The Structure of N1'-Methylthiaminium Diiodide*

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Abstract. $C_{13}H_{20}N_4OS^{2+}.2I^-$, monoclinic, $P2_1$, $a = 6.519$ (1), $b = 10.257$ (2), $c = 13.901$ (2) Å, $\beta = 91.91$ (1)°, $V = 929.0$ (3) Å^3, $D_m = 1.88$ (2) (flotation in $CCl_4/CBrCl_3$), $D_c = 1.91$ Mg m^{-3}, $Z = 2$. The crystal structure was solved by a combination of direct methods (heavy-atom positions) and Fourier maps (light-atom positions), and refined by full-matrix least-squares methods to $R = 0.066$ for 1182 reflections. The conformation of the dipositive N1'-methyl-thiaminium ion is that commonly observed for unsubstituted thiamins. The two torsional angles, $\varphi_T = 4.3°$ and $\varphi_P = 84.1°$, agree within 10° with the closely related compound thiaminium diiodide. This is the first reported occurrence of a thiamin crystallizing in a non-centrosymmetric space group.

Introduction. Thiamin diphosphate functions as a coenzyme in conjunction with a number of α-ketoacid decarboxylases and transketolase (Krampitz, 1969). The conformation of this coenzyme has been the subject of several studies using X-ray crystallography, NMR and semi-empirical theoretical methods. The crystallographic data have been reviewed and the conformational angles have been defined (Shin, Pletcher, Blank & Sax, 1977). NMR work on the conformational aspects of thiamin has been summarized recently by Gallo & Sable (1976). Semi-empirical calculations have indicated reasons for the observation of different conformations in unsubstituted thiamins (Jordan, 1974) compared to 2-(α-hydroxyethyl)thiamin (Jordan, 1976).

Recently, Jordan & Mariam (1978) postulated a catalytic role for the pyrimidine ring of the coenzyme. This was based on the finding that the title compound N1'-methylthiaminium diiodide (MTDI) in several model reactions acted as a superior catalyst to the natural vitamin. The X-ray structure of MTDI was determined in order to decide if the source of the

enhanced catalytic activity of MTDI over thiamin resides in differences in the conformational disposition of the two aromatic rings with respect to each other or in electrostatic factors (field or inductive effects).

Preliminary Weissenberg and precession photographs determined the space group to be either $P2_1$ or $P2_1/m$. A clear plate-like crystal of dimensions 0.30 × 0.15 × 0.05 mm was used for data collection on a Syntex $P2_1$ diffractometer. The cell parameters were obtained using 15 centered reflections and graphite-monochromatized Mo $K\alpha$ radiation. Space group $P2_1$ was confirmed by the structure analysis. This is the first known occurrence of a thiamin crystallizing in a non-centrosymmetric space group.

Intensity data were collected at 297 (1) K by the θ–2θ scan technique out to a maximum 2θ value of 50°. Three standard reflections were recorded every 47 reflections throughout the data collection and showed random variations of ±3% with no significant trends. Of the 2873 unique reflections collected, 1182 had values of $I \geq 3\sigma(I)$ and were subsequently used in the solution and refinement of the structure. Nine reflections were rejected on the basis of a profile scan (Glick, 1975) and all intensities were corrected by application of scale factors based on the standard-reflection intensities. Absorption corrections ($\mu = 3.542$ mm^{-1}) were made using a six-face approximation of the crystal boundary surfaces. The minimum and maximum absorption corrections were 1.412 and 1.802, respectively. Lorentz and polarization corrections were applied and the corrected data were then used to obtain estimates of the overall temperature and scale factors by Wilson's (1942) method.

The structure was solved by a combination of direct methods and heavy-atom techniques. Phases were developed for 300 reflections with the largest E magnitudes from 64 starting sets, using the program *MULTAN* (Germain, Main & Woolfson, 1971), and an E map was calculated with the most promising phase set. The map did not reveal any recognizable segment of the molecule; E maps calculated with phase sets further down the list in figures of merit also failed to solve the structure. Since the three largest peaks on any of the E maps were internally consistent and agreed

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well with a Patterson-like map (Patterson, 1935) calculated with $E^2 - 1$ values as coefficients, they were assumed to represent the two I atoms and the one S atom. The I atoms were found to be related by a pseudo n glide. A structure factor calculation based on these three heavy-atom coordinates gave $R_F = \sum ||F_o| - |F_c||/\sum |F_o| = 0.28$. An electron density map based on the phases from the three heavy atoms yielded all the remaining non-hydrogen atoms. After several cycles of refinement minimizing the quantity $\sum w_i(|F_{oi}| - |F_{ci}|)^2$, an attempt was made to locate H atom positions *via* a difference electron density synthesis. No reasonable H atom coordinates were obtained; thus, the ten H atoms were calculated with fixed geometry and were included in subsequent cycles of refinement with their coordinates held invariant and with $B_{iso} = 4.0$ Å^2 assigned to each H atom. All non-hydrogen atoms were allowed anisotropic thermal motion in the following cycles of full-matrix least-squares refinement (Busing, Martin & Levy, 1962).

At this stage of refinement, the counting-statistics weighting scheme was replaced by a scheme determined by an analysis of variance (Lalancette, Cefola,

Table 1. *Positional parameters* $(\times 10^4)$ *for $N1'$-methylthiaminium diiodide*

	x	y	z	B_{eq}* (Å^2)
I(1)	3376 (2)	2196	3400 (1)	3.47
I(2)	8162 (3)	3468 (3)	8667 (1)	3.90
S(1)	1769 (11)	−858 (7)	4806 (5)	3.18
O(5γ)	−1402 (32)	−1999 (21)	3185 (16)	4.13
N(1')	3196 (31)	−2467 (28)	8815 (16)	3.22
N(3')	5155 (30)	−579 (20)	8796 (14)	2.28
N(4'α)	3935 (26)	1248 (21)	7970 (14)	2.42
N(3)	370 (25)	−163 (21)	6382 (14)	2.39
C(2')	4936 (34)	−1798 (27)	9036 (16)	2.45
C(4')	3617 (38)	39 (28)	8272 (17)	3.11
C(5')	1770 (34)	−640 (24)	8029 (14)	2.27
C(6')	1599 (41)	−1946 (33)	8297 (23)	3.62
C(3,5'α)	25 (33)	−50 (25)	7439 (15)	2.46
C(4)	−1100 (30)	268 (22)	5690 (20)	2.21
C(5)	−503 (37)	−15 (20)	4793 (15)	2.12
C(2)	1965 (47)	−778 (32)	6031 (22)	3.56
C(4α)	−3037 (37)	952 (26)	6023 (20)	3.07
C(5α)	−1729 (49)	142 (32)	3863 (21)	3.54
C(5β)	−2763 (44)	−1041 (31)	3512 (19)	3.53
C(1'α)	3029 (57)	−3902 (33)	9085 (29)	4.65
C(2'α)	6701 (46)	−2424 (38)	9581 (18)	4.78
H(C6')	347	−2453	8123	
H(1C3,5'α)	−73	933	7629	
H(2C3,5'α)	−1290	−459	7629	
H(C2)	3129	−1275	6419	
H(1C5α)	−788	459	3350	
H(2C5α)	−2796	882	3949	
H(1C5β)	−3797	−751	2972	
H(2C5β)	−3611	−1364	4056	
H(1N4'α)	2822	1724	7587	
H(2N4'α)	5236	1755	8135	

* The equivalent isotropic thermal factor (Hamilton, 1959) B_{eq} is defined as $B_{eq} = \frac{4}{3} \sum_{i=1}^{3} \sum_{j=1}^{3} \beta_{ij}(\mathbf{a}_i \cdot \mathbf{a}_j)$.

Hamilton & LaPlaca, 1967). The new $\sigma(i)$ values were determined according to the following:

$$|F| < 11.35, \sigma(i) = -0.3125|F| + 5.50;$$

$$11.35 \leq |F| < 23.5, \sigma(i) = 0.0018|F| + 1.957;$$

$$|F| \geq 23.5, \sigma(i) = 0.068|F| + 0.426.$$

Using this weighting scheme, the refinement converged to $R_F = 0.066$, $R_w = (\sum w||F_o| - |F_c||^2/\sum w|F_o|^2)^{1/2} = 0.084$, the e.s.d. of an observation of unit weight = 1.02, and a scale factor of 0.921.

The atomic scattering factors for all non-hydrogen atoms were taken from the Dirac–Slater calculations of Cromer & Waber (1965); I⁻ scattering factors were used for the halogens; H atom scattering factors as well as the anomalous parts of the I and S scattering factors were obtained from *International Tables for X-ray Crystallography* (1962). Table 1 lists the final parameters from the least-squares refinement along with their e.s.d.'s.* A final difference Fourier map had no peak larger than 0.60 e Å^{-3}.

Discussion. The structure consists of an ionic network of iodide ions interspersed with dipositive $N1'$-methylthiaminium ions. Each iodide is associated with one of the rings, as would be expected since each ring bears a formal positive charge. I(1) forms close contacts with the thiazolium ring with distances of 3.67 (2) to N(3), 3.72 (2) to C(4), 3.74 (3) to C(2), 3.856 (8) to S(1) and 3.95 (2) Å to C(5) (see Fig. 1). I(1) also forms close contacts of 3.74 (2) to C(2'α), 3.88 (2) to N(1') and 3.95 (2) Å to N(3') of the pyrimidine ring. I(2) forms close contacts with the pyrimidine ring: 3.68 (2) to N(4'α), 3.76 (2) to N(1') and 3.84 (2) Å to C(2'α). I(2) also has a short contact to O(5γ) of 3.42 (2) Å.

H atom positions were not determined in this study. Close non-bonded contacts between non-hydrogen atoms are: N(4'α)$\cdots$O(5γ) (in a symmetry-related molecule) of 2.89 (3) Å, and O(5γ)$\cdots$S(1) of 3.23 (2) Å. This latter contact is a fairly standard feature in thiamin structures and indicates a fractional positive charge on the S atom. This S$\cdots$O distance is considerably longer than the 2.97 Å reported by Lee & Richardson (1976) for thiaminium diiodide.

Both the thiazolium and pyrimidine rings exhibit slight out-of-plane distortions due primarily to the positive charge believed to be residing on the N(1') and N(3) atoms. The dihedral angle between the planes defined by the thiazolium and pyrimidine rings is 89.6 (9)°. Neither C(1'α) nor C(3,5'α) (each bonded to a N believed to bear a positive charge) is coplanar
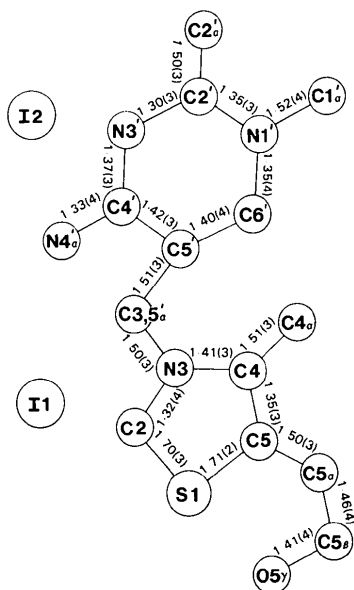
Fig. 1. Labelling scheme for $N1'$-methylthiaminium diiodide, and bond distances (Å).

with the aromatic ring to which it is attached: $C(1'a)$ is 0·03 Å above the plane of the pyrimidine ring and $C(3,5'a)$ is 0·11 Å below the plane of the thiazolium ring. In thiaminium diiodide, this latter observation (0·06 Å deviation) was also reported (Lee & Richardson, 1976).

The conformation of the two aromatic rings with respect to the bridge methylene C atom can be described in terms of two torsional angles φ_T and φ_P (Pletcher, Sax, Blank & Wood, 1977). These represent rotations about the bonds from $C(3,5'a)$ to the thiazolium and pyrimidine rings, respectively. In the present structure, $\varphi_T = 4\cdot3°$ and is the dihedral angle between the planes defined by $C(5')-C(3,5'a)-N(3)$ and $C(3,5'a)-N(3)-C(2)$, and $\varphi_P = 84\cdot1°$ and is the dihedral angle between the planes defined by $C(5')-C(3,5'a)-N(3)$ and $C(3,5'a)-C(5')-C(4')$. Lee & Richardson (1976) found $\varphi_T = -5\cdot9°$ and $\varphi_P = 81°$ for the closely related thiaminium diiodide. The conformation found falls into the class F commonly observed for unsubstituted thiamins (Pletcher, Sax, Blank & Wood, 1977).

In view of the fact that the conformation observed in $N1'$-methylthiaminium diiodide is the same as that found in a variety of other thiamin structures, one can only conclude that at least in the crystal structures there is an inherent stability associated with values of φ_T near 0° and φ_P near 90°. It appears that the rate accelerations observed in thiamin model reactions when $N1'$-methylthiaminium diiodide is employed instead of thiamin (Jordan & Mariam, 1978) are very likely not to be the result of gross conformational differences between the modified and the natural vitamin B₁.

A final comment concerning the unexpected ^{1}H NMR behavior of MTDI is in order. It was observed that in $Me_2SO-^2H_6$ (but not in H_2O) there are two $N(4'a)$ amino proton peaks (Jordan & Mariam, 1978). These peaks persist upon the addition of vast excess of I^-, or upon dilution (i.e. the observation is not due to intermolecular hydrogen bonding), although both these conditions do cause chemical-shift changes. More curious is the fact that the corresponding amino proton resonance in thiaminium dichloride (the commercial vitamin B₁ derivative) is a singlet under the same conditions even in the presence of vast excess of I^-. Of the intermolecular hydrogen bonds postulated from $N(4'a)$ to the receptors $O(5\gamma)$ and I^- in MTDI, only the one to the I^- was detected in thiaminium diiodide (Lee & Richardson, 1976). The observation of two $N(4'a)$ amino proton peaks in MTDI could be due to hydrogen bonding to two different acceptors, i.e. $O(5\gamma)$ and I^-. More than likely, aside from such intermolecular hydrogen bonds, the rotation around the $C(4')-N(4'a)$ bond is slower in MTDI than in thiaminium dichloride (vitamin B₁). As there may be several reasons for this, further speculation is unwarranted, but clearly the crystallographic data do not pinpoint a single reason for this phenomenon.

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Structure de l'Acide *tert*-Butoxycarbonyl-D-alanyl-D-glutamique Monohydraté

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Abstract. $C_{13}H_{22}N_2O_7.H_2O$ (grown from ligroin–ethyl acetate), $M_r = 336 \cdot 18$, orthorhombic, $P2_12_12_1$, $a = 9 \cdot 604$ (2), $b = 18 \cdot 242$ (4), $c = 10 \cdot 097$ (2) Å, $V = 1768 \cdot 95$ Å^3, $D_x = 1 \cdot 262$ Mg m^{-3}, $Z = 4$. The structure has been solved by direct methods. The final reliability index R is 0·055 for 1740 reflexions. The torsion angles of the polypeptide chain (φ, ψ) are in the α region on the Ramachandran plot [Ramachandran, Ramakrishnan & Sasisekharan (1963). *J. Mol. Biol.* **7**, 95]. Each molecule is involved in four hydrogen bonds.

Introduction. Dans le cadre de nos recherches sur le mode d'action des antibiotiques du type β-lactame, nous avons cristallisé une D-alanyl-D-alanine carboxy-peptidase (Dideberg, Frère & Ghuysen, 1979). Afin de localiser le site catalytique, des complexes enzyme-inhibiteur et enzyme-drogue ont été préparés. Un de ces inhibiteurs est le Boc-D-Ala-D-Glu.

Les intensités diffractées ont été mesurées sur un monocristal sensiblement cubique d'arête $a = 0,4$ mm. Le diffractomètre Hilger & Watts a été utilisé pour mesurer 1919 réflexions ($\theta < 70°$) (radiation Cu $K\alpha$ filtrée) et le balayage ω–2θ a été choisi. Parmi celles-ci, 1740 ont été conservées pour résoudre la structure [$I > 2\sigma(I)$]. Les intensités ont été corrigées des facteurs de Lorentz et de polarisation, mais pas d'absorption ($\mu r = 0,32$).

La structure a été déterminée à l'aide du programme *MULTAN* 77 (Main, Lessinger, Woolfson, Germain & Declercq, 1977). La meilleure des trente solutions générées par le programme fait apparaître l'ensemble des atomes de carbone, oxygène et azote. Les positions des atomes d'hydrogène ont été trouvées en combinant les résultats de synthèses de Fourier et les données géométriques relatives aux liaisons C–H et O–H. La structure a été affinée avec le programme *SHELX* 76 (Sheldrick, 1976) jusqu'à un facteur R égal à 0,055 pour les 1740 réflexions observées,* en gardant fixes pour les hydrogènes les distances C–H et O–H. Le schéma de pondération final est de la forme: $w = 1/(\sigma^2|F| + 0,1153|F|^2)$.

Les coordonnées atomiques et les facteurs B équivalents finaux sont donnés dans le Tableau 1. Les longueurs et les angles des liaisons intramoléculaires sont repris sur les Figs. 1 et 2. Les angles de torsion principaux de la chaîne peptidique ainsi que de la chaîne latérale sont indiqués sur la Fig. 3. Le Tableau 2 donne

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* Les listes des facteurs de structure, des facteurs d'agitation thermique anisotrope, et les positions des atomes d'hydrogène ont été déposées au dépôt d'archives de la British Library Lending Division (Supplementary Publication No. SUP 35887: 11 pp.). On peut en obtenir des copies en s'adressant à: The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, Angleterre.