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Studies on Pyrimidine Derivatives and Related Compounds. LX.[1] Synthesis of Hydroxymethylthiamine[2]

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Hydroxymethylthiamine, phosphate free "active formaldehyde," was synthesized by the acid treatment of 2-phenyloxalylthiamine-O,O′-diacetate or 2-α-furyloxalylthiamine-O,O′-diacetate, and on alkaline treatment it was converted into a dimeric 1,4-oxazine derivative (X) involving a ring expansion and auto-oxidation.

The biochemical condensation reaction of glyoxylic acid to tartronic semialdehyde involving decarboxylation has been found to be catalyzed by an enzyme called glyoxylate carboligase of which coenzyme is thiamine diphosphate (TDP).[4] The intermediacy of hydroxymethylthiamine diphosphate (HMT–DP) or "active formaldehyde" in this metabolic reaction has been proved previously by Holzer and coworkers[5] on the basis of isotope experiments. They attempted to synthesize "active formaldehyde" enzymatically according to the biochemical model illustrated in Chart 1, however, the desired substance was not isolated.

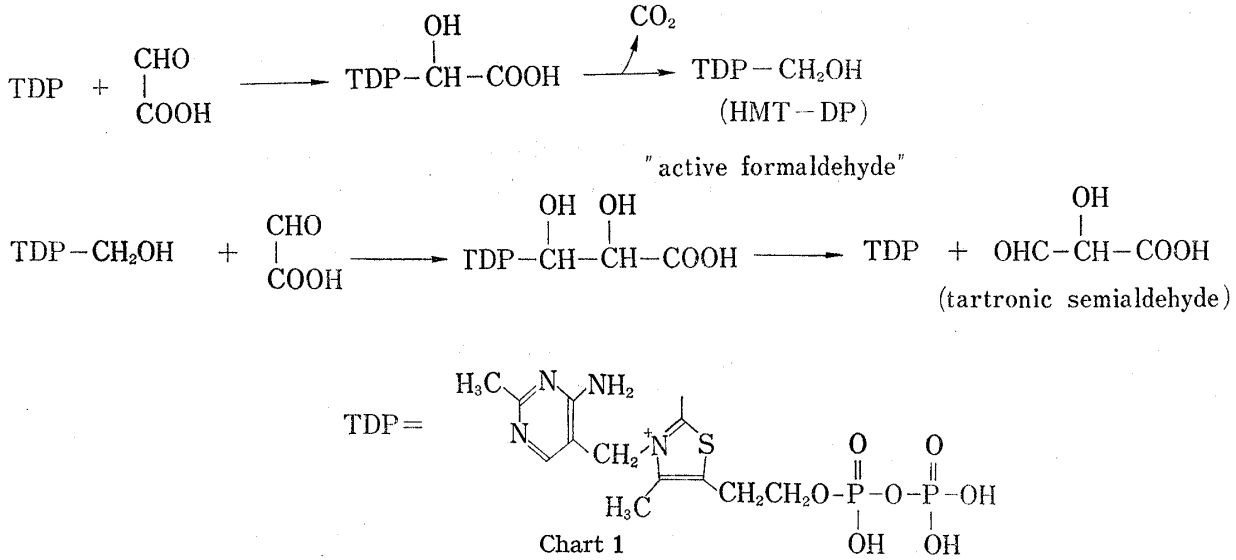


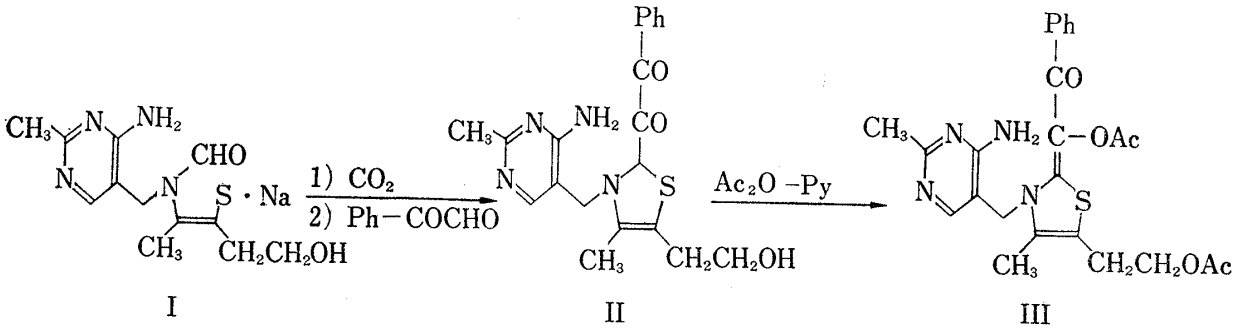
Chart 1

On the other hand, Krampitz and coworkers[6] have recently tried a direct reaction of TDP with formaldehyde in their attempted synthesis of "active glycolaldehyde," a key intermediate in the transketolase reaction,[7] but both the desired product and HMT–DP which might be the expected primary product of the reaction could not be isolated.

1) Part LIX: A. Takamizawa, Y. Hamashima, S. Hayashi, and S. Sakai, *Chem. Pharm. Bull.* (Tokyo), **16**, 2195 (1968).
2) A part of this work was reported preliminarily in *Tetrahedron Letters*, **1968**, 2189.
3) Location: *Fukushima-ku, Osaka.*
4) G. Krakow and S.S. Barkulis, *Biochim. Biophys. Acta*, **21**, 593 (1956); G. Krakow, S.S. Barkulis, and J.A. Hayashi, *J. Bacteriol.*, **81**, 509 (1961).
5) G. Kohlhaw, B. Deus, and H. Holzer, *J. Biol. Chem.*, **240**, 2135 (1965).
6) L.O. Krampitz, I. Suzuki, and G. Greull, *Ann. N.Y. Acad. Sci.*, **98**, 466 (1962).
7) R. Breslow, *J. Am. Chem. Soc.*, **80**, 3719 (1958).

The present paper describes the chemical synthesis of hydroxymethylthiamine.

In the preceding papers,[8] the authors have reported that the reaction of thiamine–sodium salt (I) with phenylglyoxal in the presence of carbon dioxide afforded 2-phenyloxalylthiamine (II) which was found to be very vulnerable on the aerial oxidation, whereas on acetylation it was convertible into a stable diacetate (III). The diacetate (III) exhibited ultraviolet

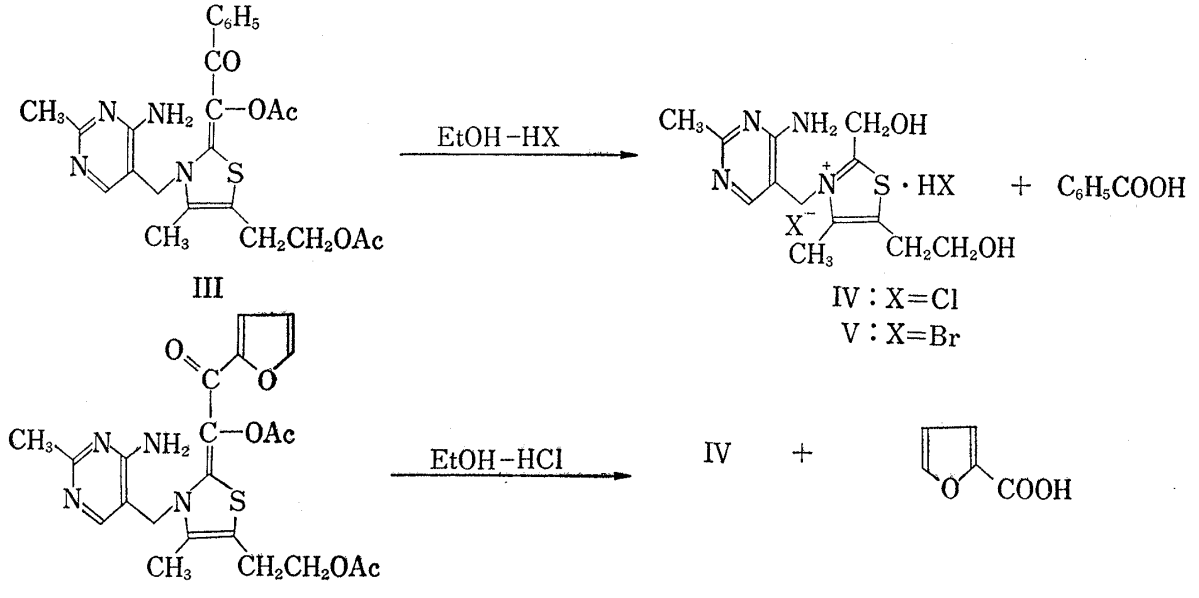


I II III

Chart 2

(UV) absorption maxima at 234 mμ (log ε=4.32), 272 mμ (log ε=3.90) and 391 mμ (log ε=4.40), but in ethanolic hydrogen chloride a new maximum was gradually appeared at 265 mμ, while the maximum at 391 mμ was disappeared. On standing in ethanolic hydrogen chloride for two days at room temperature, it was converted into a new compound (IV), $C_{13}H_{20}O_2N_4SCl_2 \cdot H_2O$, mp 225—226° (decomp.), in 48% yield accompanied with benzoic acid. UV spectrum of the new compound showed maxima at 239 mμ (log ε=4.12) and 265 mμ log ε=4.20), which was quite analogous to that of hydroxyethylthiamine (HET).[9] The nuclear magnetic resonance (NMR) spectrum (Fig. 1) of IV showed a singlet attributable to the protons of $^{+}N\!\!>\!\!S$–CH_2-O- grouping at 4.80 τ.

These spectral properties indicated that IV should be assigned as hydroxymethylthiamine hydrochloride. By the action of ethanolic hydrogen bromide, III was also converted into



III VI

Chart 3

8) A. Takamizawa, S. Matsumoto, and S. Sakai, *Tetrahedron Letters*, **1968**, 2189; *idem*, *Chem. Pharm. Bull.* (Tokyo), **17**, 128 (1969).

9) C.S. Miller, J.M. Sprague, and L.O. Krampitz, *Ann. N.Y. Acad. Sci.*, **98**, 401 (1962); L.O. Krampitz, G. Gruell, C.S. Miller, J.B. Bicking, H.R. Skeggs, and J.M. Sprague, *J. Am. Chem. Soc.*, **80**, 5893 (1958).

V, $C_{13}H_{20}O_2N_4SBr_2$, mp 231—231.5° (decomp.), of which spectral data were perfectly similar to those of IV indicating that it was assignable to hydroxymethylthiamine hydrobromide.[10] Treatment of 2α-furyloxalylthiamine O,O'-diacetate (VI)[8] with ethanolic hydrogen chloride gave similarly IV in 45% yield accompanied with α-furoic acid. On the treatment with benzoyl chloride in aqueous alkaline solution, both IV and V afforded a same tribenzoate VII, $C_{34}H_{32}O_6N_4S$, mp 160—162.5°. Its infrared (IR) spectrum showed carbonyl absorptions at 1725 cm^{-1} and 1669 cm^{-1}, and NMR spectrum showed a singlet at 5.33τ apparently attributable to the protons of -N-CO-CH_2-OCO- grouping, and other signal patterns were quite compatible with the proposed structure. The fact that the hydroxymethyl substituent is located at the 2-position of thiazolium moiety of thiamine was thus confirmed. IV was slowly

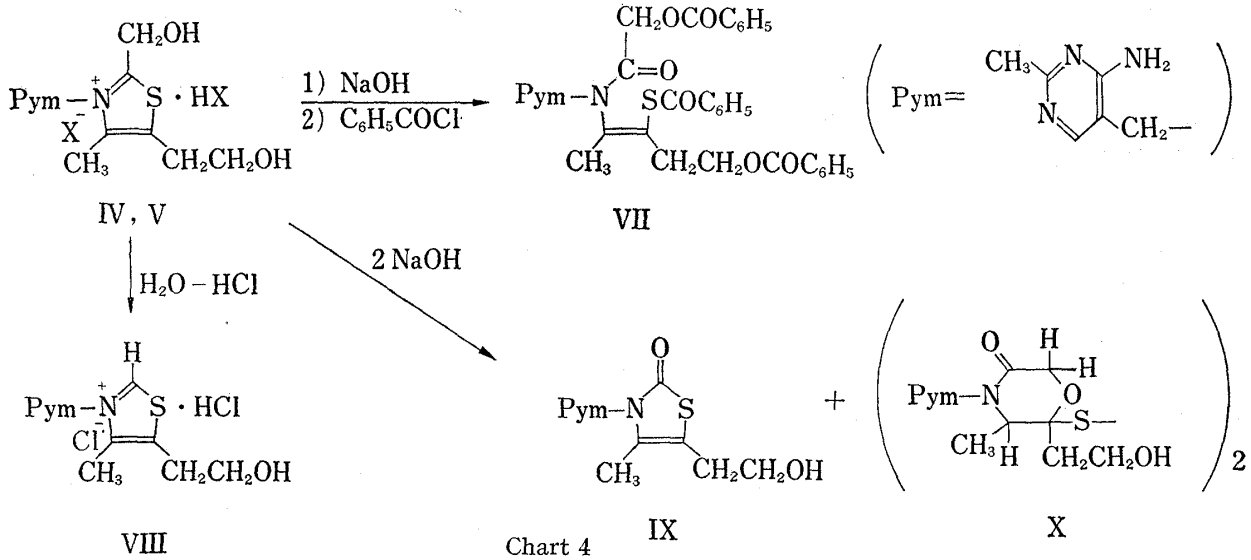


Chart 4

decomposed to thiamine hydrochloride (VIII) by the action of dilute hydrochloric acid, whereas on stirring for two days at room temperature in the presence of two equimolar amounts of a aqueous sodium hydroxide it was converted into thiamine thiazolone (IX) in 25% yield accompanied with a new compound (X), $(C_{13}H_{19}O_3N_4S)_2 \cdot H_2O$, mp 232° (decomp.) (12%). The molecular weight determination of X indicated the dimeric nature and its IR spectrum showed an absorption band at 1660 cm^{-1} indicating the presence of amide function.[11] Its NMR spectrum (Fig. 1) exhibited a doublet at 8.72 τ (J=6 cps) attributable to a secondary methyl group, and a pair of doublets at 5.29τ and 6.04τ due to the protons of N-CO-$\underset{\underset{H}{|}}{C}$-O- grouping with a

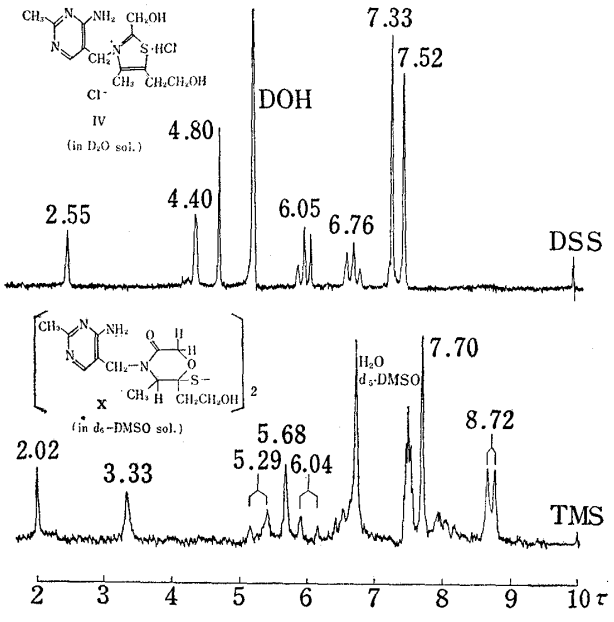


Fig. 1. NMR Spectra of Hydroxymethylthiamine Hydrochloride (IV) and X

10) Recently, hydroxymethylthiamine hydrobromide has been synthesized by a different route. J. Kiss, R. D'Souza, and H. Spiegelberg, *Helv. Chim. Acta*, **51**, 325 (1968).

11) The absorption band due to N–H deformation of the primary amine located on the pyrimidine nucleus was observed at 1645 cm^{-1}.

large geminal coupling constant (J=15 cps). These findings apparently led to the conclusion that the structure of X should be assigned as bis[3-oxo-4-(2-methyl-4-aminopyrimidin-5-yl)methyl-5-methyl-6β-hydroxyethyl-1,4-oxazine]-6-disulfide. An analogous compound has been also obtained from hydroxyethylthiamine (HET),[12] therefore it provides an additional support for the structure of IV.

The formation of hydroxymethylthiamine (HMT) from 2-oxyalylthiamine-O,O'-diacetate and its transformation into the dimeric oxazine derivative (X) will be explained by the following scheme. Thiazolium ketone (XI) will initially result from the protonation followed by hydrolysis of acetyl groups of the starting diacetate, then acid cleavage of XI will lead to HMT and carboxylic acid. Neutralization of HMT with alkali will then lead to the thioketone (XIII) *via* the thiol form XII, and the recyclization followed by the oxidative dimerization of XIII will give X.

In the preliminary examination of the biological activity, hydroxymethylthiamine hydrochloride (IV) was found to increase plasma phospholipid level and to decrease cholesterol; phospholipid (c/p) ratio in rat.

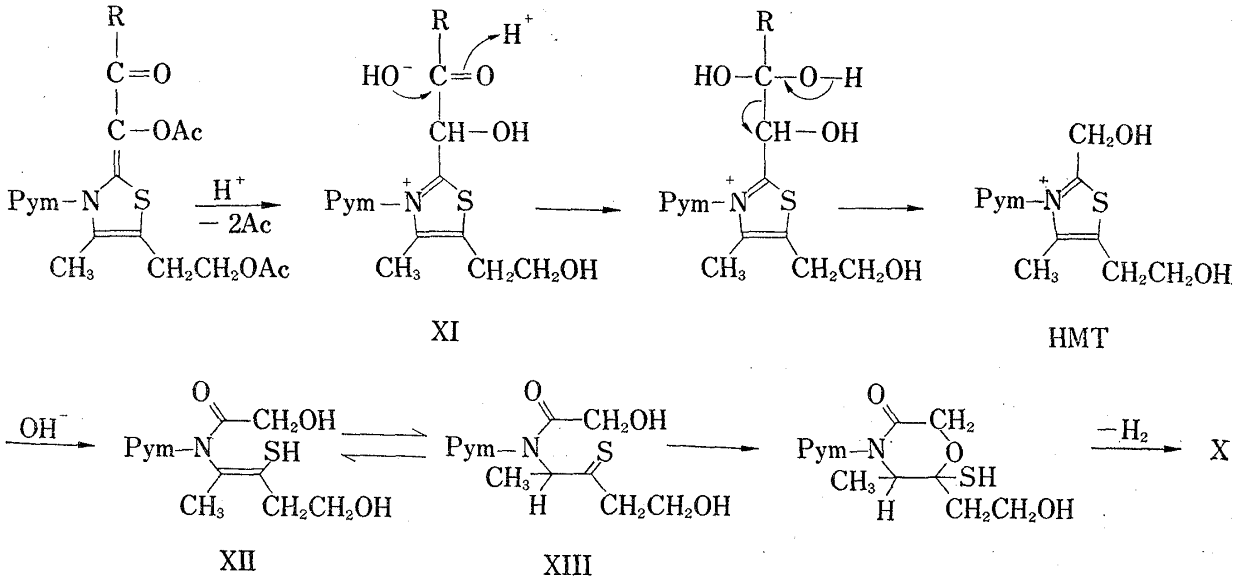


Chart 5

Experimental[13]

Hydroxymethylthiamine (HMT) Hydrochloride (IV)——a) 2-Phenyloxalylthiamine-O,O'-diacetate (III)[8] (4.82 g, 0.01 M) was dissolved in 100 ml of 10% EtOH–HCl and the solution was heated for 30 min at 60—70°, then it was allowed to stand overnight at room temperature. The deposited crystals were collected by filtration and recrystallized from MeOH containing a small amount of acetone to give IV as colorless prisms, mp 225—226° (decomp.). Yield 1.76 g (48%). *Anal.* Calcd. for $C_{13}H_{20}O_2N_4SCl_2 \cdot H_2O$: C, 42.54; H, 5.76; N, 14.55; S, 8.33; Cl, 18.42. Found: C, 42.67; H, 5.69; N, 14.23; S, 8.28; Cl, 17.97. UV λ_{max}^{EtOH} mμ (log ε): 239 (4.12), 265 (4.20). NMR (D_2O, DSS)τ: 2.55 (1H, singlet) (pyrimidine-6-H), 4.40 (2H, singlet) (pyrimidine-5-CH_2), 4.80 (2H, singlet) $\left(\overset{+}{}\genfrac{}{}{0pt}{}{N}{S}\rangle\text{-}CH_2\text{-}O\right)$, 6.05 and 6.76 (each 2H, triplet) $\left(=\!\langle CH_2CH_2O\right)$, 7.33 (3H, singlet) (pyrimidine-2-CH_3), 7.52 (3H, singlet) (thiazolium-4-CH_3). The filtrate on evaporation under reduced pressure gave a crystalline residue which was dissolved in water and extracted with ether. The ether layer gave 120 mg of benzoic acid after washing with water, drying over anhyd. $MgSO_4$ and evaporation to dryness.

12) M. Sunagawa and coworkers (Sankyo & Co., Ltd.), announced at the 18th Annual Meeting of the Society of Vitaminology (Japan), Osaka, 1966.

13) All melting points were determined in capillary tube and are uncorrected. NMR spectra were taken on a Varian A-60 recording spectrometer with tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal standard. IR spectra were taken on a Japan Spectroscopic Company IR-S instrument in Nujol mull. UV spectra were taken on Hitachi EPS-3 instrument in 99% EtOH.

b) 2-α-Furyloxalylthialmine O,O′-diacetate (VI)[8] 472 mg (0.001 M) was dissolved in 20 ml of 10% EtOH–HCl and the solution was heated for 30 min at 60—70°, then it was allowed to stand for 2 days at room temperature. Similar treatment of the reaction mixture as stated above gave colorless prisms which were identified as IV by mixed melting point and IR comparison. Yield 166 mg (45%). 16 mg of α-furoic acid was obtained from the filtrate of the reaction mixture.

Hydroxymethylthiamine Hydrobromide (V)——2-Phenyloxalylthiamine-O,O′-diacetate (III) (4.82 g, 0.01 M) was dissolved in 50 ml of saturated EtOH–HBr and the solution was heated for 30 min at 60—70°, then it was allowed to stand for two days at room temperature. The deposited crystals were collected by filtration and recrystallized from MeOH to give colorless prisms, mp 231—231.5° (decomp.). Yield 2.08 g (56.5%). *Anal.* Calcd. for $C_{13}H_{20}O_2N_4SBr_2$: C, 34.22; H, 4.42; N, 12.28; S, 7.03; Br, 35.03. Found: C, 34.53; H, 4.65; N, 12.03; S, 7.20; Br, 35.00. UV λ_{max}^{EtOH} mμ (log ε): 239 (4.10), 265 (4.19). NMR (D_2O, DSS)τ: 2.57 (1H, singlet) (pyrimidine-6-H), 4.47 (2H, singlet) (pyrimidine-5-CH_2), 4.82 (2H, singlet) $\left(\overset{+}{N}\!\!\underset{S}{>}\!\!-CH_2-O\right)$, 6.05 and 6.80 (each 2H, triplet) $\left(=\!\!<\!CH_2CH_2O\right)$, 7.37 (3H, singlet) (pyrimidine-2-CH_3), 7.53 (3H, singlet) (thiazolium-4-CH_3).

Hydroxymethylthiamine-O,O′,O″-tribenzoate (VII)——To a solution of 367 mg (0.001 M) of IV in 2 ml of 10% aqueous NaOH was added dropwise 700 mg of benzoyl chloride while cooling in an ice bath, then stirred for 1 hr at room temperature and the reaction mixture was extracted with $CHCl_3$. The combined $CHCl_3$ extract was washed, dried over anhyd. Na_2SO_4 and evaporated under reduced pressure to give an oily residue which was then chromatographed over silica gel (Davision Chemical Co., 60—200 mesh) column and eluted with acetone. The first fraction gave an unidentified oily substance (210 mg) and the second gave the tribenzoate VII, which was recrystallized from acetone containing a small amount of ether to give colorless prisms, mp 162—162.5° (decomp.). Yield 125 mg (20%). *Anal.* Calcd. for $C_{34}H_{32}O_6N_4S$: C, 65.37; H, 5.16; N, 8.97; S, 5.12. Found: C, 65.44; H, 5.22; N, 8.72; S, 5.00. IR ν_{max}^{Nujol} cm^{-1}: 1725 (C=O), 1669 (C=O). UV λ_{max}^{EtOH} mμ (log ε): 232.5 (4.19), 275 (3.68). NMR ($CDCl_3$, TMS)τ: 1.9—2.8 (16H, multiplets) (3× phenyl protons and pyrimidine-6-H), 4.2 (2H, broad) (pyrimidine-4-NH_2), 5.33 (2H, singlet) (N-CO-CH_2-O-CO-), 5.2—5.8, (4H, multiplets) (pyrimidine-5-CH_2 and CH_2-$\underline{CH_2}$-$OCOC_6H_5$), 6.92 (2H, triplet) (=C–CH_2–), 7.70 (3H, singlet) (pyrimidine-2-CH_3), 7.81 (3H, singlet) (=C–CH_3). Similarly, 4.55 mg of V (0.001 M) was benzoylated to give VII which was identified with the tribenzoate obtained above by IR comparison. Yield 180 mg (29%).

Treatment of IV with Hydrochloric Acid——367 mg (0.001 M) of IV was dissolved in 10 ml of 2% aqueous HCl solution, then the solution was allowed to stand for 5 days at room temperature. The reaction mixture was concentrated to dryness under reduced pressure and the resulting residue was washed with warm MeOH, then recrystallized repeatedly from hot MeOH to give thiamine hydrochloride (VIII) (120 mg) which was identified with authentic sample by mixed melting point and IR comparison.

Treatment of IV with Sodium Hydroxide——To a solution of IV (734 mg, 0.002 M) in 20 ml of water was added 16 ml of 1% aqueous NaOH solution (NaOH 0.004 M) while cooling in an ice bath, then the mixture was stirred for 2 days at room temperature. The reaction mixture was concentrated under reduced pressure to dryness and the resulting residue was washed with MeOH. The MeOH layer after evaporation under reduced pressure gave a gummy substance which was allowed to be chromatographed over silica gel column and eluted with MeOH. The first fraction gave thiamine thiazolone (IX) (140 mg, 25 %) which was identified with authentic sample by IR comparison, and the second fraction gave (X) which was recrystallized from acetone to give colorless prisms, mp 232° (decomp.). Yield 73 mg (12%). *Anal.* Calcd. for $(C_{13}H_{19}O_3N_4S)_2\cdot H_2O$: C, 47.67; H, 6.15; N, 17.11; S, 9.79. Found: C, 47.42; H, 5.95; N, 16.86; S, 9.51. Molecular weight Calcd. for $(C_{13}H_{19}O_3N_4S)_2$: 622.76. Found: 664 (vapor pressure lowering method). IR ν_{max}^{Nujol} cm^{-1}: 1660 (C=O). UV λ_{max}^{EtOH} mμ (log ε): 239 (4.21), 278 (3.81). NMR (d_6-DMSO, TMS) τ: 2.02 (1H, singlet) (pyrimidine-6-H), 3.33 (2H, broad) (pyrimidine-4-NH_2), 5.29 and 6.04 (each 1H, doublet, J=15 cps) (–N–CO–CH_2–OCO–), 5.68 (2H, singlet) (pyrimidine-5-CH_2), 7.70 (3H, singlet) (pyrimidine-2-CH_3), 8.72 (3H, doublet, J=6.5 cps) (–CH–$\underline{CH_3}$).

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