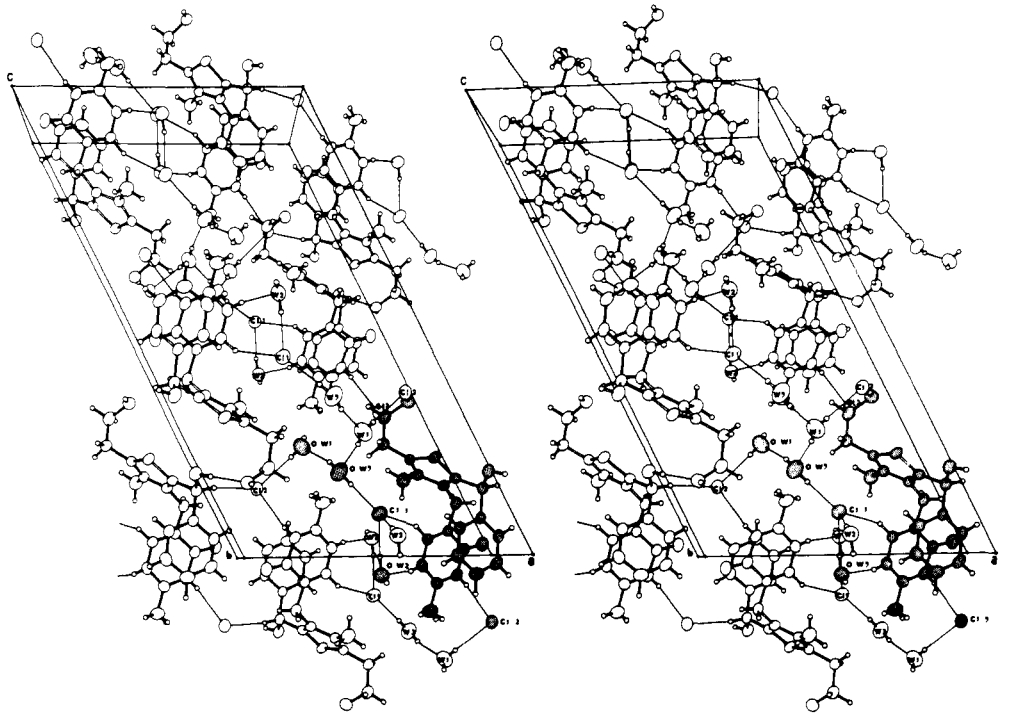

Figure 4. Stereopacking diagram for DL-2-(α-hydroxybenzyl)oxythiamin chloride hydrochloride trihydrate. Hydrogen bonds are shown as single solid lines. The shaded atoms correspond to those for which coordinates are listed in Table II.

Table IV. Comparative Parameters of the Thiazolium Ring[a]

	distances, Å				
	S(1)-C(5)	S(1)-C(2)	C(2)-N(3)	N(3)-C(4)	C(4)-C(5)
C(2)-H structures[b]	1.723 (7)	1.672 (4)	1.317 (4)	1.394 (5)	1.357 (5)
C(2)-R structures[c]	1.721 (8)	1.685 (8)	1.330 (5)	1.398 (8)	1.350 (7)
	angles, deg				
	C(5)-S(1)-C(2)	S(1)-C(2)-N(3)	C(2)-N(3)-C(4)	N(3)-C(4)-C(5)	C(4)-C(5)-S(1)
C(2)-H structures	91.4 (1)	112.4 (3)	114.1 (3)	111.6 (4)	110.5 (4)
C(2)-R structures	91.7 (7)	111.4 (4)	114.3 (1)	111.8 (6)	110.8 (8)

[a] The root mean square deviations in parentheses refer to the least significant position. [b] Structures included in the average are: thiamin pyrophosphate·HCl,[14] thiamin·Cl,[16] thiamin picrolonate,[17] thiamin·$CdCl_4$,[18] thiamin pyrophosphate·$4H_2O$,[19] and thiamin pyrophosphate·$4.5H_2O$.[20] [c] Structures included in the average are: HBOT, 2-(α-hydroxyethyl)thiamin·HCl[3] and 2-(α-hydroxyethyl)thiazolium·Br.[3]

Table V. Least-Squares Planes[a] and Dihedral Angles in HBOT

plane	*A*	*B*	*C*	*D*	σ	displacements
thiazolium	505	303	512	8967	14	**S(1) 11, C(2)-9, N(3) 3, C(4) 7, C(5) − 12,** C(2α) − 118, O(2α1) 95, H(2α1) 199, C(4α) 41, C(5α) − 42, C(5β) 1325, O(5γ) 2241, C(3,5′) −39, Cl(1) −3500
pyrimidinium	418	−906	−243	1064	16	**N(1′) −14, C(2′) 10, N(3′) 6, C(4′) −16, C(5′) 13, C(6′) 2,** Cl(1) 522, Cl(2) 535, C(3,5′) 87, C(2′α) 9, O(4′α) −5, O(W2) 273, H(1′) 67, H(3′) 127, H(6′) 52
phenyl	431	−900	−134	4608	3	**C(2β1) −1, C(2β2) −2, C(2β3) 4, C(2β4) −3, C(2β5) 0,** C(2β6) 2, C(2α) −30, H(2α) −64, H(2β2) 67, H(2β3) 44, H(2β4) 7, H(2β5) 44, H(2β6) −39

dihedral angles	deg
thiazolium-pyrimidinium	96.9
thiazolium-phenyl	90.5
pyrimidinium-phenyl	7.3

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

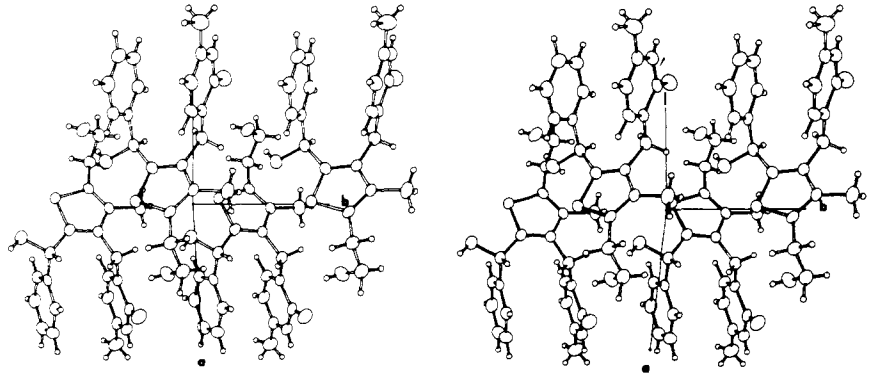

Figure 5. ORTEP drawing showing the ring stacking and the arrangement of the thiazolium ring in HBOT.

Shin et al.[17] While HBT has an extreme $\varphi_{5\alpha}$ value of 3.0°, HBOT shows the most frequently observed conformation, with $\varphi_{5\alpha}$ = 91.8 (3) and $\varphi_{5\beta}$ = −48.9 (4)°. As a result, in HBOT there is no close intramolecular contact between S(1) and O(5γ) (3.352 Å compared with 3.003 Å in HBT). However, the short S(1)···O(2α1) contact observed in HBT and HET also exists in HBOT with a value of 2.749 Å, which is 0.015 Å shorter than that in HBT and 0.152 Å shorter than that in HET. The spatial disposition of O(2α1) and H(2α1) is nearly identical with that in HBT, with both atoms located close to the plane of the thiazolium ring and the hydrogen directed away from S(1).

The stereoscopic ORTEP[22] packing drawing of the structure is shown in Figure 4. The characteristic ring stacking interaction of the 4′-aminopyrimidine ring shown in HBT and thiamin-picrolonate dihydrate[17] is also retained in HBOT which contains a 4′-oxopyrimidine ring. The stacking interactions between the phenyl and the pyrimidine rings actually form a continuous column extending along the *b* axis of the crystal (Figure 5) in exactly the same manner as in HBT. The detailed overlaps of the intra- and intermolecular ring stackings are shown in Figure 6. The closest intermolecular ring contact occurs between C(4′) and C(2β5) at a distance which is 0.05 Å less than the closest contact in HBT. The closest intramolecular ring contact occurs between C(5′) and C(2β1) with a separation of 0.02 Å less than the minimum for HBT. (A complete table of contact distances is available as supplementary material.) A comparison of the displacement from the least-squares planes also shows a closer association in HBOT. These contact distances imply that the ring stacking interaction in HBOT is slightly stronger than that in HBT. The suggestion of the stronger ring stacking with the oxypyrimidine

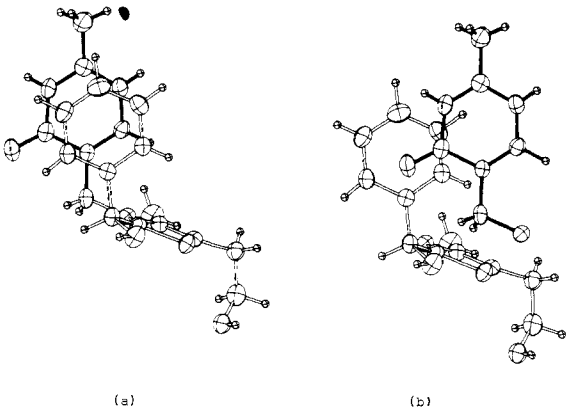

Figure 6. ORTEP drawing showing the ring stacking in HBOT. View along the normal to the pyrimidine ring. Black bonds for the pyrimidine ring: (a) intramolecular ring stacking; (b) intermolecular ring stacking.

ring is supported by the observed close chloride ion contact with C(2′) in oxythiamin,[5] an interaction not previously observed in any thiamin structure. This intermolecular ring stacking is typical of dipole-induced dipole interactions of pyrimidine rings as described by Bugg et al.[23]

In this crystal structure there are 12 unique hydrogen bonding schemes which are listed in Table III. Ten of those hydrogen bonds utilize all the atoms capable of forming strong hydrogen bonds except for the O(4′α) atom. The remaining two are relatively weak C–H···X type hydrogen bonds. Three water molecules and Cl(1) form a hydrogen-bonded sheet parallel to the xz plane. Between these sheets there are two layers of HBOT molecules running along the direction of the c axis. Within the HBOT layers a Cl(2) atom connects two centrosymmetrically related molecules through N(3′)→Cl(2)←C(2α) hydrogen bonds and two c-glide related molecules through N(3′)←Cl(2)→O(5γ) hydrogen bonds. The only direct hydrogen bond between HBOT molecules is O(2α1)–H···O(5γ), which connects HBOT molecules in a spiral along the twofold screw axis within the HBOT layers. There are many similarities in the packing of HBOT and HBT, which include the ring stacking along the direction of the b axis, the formation of the extensive hydrogen-bonded sheet, and the hydrogen bonding network between three water molecules and Cl(1) atom in that sheet. (The atom designations for the water molecules and Cl atoms are directly comparable in HBOT and HBT.) H(2α), which is known to be acidic, also forms a hydrogen bond with Cl(2). In HBT H(2α) is a hydrogen-bond donor to O(5γ). However, the difference in 4′ substituent results in an altered hydrogen bonding scheme, especially around Cl(2). Furthermore, the two structures have different space group symmetry. It is especially interesting to note that O(4′α) is always in the hydrophobic pocket rather than being exposed to a hydrogen-bonded sheet. Instead C(6′), which is exposed to that sheet, forms a hydrogen bond with Cl(1). In oxythiamin the C(6′) atoms in both independent molecules donate a hydrogen bond to O(4′α) atoms.

Discussion

The most surprising aspect of this analysis is the finding that the C(2) derivative of oxythiamin assumes an **S** conformation like the C(2) derivative of thiamin even though oxythiamin itself is unique in assuming a **V** conformation. There are two structural features that are evidently important for selecting the conformation observed in HBOT, namely, the intramolecular interaction between S(1) and the O(2α1) of the C(2) substituent and, secondly, the intramolecular ring stacking between the pyrimidine and the phenyl rings. It is noteworthy that both H(2α) and H(2α1) are potential intramolecular hydrogen bond donors to O(4′α) if the molecule were to assume a **V** conformation with the C(2) substituent in the same configuration as that observed in the hydroxyethyl derivative,[3] where there cannot be intramolecular ring stacking. A modification of Jordan's[24] conformational energy diagram for HET as shown in Figure 7 indicates that the **V** conformation is a potentially stable one when the C(2) derivative assumes the configuration observed in HET.[3] However, from the observed HBOT structure, it appears that the intramolecular ring stacking is preferred over an intramolecular hydrogen bond. It is significant in this regard that O(4′α) does not participate as a hydrogen bond acceptor in this structure although both H(2α) and H(2α1) are involved as intermolecular hydrogen bond donors. It is of further interest that in spite of the striking molecular similarity between HBOT and HBT, there are substantial differences in their respective crystal structures including different space group symmetry. These differences in their molecular environments are a result of the altered bonding characteristics of their respective pyrimidine rings. Even the change in the conformation of the C(5) substituent, the only aspect of their molecular structures that is substantially different, is made to accommodate their specific environments. It is apparent that, although the primary difference between thiamin and oxythiamin is the replacement of the 4′-amino substituent with an oxo group, the effects of this change are not confined to the substituent, but extend throughout the pyrimidine ring and its environment. Thus, although the inhibitory action of oxythiamin may be related to this primary structural change, the resulting secondary effects may actually be the principal source of this function!

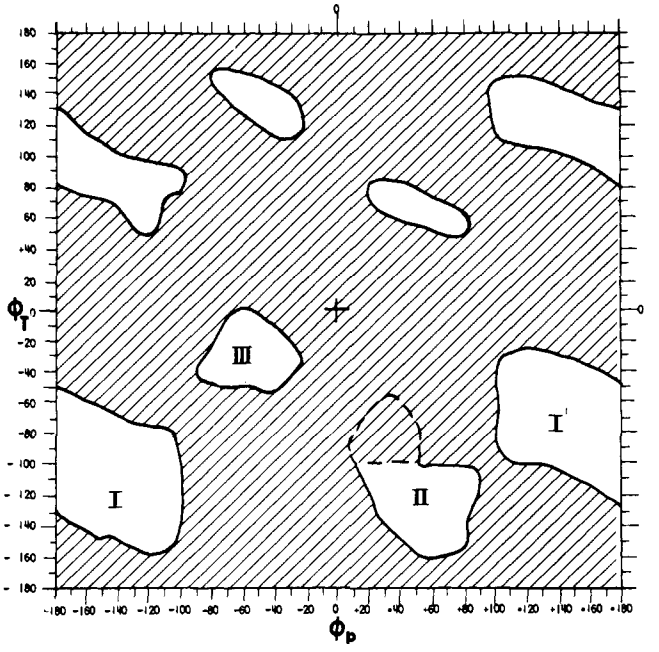

Figure 7. Conformational energy maps for HET. The 18-kcal contour from the conformational energy map of Jordan (Figure 5)[24] has been replotted in terms of φ_T and φ_P as defined in ref 21 with the origin $\varphi_T = \varphi_P = 0$ at the center of the map. The coordinates in this figure correspond directly with the modified plot for thiamin, ref 5. The cross-hatched area represents conformations with energies greater than 18 kcal. The largest continuous area, marked with I and I′, contains the S conformations which have been observed with all the C(2)-substituted structures. The region marked II contains the **V** conformation observed in oxythiamin. The small area labeled III is adjacent to the region containing the **F** conformations and corresponds to the conformation originally predicted[14b] for HET but never observed.

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Supplementary Material Available: Structure factor table for DL-2-(α-hydroxybenzyl)oxythiamin chloride hydrochloride trihydrate, anisotropic thermal parameters, and table of interring separations (6 pages). Ordering information is given on any current masthead page.

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(21) (a) The torsion angle φ_T = C(5′)–C(3,5′)–N(3)–C(2) and φ_P = N(3)–C(3,5′)–C(5′)–C(4′). An **S** conformation is specified by the torsion angles ($\varphi_T \approx \pm 100°$, $\varphi_P \approx \pm 150°$). For more complete details see footnote 13 in ref 4. (b) $\varphi_{5\alpha}$ = S(1)–C(5)–C(5α)–C(5β) and $\varphi_{5\beta}$ = C(5)–C(5α)–C(5β)–O(5γ).
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Communications to the Editor

Synthesis and Characterization of Bis(fulvalene)-divanadium and the Crystal Structure of Its Oxidation Product, Bis(fulvalene)bis(acetonitrile)divanadium(III)-(*V*–*V*) Bis(hexafluorophosphate)–Acetonitrile (1/1)

Sir:

Because of the current interest in the structure and reactivity of coordinatively unsaturated metallocenes[1] and the formally analogous metallofulvalene derivatives,[2] we wish to report our initial synthetic, physical, and X-ray structural studies of the bis(fulvalene)divanadium system.

The neutral $(C_{10}H_8)_2V_2$ (**1**) was obtained by the reaction of VCl_2·2THF[3] with 1 equiv of fulvalene dianion[2d] in refluxing THF. The dark purple air-sensitive product was isolated and purified by repeated sublimation under high vacuum at 230 °C.[4] The solubility of **1** in polar and nonpolar solvents is very low. The mass spectrum[5] and infrared spectrum of **1** are similar to those for the neutral bis(fulvalene)dimetal derivatives of Ni,[6a] Co,[6b] Fe,[6c] and Cr.[6b] Magnetic susceptibility measurements on solid samples indicate that **1** is diamagnetic from 4.2 to 100 K. This is consistent with the observed diamagnetism of both the dicationic and neutral derivatives of all bis(fulvalene)dimetal complexes studied to date, from vanadium, with 28[6b] and 30 valence electrons, respectively, to nickel, with 38 and 40[6a] valence electrons. The complete magnetic coupling found in **1**, compared to the "parent" vanadocene with an open-shell 15-electron structure and three unpaired electrons, can be rationalized by either a direct metal–metal interaction (bond) or by ligand-propagated exchange. Although X-ray crystallographic data for neutral $(C_{10}H_8)_2Ni_2$ clearly indicate the presence of a ligand-propagated exchange,[7] an assessment of the relative contributions from each exchange mechanism for electron-deficient early transition metal derivatives must await similar detailed structural information.

The oxidation of **1** with either 1 or 2 equiv of ferrocenium hexafluorophosphate per dinuclear vanadium complex in dry oxygen-free acetonitrile yields only a two-electron product, $[(\eta^5{:}\eta^5\text{-}C_{10}H_8)_2(CH_3CN)_2V_2(III)(V\text{–}V)^{2+}][PF_6^-]_2\cdot CH_3CN$ (**2**), that was isolated as brown microcrystals by the slow addition of toluene.[8] Magnetic susceptibility measurements in acetonitrile at 310 K by the Evans NMR method[9] indicate that **2** is paramagnetic with μ_{eff} = 2.9 BM per dinuclear vanadium complex. Measurements on solid samples from 4 to 80 K indicate a μ_{eff} = 3.0 BM. These results establish the presence of two unpaired electrons for the dinuclear metal complex. The infrared spectrum of **2** exhibits terminal nitrile stretches at 2320 and 2290 cm^{-1}.

Hygroscopic and air-sensitive single crystals of the acetonitrile solvate **2**, obtained by recrystallization from acetonitrile/benzene, are orthorhombic and noncentrosymmetric, space group $Cmc2_1$ (C_{2v}^{12}, No. 36), with a = 7.771 (1) Å, b = 20.556 (3) Å, c = 18.324 (3) Å, and Z = 4 ($[(C_{10}H_8)_2V_2(NCCH_3)_2][PF_6]_2\cdot CH_3CN$ formula units). The various statistical indicators calculated with normalized structure factors, as well as all stages of the structure solution and refinement, were in agreement with the choice of a noncentrosymmetric space group. Diffracted intensities were measured for 2193 independent reflections having $2\theta_{MoK\bar{\alpha}} < 58.7°$ on a computer-controlled Syntex $P\bar{1}$ autodiffractometer using graphite-monochromated Mo $K\bar{\alpha}$ radiation and full (1° wide) ω scans. The structural parameters[10] have been refined to convergence [R = 0.045 for 949 independent reflections having $2\theta_{MoK\bar{\alpha}} < 43°$ and $I > 3\sigma(I)$] in cycles of unit-weighted, full-matrix, least-squares refinement which employed anisotropic thermal parameters for all nonhydrogen atoms.

The structural analysis reveals that the crystal is composed of bis(fulvalene)bis(acetonitrile)divanadium(III)(*V*–*V*) dications (Figure 1), hexafluorophosphate anions, and acetonitrile molecules of crystallization. Although each $[(\eta^5{:}\eta^5\text{-}C_{10}H_8)_2(CH_3CN)_2V_2]^{2+}$ unit is required to possess only crystallographic C_s-m symmetry (with the two vanadium atoms and the nonhydrogen atoms of their coordinated acetonitrile ligands lying in the mirror plane), the dication as a whole approximates rather closely idealized C_{2v} site symmetry with the pseudo-C_2 axis passing through the midpoints of the C_9–C_{10}' and V_a–V_b vectors of Figure 1. Bond lengths and angles for chemically equivalent groupings, averaged in accord with approximate C_{2v} molecular symmetry, include: V_a–V_b, 3.329 (4) Å; V–N, 2.09 (1,2,2,2) Å;[11] V–C, 2.28 (1,2,3,10)

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