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Crystal and Molecular Structure of Oxythiamin Chloride Hydrochloride Monohydrate. A Thiamin Antagonist with a Conformation That Differs from Thiamin

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Abstract: The hydrochloride salt of oxythiamin assumes a conformation with respect to its C(3,5′) methylene bridge that differs substantially from the characteristic conformations of thiamin or its C(2) substituted derivatives. Its intermolecular bonding patterns are very similar to those observed in thiamin structures, although N(3′) is now a hydrogen bond donor and the 4′-oxo group is a hydrogen bond acceptor. There is also a close contact between a chloride ion and the oxopyrimidine ring normal to the plane of the ring. The crystal structure was determined using diffractometer data obtained by the θ:2θ scan technique with Cu radiation from a crystal having $P2_1$ space group symmetry and unit cell parameters a = 13.072 (4), b = 8.977 (3), c = 15.097 (4) Å, and β = 110.17 (2)°. The structure was solved by direct methods and refined by least squares to an R = 0.104 for all 3039 independent reflections and an R = 0.041 for the 1788 observed reflections.

Oxythiamin is a broadly active and potent antagonist of thiamin[2] (vitamin B_1), which differs from thiamin only in that an oxygen atom replaces the 4′-amino group. Oxythiamin can be readily converted to its pyrophosphate ester, since it is a suitable substrate for thiamin kinase, the enzyme that catalyzes the phosphorylation of thiamin into the active coenzyme, thiamin pyrophosphate. Furthermore, it has been found that oxythiamin pyrophosphate, OTPP, is as acceptable as thiamin pyrophosphate at the binding site of pyruvate decarboxylase, and that pyruvate reacts with the OTPP holoenzyme to form the C(2) adduct intermediate, but the reaction does not proceed to release the acetaldehyde product.[3] The inhibitory properties of oxythiamin have been attributed to its inability to form the intramolecular N···O hydrogen bond between the 2α-hydroxyl group and the 4′ substituent. This intramolecular hydrogen bond has been proposed to both stabilize the adduct and assist in proton removal. In order to achieve this interaction, the C(2) adduct of thiamin is depicted as assuming a **V** conformation. (See ref 4 for a description of several conformations.) However, this is not supported by the crystal-

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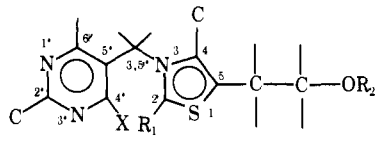
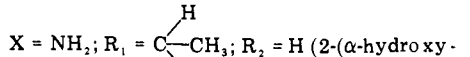
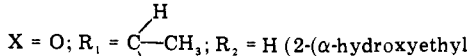


X = NH_2; R_1 = R_2 = H (thiamin); R_2 = $P_2O_7H_3$ (thiamin pyrophosphate)

X = O; R_1 = R_2 = H (oxythiamin); R_2 = $P_2O_7H_3$ (oxythiamin pyrophosphate)

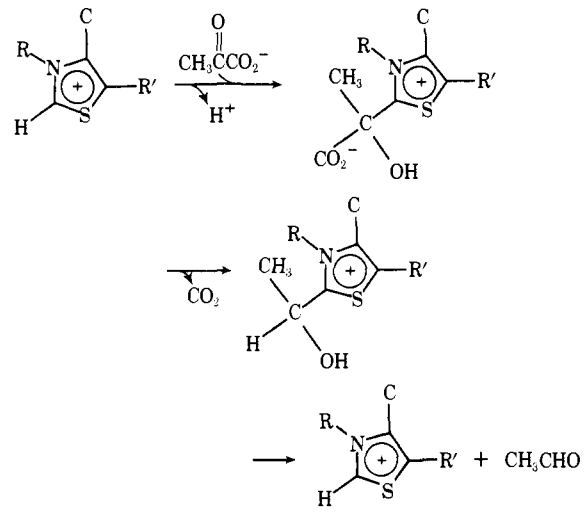


X = NH_2; R_1 = $-CH(OH)-CH_3$; R_2 = H (2-(α-hydroxyethyl)thiamin)



X = O; R_1 = $-CH(OH)-CH_3$; R_2 = H (2-(α-hydroxyethyl)-oxythiamin)

structure analysis of 2-(α-hydroxyethyl)thiamin, which displays the **S** conformation. In the **S** form, there is no possibility of an intramolecular interaction of the 4′-amino group with the hydroxyl group of the C(2) substituent. Hence the function of the 4′-aminopyrimidine ring of thiamin still remains an open question.



Whatever the function of the 4′-aminopyrimidine ring may be, the replacement of the 4′-amino group with a 4′-oxo may introduce structural changes (electronic and conformational) which abolish or greatly diminish the catalytic properties of thiamin. Although some of the changes may be related to the active apoenzyme-coenzyme complex, even those factors are ultimately dependent upon the chemical differences between thiamin and oxythiamin.

The primary purpose of this study is to determine whether there are any structural differences between thiamin and oxythiamin as a result of this single-site substitution. This is particularly important since there is a conflicting viewpoint about the preferred conformation of the free thiamin. There are numerous crystal-structure analyses indicating that the **F** form is the preferred conformation, while the **V** form has been proposed from the NMR studies of thiamin-indole complexes.[5] Theoretical calculations[6] and ^{13}C magnetic relaxation studies[7] suggest that there are a whole spectra of isoenergetic conformations. In addition to this conformational aspect, the crystal-structure analysis of oxythiamin may not only give clues to its inhibitory properties but may also indicate possible catalytic function(s) for the 4′-aminopyrimidine ring.

Experimental Section

This structure analysis is based on two diffraction experiments. For the first of these, transparent tabular single crystals were grown from aqueous ethanol solution by a vapor diffusion method. Since microcrystals were rapidly deposited on the surface of the crystal upon exposure to the air, the crystals were transferred to mineral oil and then mounted in thin-walled glass capillaries. A crystal having dimensions 0.48 × 0.24 × 0.08 mm along the a^*, b^*, and c^* axes, respectively, was used in the experiment.

Table I. Crystal Data for Oxythiamin Chloride Hydrochloride Monohydrate

formula: $C_{12}H_{16}N_3O_2S\cdot Cl\cdot HCl\cdot H_2O$
mol wt: 356.26
space group: $P2_1$, $Z = 4$
$F(000)$: 744
μ(Cu Kα): 47.6 cm^{-1}
ρ_{obsd}: 1.408 g/cm^3 in CCl_4-benzene mixture (first crystal)

	first crystal	second crystal
a, Å	13.059 (9)	13.072 (4)
b, Å	8.959 (4)	8.977 (3)
c, Å	15.089 (8)	15.097 (4)
β, deg	110.27 (2)	110.17 (2)
V, Å^3	1656.0	1662.9
ρ_{calcd}, g/cm^3	1.429	1.423

Oscillation and Weissenberg photographs, which showed systematically absent reflections for $0k0$ when k is odd, indicated the crystals were monoclinic with space group symmetry $P2_1/m$ or $P2_1$. The space group $P2_1$ was confirmed later in the structure determination. The crystal data are given in Table I. The unit cell parameters were determined by a least-squares fit of the orientation and 2θ angles for 12 reflections[8] measured with Cu Kα radiation on a diffractometer. The values used for each reflection were the average of four separate measurements taken at $(\pm 2\theta, \chi)$ and $(\pm 2\theta, 180° + \chi)$. The intensity data were collected with graphite-monochromated Cu Kα radiation on a Picker FACS-1 X-ray diffractometer to a 2θ limit of 127°. 2867 independent reflections were measured by the θ:2θ scan technique over a scan range of 1.7° at a scan rate of 1° per min. The background was counted for 20 s at each end of the scan range. Three standard reflections were monitored after each of 50 data reflections. Their intensities, which showed a rapid decrease of up to 25% for the last 10% of the reflections, were used to place the reflection data on a common scale. The intensities were converted to relative structure amplitudes after correction for Lorentz and polarization effects appropriate for graphite-monochromated ($2\theta_m$ = 26.16°) radiation. Of the independent reflections, 338 (11.8%) were unobserved, as defined by $|F| \leq 6\sigma(F)$.[9] No correction for the absorption and the extinction effects was made.

Since the data from this crystal could not be refined below an R factor of 11% and no other good single crystals were available, crystallization was undertaken again. The second crystals were grown from aqueous acetone solution by adding acetone gradually over a period of time. These crystals appeared to be more stable than the first ones. A 0.3-mm long needle-shaped crystal was mounted on a glass fiber with the b^* axis parallel to the φ axis. No differences were observed between the Weissenberg photographs of the two crystalline samples. The crystal data for the second crystal are also presented in Table I. The unit cell parameters were determined by a least-squares refinement of 2θ angles for 24 reflections (2θ range 26–48°) measured on a Nonius CAD-4 diffractometer. The intensity data were collected by θ:2θ scan method on the CAD-4 using graphite-monochromated Cu Kα radiation to a 2θ limit of 130°. After the data reduction, 1251 (41.2%) of the 3039 measured reflections were considered unobserved with the criteria $|F| \leq 6\sigma(F)$. No correction for the absorption and the extinction effects was made.

Structure Determination and Refinement. The structure was solved with MULTAN[10] using the first data set. The space group was assumed to be $P2_1$ from packing considerations. From the initial E map,[11] 34 atoms could be identified out of 42 nonhydrogen atoms. The initial R factor ($R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$) was 0.37. A difference Fourier synthesis revealed the remaining atoms except two water oxygens. Three cycles of isotropic full-matrix least-squares refinement lowered the R factor to 0.26, and two water oxygens were located in a difference Fourier synthesis. Although very painstaking refinement procedures were followed, the refinement converged at R = 0.11 and most of the hydrogen atoms could not be located.

Table II. Fractional Coordinates and Temperature Factors for Oxythiamin Cl·HCl Hydrate

A. Nonhydrogen Atoms[a]

atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
				Molecule A					
S(1)	1295 (1)	4043 (2)	810 (1)	40 (1)	59 (1)	66 (1)	13 (1)	24 (1)	−12 (1)
C(2)	1392 (5)	4005 (8)	1929 (5)	32 (3)	48 (4)	58 (4)	6 (3)	8 (3)	−1 (4)
N(3)	474 (3)	4444 (5)	2069 (3)	30 (2)	30 (3)	45 (3)	−1 (2)	17 (2)	−9 (2)
C(4)	−357 (4)	4798 (7)	1238 (5)	28 (3)	41 (4)	50 (4)	5 (3)	16 (3)	−8 (3)
C(4α)	−1453 (5)	5233 (11)	1258 (6)	18 (3)	90 (7)	60 (5)	7 (4)	10 (3)	−2 (5)
C(5)	−48 (5)	4708 (8)	458 (5)	41 (3)	53 (4)	47 (4)	7 (3)	15 (3)	−11 (3)
C(5α)	−669 (6)	5116 (10)	−521 (5)	49 (4)	67 (6)	52 (5)	13 (4)	11 (4)	−8 (4)
C(5β)	−843 (6)	3806 (14)	−1199 (6)	56 (5)	118 (9)	52 (5)	18 (5)	14 (4)	3 (5)
O(5γ)	152 (5)	3305 (8)	−1286 (4)	88 (4)	78 (4)	89 (4)	8 (4)	48 (3)	−21 (4)
C(3,5′)	418 (5)	4602 (8)	3017 (5)	32 (3)	38 (4)	59 (4)	−3 (3)	13 (3)	1 (3)
N(1′)	−373 (4)	8377 (7)	3659 (4)	27 (3)	46 (3)	49 (3)	5 (3)	21 (2)	−2 (3)
C(2′)	473 (5)	9225 (7)	3716 (4)	33 (3)	25 (3)	44 (3)	6 (2)	16 (3)	7 (3)
C(2′α)	502 (6)	10815 (8)	3936 (6)	40 (4)	31 (4)	77 (5)	12 (3)	21 (4)	1 (4)
N(3′)	1299 (3)	8584 (7)	3530 (3)	25 (2)	47 (3)	38 (3)	−6 (2)	11 (2)	−6 (3)
C(4′)	1381 (5)	7101 (7)	3318 (4)	35 (3)	30 (3)	42 (4)	2 (3)	16 (3)	−3 (3)
O(4′α)	2184 (3)	6659 (6)	3170 (3)	21 (2)	57 (3)	78 (3)	−1 (2)	28 (2)	−8 (3)
C(5′)	426 (4)	6237 (6)	3262 (4)	26 (3)	30 (3)	29 (3)	−2 (2)	11 (2)	9 (2)
C(6′)	−397 (5)	6873 (7)	3457 (4)	33 (3)	37 (4)	40 (3)	1 (3)	14 (3)	0 (3)
				Molecule B					
S(1)	3696 (1)	3673 (2)	−512 (1)	44 (1)	61 (1)	62 (1)	5 (1)	29 (1)	−8 (1)
C(2)	3632 (5)	3946 (7)	−1637 (5)	37 (3)	33 (4)	66 (4)	7 (3)	20 (3)	−3 (3)
N(3)	4580 (3)	3577 (6)	−1732 (3)	26 (2)	33 (3)	46 (3)	0 (2)	11 (2)	−9 (2)
C(4)	5381 (4)	3061 (7)	−937 (4)	29 (3)	33 (3)	41 (3)	−4 (2)	17 (2)	7 (3)
C(4α)	6483 (6)	2644 (11)	−921 (5)	45 (4)	79 (6)	41 (5)	−5 (4)	18 (4)	0 (4)
C(5)	5033 (5)	3065 (8)	−168 (5)	45 (3)	41 (4)	54 (4)	7 (3)	13 (3)	−3 (3)
C(5α)	5613 (7)	2561 (10)	807 (6)	63 (5)	70 (6)	56 (5)	30 (4)	26 (4)	10 (4)
C(5β)	5848 (7)	3810 (13)	1522 (6)	70 (5)	107 (8)	48 (4)	18 (6)	12 (4)	−29 (5)
O(5γ)	4848 (5)	4401 (8)	1595 (4)	79 (4)	80 (4)	67 (4)	15 (3)	36 (3)	−6 (3)
C(3,5′)	4698 (5)	3639 (8)	−2681 (5)	32 (3)	43 (4)	56 (4)	−3 (3)	15 (3)	24 (3)
N(1′)	5309 (4)	180 (7)	−3797 (3)	23 (2)	57 (4)	39 (3)	9 (2)	16 (2)	10 (3)
C(2′)	4402 (4)	−618 (7)	−3998 (4)	27 (3)	44 (4)	39 (3)	4 (3)	11 (3)	1 (3)
C(2′α)	4280 (8)	−2131 (10)	−4420 (7)	57 (5)	52 (5)	75 (7)	7 (4)	30 (4)	−4 (5)
N(3′)	3586 (4)	−35 (6)	−3774 (3)	28 (2)	43 (3)	42 (3)	0 (2)	16 (2)	−2 (2)
C(4′)	3603 (5)	1328 (7)	−3345 (4)	29 (3)	46 (4)	41 (3)	2 (3)	18 (3)	−3 (3)
O(4′α)	2840 (3)	1776 (5)	−3137 (3)	28 (2)	50 (3)	76 (3)	−5 (2)	30 (2)	−19 (3)
C(5′)	4621 (5)	2126 (7)	−3116 (4)	29 (3)	44 (4)	28 (3)	−1 (3)	12 (2)	0 (3)
C(6′)	5438 (5)	1547 (8)	−3371 (4)	27 (3)	55 (4)	41 (3)	−11 (3)	15 (3)	0 (3)
				Counterions and Water Molecules					
Cl(1A)	3107 (1)	3500 (0)	4649 (1)	42 (1)	63 (1)	53 (1)	1 (1)	23 (1)	12 (1)
Cl(1B)	3522 (2)	1671 (3)	1982 (2)	63 (1)	68 (1)	91 (1)	24 (1)	41 (1)	21 (1)
Cl(2A)	1524 (1)	−1770 (2)	−4130 (1)	40 (1)	42 (1)	63 (1)	−12 (1)	22 (1)	−5 (1)
Cl(2B)	1464 (2)	5942 (3)	−1555 (2)	56 (1)	94 (2)	96 (2)	4 (1)	26 (1)	−12 (1)
O(W1)	3123 (5)	368 (7)	3753 (5)	67 (3)	44 (3)	110 (5)	−16 (3)	44 (3)	15 (3)
O(W2)	2074 (4)	4912 (7)	−3903 (4)	55 (3)	51 (3)	100 (4)	4 (3)	48 (3)	11 (3)

B. Hydrogen Atoms[b]

atom	x	y	z	U	atom	x	y	z	U
		Molecule A					Molecule B		
H(2)	197 (4)	389 (8)	248 (4)	5	H(2)	292 (5)	430 (8)	−218 (4)	5
H(4α1)	−194 (5)	554 (8)	62 (4)	5	H(4α1)	687 (5)	242 (7)	−26 (4)	5
H(4α2)	−159 (5)	568 (9)	197 (5)	5	H(4α2)	682 (4)	379 (8)	−109 (4)	5
H(4α3)	−181 (5)	468 (8)	134 (5)	5	H(4α3)	643 (5)	218 (8)	−129 (4)	5
H(5α1)	−24 (5)	577 (8)	−83 (4)	6	H(5α1)	625 (6)	215 (9)	87 (5)	6
H(5α2)	−155 (5)	544 (8)	−57 (4)	6	H(5α2)	510 (5)	177 (9)	87 (5)	6
H(5β1)	−128 (6)	465 (10)	−188 (5)	8	H(5β1)	619 (6)	498 (9)	137 (5)	8
H(5β2)	−127 (6)	322 (10)	−91 (5)	8	H(5β2)	633 (5)	369 (10)	215 (5)	8
H(5γ)	54 (6)	433 (10)	−133 (5)	9	H(5γ)	454 (5)	379 (9)	183 (5)	8
H(3,5′1)	111 (4)	426 (7)	352 (4)	5	H(3,5′1)	530 (5)	422 (7)	−259 (4)	5
H(3,5′2)	−31 (4)	398 (7)	291 (4)	5	H(3,5′2)	415 (4)	429 (7)	−305 (4)	5
H(1′)	−85 (4)	865 (7)	367 (4)	4	H(1′)	576 (4)	−31 (7)	−400 (4)	4
H(2′α1)	123 (5)	1127 (8)	416 (4)	5	H(2′α1)	477 (5)	−259 (8)	−391 (5)	6
H(2′α2)	2 (5)	1127 (8)	361 (4)	5	H(2′α2)	450 (5)	−206 (9)	−476 (5)	6
H(2′α3)	26 (5)	1096 (8)	447 (4)	5	H(2′α3)	354 (6)	−260 (8)	−459 (4)	6
H(3′)	178 (4)	890 (7)	380 (4)	4	H(3′)	279 (4)	−75 (7)	−405 (4)	4
H(6′)	−113 (4)	631 (7)	335 (4)	4	H(6′)	616 (5)	224 (7)	−321 (4)	4
		Water					Water		
H(W11)	301 (7)	161 (12)	372 (6)	8	H(W21)[c]	150	530	−420	8
H(W12)	300 (7)	43 (14)	333 (6)	8	H(W22)	175 (7)	448 (10)	−463 (7)	8

[a] Positional parameters × 10^4; thermal parameters × 10^3; the expression used for the anisotropic temperature factor is $\exp(-[2\pi^2(h^2a^{*2}U_{11} + \cdots 2hka^*b^*U_{12})])$. Estimated standard deviation in parentheses is for least significant figure. [b] Positional parameters × 10^3; thermal parameter × 10^2; the expression used for isotropic temperature factor is $\exp(-[(8\pi^2U)\sin^2\theta/\lambda^2])$. [c] Coordinates from a difference Fourier map.

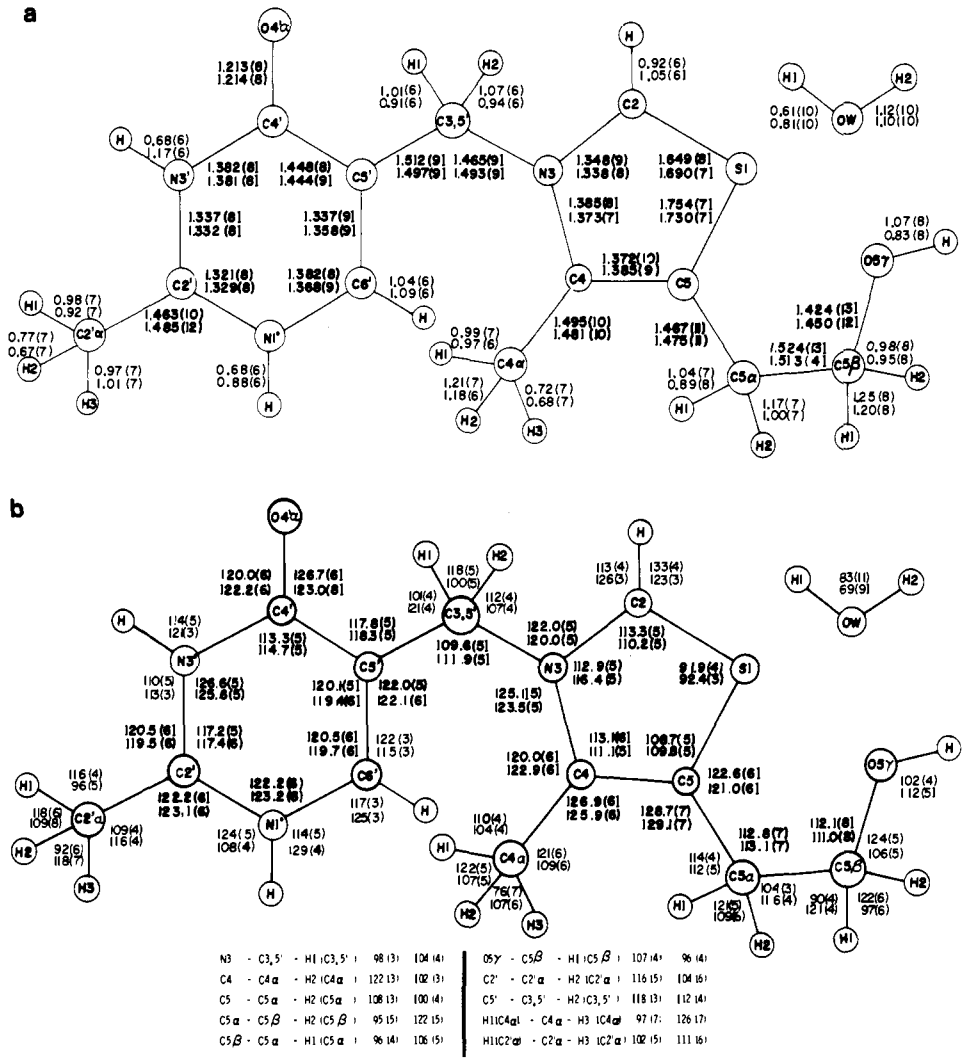


Figure 1. (a) Bond distances (angstroms) in oxythiamin chloride hydrochloride monohydrate. The first numbers refer to molecule A, the second to molecule B. The estimated standard deviations are given in parentheses. (b) Bond angles (degrees) in oxythiamin chloride hydrochloride monohydrate. The first numbers refer to molecule A, the second to molecule B. The estimated standard deviations are given in parentheses.

The second data set with new unit cell dimensions was used to refine the final coordinates from the first data set. The initial R factor was 0.12. The coordinates of some hydrogen atoms were generated and introduced into the refinement. Numerous cycles of refinement and subsequent difference Fourier synthesis revealed the locations of all of the hydrogen atoms. The refinement converged at $R = 0.041$ for 1788 observed reflections and 0.104 for all 3039 reflections. The weighted R was 0.057. The function $\Sigma\omega(k|F_o| - |F_c|)^2$ was minimized, where k is a single scale factor. ω, the weight of the reflection, was defined by $1/(A + B|F_o| + C|F_o|^2)$, where $A = 5.0$, $B = 0.9$, and $C = 0.005$ were empirically adjusted. The atomic scattering factors for Cl^-, S, O, N, and C are from Cromer and Waber[12] and that for H is from Stewart, Davidson, and Simpson.[13] The real and imaginary terms of the anomalous-dispersion correction for Cl and S are from the International Tables for X-ray Crystallography.[14] As a result of the large number of parameters involved in the refinement, the parameters were divided into two blocks.

Throughout the refinement, the y coordinate of the Cl(1A) atom was fixed at 0.35. The temperature factors for H atoms were assigned as the isotropic equivalents of the atoms to which they were bonded and were not refined. In the final cycle of refinement the parameters, except for O(W1), shifted by less than 0.5 esd. The y and z coordinates of O(W1) shifted by 1.29 and 1.37 esd, respectively. Since the coordinates of H(W21) converged to a bad position and the difference Fourier gave a definitely better position, the positional parameters for H(W21) from a final difference Fourier map were used for subsequent calculations. Due to the high portion of the unobserved reflections, the final difference Fourier was not clean, but it was featureless except for the peak for H(W21) and an antisymmetric diffraction ripple associated with each of the six heavier atoms for which anomalous-dispersion corrections were applied. The final atomic coordinates and thermal parameters are listed in Table II. The structure factor table is available as supplementary material. (See paragraph at end of paper regarding supplementary material.)

Description of the Structure

Oxythiamin Molecule. The atomic numbering scheme, the bond distances, and the bond angles of two independent molecules (A and B) are presented in Figures 1a and 1b. A comparison of the bond distances and angles in the two independent molecules was made using the half-normal probability distribution[15] (details in figure available as supplementary material). The linear plot with a slope of 1.72 deviates significantly from unity, which is obtained for ideal coincidence. This deviation indicates that, either (i) the differences are larger than expected for purely random errors, or (ii) the estimated standard deviations in the parameters are too small, or (iii) both conditions exist. The last most likely represents the true situation. The different molecular environments for the two molecules in the crystal could account for the nonrandom contribution to the observed differences in the parameters. (Agreement between pyrimidine rings is better than that of the thiazolium rings.) The S(1)-C(2) bond distances show the largest difference (6σ). However, all of the other dimensions agree within 3σ and all but 5 agree within less than 2σ.

Table III. Least-Squares Planes[a]

plane	A	B	C	D	σ	displacements
			Molecule A			
thiazolium	303	946	4	3962	23	**S(1) −11, C(2) 2, N(3) 11, C(4) −21, C(5) 20,** C(4α) −87, C(5α) 114, C(5β) −1072, O(5γ) −1103, C(3,5′) 129, H(2) 138, H(5γ) −79, Cl(2A) −1135
pyrimidinium	98	−218	878	3171	16	**N(1′) −10, C(2′) 7, N(3′) −8,** C(4′) **12, C(5′) −15, C(6′) 14,** C(2′α) −9, O(4′α) 5, C(3,5′) −21, H(1′) −112, H(3′) 350, H(6′) −107, O(4′αB)[ii] −704, O(W1)[ii] 170, O(W2)[i] −204, Cl(1B)[iv] 3191
			Molecule B			
thiazolium	267	938	118	4294	11	**S(1) −6, C(2) 2, N(3) 5,** C(4) **−10, C(5) 9,** C(4α) 27, C(5α) −39, C(5β) 1221, O(5γ) 1384, C(3,5′) −71, H(2) −46, H(5γ) 802, Cl(2B) 941
pyrimidinium	−50	445	−822	4433	16	**N(1′) 2, C(2′) −8, N(3′) 0,** C(4′) **13, C(5′) −20, C(6′) 13,** C(2′α) −81, O(4′α) −17, C(3,5′) 41, H(1′) 32, H(3′) 105, H(6′) 49, O(4′αA)[iii] −348, Cl(1A)[iv] 3403
		Molecule A		Molecule B		
dihedral angle		91.7°		78.2°		

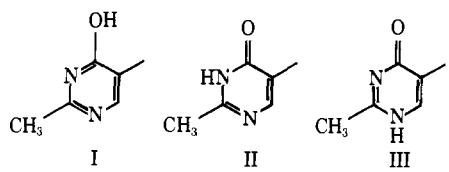
[a] Coefficients × 10^3 in $Ax + By + Cz = D$ are referred to crystallographic axes in angstroms. Standard deviations in least-squares planes and the displacements of atoms from the planes are in Å × 10^3. Boldface type designates atoms used to define the planes. Symmetry code as in Table V.

Table IV. Torsion Angles[a]

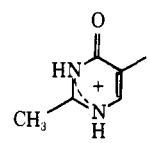
molecule A	φ_T = 105.5 (7)	φ_P = −62.8 (7)	$\varphi_{5\alpha}$ = 61.9 (8)	$\varphi_{5\beta}$ = −66.8 (9)
molecule B	φ_T' = −101.5 (7)	φ_P' = 64.2 (7)	$\varphi_{5\alpha}'$ = −68.7 (9)	$\varphi_{5\beta}'$ = 66.3 (10)

[a] The estimated standard deviations in parentheses refer to the least significant digit.

This structure analysis confirms that the O atom at the 4′ position exists as the keto form instead of the formally depicted hydroxyl form (I). From an NMR study of pyrimidines and nucleosides, Jardetzky, Pappas, and Wade[16] found that the order of basicity is ring N > amino N > oxygen. Although it is quite clear that oxythiamin is in the keto form, it is hard to predict from this structure whether the H atom is on N(3′) (II) or N(1′) (III) when it is in the unprotonated state, since both N atoms are bonded to H in this protonated structure (IV). In thiamin the basicity of N(1′) is greater than N(3′) and N(1′) is protonated preferentially.[17,18]



I II III



IV

In comparison with thiamin there is a general lengthening of the N(1′)-C(6′) and N(3′)-C(4′) bonds by ~0.03 Å. An inspection of all of the bond distances in the pyrimidine ring indicates that formula IV provides the major contribution to the various resonance forms. The S(1)-C(2), N(3)-C(3,5′), and C(5)-C(5α) distances in molecule A are anomalously shorter than the normal distances in thiamin. Although the differences in inductive effects of their respective pyrimidine rings may affect the electronic configuration in the thiazolium ring, no critical evaluation can be made, since intrinsic experimental errors due to poor diffracting power of the crystal are relatively large in comparison with thiamin. It is interesting, however, that the C(2′)-C(2′α) bonds appear to exhibit the correlation between bond length and the methyl group environment which has been seen in other thiamin structures.[19,20] It has been observed that an environment of negative ions or electronegative atoms around the methyl groups (particularly C(2′α)) results in a shorter C-C bond, possibly through stabilization by hyperconjugational resonance forms.[19] In molecule A, where this distance is shorter than the normal sp^2-sp^3 distance, the methyl group has close contacts with three different chloride ions. In molecule B, where the bond is closer to the expected distance (1.50 Å), there is only one close contact with a chloride ion.

The least-squares planes for the thiazolium and pyrimidine rings are listed in Table III. The planarities are all quite good relative to the magnitude of the errors in the final positional parameters. The magnitudes of the deviations are similar for the pyrimidine and thiazolium rings. This differs from most thiamin structures in which the deviations from the pyrimidine ring are usually larger than those for the thiazolium ring. The torsion angles defining the conformation with respect to the C(3,5′) bridge and the conformation of the C(5) side chain are given in Table IV.[4b,21] In this structure O(5γ) and the C(4′) substitutent are "syn" related, which is commonly observed in thiamin structures. The small differences in torsion angles for the two independent molecules appear to be derived from differences in their hydrogen bonding schemes and other crystal packing interactions.

Packing and Hydrogen Bonding. Figure 2 shows the stereoscopic ORTEP[22] packing drawing of the structure. The crystal packing consists of 14 unique hydrogen bonds and some unusually close contacts less than the sums of van der Waals' radii (Table V). The two independent molecules in each asymmetric unit are related by a pseudocenter of symmetry. This accounts for the way that achiral oxythiamin molecules crystallize in the noncentrosymmetric space group. A pseudocenter of symmetry relating the oxythiamin molecules whose coordinates are given in Table II is located at (0.249 ± 0.004, 0.407 ± 0.019, 0.006 ± 0.013). The Cl atoms, Cl(1B) and Cl(2B), have the same mean coordinates. Also, Cl(1A) and Cl(2A) and O(W1) and O(W2) have almost the same mean x and z coordinates, but they have different mean y coordi-

Table V. Inter- and Intramolecular Contacts for Oxythiamin Cl·HCl·H_2O

atom a	atom b	atom c	distance, Å a-c	distance, Å b-c	angle, deg a-b-c
			A. Hydrogen Bonds		
O(5γA)-H···		Cl(2B)	3.032 (7)	1.99 (8)	166 (7)
N(1′A)-H···		O(W2)[i]	2.746 (8)	2.09 (6)	165 (7)
N(3′A)-H···		O(W1)[ii]	2.794 (9)	2.22 (6)	143 (6)
C(2A)-H···		Cl(2A)	3.463 (8)	3.12 (6)	105 (4)
C(6′A)-H···		O(4′αB)[ii]	3.062 (8)	2.19 (6)	140 (5)
O(5γB)-H···		Cl(2A)	3.171 (7)	2.37 (8)	160 (7)
N(1′B)-H···		Cl(1A)[iii]	3.171 (6)	2.29 (6)	176 (5)
N(3′B)-H···		Cl(1B)	2.997 (5)	1.85 (6)	164 (5)
C(2B)-H···		Cl(2B)	3.391 (7)	2.82 (6)	114 (4)
C(6′B)-H···		O(4′αA)[iii]	3.019 (8)	2.21 (6)	130 (4)
O(W1)-H(1)···		Cl(1A)	3.123 (7)	2.18 (10)	140 (7)
O(W1)-H(2)···		Cl(2A)	3.123 (7)	2.60 (10)	145 (11)
O(W2)-H(1)···		Cl(1B)[ii]	3.056 (6)	2.63 (10)	114 (9)
O(W2)-H(2)···		Cl(1A)[iv]	3.201 (6)	2.54 (10)	118 (6)
			B. Close Contacts around S(1)		
C(2A)-S(1A)···		O(5γA)		3.070 (7)	152.8 (3)
C(5A)-S(1A)···		O(5γA)			71.4 (3)
C(2A)-S(1A)···		Cl(2A)		3.548 (3)	73.5 (3)
C(5A)-S(1A)···		Cl(2A)			156.8 (3)
C(2B)-S(1B)···		O(5γB)		3.083 (7)	147.5 (3)
C(5B)-S(1B)···		O(5γB)			71.0 (3)
C(2B)-S(1B)···		Cl(2B)		3.460 (3)	73.5 (2)
C(5B)-S(1B)···		Cl(2B)			158.1 (3)
			C. Miscellaneous Contacts		
C(2′αA)-H(2)···		Cl(1B)[viii]	3.512 (8)	2.98 (7)	129 (6)
C(2′αA)-H(3)···		Cl(1B)[viii]	3.607 (8)	3.01 (7)	114 (5)
C(2′αA)-H(2)···		Cl(2B)	3.629 (9)	3.06 (7)	134 (6)
C(2′αB)-H(2)···		Cl(1A)[iii]	3.594 (10)	3.12 (7)	131 (7)
C(2′αB)-H(3)···		Cl(1B)	3.791 (10)	3.04 (7)	132 (5)
C(2′αB)-H(1)···		Cl(2A)[iii]	3.958 (10)	3.06 (7)	165 (6)
C(3,5′A)-H(1)···		Cl(1A)	3.665 (7)	2.66 (6)	170 (5)
C(3,5′B)-H(1)···		Cl(2A)[vii]	3.499 (7)	2.67 (6)	153 (5)
			D. Close Contacts on the Pyrimidine Rings		
Cl(1A)		C(4′B)[v]	3.469 (7)		
Cl(1A)		C(5′B)[v]	3.496 (6)		
Cl(1B)		C(2′A)[vi]	3.191 (6)		
Cl(1B)		N(1′A)[vi]	3.406 (6)		
Cl(1B)		N(3′A)[vi]	3.458 (6)		

Symmetry Code

none	x, y, z	iii	$1-x, -\frac{1}{2}+y, -z$	vi	$x, -1+y, -1+z$
i	$-x, \frac{1}{2}+y, -z$	iv	$x, y, -1+z$	vii	$1-x, \frac{1}{2}+y, -z$
ii	$x, 1+y, z$	v	$x, y, 1+z$	viii	$-x, \frac{3}{2}+y, -z$

nates (i.e., 0.087 and 0.264, respectively). If the pseudocenter is taken as the unit cell origin, then the oxythiamin molecules and Cl(1B and 2B) are approximately related by $P2_1/a$ space group symmetry. However, Cl(1A and 2A) and O(W1 and W2) are only related by the twofold screw axis. It is this asymmetry that is responsible for the asymmetric hydrogen-bonding schemes around Cl atoms and hence its crystallization in the noncentrosymmetric space group. The major crystal packing utilizes the Cl atoms and water molecules to form three-dimensional hydrogen bonding networks. The number of hydrogen bonds around the four Cl atoms is different. It is interesting to note that both N atoms of the pyrimidine ring are hydrogen bonded to water molecules in molecule A, whereas in molecule B they are bonded to Cl atoms. Around the pseudocenter of symmetry the two molecules are associated through O(5γ)-H···Cl hydrogen bonds and S···Cl close contacts (Figure 2).

In thiamin there is a characteristic tendency for negative ions or electronegative atoms to form close contacts with S(1) in the plane of the thiazolium ring along the directions of the C(2)-S(1) and C(5)-S(1) bonds. This close contact has been suggested to have mechanistic importance in C(2) substituted thiamin.[23] This property is retained in the present oxythiamin structure. For both A and B molecules there are S(1)···O(5γ) intramolecular contacts along the C(2)-S(1) bond and S(1)···Cl contacts along the C(5)-S(1) bond. There are other interesting intermolecular short contacts. The keto oxygens, O(4′αA) and O(4′αB), are very close to C(6′B) and C(6′A) of symmetry-related molecules (see Table V) with values of 3.019 and 3.062 Å, respectively. These short contacts may be unusual C-H···O type hydrogen bonds, but close contacts with C(6′)-H have also been observed in some other thiamin structures. A possible hydrogen bond from C(2) to a suitable acceptor is a characteristic feature in structures containing the thiazolium ring. This feature is in accord with the acidic nature of the C(2) hydrogen atom, an important property in the Breslow's mechanism for thiamin catalysis.[24] In oxythiamin there are also close contacts or possible hydrogen bonds with Cl atoms with separations of 3.463 and 3.391 Å in molecules A and B, respectively. Another interesting feature is that Cl(1B) lies on top of the pyrimidine ring of molecule A to form a very short contact of 3.191 Å with C(2′). There is a possibility

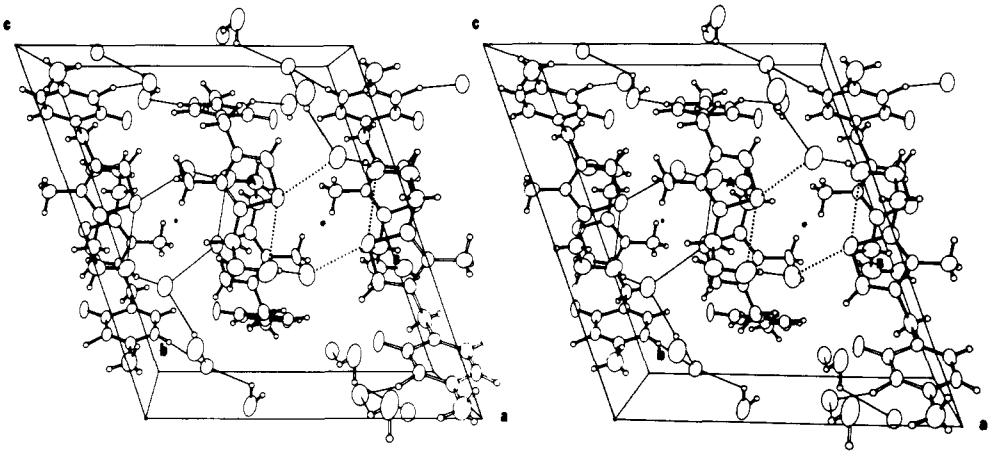


Figure 2. ORTEP drawing of crystal packing in oxythiamin chloride hydrochloride monohydrate viewed down the *b* axis. The origin for the fractional coordinates of molecules A and B listed in Table II is located at ($\frac{1}{2}$, 0, $\frac{1}{2}$) with respect to the cell outlined in the figure. Hydrogen bonds and close contacts with S are shown as single light or dotted lines (hydrogen bonds to C(2)H and C(6′)H are not shown). The location of the pseudocenter of symmetry relating molecules A and B is indicated by a small circle at the center of the six-sided array of intermolecular interactions. The molecule labeled "B" clearly shows the V conformation with C(2)–H adjacent to O(4′α). If it were in the F conformation, the thiazolium ring would be rotated such that C(2)H would be positioned "over" the pyrimidinium ring. For the S conformation the pyrimidinum ring would be rotated such that C(6′)H would be positioned "over" the thiazolium ring.

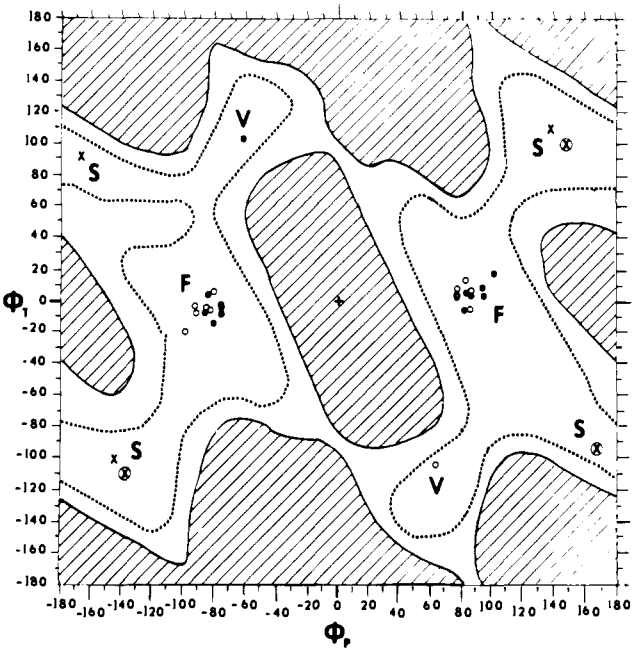


Figure 3. Conformational energy map and observed structures of thiamin. Limited contours from the conformation energy diagram of Jordan[6a] have been replotted in terms of the torsion angles φ_T and φ_P, as defined in ref 25. The map was also rearranged so that the origin, $\varphi_T = \varphi_P = 0$, is at the center of the map to more clearly portray the centrosymmetric relationship. The solid contour represents the 18-kcal level, while the dotted contour shows the 6-kcal level. The cross-hatched areas correspond to conformational energies above the 18-kcal level. The structures exhibiting the F conformation are clustered in two symmetry-related regions along the $\varphi_T = 0$ axis. The individual structures are represented in pairs; the open circle corresponds to the molecule with torsion angles that conform to the definition as cited above, while the solid circle corresponds to its centrosymmetrically related mate (φ').[4b] Structures with an S conformation are clustered in four regions of the map with the structures again represented in pairs. The molecule that conforms to the definition and its centrosymmetric mate are represented by Ⓧ and X, respectively. The two oxythiamin molecules, which are only pseudosymmetrically related, display the V conformation. The open square represents the molecule that conforms to the definition, while the solid square represents the one with φ' angles. It is important to note that there are only two V conformations that conform to its descriptive definition; when defined in terms of the torsions angles, φ_T and φ_P are always required to have opposite signs.

that this contact may actually be a weak charge-transfer phenomenon, which has not been reported in oxythiamin. Cl(1A) also lies on top of the pyrimidine ring of molecule B with a distance of less than 3.5 Å to C(4′) and C(5′). This type of interaction has not been observed with the aminopyrimidine ring of thiamin, although ring stacking interactions occur in several structures.[20a,25,26]

Discussion

The most striking result of this study is that oxythiamin assumes a conformation that is different from that of either thiamin or C(2)-substituted thiamin. In over a dozen different crystal structures, thiamin has been observed to assume only two basic conformations,[20a] namely, **F** when it is free of substituents on C(2) and **S** when it is substituted on C(2).[27] None of the crystal structures of thiamin shows the proposed V conformation. As discussed in Shin et al.,[20a] it is very unlikely that these structural results can be attributed to lattice forces. In contrast to thiamin, oxythiamin does favor a V conformation (Figure 2), although its conformation angles do not correspond exactly with the formally "idealized" magnitudes of 90°. Structural analysis of oxythiamin also shows that it is very unlikely that the conformation of oxythiamin is significantly influenced by the crystal packing forces. This is supported by the fact that the crystal structure maintains some unusual contacts. In addition, the substantial underutilization of hydrogen bonding potential by the chloride ions is contraindicative of confining lattice forces. Furthermore, it is highly significant that the intermolecular bonding for oxythiamin is very similar to that of thiamin with major differences related to the donor/acceptor properties of the hydrogen bonding positions in the pyrimidine ring. The significance of the **F** form of thiamin has been enhanced by the occurrence of oxythiamin in the **V** conformation, since small changes in the structure (i.e., the replacement of the 4′-amino group with an oxo group) result in a large difference in its conformation.

From molecular orbital calculations of thiamin, Jordan has constructed a conformational map of thiamin.[6a] Two contour levels from his map have been replotted in Figure 3 in terms of the torsion angles as defined in ref 25 and by placing the coordinate origin, which corresponds to a conformational center of symmetry, in the center of the map. The conformation angles of the known structures of thiamin, its derivatives, and

analogues are superimposed on this map. The features of the map at the 18-kcal level in general conform to the required centrosymmetry. However, the dotted contour at the 6-kcal level shows substantial deviations from the required centrosymmetry. These deviations indicate that significant errors have been introduced at this level by the simplifying assumptions employed in the calculations. Thus, energy differences of this magnitude may not be presented correctly in the map. However, the gross features of the map are reasonably consistent with the observed structures. It is clear that there must be a relatively low barrier between a whole spectrum of conformations, since centrosymmetrically related molecules are readily interchangeable. The map shows that a rather broad continuous pathway connects centrosymmetrically related structures. It also shows that continuous rotation of either φ_T or φ_P is not very favorable, but instead a coordinated oscillation about both bonds is preferable. However, the clustering of the observed structures in specific regions of this broad pathway indicates that all points on the path are not isoenergetic, but that small but significant differences do exist. The differences appear to be related to inherent structural features, in that thiamin, its C(2) derivatives, and oxythiamin favor different conformations. It is interesting to note that conformation angles of oxythiamin fall well inside an enlarged low-energy region appended to the main circular path, while the "idealized" values fall at the more restricted neck of this appended region.

It is increasingly clear that the energy differences in the various conformations are small but significant enough to be resolved in the crystal structures. However it is not yet certain what factors govern these differences in the conformations. From the point of view of the mechanism of thiamin catalysis, it appears worthwhile to consider that oxythiamin differs from thiamin with respect to its preferred conformation as well as its electronic structure. Even if the conformational differences per se are not important to the catalytic function, the properties that give rise to these differences may very well be.

Acknowledgment. We wish to acknowledge the observation by Brian Craven that the independent molecules are approximately related by $P2_1/a$ space group symmetry. We also thank the Computation Center for providing the computing facilities used in this study. This research was supported in part by National Institutes of Health Grant No. GM 23609.

Supplementary Material Available: Structure factor table for oxythiamin Cl·HCl·H_2O and plot of the half-normal probability distribution of bond distances and angles for molecules A and B (19 pages). Ordering information is given on any current masthead page.

References and Notes

(1) In partial fulfillment of requirements for the Ph.D. degree, University of Pittsburgh.
(2) E. F. Rogers, *Methods Enzymol.*, **18A**, 245 (1970).
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(4) (a) The relative orientations of the thiazolium and pyrimidine rings with respect to the C(3,5′) bridging methylene are specified in terms of the torsion angles φ_T = C(5′)–C(3,5′)–N(3)–C(2) and φ_P = N(3)–C(3,5′)–C(5′)–C(4′). The reference conformation ($\varphi_T = \varphi_P = 0$) is defined by the hypothetical planar molecule with C(4′) "syn" to C(2). This conformation resembles the structure of thiochrome (J. Pletcher, M. Sax, C. S. Yoo, J. Chu, and L. Power, *Acta Crystallogr., Sect. B*, **30**, 496 (1974)), the tricyclic oxidation product of thiamin. The conformations which correspond to ($\varphi_T \approx 0°$, $\varphi_P \approx \pm 90°$) have been designated **F**. Those corresponding to ($\varphi_T \approx \pm 100°$, $\varphi_P \approx \pm 150°$) have been designated **S**. The conformations which have been referred to as **V** correspond to ($\varphi_T \approx \pm 90°$, $\varphi_P \mp 90°$) (see Figure 2). For a more extensive discussion see footnote 13 in Pletcher, Sax, Blank, and Wood.[25] (b) In the definition of the torsion angles referred to above, a reference orientation is specified in order to distinguish between centrosymmetrically related molecules which have torsion angles with identical magnitudes but opposite signs. The reference orientation is determined by the C(5) side chain and corresponds to the one for which $\varphi_{5\alpha}$ is positive. In structures which do not contain molecules in centrosymmetrically related pairs (such as the present structure), it may not be possible to specify torsion angles that conform to the reference orientation. In those cases where $\varphi_{5\alpha}$ is negative, the torsion angles will now be specified as primed values, i.e., φ_T', φ_P', $\varphi_{5\alpha}'$, and $\varphi_{5\beta}'$.
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