# THE CRYSTAL AND MOLECULAR STRUCTURE OF THIAMINE HYDROIODIDE 

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## ABSTRACT

The x-ray crystal structure of thiamine hydroiodide, $\mathrm{C}_{12} \mathrm{H}_{18} \mathrm{~N}_{4} \mathrm{OSI}_{2}$, has been determined. The unit cell parameters are $a=13.84 \pm 0.03$, $b=7.44 \pm 0.01, c=20.24 \pm 0.02 \stackrel{\circ}{A}, \beta=120.52 \pm 0.07^{\circ}$, space group $P 2_{1} / c, z=4$. A total of 1445 reflections having $F^{2}>2 \sigma\left(F^{2}\right), 2 \theta<40^{\circ}$ were collected on a Picker four-circle diffractometer with MoK $\alpha$ radiation by the $2 \theta$ scan technique. The structure was solved by the heavy atom method. The iodine and sulphur atoms were refined anisotropically; only the positional parameters were refined for the hydrogen atoms. Successive least squares cycles yielded an unweighted $R$ factor of 0.054 . The site of protonation of the pyrimidine ring is the nitrogen opposite the amino group. The overall structure conforms very closely to the structures of other related thiamine compounds. The bonding surrounding the iodine atoms is distorted tetrahedral. The iodine atoms make several contacts with surrounding atoms, most of them at or near the van der Waal's distances.

A thiaminium tetrachlorocobaltate salt was produced whose molecular and crystal structure was judged to be isomorphous to thiaminium tetrachlorocadmate.

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#### Abstract

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## CHAPTER I

## INTRODUCTION

Vitamin $B_{1}$ was first recognized in the early $1890^{\prime}$ s when it was observed that hens fed exclusively on polished rice developed polyneuritic symptoms similar to those of human beriberi patients. Ensuing work showed that these induced paralytic symptoms could be alleviated by the addition of rice bran to the diet. This discovery led researchers to speculate rightly that the cause of the disease was a nutritional deficiency. In the following years efforts were directed towards isolating the active factor from rice bran, but it remained elusive until the 1920's. The year 1926 marked the report by Jansen and Donath (la) of a crystalline hydrochloride of the active principle, however several years elapsed before the substance was successfully isolated in other laboratories. Numerous refinements in the technique of isolation were necessary before a sufficient quantity of the vitamin could be produced for structure elucidation. By 1934 an improved process was reported for the isolation of thiamine from rice bran which yielded 5 grams of thiamine hydrochloride, I,from each ton of rice bran processed (1b) ! This was a yield of about $25 \%$ (1).


I, $R_{1}=H ; R_{2}=H$, thiamine
Ia, $\mathrm{R}_{1}=\mathrm{H} ; \mathrm{R}_{2}=\mathrm{P}_{2} \mathrm{O}_{6}{ }^{3-}$, thiamine pyrophosphate, TPP

II, $\mathrm{R}_{1}=\mathrm{H}_{3} \mathrm{CC}(\mathrm{H}) \mathrm{OH} ; \mathrm{R}_{2}=\mathrm{H}, 2-\alpha$ (Hydroxyethylthiamine)

In its biologically active coenzyme form thiamine exists as an ester of pyrophosphoric acid, thiamine pyrophosphate,Ia. The enzyme systems for which thiamine pyrophosphate (TPP) is a cofactor include the $\alpha$-keto acid decarboxylases and the transketolases (2). As far as it is known TPP or cocarboxylase, as it is sometimes called, is exclusively a coenzyme in decarboxylating systems (3). Studies on the enzyme pyruvate decarboxylase (PDC) have led to a proposal of a mechanism for the decarboxylation of pyruvic acid,III, to acetaldehyde via a 2-(1-Carboxy -1-Hydroxyethy1) thiamine pyrophosphate intermediate (4,5).


III
pyruvic
acid



For enzyme activity PDC requires in addition to TPP a divalent metal ion such as $\mathrm{Mg}^{2+}$. The process by which the metal ion, coenzyme and apoenzyme bind together to form a holoenzyme is not yet known, but there are several ways in which this could occur. First the metal ion could bind TPP to the enzyme,


Second, TPP could be bound directly to the enzyme and the metal ion could function to induce conformational changes by fixing itself elsewhere on the enzyme's surface,


In any case the conformation of the thiamine residue of the coenzyme is important to the functioning of the enzyme. To date there have been no examples of direct thiamine-metal bonding in any of the reported structures. Species such as $\mathrm{HThiCdCl}_{4}$ (6), $\mathrm{HThiCuC1}_{4}$ (7), and $\mathrm{HThiMgC1}_{4}$ (8) exist as complex salts [HThi] ${ }^{2 \boldsymbol{q}}\left[\mathrm{NCI}_{4}\right]^{2-}$ with no metal-sulphur or metal-nitrogen interactions.

The conformation of the thiamine cation, that is the manner in which the pyrimidine and thiazolium rings are oriented around the bridging methylene group, can be easily defined in terms of the torsional angles $\phi_{T}, \mathrm{C} 8-\mathrm{C} 7-\mathrm{N} 1-\mathrm{C} 6$ and $\phi_{\mathrm{P}}$, N1-C 7-C $8-\mathrm{C} 9$ (9). Projections of $\phi_{\mathrm{T}}$ and $\phi_{\mathrm{P}}$ are shown in Figure 1. A particular $\phi$, for example $\phi_{\mathrm{P}}$, angle equals zero when the normal to the plane of the pyrimidine ring is parallel to the normal of the plane defined by N1-C7-C8. A counterclockwise rotation corresponds to a positive angle. The mirror image of a particular conformation will have opposite signs for both $\phi_{T}$ and $\phi_{P}$. Table 1 lists the torsional angles for the accurately determined structures containing the thiamine cation.

Recent calculations on the electronic structure and preferred conformations of thiamine (2) have shown that by concerted rotations about $\phi_{\mathrm{T}}$ and $\phi_{\mathrm{P}}$ there is a nearly continuous range of conformations which will


Projection along N1-C7 bond ( $\phi_{\mathrm{T}}$ )


Projection along C7-C8 bond ( $\phi_{\mathrm{P}}$ )

Figure 1. Newman projections for the torsional angles $\phi_{T}$ and $\phi_{P}$. in thiamine hydroiodide.

| Compound | $\phi_{\mathrm{P}}$ | $\phi_{\mathrm{T}}$ | Ref. |
| :---: | :---: | :---: | :---: |
| Thiamine hydroiodide | $81.0 \pm 1.5^{\circ}$ | $-5.9 \pm 1.2^{\circ}$ | 11 |
| Thiamine hydrochloride | 76.1 | 9.0 | 10 |
| Thiamine chloride. $\mathrm{H}_{2} \mathrm{O}$ | 76.2 | 2.6 | 17 |
| $\mathrm{HThiCuC1}_{4}$ | 82.6 | 14.1 | 7 |
| (HThi) $2^{\mathrm{UO}} 2^{\mathrm{Cl}} 4$ | 83.7 | -5.4 | 19 |
| Hydrolyzed cocarboxylase | 85.4 | 6.6 | 16 |
| Thiamine pyrophosphate. HC1 | 93.1 | 2.7 | 21 |
| $\mathrm{HThiCdCl}_{4} \cdot \mathrm{H}_{2} \mathrm{O}$ | 137.3 | 110.4 | 6 |
| 2-( $\alpha$-Hydroxyethyl) thiamine chloride. HC1 |  |  |  |
|  | 145.6 | 100.3 | 9 |

* $\phi_{\mathrm{P}}$ and $\phi_{\mathrm{T}}$ are the N1-C7-C8-C9 and C8-C7-N1-C6 torsional angles, respectively. The angle $\phi$ equals zero when the normal to the plane is parallel to the normal of the plane defined by N1-C7-C8. A counter clockwise rotation corresponds to a positive angle.
be accessible to the molecule at normal temperatures provided that the rings are not coplanar. However, despite the large number of conformations predicted to be stable, the crystalline structures for thiamine compounds have so far fallen into two general conformations, one with $\left|\phi_{P}\right|=85 \pm 1 \theta^{\circ}$, $\left|\phi_{T}\right|=5 \pm 10^{\circ}$ and the other with $\phi_{p}=140 \pm 5, \phi_{\mathrm{T}}=105 \pm 5^{\circ}$. The former category which includes thiamine hydrochloride is the more common of the two. The latter conformation was originally reported by Sax et al. (9) in the structure of 2 -( $\alpha$-Hydroxyethyl) thiamine (II). It was claimed that this new conformation was due to the presence of the $\alpha$-hydroxyethyl side chain. Richardson et al.in their thiaminium tetrachlorocadmate structure (6) also observed a conformation similar to that of 2 ( $\alpha$-Hydroxyethyl) thiamine on a molecule without a substituent in the 2-position. Thus the $2-(\alpha-H y d r o x y e t h y l)$ side chain may be a contributory factor for this particular conformation, but it is by no means crucial. That only a limited number of conformations occur in the solid state is probably the result of the favourable packing arrangements which may be achieved for select values of $\phi_{T}$ and $\phi_{P}$. Other conformations which are also energetically stable from a torsional point of view (2) may not offer the proper orientations for the extensive hydrogen bonding which is generally observed in this class of compounds.

In the course of some general investigative studies with thiamine, crystals of thiamine hydroiodide, THI, were produced. Preliminary x-ray work showed that this structure was not isomorphous to thiamine hydrochloride (10). In the light of two "odd" structures, 2-( $\alpha$-Hydroxyethy1)
thiamine and thiaminium tetrachlorocadmate, in which similar conformations were observed for apparently different reasons, a complete structure determination of thiamine hydroiodide seemed a worthwhile avenue of pursuit.

The problem stated briefly is: what would be the effect upon the basal THC conformation by the substitution of the chloride anions with significantly larger iodide ions?

## CHAPTER II

EXPERIMENTAL SECTION
A. Reagents and Film

Thiamine was obtained as Aneurine hydrochloride $\mathrm{C}_{12} \mathrm{H}_{17} \mathrm{~N}_{4} \mathrm{OSCl} \cdot \mathrm{HCl}$ from BDH Chemicals. Sodium Iodide was of reagent grade conforming to A.C.S. specifications. These chemicals were used without any further purification.

The film used was Kodak No-Screen Medical X-ray film.
B. Preparation of Thiamine Hydroiodide

Thiamine hydrochloride, 3.37 g ( 0.01 mole ) and sodium iodide, 3.20 g ( 0.02 mole) were diluted to 60 ml in distilled water. The mixture was vacuum filtered and the filtrate was placed in a petri dish overnight, producing a large quantity of colourless crystals. The crystals were collected by the Pasteur method, washed with water and left to dry in the air. Micro analysis indicated: $\mathrm{C}, 27.79$; $\mathrm{H}, 3.47$; $\mathrm{N}, 10.90$; S, 6.36\%. Thiamine hydroiodide as $\mathrm{C}_{12} \mathrm{H}_{18} \mathrm{~N}_{4} \mathrm{OSI}_{2}$ requires $\mathrm{C}, 27.70 ; \mathrm{H}, 3.49$; $\mathrm{N}, 10.77$; S, 6.17\%. This compound melted with decomposition at $230^{\circ}$ (1it. $229^{\circ}$, ref 23).
C. Density Measurements

The density of thiamine hydroiodide was measured by the flotation method. A crystal of this compound was placed in a mixture of benzene, $\rho=0.879 \mathrm{~g} \mathrm{~cm}^{-3}$ and 1,2 -dibromoethane (ethylene bromide), $\rho=2.179 \mathrm{~g} \mathrm{~cm}^{-3}$. When the crystal remained more or less suspended, that is, it neither sank to the bottom of the vessel nor floated to the surface of the liquid, the density of the crystal was assumed to be equal to the density of the liquid. A 1 ml sample of the mixture was extracted
volumetrically, transferred to a screw cap vial and weighed. The density of the crystal was $1.956 \mathrm{~g} \mathrm{~cm}^{-3}$.
D. Crystal Selection

Suitable crystals were chosen with the aid of a polarizing microscope. The quality of the crystals was based on the size, regularity in shape and by the sharpness of the extinctions when the crystal was rotated in plane polarized light. Crystals judged as suitable were mounted on thin glass fibres with "Five Minute" epoxy cement.

## E. Diffraction Studies

1. Oscillation Photographs

Oscillation photographs were taken on a Picker X-ray diffraction instrument using a Charles Supper Co., Weissenberg camera. The crystal was allowed to oscillate through an arc of $15^{\circ}$. Unfiltered $\mathrm{CuK} \alpha$ radiation was used for all photographs. The exposure time was of a $20-30$ minute range. The alignment of the crystal was accomplished by the methods outlined in Stout and Jensen (12a). A right-1eft symmetry pattern was observed on the oscillation photographs.

## 2. Weissenberg Photographs

Equi-inclination Weissenberg photographs were taken using the same alignment obtained from the oscillation photographs. Nickel filtered CuK $\alpha$ radiation ( $\lambda=1.5481 \mathrm{~A}^{\circ}$ ) and an exposure time of about 12 hours were used for all photographs. The layers zero through four were photographed using camera settings based on the lattice dimensions obtained from the oscillation photographs (12b).

## 3.Diffractometer Studies

A Picker four-circle diffractometer was used for the data collection. A flat single crystal of thiamine hydroiodide ( $1.0 \times 0.5 \times 0.2 \mathrm{~mm}$ ) was mounted on a eucentric goniometer head, aligned by oscillation photography on the Weissenberg camera, and transferred to the diffractometer. Accurate unit cell dimensions and all intensity data were obtained using zirconium filtered $\operatorname{MoK} \alpha_{1}$ radiation ( $\lambda=0.7093 A^{\circ}$ ). The methods of crystal alignment for the four-circle diffractometer given in Stout and Jensen (12c) were employed.
4. Unit Cell
(a) Film

On the oscillation photographs a diffraction pattern with right-1eft symmetry was observed. The axis about which the crystal rotated was designated as $\mathrm{b} \%$. Systematic absences for $0 \mathrm{k} 0, \mathrm{k}=2 \mathrm{n}+1$; and h0l, $\ell=2 \mathrm{n}+1$ indicated space group $\mathrm{P}_{1} / \mathrm{c}$. This monoclinic space group is completely determined by its systematic absences (12d).
(b) Diffractometer

The monoclinic unit cell dimensions were calculated from the axial reflections. The angle $\beta^{*}$ was determined from the locations of the $a^{*}$ and $c^{*}$ axes $\left(\beta^{*}=\phi_{h 00}-\phi_{001}\right)$; a second determination was made from the d-spacings of the $6,0,12$ and $7,0,14$ reflections. From $\beta^{*}, \beta$ was then obtained. The cell constants are $a=13.84 \pm 0.03$, $b=7.44 \pm 0.01, c=20.24 \pm 0.02 \AA ; \beta=120.52 \pm 0.07^{\circ}$ (Table 2). The calculated density based on 4 molecules per unit cell is $2.017 \mathrm{~g} \mathrm{~cm}^{-3}$.

Table 2. The $2 \theta$ angles and interplanar spacing from which the cell constants were obtained.
a* axis

| n00 reflection | $2 \theta$ | $\mathrm{~d}_{\mathrm{n} 00}$ | $\mathrm{~d}_{100}$ |
| :--- | :--- | :--- | :--- |
| 100 | 3.425 | 11.891 | 11.891 |
| 200 | 6.845 | 5.952 | 11.904 |
| 300 | 10.255 | 3.976 | 11.928 |
| 400 | 13.730 | 2.973 | 11.891 |
| 600 | 20.585 | 1.989 | 11.933 |
| 700 | 23.569 | 1.740 | 12.179 |
| 800 | 27.665 | 1.486 | 11.890 |
| 900 | 31.105 | 1.325 | 11.928 |
| 10,00 | 34.665 | 1.193 | 11.931 |
| 13,000 | 45.560 | 0.918 | 11.931 |
| 14,00 | 49.450 | 0.850 | 11.894 |

$\overline{\mathrm{d}}_{100}=11.930 \mathrm{~A}^{\circ}$
Std. Dev. $=0.03 \mathrm{~A}^{\circ}$
b* axis
On0 reflection 2
$020 \quad 10.975$
040
22.025
${ }^{\mathrm{d}} \mathrm{OnO}$
${ }^{\mathrm{d}} 010$
3.716
7.432
33.260
1.860
7.441

060
44.895
1.242
7.450

080
0.931
7.445
$\overline{\mathrm{d}}_{010}=7.442 \mathrm{~A}^{\circ}$
Std. Dev. $=0.01 \mathrm{~A}^{\circ}$

Table 2. (continued)
c* axis
00
002
004
006
00
00
00
00
$\beta$
reflection $2 \theta$
6, 0,12
43.19
$\beta *{ }^{\circ}$
59.41

7,0,14
50.86
59.44
axial measurement
59.58
$\bar{\beta}^{*}=59.48^{\circ}$
Std. Dev. $=0.07^{\circ}$
For a monoclinic system
thus

$$
\begin{aligned}
& a=d_{100} / \sin \beta \\
& b=d_{010} \\
& c=d_{001} / \sin \beta \\
& \beta=180^{\circ}-\beta^{*} \\
& a=13.84 \pm 0.03 \mathrm{~A}^{\circ} \\
& b=7.44 \pm 0.01 \mathrm{~A}^{\circ} \\
& c=20.24 \pm 0.02 \mathrm{~A}^{\circ} \\
& \beta=120.52^{\circ}
\end{aligned}
$$

## 5. Intensity Measurements

The $\omega-2 \theta$ scan technique was used, with $\omega$ changing at one half the rate of $2 \theta$.

The scan rate was $2^{\circ} / \mathrm{min}$. over a $2^{\circ}$ range in $2 \theta$, from $1^{\circ}$ below to $1^{\circ}$ above the calculated peak positions. Background was counted for 10 sec. at each end of the scan. Zirconium filters . $0005^{\prime \prime}$ thick were inserted in the reflected beam whenever the peak counting rate exceeded $40,000 \mathrm{cps}$. A standard reflection $(6,1,-2)$ was monitored at 2 hour intervals in order to correct for instrumental fluctuations and possible crystal decomposition. The reflection data between two successive measurements of the standard reflections were corrected by a linear interpolation according to the number of intervening reflections. During the course of the data collection there was a slight browning of the crystal along the face which was incident to the $x$-ray beam; however the intensity measurements of the standard reflection were not noticeably affected. The intensities were corrected for background, for variation in the standard reflection and for extra filters.

Lorentz and polarizing corrections were applied to convert the net intensities $I$, into structure factors by:

$$
\begin{equation*}
|F(h k 1)|=\left(\frac{I(h k 1)}{L p}\right) 1 / 2 \tag{1}
\end{equation*}
$$

The combined form of the Lorentz and polarizing factors, Lp,is:

$$
\begin{equation*}
\mathrm{Lp}=\frac{1+\cos ^{2} 2 \theta}{\sin 2 \theta} \tag{2}
\end{equation*}
$$

where $\theta$ is the Bragg angle of the hkl reflection. No corrections were made for absorption or extinction ( $\mu=37.2 \mathrm{~cm}^{-1}$ ). Standard deviations on the structure factors, $\sigma(F)$ were calculated by the procedure detailed in Stout and Jensen (12e):

$$
\begin{equation*}
\sigma(F)=0.5(\mathrm{Lp})^{-1 / 2}\left|\frac{\mathrm{~N}_{\mathrm{T}}+\mathrm{N}_{\mathrm{B}}+\left(0.05 \mathrm{~N}_{\mathrm{P}}\right)^{2}}{\mathrm{~N}_{\mathrm{T}}-\mathrm{N}_{\mathrm{B}}}\right|^{1 / 2} \tag{3}
\end{equation*}
$$

where $N_{T}, N_{B}$ and $N_{P}$ are the total counts, the background and the peak counts respectively. A total of 1865 reflections were measured having $2 \theta<40^{\circ}$. Of these 1445 had $I \geqslant 2 \sigma(I)$ and were classified as observable. The remaining 420 unobserved reflections were assigned zero weights during the refinement procedures and were not included in the R-factor calculations.

Scattering factors for neutral I, S, O, N, C and. H atoms as well as the real and imaginary parts of the anomalous scattering of iodine were taken from the International Tables for X-ray Crystallography (13).

CHAPTER III

STRUCTURE DETERMINATION AND REFINEMENT

## A. Patterson Function

A three dimensional Patterson function was calculated by:

$$
\begin{equation*}
P(U, V, W)=\sum \sum \sum|F o(h k I)|^{2} \cos 2 \pi(h U+k V+1 W) \tag{4}
\end{equation*}
$$

where $U, V, W$ are the fractional coordinates of the cell at which the function was calculated, and $\mid$ Fo(hkl) | is the absolute value of the observed structure factor for the hkI plane. The iodine atoms occupy the $\mathrm{P} 2_{1} / \mathrm{c}$ general positions, $4(\mathrm{e}): \mathrm{x}, \mathrm{y}, \mathrm{z} ;-\mathrm{x},-\mathrm{y},-\mathrm{z} ;-\mathrm{x}, 1 / 2+\mathrm{y}, 1 / 2-\mathrm{z}$; $\mathrm{x}, 1 / 2-\mathrm{y}, 1 / 2-\mathrm{z}$.

The coordinates for the iodine atoms were found to be $x=0.61$, $y=0.12, z=0.89$ for $I 1$ and $x=0.08, y=0.66, z=0.21$ for $I 2$ (Table 3 ).
B. Least Squares Refinement and Fourier Synthesis

The contributions to the structure factors were calculated by:

$$
\begin{equation*}
F(h k 1)=\Sigma f_{j} \cos 2 \pi\left(h x_{j}+k y_{j}+1 z_{j}\right) \exp -\left[B_{j}(\sin \theta / \lambda)^{2}\right] \tag{5}
\end{equation*}
$$

where $X_{j}, y_{j}, z_{j}$ are the fractional coordinates, $f_{j}$ is the scattering factor and $B_{j}$ is the isotropic thermal parameter of the $j$ th atom. The wavelength of the radiation and the Bragg angle of the hkl reflection are respectively $\lambda$ and $\theta$. Ibers andCorfield's version of Busing and

Table 3. The Patterson Map

| No. | Peak Position |  |  | Relative height to origin* | Assignment** of I-I vector | Multiplicity |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | u | V | W |  |  |  |
| 1 | 0.0000 | 0.0000 | 0.0000 | 999 |  |  |
| 2 | 0.5208 | 0.4836 | -0.324 | 407 | $\begin{aligned} & x-x^{\prime} y-y^{\prime} z-z^{\prime} \\ & x-x^{\prime}-y+y^{\prime} z-z^{\prime} \end{aligned}$ | $\left.\begin{array}{l}2 \\ 2\end{array}\right\} 4$ |
| 3 | 0.0000 | 0.2418 | -0. 5000 | 406 | $\begin{array}{lll} 0 \frac{1}{2}-2 y & \frac{1}{2} \\ 0 & \frac{1}{2}-2 y^{\prime} & \frac{1}{2} \end{array}$ | $\left.\begin{array}{l}2 \\ 2\end{array}\right\} 4$ |
| 4 | 0.4774 | 0.4836 | 0.3288 | 389 |  | $\left.\begin{array}{l}2 \\ 2\end{array}\right\} \quad 4$ |
| 5 | 0.000 | 0.2418 | 0.5064 | 388 | $\begin{aligned} & 0 \frac{1}{2}+2 y^{\frac{1}{2}} \\ & 0 \frac{1}{2}+2 y^{\prime} \frac{1}{2} \end{aligned}$ | $\left.\begin{array}{l}2 \\ 2\end{array}\right\} 4$ |
| 6 | 0.3038 | 0.000 | 0.4176 | 356 | $\begin{aligned} & -x-x^{\prime} \frac{1}{2}-y+y^{\prime} \frac{1}{2}-z-z^{\prime} \\ & -x-x^{\prime} \frac{1}{2}+y-y^{\prime} \frac{1}{2}-z-z^{\prime} \end{aligned}$ | $\left.\begin{array}{l}2 \\ 2\end{array}\right\} 4$ |
| 7 | 0.2170 | 0.4836 | 0.2696 | 213 | $2 \mathrm{x} \frac{1}{2} \frac{1}{2}+2 z$ | 2 |
| 8 | 0.3038 | 0.2418 | -0.0856 | 193 | $-x-x^{\prime}-y-y^{\prime}-z-z^{\prime}$ | 2 |
| 9 | 0.1736 | 0.4836 | -0.0856 | 191 | $2 x^{\prime} \frac{1}{2} \frac{1}{2}+2 z^{\prime}$ | 2 |
| 10 | 0.5208 | 0.2418 | 0.1808 | 183 | $x-x^{\prime} \frac{1}{2}+y+y^{\prime} \frac{1}{2}+z-z^{\prime}$ | 2 |
| 11 | 0.4774 | 0.2418 | -0.1744 | 182 | $-x+x^{\prime} \frac{1}{2}+y^{\prime} y^{\prime} \quad \frac{1}{2}-z+z^{\prime}$ | 2 |
| 12 | 0.2170 | 0.2418 | -0.2336 | 95 | $2 x^{\prime} 2 y^{\prime} 2 z^{\prime}$ | 1 |
| 13 | 0.1736 | 0.3224 | 0.4176 | 92 | 2 x 2 y 2 z | 1 |

* The calculated height of one I-I vector is 107 , relative to the origin. Thus peak 2 with peak height of 407 is equivalent to four I-I vectors.
** The assignment of the $I-I$ vectors is based on the coordinates:

|  | x | y | z |
| :--- | :--- | :--- | :--- |
| I | 0.61 | 0.12 | 0.89 |
|  |  |  |  |
| $I^{\prime}$ | 0.08 | 0.66 | 0.21 |

Levy's ORFLS program (14) was used for the least squares refinement. The computations were performed by a Burroughs 5500 computer. During the refinements the function that was minimized was $\Sigma \mathrm{w} \Delta^{2}$, where the weight factor w is

$$
\mathrm{w}=1 / \sigma^{2}(F)
$$

and

$$
\Delta=||F o|-|F c||
$$

An initial refinement of the iodine positions was made with $\mathrm{B}_{\mathrm{j}}$ set equal to $3.5 \mathrm{~A}^{\circ}$.

From the 1445 observable reflections the Fourier syntheses were calculated by:

$$
\begin{equation*}
\rho(x, y, z)=\sum \sum \sum F(h k 1) \cos 2 \pi(h x+k y+1 z) \tag{6}
\end{equation*}
$$

where $\rho(x, y, z)$ is the electron density at point $x, y, z$ in the unit cell and $F(h k l)$ is the observed value of the structure factor of the hkl reflection. A difference Fourier synthesis was computed by subtracting the contributions of the iodine atoms from the overall electron density Fourier synthesis. From this first Fourier difference map the positions of all of the atoms heavier than hydrogen were ascertained.

Thermal parameters were converted from isotropic to anisotropic for iodine and sulphur, and anomalous scattering factors were introduced for iodine. Two successive cycles of least squares refinement of the atomic positions, temperature factors and scale factor converged to a value of 0.068 for the unweighted $R$ factor $\left(R_{1}\right)$ and 0.087 for the weighted $R$ factor ( $R_{2}$ ) where:

$$
\begin{align*}
& \mathrm{R}_{1}=\Sigma \Delta / \Sigma|\mathrm{Fo}|  \tag{7}\\
& \mathrm{R}_{2}=\Sigma \mathrm{w} \Delta^{2} / \Sigma \mathrm{wFo} \tag{8}
\end{align*}
$$

Another difference Fourier synthesis yielded the positions of 14 of the 18 hydrogen atoms. The atoms which were not located at this point were $\mathrm{H} 1 \mathrm{Cl}, \mathrm{H} 2 \mathrm{C} 2, \mathrm{H} 2 \mathrm{~N} 2, \mathrm{H} 3 \mathrm{C} 11$. The positions of these hydrogen atoms were calculated by assuming $d(C-H)=d(N-H)=1 A^{\circ}$ and the appropriate angles for tetrahedral carbon and trigonal nitrogen. The isotropic thermal parameter for each hydrogen atom was fixed at $1 A^{\circ}$ greater than the thermal parameter of the atom to which it was bonded.

By a weighting analysis it was observed that a number of strong reflections with low $\sin \theta / \lambda$ values were somewhat overweighted. For $(2,0,-6)$, $(3,0,-2),(3,0,2),(3,1,0)$ the weighting scheme was changed so that

$$
\begin{equation*}
\sigma(F)=(1 /|\mathrm{Fo}-\mathrm{Fc}|) \cdot \text { scale factor } \tag{9}
\end{equation*}
$$

The weights of these reflections were changed because they were probably
affected by extinction. As no corrections were made for extinction, the accuracy of these measurements is diminished so less weight was given to them. The hydrogen positions were included but not refined in the next least squares cycle. The $R$ factors resulting from this refinement were $R_{1}=0.055$ and $R_{2}=0.069$. In the last cycle the scale factor, positional parameters for all 38 atoms, anisotropic thermal parameters for iodine and sulphur and isotropic thermal parameters for the other 17 atoms heavier than hydrogen were refined: a total of 150 parameters. The R factors were $R_{1}=0.054$ and $R_{2}=0.066$. The average (parameter shift)/(sigma) on this cycle was equal to 0.6. A final difference Fourier synthesis was computed with the four bad reflections $(2,0,-6),(3,0,-2),(3,1,0)$ (vide supra) omitted. The highest peak positions corresponding to ripples around the iodine atoms were approximately $1 \mathrm{e} / \mathrm{A}^{{ }^{3}}$. There were no positive holes in the vicinity of the hydrogen atoms whose positions were calculated. At this point the refinement was considered complete.

The appendix contains the structure factors for the observed and unobserved reflections. The refined atomic parameters are given in Tables 4, 5 and 6.

Table 4 Positional Parameters of Non-Hydrogen Atoms in Fractional Co-ordinates and Their Estimated Standard Deviations in Thiamine Hydroiodide


Table 5. Thermal Parameters for the Non-Hydrogen Atoms and Their Estimated Standard Deviations in Thiamine Hydroiodide
(a) Anisotropic *

|  | $\beta_{11}$ | $\beta_{22}$ | $\beta_{33}$ | $\beta_{12}$ | $\beta_{13}$ | $\beta_{23}$ | $\times 10^{4}$ |
| :--- | :--- | :--- | :--- | ---: | :--- | ---: | :--- |
| I1 | $58 \pm 1$ | $199 \pm 3$ | $27 \pm 1$ | $5 \pm 1$ | $20 \pm 1$ | $5 \pm 1$ |  |
| I2 | $93 \pm 1$ | $140 \pm 2$ | $20 \pm 1$ | $-20 \pm 1$ | $26 \pm 1$ | $4 \pm 1$ |  |
| S | $76 \pm 3$ | $104 \pm 7$ | $46 \pm 2$ | $0 \pm 4$ | $44 \pm 2$ | $-4 \pm 3$ |  |

(b) Isotropic **

|  | B |  | B |
| :---: | :---: | :---: | :---: |
| 0 | $3.6 \pm 0.2$ | C8 | $2.7 \pm 0.3$ |
| C1 | $4.1 \pm 0.3$ | C9 | $2.2 \pm 0.2$ |
| C2 | $3.1 \pm 0.3$ | N2 | $3.0 \pm 0.2$ |
| C3 | $2.7 \pm 0.3$ | N3 | $3.0 \pm 0.2$ |
| C4 | $2.4 \pm 0.2$ | C10 | $3.2 \pm 0.3$ |
| C5 | $3.7 \pm 0.3$ | C11 | $3.9 \pm 0.4$ |
| C6 | $2.8 \pm 0.2$ | N4 | $2.9 \pm 0.2$ |
| N1 | $2.3 \pm 0.2$ | C12 | $2.5 \pm 0.2$ |
| C7 | $2.4 \pm 0.2$ |  |  |

* The expression for the anisotropic temperature factor is

$$
T_{i}=\exp -\left(\beta_{11} h^{2}+\beta_{22} k^{2}+\beta_{33} 1^{2}+\beta_{12} h k+\beta_{13} h 1+\beta_{23} k I\right)
$$

** The expression for the isotropic temperature factor is

$$
T_{i}=\exp \left(-B_{i} \sin ^{2} \theta / \lambda^{2}\right)
$$

Table 6. Positional Parameters of Hydrogen Atoms and Their Estimated Standard Deviation in Thiamine Hydroiodide

|  | $x / a$ | $y / b$ | $z / \mathrm{c}$ |
| :---: | :---: | :---: | :---: |
| Но | $-43 \pm 1$ | $74 \pm 2$ | $19 \pm 1$ |
| H1C1 | $-48 \pm 1$ | $44 \pm 2$ | $19 \pm 1$ |
| H2C1 | $-36 \pm 1$ | $33 \pm 2$ | $22 \pm 1$ |
| H1C2 | $-48 \pm 1$ | $26 \pm 2$ | $10 \pm 1$ |
| H2C2 | $-51 \pm 1$ | $46 \pm 2$ | $6 \pm 1$ |
| H1C5 | $-24 \pm 1$ | $-11 \pm 2$ | $15 \pm 1$ |
| H2C5 | $-35 \pm 1$ | $-7 \pm 2$ | $4 \pm 1$ |
| H3C5 | $-39 \pm 1$ | $-11 \pm 2$ | $10 \pm 1$ |
| HC6 | $-9 \pm 1$ | $46 \pm 2$ | $12 \pm 1$ |
| H1C7 | $-18 \pm 1$ | $-2 \pm 2$ | $4 \pm 1$ |
| H2C7 | $-10 \pm 1$ | $0 \pm 2$ | $14 \pm 1$ |
| H1N2 | $-17 \pm 1$ | $18 \pm 2$ | $-4 \pm 1$ |
| H2N2 | $-9 \pm 1$ | $24 \pm 2$ | $-9 \pm 1$ |
| H1C11 | $28 \pm 1$ | $36 \pm 2$ | $6 \pm 1$ |
| H2C11 | $33 \pm 1$ | $37 \pm 2$ | $17 \pm 1$ |
| H3C11 | $32 \pm 1$ | $26 \pm 2$ | $13 \pm 1$ |
| HN4 | $25 \pm 1$ | $18 \pm 2$ | $23 \pm 1$ |
| HC12 | $9 \pm 1$ | $11 \pm 2$ | $2 \pm 1$ |

## CHAPTER IV

DISCUSSION AND RESULTS

Thiamine hydroiodide (THI) is composed of a divalent thiaminium cation and two monovalent iodide anions. One positive charge is associated with the aromatic thiazolium ring; the other results from protonation of N 4 in the pyrimidine ring. The molecular structure of thiamine hydroiodide resembles that of thiamine hydrochloride (THC) (10). The bond distances and angles of the thiazolium and pyrimidine rings in each structure are similar. The increased size of the anions in THI has only a minor effect on the general molecular conformation, although the crystal structure itself is different. The bond distances and angles of THI and THC are given in Tables 7 and 8. Hydrogen bonds and close contacts are listed in Tables 10 and 11. Figure 2 shows a single molecule of thiamine hydroiodide projected along 010 and 001. A 100 projection of THC is shown in Figure 3. The same conformation at the bridging methylene carbon is present in both THI and THC. In THI the dimethylene side chain is "trans" to amino group (N2) where as in THC it is "cis". The protonation site of the pyrimidine ring is the N4. This is consistent with what has been observed in other structures containing protonated thiaminium cations $(6,7,8,10,11,16,17)$. If the amino nitrogen, $N 2$, were the protonation site, the $\pi$ system of the pyrimidine would be greatly disturbed and the resonance energy

Table 7a. Bond Lengths of Covalent Bonds Not Involving Hydrogen Atoms and Their Estimated Standard Deviations for Thiamine Hydroiodide and Thiamine Hydrochloride

| Bond | Length |  |
| :--- | :--- | :--- |
|  | THI (11) | THC (10) |
| O-C1 | $1.38 \pm 0.02 \mathrm{~A}$ | $1.417 \pm 0.009 \mathrm{~A}$ |
| C1-C2 | $1.57 \pm 0.02$ | $1.542 \pm 0.010$ |
| C2-C3 | $1.51 \pm 0.02$ | $1.499 \pm 0.008$ |
| C3-C4 | $1.33 \pm 0.02$ | $1.349 \pm 0.007$ |
| C3-S | $1.74 \pm 0.01$ | $1.718 \pm 0.006$ |
| C4-C5 | $1.49 \pm 0.02$ | $1.479 \pm 0.008$ |
| C4-N1 | $1.38 \pm 0.02$ | $1.401 \pm 0.007$ |
| C6-S | $1.68 \pm 0.015$ | $1.667 \pm 0.006$ |
| C6-N1 | $1.33 \pm 0.015$ | $1.332 \pm 0.007$ |
| N1-C7 | $1.51 \pm 0.02$ | $1.476 \pm 0.007$ |
| C7-C8 | $1.50 \pm 0.02$ | $1.496 \pm 0.008$ |
| C8-C9 | $1.45 \pm 0.02$ | $1.434 \pm 0.007$ |
| C8-C12 | $1.32 \pm 0.01$ | $1.354 \pm 0.007$ |
| C9-N2 | $1.32 \pm 0.01$ | $1.316 \pm 0.007$ |
| C9-N3 | $1.36 \pm 0.02$ | $1.367 \pm 0.007$ |
| N3-C10 | $1.31 \pm 0.01$ | $1.306 \pm 0.007$ |
| C10-C11 | $1.30 \pm 0.03$ | $1.492 \pm 0.008$ |
| C10-N4 | $1.333 \pm 0.007$ |  |
| N4-C12 | $1.362 \pm 0.007$ |  |

Table 7b. Bond Lengths of Covalent Bonds Involving Hydrogen Atoms for Thiamine Hydroiodide and Thiamine Hydrochloride

Bond
THI (11)
$1.2 \pm 0.2 \AA$
$1.0 \pm 0.2$
$1.1 \pm 0.2$
$0.8 \pm 0.2$
$1.2 \pm 0.1$
$1.5 \pm 0.1$
$1.0 \pm 0.2$
$1.2 \pm 0.2$
$1.2 \pm 0.1$
$1.0 \pm 0.1$
$1.0 \pm 0.2$
$1.0 \pm 0.2$
$1.1 \pm 0.2$
$1.2 \pm 0.2$
$0.8 \pm 0.2$
$0.9 \pm 0.2$
$1.1 \pm 0.1$
$1.1 \pm 0.2$
$0.9 \pm 0.1$
THC (10)
$1.1 \pm 0.1 \AA$
$1.3 \pm 0.1$
$1.1 \pm 0.1$
$0.9 \pm 0.1$
$1.0 \pm 0.1$
$1.0 \pm 0.1$
$0.9 \pm 0.1$
$1.0 \pm 0.1$
$1.0 \pm 0.1$
$0.9 \pm 0.1$
$0.9 \pm 0.1$
$1.0 \pm 0.1$
$1.0 \pm 0.1$
$1.1 \pm 0.1$
$0.7 \pm 0.1$
$1.1 \pm 0.1$

Table 8. Bond Angles for Covalent Bonds Not Involving Hydrogen Atoms and Their Estimated Standard Deviation for Thiamine Hydroiodide and Thiamine Hydrochloride.

| Angle | Degrees |  |
| :---: | :---: | :---: |
|  | THI (11) | THC (10) |
| 0-C1-C2 | $112.0 \pm 1.5$ | $113.0 \pm 0.5$ |
| C1-C2-C3 | $110.4 \pm 1.1$ | $110.8 \pm 0.5$ |
| C2-C3-C4 | $130.1 \pm 1.3$ | $128.1 \pm 0.5$ |
| C2-C3-S1 | $119.8 \pm 0.9$ | $121.1 \pm 0.4$ |
| C3-C4-C5 | $127.5 \pm 1.5$ | $127.9 \pm 0.5$ |
| C3-C4-N1 | $112.9 \pm 1.2$ | $111.9 \pm 0.4$ |
| C5-C4-N1 | $119.6 \pm 1.3$ | $120.2 \pm 0.4$ |
| S-C3-C4 | $109.9 \pm 1.0$ | $110.7 \pm 0.4$ |
| S-C6-N1 | $111.0 \pm 1.1$ | $112.3 \pm 0.4$ |
| C3-S1-C6 | $91.7 \pm 0.7$ | $91.8 \pm 0.3$ |
| C4-N1-C6 | $114.6 \pm 1.1$ | $113.3 \pm 0.4$ |
| C4-N1-C7 | $123.2 \pm 1.0$ | $121.8 \pm 0.4$ |
| C6-N1-C7 | $122.1 \pm 1.2$ | $124.9 \pm 0.4$ |
| N1-C7-C8 | $112.8 \pm 1.0$ | $113.5 \pm 0.4$ |
| C7-C8-C9 | $122.7 \pm 0.9$ | $123.5 \pm 0.4$ |
| C7-C8-C12 | $119.8 \pm 1.4$ | $119.6 \pm 0.4$ |
| C8-C9-N2 | $123.0 \pm 1.2$ | $122.5 \pm 0.4$ |
| C8-C9-N3 | $120.3 \pm 0.9$ | $120.5 \pm 0.4$ |
| C9-N3-C10 | $118.7 \pm 1.3$ | $118.8 \pm 0.4$ |
| N3-C10-C11 | $118.6 \pm 1.5$ | $116.9 \pm 0.4$ |
| N3-C10-N4 | $122.9 \pm 1.3$ | $122.9 \pm 0.4$ |
| C11-C10-N4 | $118.5 \pm 1.1$ | $117.2 \pm 0.4$ |
| C10-N4-C12 | $119.0 \pm 0.9$ | $120.7 \pm 0.4$ |

Table 8. (continued)

| Angle | Degrees | THC (10) |
| :--- | :--- | :--- |
| C8-C12-N4 | $121.3 \pm 1.4$ | $120.3 \pm 0.4$ |
| N2-C9-N3 | $116.8 \pm 1.3$ | $116.9 \pm 0.4$ |
| C9-C8-C12 | $117.5 \pm 1.3$ | $116.8 \pm 0.4$ |



Figurè 2a. Projection of thiamine hydroiodide along 010
Symmetry code:none, $x, y, z ; 1, x-1, y, z ; 2,-x, 1-y,-z ; 3, x, v-1, z ;$
$4,-x,(1 / 2=y)-1,1 / 2-z ; 5,-x, 1 / 2+y, 1 / 2-z$.


Figure 2b. Projection of thiamine hydroiodide along 001
Symmetry cole: none, $x, y, z ; 1, x-1, y,-z ; 2,-x, 1-y,-z$;
3, $-\mathrm{x},(\mathrm{n} / 2+\mathrm{y})-1,1 / 2-\mathrm{z}$;


Figure 3. Projection of thiamine hydrochloride along 100
decreased. The separation of charges is more favourably accomplished by protonation at N 4 rather than the other pyrimidine nitrogen N 3 . The resonance forms of the pyrimidine and thiazolium rings are shown in Figure 4.

The aromatic rings in the molecule are virtually planar. Table 9 lists the equations of the least squares planes and the perpendicular distances from these planes of pertinent atoms. The appearance of the thiazolium ring plus substituents is somewhat bowl shaped; that is, the substituents all lie out of the plane and to the same side of the ring. This is a result of the position of the $I 2$ atom in the lattice and the fact that hydrogen bonding occurs only between molecules having the same "handedness" (vide infra).

The dihedral angle between the plane of the pyrimidine ring and the plane of the amino group (C9, N2, H1N2, H2N2) is $10.9^{\circ}$. Atoms H1N2 and H2N2 are both hydrogen bonded to iodine atoms so this slight till is due to packing effects. The overlapping of the filled $p_{z}$ orbital on $N 2$ with the $\pi$ system of the pyrimidine ring (Fig. 4) is demonstrated by the planarity of the amino group. The C $9-\mathrm{N} 2$ bond distance of $1.32 \AA$ is comparable to the C-N distances of the pyrimidine ring. The bond order C9-N2 is 1.6 (18).

There are a number of contacts occurring on the thiazolium nitrogen and sulphur atoms. I1 and I2 sit almost directly above and below N1 at the van der Waal's radius for I-N ( 3.70 A ). The distances for $I 1-N 1$ and $I 2-N 2$ are 3.69 and 3.67 A respectively. The angle subtended by $\mathrm{I} 1-\mathrm{N} 1-\mathrm{I} 2$ is $155.5^{\circ}$. Angles $11-\mathrm{N} 1-\mathrm{C} 6$ and I2-N1-C6 are $103.5^{\circ}$ and $97.9^{\circ}$. The $p_{z}$ orbital on the trigonal N1 is suitably oriented for accepting negative charge from






## THIAZOLIUM





Figure 4. The major contributing resonance forms for thiamine hydroiodide.

Table 9. Deviations from Least Squares Planes*

| Atoms used in | Deviation | Atoms not used | Deviation |
| :--- | :---: | :--- | :---: |
| calculating | o | in calculating | $\circ$ |
| plane | A | plane | A |

Pyrimidine Ring
$0.372 x-0.897-0.238=-1.77$

| C8 | 0.01 | C 7 | 0.03 |
| :--- | :--- | :--- | :--- |
| C9 | -0.02 | N 2 | -0.07 |
| N3 | 0.001 | H 1 N 2 | -0.06 |
| C10 | 0.02 | H 2 N 2 | -0.36 |
| N4 | -0.03 | C 11 | 0.14 |
| C12 | 0.01 | HN 4 | 0.01 |
|  |  | $\mathrm{HC12}$ | 0.27 |

Thiazolium Ring
$-0.0558 x+0.0352 y-0.998 z=-1.30$

| C3 | -0.01 | C2 | 0.06 |
| :--- | ---: | :--- | :--- |
| C4 | 0.01 | C5 | 0.01 |
| C6 | -0.01 | C7 | 0.06 |
| S | 0.01 | HC6 | 0.53 |
| N1 | -0.01 | 0 | 1.49 |

C9-N2-H1N2-H2N2 (amino) plane
$0.271 x-0.958 y-0.0890 z=-1.73$
C9 $\quad-0.02$
$\mathrm{N} 2 \quad 0.07$
H1N2 -0.02
$\mathrm{H} 2 \mathrm{~N} 2 \quad-0.03$

Table 9. (continued)

* The equations of these unweighted least squares planes have the form $a x+b y+c z=d$ where $x, y$ and $z$ are the orthogonal coordinates (in $\AA$ ) of the atoms. These orthogonal coordinates are related to the fractional coordinates of the unit cell ( $\mathrm{a}, \mathrm{b}, \mathrm{c}$ ) by the transformations: $\mathrm{x}=\mathrm{a}+\cos \beta$ $y=b$ $z=c \sin \beta$

Table 10. Hydrogen Bond and Short Contact Distances
a b
c
$<a b c,{ }^{\circ} d_{b-c} \stackrel{\circ}{A}$
$d_{a-c^{A}} \quad$ Sum of van der Waal's radii (a-c) ${ }^{\circ}$
O-HO ...II ${ }^{\text {i }}$
$145.4 \quad 2.32 \pm 0.2$
3.42
3.55
N2-H1N2...I1 ${ }^{\text {ii }}$
$152.7 \quad 2.70 \pm 0.2 \quad 3.64$
3.65
C11-H3C11...I1 ${ }^{\text {iii }}$
$162.43 .09 \pm 0.2$
3.934 .15
C6-HC6...I2
$161.6 \quad 2.50 \pm 0.1$
3.61
4.0
N2-H2N2...I2 $2^{i i}$
$169.7 \quad 2.58 \pm 0.2$
3.62
3.65
C12-HC12...I2 ${ }^{\text {iii }}$
$193.3 \quad 3.40 \pm 0.1 \quad 3.65 \quad 4.00$
N4-HN4. .. $\mathrm{O}^{\mathrm{iv}}$
163.2
$1.61 \pm 0.1$
2.722 .9
S... 0
$2.97 \pm 0.01$
3.25
N1...II ${ }^{\text {ii }}$
$3.66 \pm 0.01$
3.65
N2...II $1^{i v}$
$3.67 \pm 0.01$
3.65

Symmetry code: none, $x, y, z ;$

$$
\begin{aligned}
& \text { i, } x-1, y, z \\
& \text { ii, }-x, 1-y,-z \\
& \text { iii, } x, y-1, z \\
& \text { iv, } x,\left(\frac{1}{2}+y\right)-1, \frac{1}{2}-z
\end{aligned}
$$

the iodine atoms as well as for taking part in the $\pi$ system of the thiazolium ring. There is an intramolecular contact of $2.97{ }^{\circ}$ occurring between the sulphur and oxygen atoms. The van der Waal's $0-S$ radius is 3.60 A. Because of the partial positive charge (fig. 4, Ref. 2) and the empty 3d orbitals of sulphur, S-O and/or S-C1 interactions are a common phenomenon in other structures $(6,8,9,10,19)$. In the thiamine monohydrate structure (17) sulphur is surrounded by electronegative nitrogen atoms N2 and N3 of the unprotonated pyrimidine ring from adjacent molecules, although neither of these distances is less than the sum of the van der Waal's radii. Fewer examples are to be found of interactions involving thiazolium nitrogen. Nitrogen-chlorine contacts of this type have been observed in thiaminium tetrachlorocadmate (6) and in bis (protonated thiamine) tetrachlorodioxouranium (VI) (19), and nitrogen-iodine contacts in this present work. Table 10 gives the hydrogen bond lengths, hydrogen bond ang1es and the corresponding van der Waal's distances.

The coordination geometry surrounding the iodine atoms is tetrahedral although the bond angles are rather distorted. Each of I1 and I2 is involved in two short hydrogen bonds and two longer contacts. The two iodine atoms are separated by more than $7 \AA$ so there is no contact between them. Table 11 lists the intermolecular contact angles surrounding the iodine atoms and N 1 .

Although the thiamine molecule has no centre of asymmetry, the molecules in the unit cell which are related by a glide plane are in
fact non-superimposable mirror images by virtue of their molecular conformation. Conversion of one enantiomeric form to the other is achieved by rotation about single bonds. Therefore, in solution there is rapid equilibrium between configuarations.

It can be seen (Fig. 5) that hydrogen bonding occurs only between molecules of like configuration, i.e., those related by a screw axis. This particular hydrogen bond is between 0 and HN4 (Table 7).

The conformation of the molecule around the bridging methylene carbon (vide supra) is of the same category as THC. The values of the torsional angles $\phi_{\mathrm{T}}$ and $\phi_{\mathrm{P}}$ are $-5.9^{\circ}$ and $81.0^{\circ}$. Table 1 lists these torsional angles as well as the angles for the other accurately determined structures.

One of the most interesting aspects of the thiamine molecule is its conformation. The angles which $\phi_{T}$ and $\phi_{P}$ adopt are critical to the formation and stability of the holoenzyme (enzyme plus cofactors) and of the holoenzyme substrate complex. Attempts to unravel the structure of the holoenzyme complex by x-ray diffraction have not been made. Studies so far have been limited to crystal structures of the thiamine salts and substituted thiamine derivatives.

Table 11. Angles of Hydrogen Bonds and Short Contacts Surrounding N1 and Iodine Atoms.

| Angle | degrees |  |  |
| :---: | :---: | :---: | :---: |
| $\mathrm{N} 1^{\text {i }}-\mathrm{I} 1-\mathrm{H} 1 \mathrm{~N} 2^{\text {i }}$ | 51 |  | 4 |
| N1-I1-H0 ${ }^{\text {ii }}$ | 126 |  | 5 |
| N1-I1-H3C11 ${ }^{\text {iii }}$ | 100 |  | 3 |
| H1N2-I1-H0 | 146 |  | 5 |
| H1N2-I1-H3C11 | 81 |  | 5 |
| H0-I1-H3C11 | 128 |  | 5 |
| $\mathrm{N1}{ }^{\text {iv }}-\mathrm{I} 2-\mathrm{HC12}{ }^{\text {iii }}$ | 77 |  | 3 |
| N1-I2-H2N2 ${ }^{\text {i }}$ | 143 |  | 3 |
| N1-I2-HC6 | 126 |  | 4 |
| HC12-I2-H2N2 | 77 | $\pm$ | 5 |
| HC12-I2-HC6 | 129 |  | 4 |
| H2N2-I2-HC6 | 98 | $\pm$ | 3 |
| I1 ${ }^{\text {i }}$-N1-C6 | $103.5 \pm 0.7$ |  |  |
| I2-N1-C6 | $97.9 \pm 0.4$ |  |  |
| I1-N1-I2 | $155.5 \pm 0.3$ |  |  |
| symmetry code: |  |  |  |

$$
\begin{aligned}
& \text { i, }-x, 1+y,-z \\
& \text { ii, } x+1, y, z \\
& \text { iii, } x, y+1, z \\
& \text { iv, }-x, \frac{1}{2}+y, \frac{1}{2}-z \\
& \text { v, } x,\left(\frac{1}{2}+y\right)-1, \frac{1}{2}-z
\end{aligned}
$$

$$
\begin{aligned}
& \text { (1) }=11 \\
& \text { (2) }=12
\end{aligned}
$$

Figure 5. Packing of molecules in the crystal structure of thiamine hydroiodide projected onto the 001 plane. Hydrogen atoms have been omitted from the molecular structure.

In these structures only two conformations have been observed, in spite of prediction of a wide range of stable conformations at ordinary temperature. (Ref. 2, vide supra).

Many authors ( $2,6,9,11$ ) including the present one, have attributed these observations to the presence of favourable packing interactions that may be achieved by one or the other of the conformers. Molecular models suggest that the extensive hydrogen bonding usually observed would be in part disrupted for some ring orientations. In the structures reported, intermolecular contacts have been very different (6-11,14-17). Anions have varied from chloride to tetrachlorodioxouranium (VI) but the thiamine conformations have remained more or less constant.

From a simplistic point of view one might legitimately ask whether an x-ray crystal structure of a thiamine compound casts any light upon the mechanistic behaviour of thiamine as a coenzyme in biological processes. Clearly the living system does not produce a lattice of thiamine and anions. However, by the same token, it is just as unreasonable to believe that chemical reactions in living organisms occur in a "test tube" fashion. A cell is by no means a solution of catalysts and substrates inside a miniature baggie, but rather a highly ordered system. There is a substantial amount of ice inside a cell, even at $36^{\circ}$ (20)! Because of the large degree of hydrogen bonding present in biologically active molecules many aspects of biochemistry may be considered with references to the solid state. Holoenzyme-substrate complexing is one example; diffusion of molecules through membranes is another.

In the model PDC system (4) pyruvate decarboxylase apoenzyme, thiamine pyrophosphate, $\mathrm{Mg}^{2+}$ ions, and pyruvic acid substrate are the necessary components. The structures of the thiamine-Mg ${ }^{2+}$ salt and 2-( $\alpha$-Hydroxyethy1)thiamine hydrochloride (HET) are known. HET is an intermediate in the PDC system where pyruvic acid is converted to acetaldehyde. The conformation is "regular" for Thi-Mg ${ }^{2+}$ and "odd"for HET. A structural feature of thiamine pyrophosphate hydrochloride (22) is that the three non-protonated pyrophosphate oxygens are directed away from the same side of the molecule. Since the pyrophosphate group is suspected to bind the molecule to the enzyme, an arrangement such as this allows the bound enzyme to be accessible to the substrate and permits conformational shifts between the two rings.

In the structure presented here, the atomic positions of carbon, nitrogen and oxygen were located to within $0.01 \AA$. Owing to the fact that half of the electron density of the molecule is associated with two iodine atoms, the structure factors are not very sensitive to small shifts in these atomic positions. The final $R$ factor converged to 5.4 \%.

The final $R$ factor is not always indicative of the "goodness" of the structure. The atomic positions of thiamine hydrochloride have been determined to within $0.004 \mathrm{~A}^{\circ}$ with a final R factor of 8\%. However, THC has no heavy atoms. Iodide and other heavy atoms simplify the initial tasks of the interpreting of the Patterson and the phasing of the reflections, but at the same time cause less accuracy in the light atom parameters later in the refinement.

## CHAPTER V

## THIAMINIUM TETRACHLOROCOBALTATE

A thiaminium tetrachlorocobaltate salt was produced from an aqueous 1:1 mole ratio mixture of thiamine hydrochloride and cobalt (II) chloride. A solution of thiamine chloride, 3.49 (0.01 mole) in 4 ml . of water was added dropwise to 2.99 ( 0.01 mole ) of cobalt (II) chloride ( $\mathrm{C}_{0} \mathrm{Cl}^{2} .6 \mathrm{H}_{2} \mathrm{O}$ ) dissolved in 2 ml . of water. Upon evaporation of the solvent small diamond shaped crystals were formed. Oscillation and Weissenberg photography indicated monoclinic space group $\mathrm{P}_{1} / \mathrm{c}$ and cell constants: $\mathrm{a}=16.38, \mathrm{~b}=15.39, \mathrm{c}=7.83 \mathrm{~A}$, $\beta=97.23^{\circ}$. Structure factors were calculated for a thiaminium tetrachlorocobaltate structure using the atomic and thermal parameters of thiaminium tetrachlorocadmate (6) and substituting cobalt scattering factors for cadmium. A comparison was made between the intensities of the axial reflections on the film and the calculated structure factors of these reflections. In terms of weak, moderate, strong, very strong there was a close agreement between the intensities and structure factors. On this basis thiaminium tetrachlorocobaltate was judged to be isomorphous to thiaminium tetrachlorocadmate whose space group is $\mathrm{P}^{2} / \mathrm{c}$ and ce11 constants are $\mathrm{a}=16.874, \mathrm{~b}=15.553$, $c=7.906{ }^{\circ} \mathrm{A} ; \quad \beta=97.61^{\circ}$.

## APPENDIX

## STRUCTURE FACTORS

The columns in the following tables contain

```
h k
Fo x 5
Fc x 5
\(\sigma(F) \times 5\)
```

 w





































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 377
133
71
75
59
516
537
262
353
1044
271
434
135
87
366
383
303
470
193
1013
492
306
54
125
288
57
88
124
990
101
138
445
135
140
115
287
520
274
548
268
124
213
450
93
67
123
576
237
230
197
0




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