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# 17. Yoshikazu Oka, Koichi Yoshioka and Hiroshi Hirano: Studies on Vitamin $B_1$ and Related Compounds. CVI.*1 A Novel Synthesis of Hydroxyethylthiamine.

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A dihydrothiamine derivative (Ⅵ) was prepared by the reaction of Ⅳ, Ⅴ and methylglyoxal. On treatment with hydrochloric acid Ⅵ underwent hydrolysis in the presence of water, but in non-aqueous solutions thiamine was afforded under room temperature. Treatment of Ⅵ, however, with weak acids such as phosphoric acid, formic acid or acetic acid effected the conversion to hydroxyethylthiamine in fairly good yields.

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Hydroxyethylthiamine (HET) (I) has received considerable attention in recent years because the compound, according to Breslow's theory on the mechanism of the action of thiamine, may play a vital role in the biochemical decarboxylation of pyruvate.[1]

To date only two different syntheses of HET have been reported; the one is the condensation of 4-amino-5-bromomethyl-2-methylpyrimidine and 2-(1-hydroxyethyl)-5-(2-hydroxyethyl)-3-methylthiazole,[2] and the other is the direct addition of acetaldehyde on to thiamine.[3]

From an inspection of the structure it might at first sight appear that HET is equilibrated with its enol-type and keto-type isomers (Ⅱ and Ⅲ). Contray to this assumption, neutralization of the hydrochloride of HET in aqueous media resulted in the ring opening of the thiazole moiety to yield the corresponding thiol as has been known with thiamine hydrochloride.

Our presumption, however, was that the compound (Ⅲ), if it would be adequately synthesized, might undergo molecular rearrangement to yield HET under certain acidic conditions, and this was actually shown to be the case. The present paper describes a novel synthesis of HET as well as thiamine based on the aforementioned speculations.

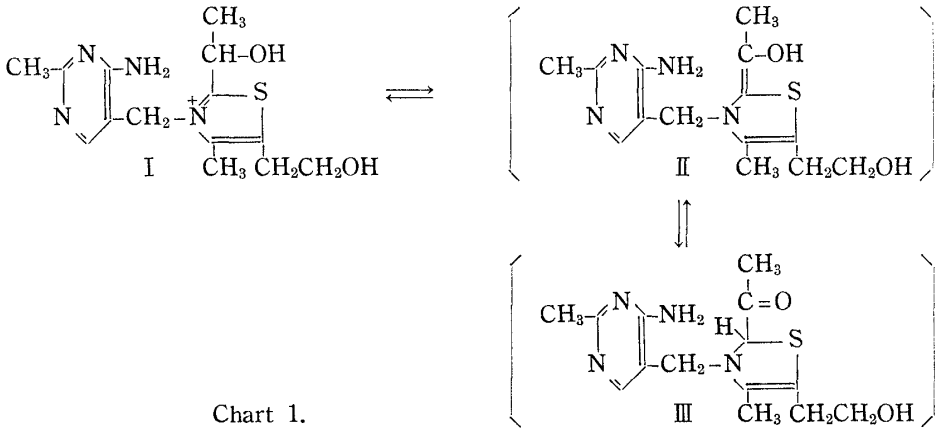


Chart 1.

*1 Part CIV: Ann. Rep. Takeda Res. Lab., **22**, 1 (1963); Part CV: Vitamins, **32**, 570 (1965).

*2 Juso, Higashiyodogawa-ku, Osaka (岡 良和, 吉岡晃一, 平野 弘).

1) R. Breslow: J. Am. Chem. Soc., **80**, 3719 (1958).

2) L. O. Krampitz, G. Greull, C. S. Miller, J. B. Bicking, H. R. Skeggs, J. M. Sprague: *Ibid.*, **80**, 5893 (1958).

3) C. S. Miller, J. M. Sprague, L. O. Krampitz: Ann. N. Y. Acad. Sci., **98**, 401 (1962).

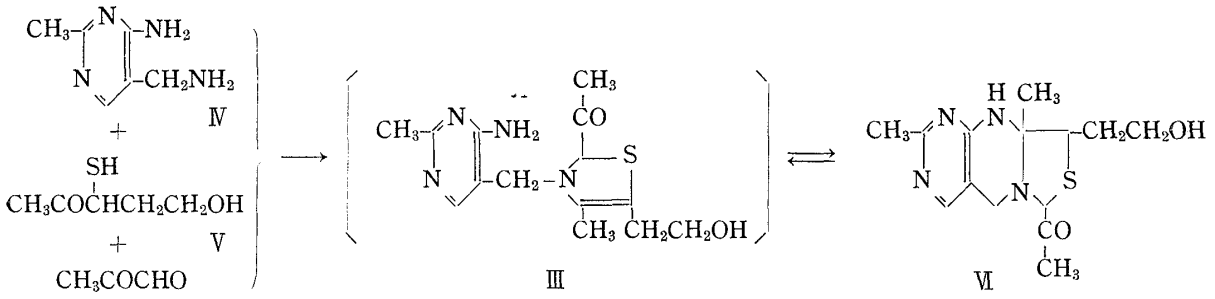


Chart 2.

When an equimolar mixture of 4-amino-5-aminomethyl-2-methylpyrimidine (Ⅳ) and 3-acetyl-3-mercapto-1-propanol (Ⅴ) in aqueous solution was treated with methylglyoxal and the reaction mixture was processed as described by Iwatsu in the notable dihydrothiamine synthesis,[4] a single compound melting at 198~200° (decomp.) was obtained as colorless needles in 30% yield. The elementary analysis indicated that the compound showed the correct analysis for the desired compound (Ⅱ or Ⅲ). However, in the NMR spectrum (Fig. 1) the methyl signal due to the one on the thiazole moiety appeared at 8.53 $\tau$, which was too much up-field shifted from that for the expected compound; moreover a signal ascribable to NH rather than $NH_2$ was observed at 2.70 $\tau$. In the IR spectrum an absorption band due to a carbonyl group appeared at 1700 $cm^{-1}$, but no band due to deformation of $NH_2$ in the pyrimidine ring which has been shown in many thiamine derivatives to appear in the region of 1637~1661 $cm^{-1}$ [5] was observed. These results strongly suggested that the compound should not have the structure Ⅱ or Ⅲ, where the methyl resides on the thiazoline ring, but it should rather have the structure Ⅵ, in which the double bond in the original thiazoline has been saturated by forming a tricyclic structure analogous to the structure of pseudo-dihydrothiamine.[6] Confirmation of the structure assigned to Ⅵ was further obtained from the comparison of the NMR data of Ⅵ with those of the related compounds whose structures have been established beyond doubt (Table I). It is apparent in the table that a methyl on a thiazolidine ring appears at 8.4~8.6 $\tau$, while the one on a thiazole or a 4-thiazoline ring at 7.4~7.8 $\tau$.

Our interest was then directed to see if the compound Ⅵ could actually be converted to HET, and a number of attempts have been made with this aim. Consequently, it was discovered that Ⅵ underwent three different reactions primarily depending on the reaction conditions employed. First, the treatment of Ⅳ with hydrochloric acid in aqueous solution gave rise to 4-amino-5-aminomethyl-2-methylpyrimidine (Ⅳ) and other unidentified fragments. Secondly, somewhat surprising was the observation that, when Ⅵ was treated with hydrochloric acid in ethanol or acetone, thiamine was obtained as a main product. Since HET has been recovered unchanged under these conditions, it is not pertinent to assume that the reaction had proceeded by way of HET. The mechanism of this unusual rearrangement, therefore, cannot be fully understood, and the future investigation should throw light on this problem. Thirdly, it was found that, when Ⅵ was warmed in anhydrous solvents in the presence of weak acids such as formic acid, acetic acid or phosphoric acid, Ⅵ had rearranged to HET in fairly good yields. For example, the treatment of Ⅵ with 20% ethanolic phosphoric acid at 60~80° for one hour afforded HET in 48% yield. The mechanism of the reaction in this case would probably be interpreted as a sequence of transformations shown in Chart 3; the

4) T. Iwatsu : Yakugaku Zasshi, **75**, 677 (1955).
5) S. Yoshida, M. Kataoka : This Bulletin, **6**, 577 (1958).
6) H. Hirano, T. Iwatsu, S. Yurugi : Yakugaku Zasshi, **77**, 244 (1957).

isomerization to Ⅲ, the protonation to the carbonyl oxygen, the enolization to Ⅱ and finally the displacement of the unshared electron on the nitrogen to give HET.

HET thus obtained was assigned by elemental analysis and completely identical with an authentic specimen prepared by Miller's method[3] in NMR and IR spectra.

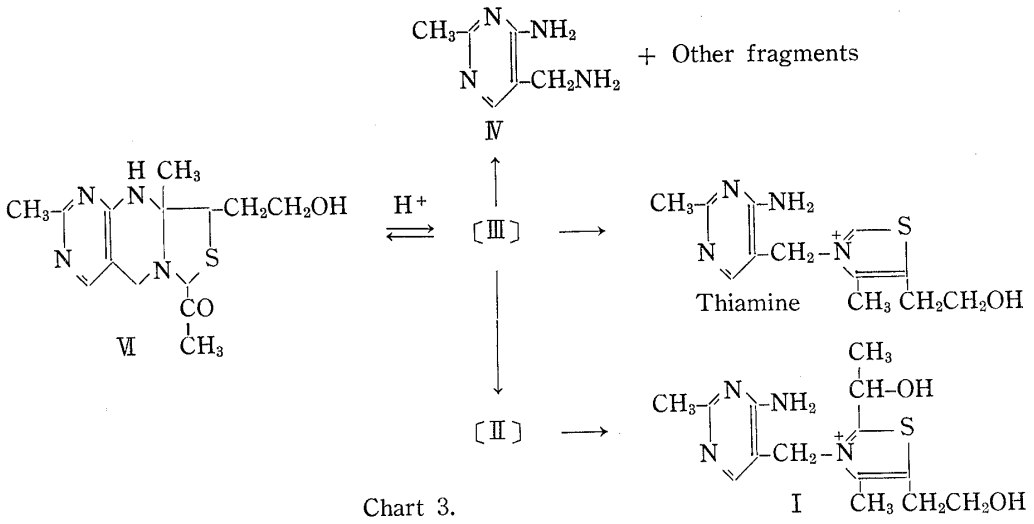
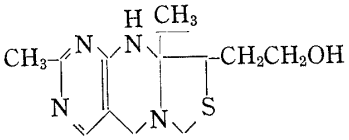
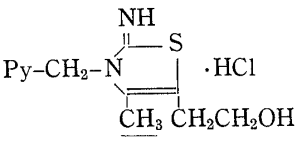
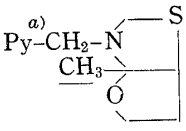
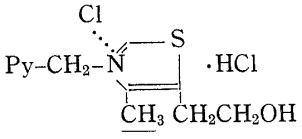
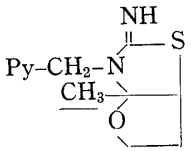
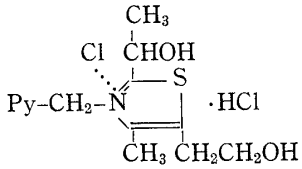
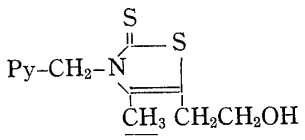
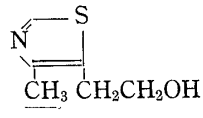


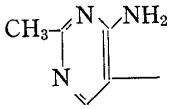

Chart 3.

## Experimental

**7-Acetyl-2,9a-dimethyl-9-(2-hydroxyethyl)-5,9,9a,10-tetrahydro-7*H*-pyrimido[4,5-*d*]thiazolo[3,4-*a*]-pyrimidine (VI)**——To an aqueous solution of 3-acetyl-3-chloro-1-propanol prepared by heating 14 g. of its dimer[7] with 50 ml. of $H_2O$ at 100° for 15 minutes, 40 ml. of 10% NaOH saturated with hydrogen sulfide and a solution of 21 g. of Ⅵ·2HCl in 30 ml. of $H_2O$ neutralized with 80 ml. of 10% NaOH were added. To

Table I. Chemical Shifts of the Methyl Groups at the 4-Positions of the Thiazole, Thiazoline or Thiazolidine Rings in Thiamine and Related Compounds

| Structure | Solvent | $CH_3$ ($\tau$) | Structure | Solvent | $CH_3$ ($\tau$) |
|---|---|---|---|---|---|
|  | $D_2O$ | 8.55 |  | $D_2O$ | 7.75 |
|  | $CDCl_3$ | 8.42 |  | 〃 | 7.43 |
|  | 〃 | 8.45 |  | 〃 | 7.57 |
|  | $CF_3COOH$ | 7.64 |  | $CDCl_3$ | 7.63 |

*a*) Py : 

7) T. Matsukawa, T. Iwatsu : Yakugaku Zasshi, **71**, 720 (1951).

this mixture was added with stirring a 30% aqueous solution of methylglyoxal (25 g.). An oily substance appeared in a few minutes and solidified gradually, which was filtered, washed with $H_2O$ and recrystallized from EtOH to give 9.2 g. (30%) of colorless needles, m.p. 198~200° (decomp.). UV $\lambda_{max}^{EtOH}$ mμ (ε): 245 (8660), 289 (6320). *Anal.* Calcd. for $C_{14}H_{20}O_2N_4S$: C, 54.52; H, 6.54; N, 18.17. Found: C, 54.74; H, 6.60; N, 17.94.

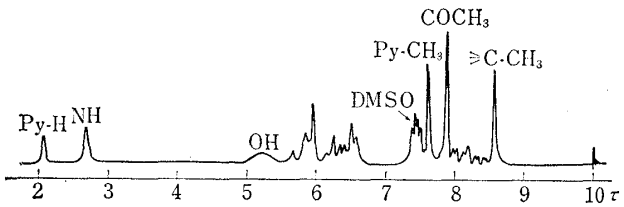



Fig. 1. Nuclear Magnetic Resonance Spectrum of Ⅵ in DMSO-$d_6$ at 60 Mc.

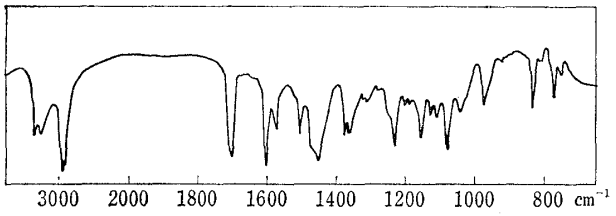



Fig. 2. Infrared Absorption Spectrum of Ⅵ in Nujol

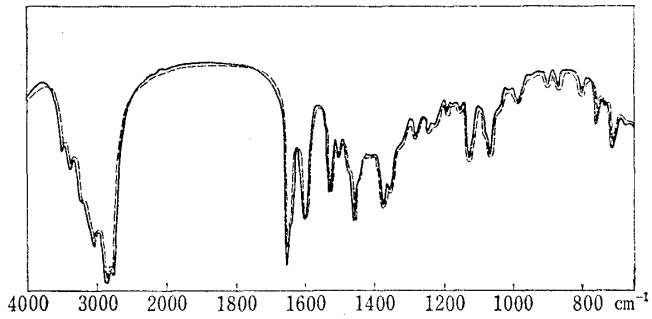



Fig. 3. Infrared Absorption Spectra of HET Hydrochloride in Nujol

———: Prepared by the present method.
- - - - - -: Prepared by the reaction of thiamine with acetaldehyde.[8]

**Acid Hydrolysis of (VI)**——To a suspension of Ⅵ (0.6 g.) in 10 ml. of $H_2O$, 1.8 ml. of 10% HCl was added and the mixture was set aside at room temperature for 4 days. The solution was extracted with 5 ml. of AcOEt and the aqueous layer was evaporated to dryness under reduced pressure. The residue was washed with EtOH and recrystallized from EtOH–$H_2O$ (5:1) to give 0.2 g. of Ⅳ·2HCl as needles, m.p. 260~263° (decomp.). *Anal.* Calcd. for $C_6H_{10}N_4 \cdot 2HCl$: C, 34.14; H, 5.73; N, 26.54. Found: C, 34.15; H, 5.85; N, 26.87.

The AcOEt extract was dried over sodium sulfate and evaporated to dryness to give 0.2 g. brown oil which showed the positive sodium nitroprusside test indicative of the presence of a mercapto compound, but no further investigation of the product was achieved.

**The Formation of Thiamine from (VI)**——To a suspension of Ⅵ (1.0 g.) in 10 ml. of EtOH was added 1.5 ml. of 20% EtOH–HCl and 40 ml. of $Me_2CO$. The mixture was allowed to stand at room temperature for 4 days and the resulting precipitate was recrystallized from EtOH–$H_2O$ (10:1) to afford 0.6 g. (55%) of thiamine hydrochloride, m.p. 245° (decomp.), which was positive for thiochrome test and showed no depression of melting point on admixture with an authentic thiamine hydrochloride. *Anal.* Calcd. for $C_{12}H_{18}ON_4SCl_2$: C, 42.73; H, 5.38; N, 16.61. Found: C, 42.63; H, 5.25; N, 16.81.

**3-(4-Amino-2-methyl-5-pyrimidinylmethyl)-2-(1-hydroxyethyl)-5-(2-hydroxyethyl)-4-methylthiazolium Chloride Hydrochloride (Hydroxyethylthiamine: HET) (I)**——i) One gram of Ⅵ was heated at 80° with 3 ml. of 20% ethanolic phosphoric acid for 1.5 hr. To the mixture was added 5 ml. of $H_2O$ and 2.23 ml. of 10% HCl, and the solution was evaporated to dryness *in vacuo*. The residue dissolved in 3 ml. of EtOH was allowed to stand overnight. The resulting crystals were filtered and recrystallized from MeOH–$Me_2CO$ to give 0.6 g. (48%) of colorless needles, m.p. 217~219° (decomp.), which showed no depression of melting point on admixture with an authentic sample prepared from thiamine and acetaldehyde.[3] *Anal.* Calcd. for $C_{14}H_{22}O_2N_4SCl_2$: C, 44.09; H, 5.82; N, 14.69. Found: C, 44.13; H, 5.90; N, 14.50.

ii) A solution of Ⅵ (2.0 g) in AcOH (20 g.) was heated at 80° for 4 hr. The reaction mixture was evaporated to dryness under reduced pressure and the residue was dissolved in 2.5 ml. of 20% EtOH–HCl. On addition of $Me_2CO$ (30 ml.) a white crystalline mass was precipitated. The precipitate was filtered to give 2.31 g. of a mixture of thiamine hydrochloride and HET hydrochloride. The mixture was dissolved in 3 ml. of $H_2O$, and to the solution was added 1.6 g. of potassium thiocyanate dissolved in 3 ml. of $H_2O$ and 0.6 g. of $NaHCO_3$. Resulting precipitate was filtered to give 0.23 g. (10%) of thiamine mono-thiocyanate. The filtrate was adjusted to pH 2~3 with HCl and evaporated to dryness *in vacuo*. To the residue was added 5 ml. of EtOH and insoluble materials were filtered off. The filtrate was evaporated again to dryness and the residue was dissolved in $Me_2CO$. The solution was acidified with 20% EtOH–HCl to precipitate crude HET·Cl·HCl (1.96 g.), which was filtered and recrystallized twice from MeOH–$Me_2CO$ to afford 1.17 g. (47%) of HET·Cl·HCl as needles, m.p. 217~219° (decomp.). This showed no depression of melting point on admixture with authentic HET hydrochloride.

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