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X-Ray Analysis of Thiamine Propyl Disulfide[1]

Masao Nishikawa, Kazuhide Kamiya,
Yutaka Asahi and Hideo Matsumaru

Chemical Research Laboratories, Research and Development Division,
Takeda Chemical Industries, Ltd.[2]

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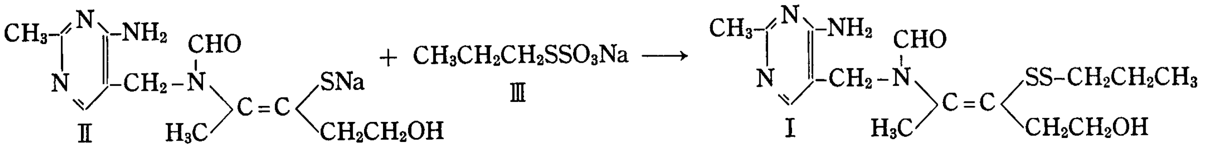
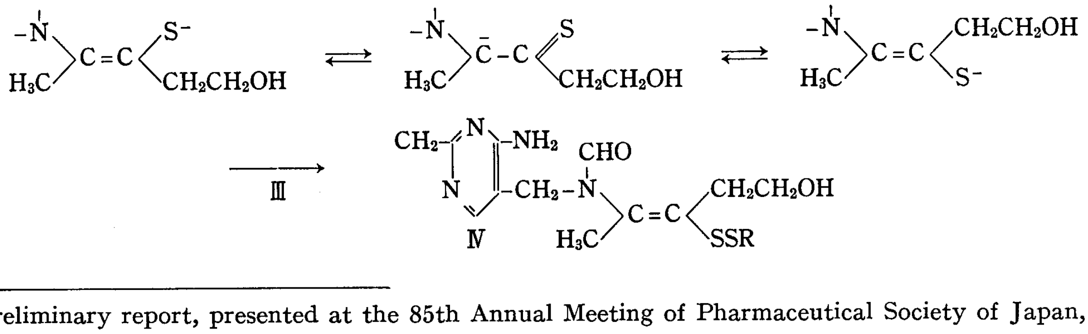
The crystal structure of thiamine propyl disulfide (TPD) was determined by the three dimensional Patterson superposition method and by application of least squares treatment and Fourier syntheses. The unit cell is monoclinic, space group $P2_1/a$, with $a=21.04$, $b=11.35$, $c=8.53$ Å and $\beta=101°36'$. The propyl disulfido group has been found to lie *cis* to the N-formyl group, the result being consistent with the hitherto proposed structure for TPD. The structure of the pyrimidine portion was discussed in comparison with the results of earlier workers. The stereochemical structure of TPD and a unique hydrogen bonding in the crystal were also discussed.

Thiamine propyl disulfide (TPD), a long acting thiamine derivative with high affinity to nervous tissues, has been assigned without definite evidence the structure (I) since it was first synthesized by Matsukawa and Kawasaki.[3] The preparation of TPD comprises reacting sodium salt of thiol thiamine (II) with sodium *n*-propyl thiosulfate (III) (Bunte' salt) in aqueous solution.[4] Masuda[5] was the first to state in his studies on the reaction of ketonic reagents with thiamine base that thiamine would at least take a thioketone form in part in aqueous solution. If such a tautomeric mechanism may operate in the course of the reaction and the both tautomers react with the Bunte' salt, two isomers of TPD should result by the reaction mechanism given below. Recently, Murakami and his collaborators[6] have found that the sodium salt of thiol thiamine reacted with *n*-butyl thiosulfate, at room temperature in the presence of sulfur, to give an isomeric disulfide, which was assigned the *trans* structure (IV: $R=CH_2CH_2CH_2CH_3$), mainly on the reason that the compound was not identical





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[1] Preliminary report, presented at the 85th Annual Meeting of Pharmaceutical Society of Japan, Tokushima, Oct. 1965.

[2] Location: *Juso-Nishinocho, Higashiyodogawa-ku, Osaka.*

[3] T. Matsukawa and H. Kawasaki, *Yakugaku Zasshi*, **73**, 216 (1953).

[4] T. Matsukawa, T. Iwatsu and H. Kawasaki, *Yakugaku Zasshi*, **73**, 497 (1953).

[5] K. Masuda, *Yakugaku Zasshi*, **81**, 533 (1961).

[6] M. Murakami, K. Takahashi, M. Iwanami and H. Iwamoto, *Yakugaku Zasshi*, **85**, 752 (1965).

with that obtained by Matsukawa's method. This implies, therefore, that a possibility of TPD having the *trans* structure has not been fully ruled out. The X-ray analysis now clearly demonstrated that the so far proposed *cis* structure was absolutely valid.

Experimental

Single crystals used in the present work were obtained from a solution of commercially available TPD in ethyl acetate. The NMR and IR spectra and chromatographic analysis of two specimens, before and after recrystallization, have shown that they were practically the same and no appreciable purification was done by the recrystallization.

Crystals of TPD were well developed colorless prisms. Oscillation and Weissenberg photographs were taken about the two principal crystal axes, using CuKα radiation ($\lambda = 1.5418$ Å). The specimens used had the following cross sections at right angle to the axis of rotation; 0.3×0.4 mm for the *c* axis and 0.3×0.3 mm for the *b* axis.

Intensities of reflection were visually estimated from multiple film integrating Weissenberg photographs (8 layers about the *c* axis and 13 layers about the *b* axis) and a total of 6600 diffraction maxima were evaluated visually, from which 3929 independent data were obtained. Absorption and extinction errors were neglected. The usual Lorentz-polarization corrections were made and the reflections were correlated to a single scale with the use of reflections common to the two films obtained about the different axes. The absolute scale was established by the use of statistical method as usual.

Results and Discussion

Unit Cell and Space Group

A preliminary study indicated that the crystal belongs to the monoclinic system, with the cell dimensions

$$a = 21.04 \pm 0.04\ \text{Å}$$
$$b = 11.35 \pm 0.03\ \text{Å}$$
$$c = 8.53 \pm 0.02\ \text{Å}$$
$$\beta = 101°36' \pm 25'$$

Systematic absences occur in the *0k0* reflections for *k* odd and in the *h0l* reflections for *h* odd. The space group is therefore $P2_1/a$, which requires four or a multiple of four molecules in the unit cell. The density, measured by floatation, was found to be 1.193 g/cm^3. There are four molecules of TPD ($C_{15}H_{24}O_2N_4S_2$, mol. wt.=356.51) in the unit cell, which gives a calculated density of 1.186 g/cm^3.

Determination of the Structure

The three-dimensional sharpened Patterson function was calculated for the first step of analysis. Harker section, H (u, 1/2, w), is shown in Fig. 1. The positions of the two sulfur atoms could not be determined definitely from this map. But assuming 1.9—2.2 Å for the length of the disulfide bond, careful inspection of the Patterson map eventually led us to afford the coordinates of the two sulfur atoms.

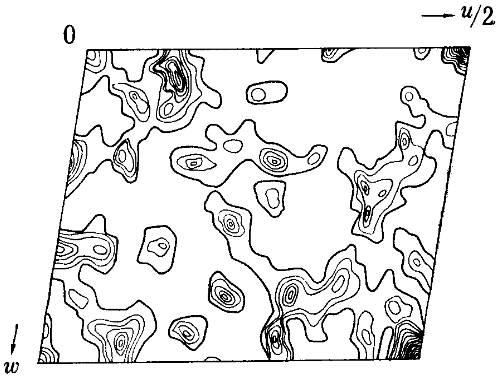
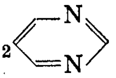


Fig. 1. Harker Section at $v = \frac{1}{2}$

Each of the heavy atom location was then used to compute a separate four term minimum function superposition. The skeleton of TPD, *i.e.*, the pyrimidine ring, the C=C bond and the two atoms (C and N) connecting pyrimidine and the C=C bond, were visualized on the minimum function maps. The *cis* arrangement of $-NCH_2$  and S–S groups on the ethylenic bond was clearly seen. Thus the primary aim of the present work was accomplished at this stage of the analysis.

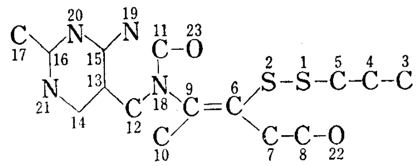
The coordinates and temperature factors of the 13 atoms obtained from the minumum function maps were subjected to two cycles of least squares with diagonal approximation. *R*–value, starting with 0.51, decreased to 0.41 in accordance. The first electron density synthesis was calculated with phase angles based on these 13 atoms. As a result, the whole structure was seen except for the two carbon atoms at the end of the *n*-propyl group.

Three cycles of least squares treatment were carried out, at this stage, so as to refine the atomic coordinates and the temperature factors of the 21 atoms thus obtained. *R*–value fell from 0.39 to 0.26. The second electron density synthesis calculated with phase angles based on these 21 atoms, however, failed to visualize the positions of the remaining two carbon atoms definitely. A difference Fourier synthesis was, therefore, computed. Careful examination of these two maps revealed a probable structure of the *n*-propyl group, which seemed to be fairly mobile in unoccupied space of the unit cell.

The final refinement was carried out by least squares treatment with diagonal matrix. After seven cycles of the treatment, *R*–value was converged to 0.15. The atomic coordinates and isotropic temperature factors obtained were listed in Table I, together with corresponding standard deviations in parentheses. Bond distances and angles calculated from these atomic parameters were shown in Fig. 2. Since the aim of this work has been achieved already, no further refinement was made.

Table I. Atomic Coordinates and Temperature Factors with Their Standard Deviations in Parentheses

Atom	*x*/*a*	*y*/*b*	*z*/*c*	B
S(1)	0.6333 (02)	0.0749 (04)	0.1115 (06)	7.96 (13)
S(2)	0.6845 (02)	0.0006 (04)	0.9584 (05)	6.15 (10)
C(3)	0.4994 (20)	0.1942 (41)	0.0786 (48)	23.65 (1.64)
C(4)	0.5476 (13)	0.2920 (28)	0.0941 (35)	15.87 (97)
C(5)	0.5867 (11)	0.1952 (22)	0.9832 (28)	12.08 (71)
C(6)	0.7535 (05)	0.0924 (11)	0.9566 (14)	3.95 (29)
C(7)	0.7844 (06)	0.1560 (13)	0.1133 (17)	5.74 (36)
C(8)	0.8200 (07)	0.0725 (15)	0.2395 (18)	6.69 (40)
C(9)	0.7751 (05)	0.0981 (11)	0.8201 (13)	3.51 (27)
C(10)	0.8364 (07)	0.1727 (14)	0.8036 (17)	5.86 (37)
C(11)	0.2531 (06)	0.0830 (12)	0.3213 (15)	4.57 (31)
C(12)	0.7153 (05)	0.1011 (11)	0.5323 (13)	3.39 (27)
C(13)	0.6525 (05)	0.1585 (10)	0.5513 (12)	2.63 (24)
C(14)	0.1483 (05)	0.2268 (11)	0.5822 (14)	3.73 (28)
C(15)	0.5925 (05)	0.0916 (11)	0.5292 (13)	3.38 (27)
C(16)	0.0386 (05)	0.2374 (11)	0.5662 (14)	3.68 (28)
C(17)	0.9746 (07)	0.1719 (14)	0.5719 (18)	6.12 (38)
N(18)	0.7444 (04)	0.0367 (08)	0.6796 (10)	3.27 (21)
N(19)	0.4104 (04)	0.0212 (09)	0.4965 (11)	4.09 (23)
N(20)	0.5377 (04)	0.1520 (08)	0.5384 (11)	3.47 (22)
N(21)	0.0919 (05)	0.1653 (10)	0.5931 (12)	4.83 (26)
O(22)	0.8749 (05)	0.0270 (10)	0.1857 (12)	7.75 (29)
O(23)	0.2812 (04)	0.1402 (08)	0.4299 (10)	5.37 (22)



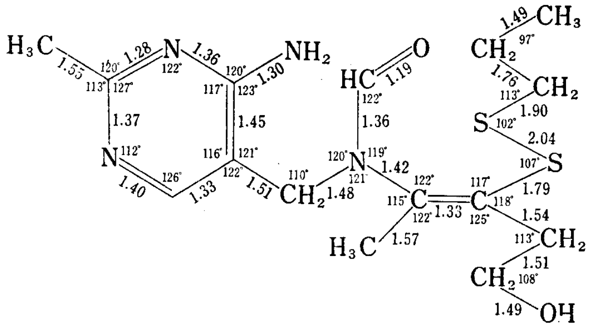


Fig. 2. Bond Distances and Angles

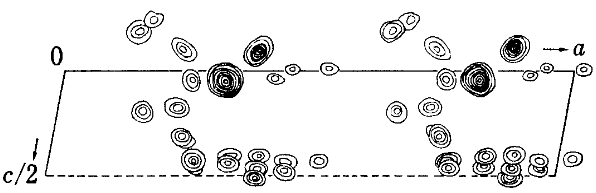


Fig. 3. The Final Three-Dimensional Electron Density Distribution projected on (010)

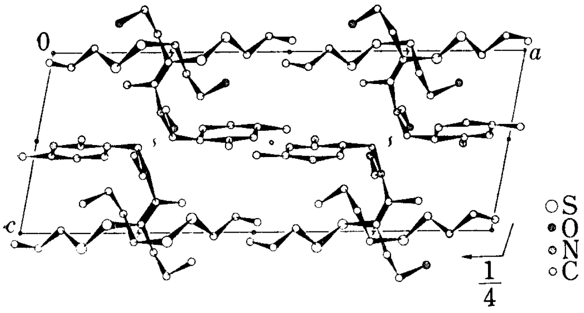


Fig. 4. Packing of the Structure seen down the *b*-Axis

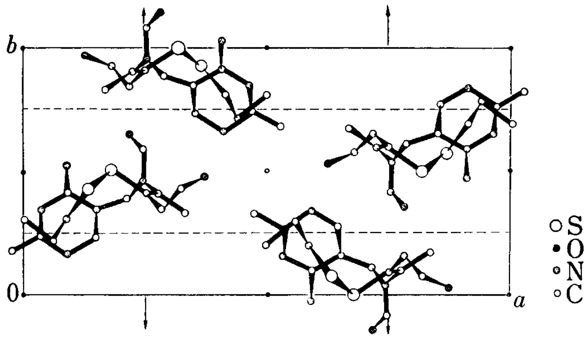


Fig. 5. Packing of the Structure seen down the *c*-Axis

The third and final electron density distribution is shown in Fig. 3 in superimposed contour sections projected on (010). The stereochemical structure and the packing of the molecules viewed along the *b* and *c* axis were shown in Fig. 4 and 5, respectively. Observed and calculated structure factors are listed in Table II.

Discussion

From Fig. 4 and 5, it is clearly seen that the *n*-propyl disulfido group lies *cis* to the N-formyl group. Isomerization in the course of Bunte's reaction, therefore, has not taken place and the original assignment of the structure (I) for TPD has been proved correct.

Hitherto, the crystal structures of thiamine and its derivatives have been investigated by three different groups. Kraut and Reed[7] analyzed thiamine dihydrochloride mainly by the superposition method. The structure of cocarboxylase, the pyrophosphoric acid ester of thiamine, was studied by Karle and Bitts[8] and by Pletcher and Sax,[9] independently. Though the degree of the refinement is not identical in each work, comparison of their results with that of ours may provide useful tools for the understanding of the thiamine structure and its mode of action in enzymic reactions.

In Table III and IV, the bond lengths and angles of the pyrimidine moiety were comparatively listed. There was observed no significant difference between the values reported by the present authors and those of the three earlier workers.

Elongation of C(13)–C(15) bond observed by these authors may be attributed to steric interaction of the *ortho* substituents (NH_2 and $-CH_2-$). This would receive further support by relatively large valence angles of C(12)–C(13)–C(15) and C(13)–C(15)–N(19). The shortest

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[7] J. Kraut and H.J. Reed, *Acta Cryst.*, **15**, 747 (1962).

[8] I.L. Karle and K. Britts, *Acta Cryst.*, **20**, 118 (1966).

[9] J. Pletcher and M. Sax, *Science*, **154**, 1331 (1966).

TABLE II. Observed and Calculated Structure Factors (a)

H	K	L	FO	FC	H	K	L	FO	FC	H	K	L	FO	FC	H	K	L	FO	FC	H	K	L	FO	FC
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table II. Observed and Calculated Structure Factors (b)

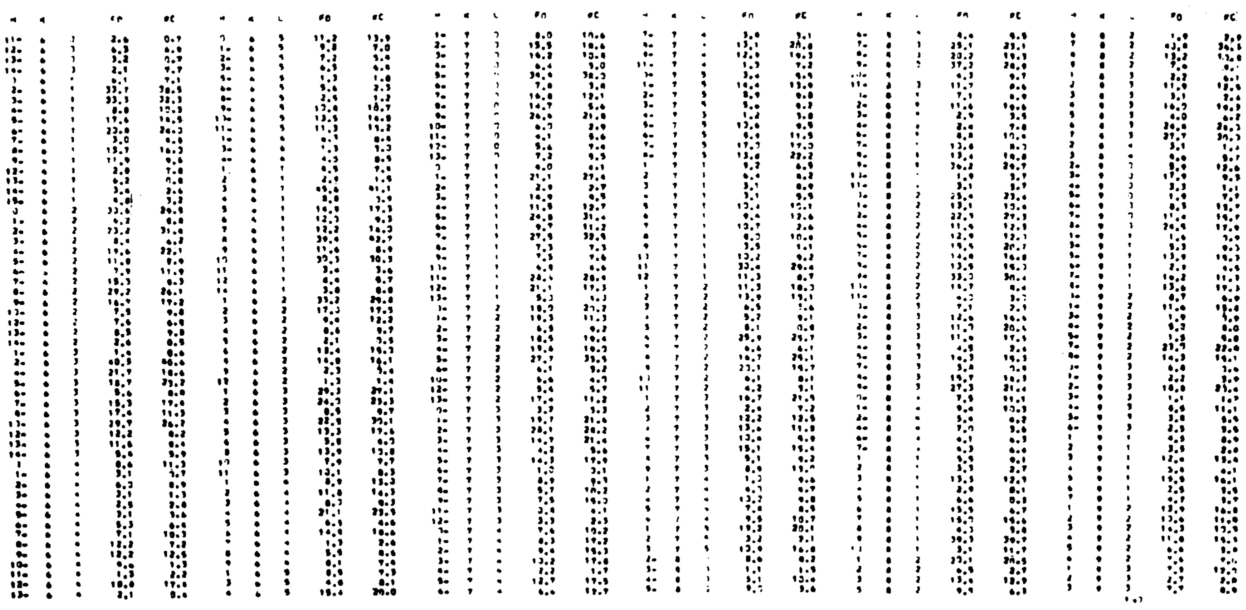


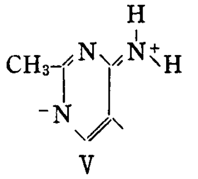
Table III. Comparison of Bond Lengths in the Pyrimidine Portion

	Present work Å	Kraut & Reed[7] Å	Karle & Britts[8] Å	Pletcher & Sax[9] Å
C(12)–C(13)	1.51	1.50	1.51	1.49
C(16)–C(17)	1.55	1.49	1.51	1.49
C(15)–N(19)	1.30	1.32	1.31	1.31
C(13)–C(15)	1.45	1.43	1.44	1.46
C(15)–N(20)	1.36	1.37	1.35	1.37
C(16)–N(20)	1.28	1.31	1.29	1.27
C(16)–N(21)	1.37	1.33	1.38	1.42
C(14)–N(21)	1.40	1.36	1.36	1.36
C(13)–C(14)	1.33	1.35	1.34	1.35
Average value of standard deviation	0.016	0.007	0.015	0.015

Table IV. Comparison of Bond Angles in the Pyrimidine Portion

	Present work	Kraut & Reed[7]	Karle & Britts[8]	Pletcher & Sax[9]
C(12)–C(13)–C(14)	122°	123°	121°	123°
C(12)–C(13)–C(15)	121	120	120	122
C(14)–C(13)–C(15)	116	117	119	116
C(13)–C(15)–N(19)	123	123	123	121
N(19)–C(15)–N(20)	120	117	118	119
C(13)–C(15)–N(20)	117	121	118	118
C(15)–N(20)–C(16)	122	119	121	123
C(17)–C(16)–N(20)	120	120	122	124
C(17)–C(16)–N(21)	113	117	115	115
N(20)–C(16)–N(21)	127	123	123	121
C(16)–N(21)–C(14)	112	121	119	116
C(13)–C(14)–N(21)	126	120	120	125
Average value of standard deviation	1.0	0.4	0.9	

bond length was found at C(16)–N(20) and the second was at C(15)–N(19) in all four papers. C(13)–C(14) was found to be the third shortest bond in three papers. These observations would suggest a large contribution of the canonical structure (V) for the ground state of thiamine derivatives.⁷⁾


V

As for the packing of molecules in the crystals, the dimeric hydrogen bond formed around the center of symmetry in the unit cell (shown in Fig. 6) would be one of the major causes of intermolecular forces in the crystal. Such dimer formation has not been observed in earlier work, where the NH_2 group was considered to lie close to the thiazol ring of the same molecule. An intermolecular hydrogen bond is also formed between hydroxyethyl group and N(21) in the pyrimidine ring, the distance of the proton donor O(22) and the acceptor N(21) being 2.88 Å. This bond connects the two molecules related by the screw axis of the crystal. The above mentioned structural similarity between the pyrimidine ring of TPD, which is a free base, and that reported in the earlier three papers, in which a positive charge is located on the ring, may be attributed to this hydrogen bond which would bestow a partial positive charge on N(21) of the TPD molecule.

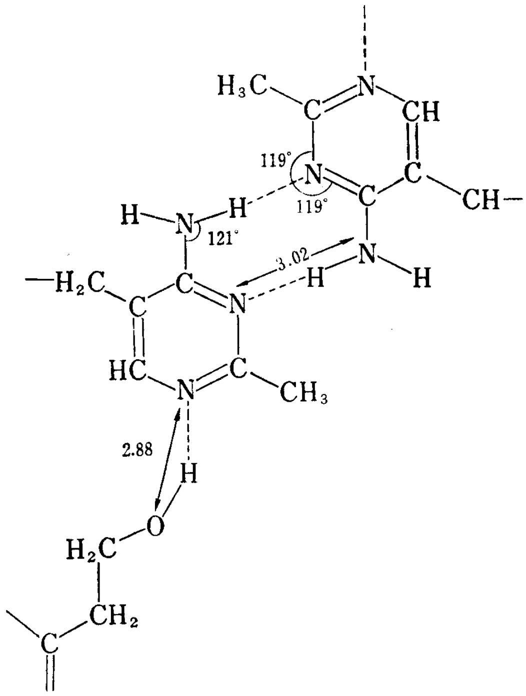
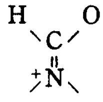


Fig. 6. Intermolecular Hydrogen Bonds in Thiamine Propyl Disulfide

The X-ray analysis of TPD crystal has also revealed that the plane constituted by N-CHO group is nearly perpendicular to the plane of the ethylene group. This would mean that the lone pair electrons on N(18) is consumed for the conjugation with the formyl group (the canonical structure (VI)) and the interaction with the ethylenic π-orbital is practically forbidden. The contribution of the canonical structure (VI) would be supported by the observation that N(18) takes a planar trivalent structure, the sum of the valence angle around N(18) being 360° (Fig. 2).


VI

The pyrimidine ring is also nearly perpendicular to the above mentioned two planar groups. It would be interesting to note that the three π-electron clouds located in close proximity in a single molecule are nearly perpendicular to each other.

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