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Decomposition of Thiamine in Alcohol Solution. II[1]

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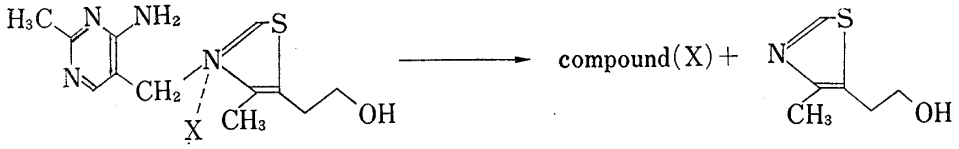
Thiamine mononitrate (Ib) was easily decomposed in methanol solution to produce crystalline compound (X') and 5-(2-hydroxyethyl)-4-methylthiazole (III). The structure of this compound (X') was presumed to be pentamethi(1,4-dihydro-4-imino-2-methyl)-pyrimidinylen-1,5-amer nitrate (IIc) from spectral and chemical data, and this was confirmed by the synthesis of 9-amino-2,7,10-trimethyl-5H-dipyrimido[1,6-*a*: 4',5'-*d*]pyrimidine derived from IIc. Compound (X), which was produced by the decomposition of thiamine monochloride (Ia) in methanol, is the hydrochloride of a base in IIc. IIc in aqueous solution reacted with acidic sodium sulfite to give 4-amino-2-methylpyrimidinyl-5-methanesulfonic acid (IX), and with III to give Ib.

In our preceding paper[1] we reported that thiamine monochloride (Ia) is easily decomposed in alcohols to produce a crystalline compound (X) (II) and 5-(2-hydroxyethyl)-4-methylthiazole (III). This paper deals with the structure of X and a few findings about it (Chart 1).



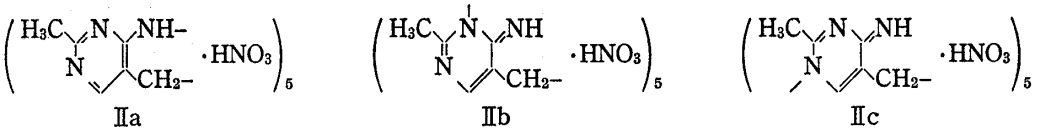
Ia : X=Cl
Ib : X=NO_3

II III

Chart 1

Compound (X) is slightly hygroscopic crystals. Since it was difficult to purify, we converted it to a nitrate (compound (X')) by treating it with silver nitrate. The infrared (IR), nuclear magnetic resonance (NMR) spectra and melting point of X' were identical with those of crystals which were obtained from thiamine mononitrate (Ib) in the same manner as Ia. Accordingly, the difference between compounds (X and X') was confirmed to be a hydrochloride or a nitrate of the same base. Based on this fact and its good crystallinity, compound (X') was used for structural studies. The composition of compound (X') was assumed to be $C_6H_7N_3 \cdot HNO_3$ from elementary analysis and measurement with thermobalance. The parent peak and main peaks in the mass spectrum were indicated at *m/e* 605 (M^+), 484 ($M^+ -121$), 363 ($M^+ -121\times2$) and 242 ($M^+ -121\times3$), respectively. As the difference [$121\times n(n=1—3)$] between the parent peak and each main peak corresponded to 4-imino-2-methyl-pyrimidinyl-5-methyl ($C_6H_7N_3$) and M^+ (605) corresponded to 121×5, compound (X') could be assumed to be a pentamer of the above-mentioned pyrimidine compound $C_6H_7N_3$. Its pyrimidine part was confirmed by the NMR signals of C_2—CH_3 (2.64 ppm), C_5–CH_2 (5.24 ppm) and C_6–H (7.90 ppm) in the pyrimidine nucleus. Accordingly, structural formulas IIa, IIb, and IIc were considered for compound (X') (Chart 2).

1) Part I: N. Shimahara, N. Nakajima, and H. Hirano, *Chem. Pharm. Bull.* (Tokyo), **22**, 2081 (1974).
2) Location: *Juso, Higashiyodogawa-ku, Osaka.*



IIa IIb IIc

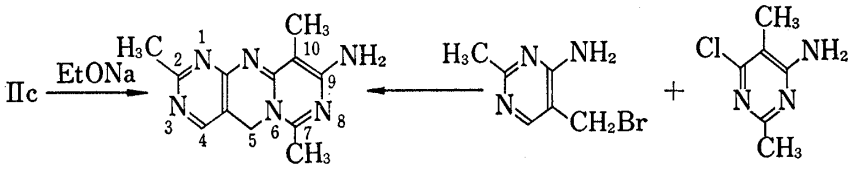
Chart 2

The facts that the signal of C_5–CH_2– in the NMR is a singlet, there is no absorption of 1600 cm^{-1} (C=C in the pyrimidine aromatic ring) and a strong absorption of 1665 cm^{-1} (imino, C=N bond) appears in IR, supported IIb and IIc and eliminated IIa. The NMR and IR spectra of both the hydronitrates of 1,4-dihydro-4-imino-5-hydroxymethyl-1,2-dimethylpyrimidine (IV) and 5-hydroxymethyl-4-methylamino-2-methylpyrimidine (V), which were synthesized according to the methods of Curd, *et al.*[3] and Matsukawa, *et al.*,[4] supported the above-mentioned information (Chart 3 and Experimental).

IV V

Chart 3

It could not be decided by p*K*a whether compound (X′) was IIb or IIc, because compound (X′) was unstable in alkali. Compound (X′) was stable as a salt of an acid, but an attempt to isolate its case was unsuccessful. A fluorescent substance like thiochrome was formed when compound (X′) was treated with sodium ethoxide in methanol, and was isolated as yellow needles, which were confirmed to be $C_{12}H_{16}N_6$ (M^+=242) by elementary analysis and mass spectrum. The presence of NH_2, CH_2, three CH_3 and an aromatic H was indicated by spectral data. From these data, the fluorescent substance was presumed to be 9-amino-2,7,10-trimethyl-5H-dipyrimido[1,6-*a*: 4′,5′-*d*]pyrimidine (VI), and was identified by elementary analysis and spectroscopic comparison with VI, which was synthesized by condensation of 4-amino-5-bromomethyl-2-methylpyrimidine (VII) and 4-amino-6-chloro-2,5-dimethylpyrimidine (VIII) (Chart 4).



VI VII VIII

Chart 4

Since it is not possible to produce VI from IIb, the structure of compound (X′) should be IIc. This agrees with the finding that N^1-methyliodide instead of the N^3-substitution product was obtained by the reaction of 4-amino-2-methylpyrimidine with methyliodide.[3] Therefore compound (X) can be considered to be the hydrochloride of the same base.

It can be inferred that thiamine is decomposed to an ammonium-type pyrimidine polymer (II′) and thiazole (III), followed by transformation of II′ into the imino type (II) as shown in Chart 5.

II was a pentamer, which can be more stable than the other polymers, and its crystallinity in alcohol was good. But it cannot be denied that other polymers are also produced.

Our preceding paper made clear that this reaction is specific for the mono-acid salt of thiamine. The decomposition rate was shown to be lower when water was present in the

3) F.H.S. Curd and Miss D.N. Richardson, *J. Chem. Soc.*, **1955**, 1853.
4) T. Matsukawa, *Yakugaku Zasshi*, **62**, 417 (1942).

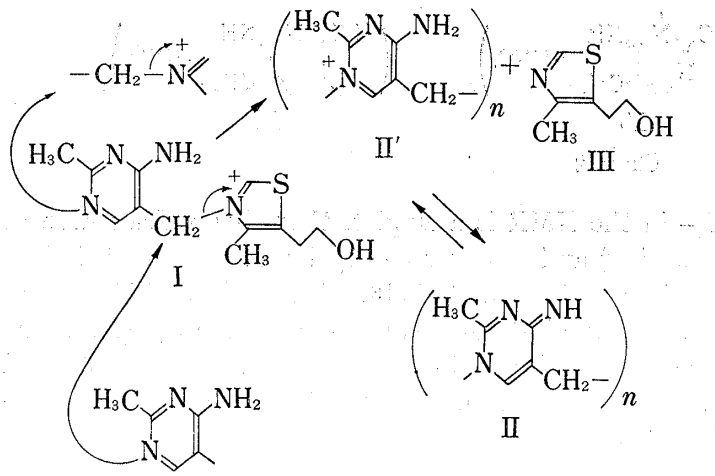


Chart 5

alcohol. This indicates that the protonation to nucleus N of the pyrimidine reduces its nucleophilicity to obstruct this decomposition reaction.

A possible reaction route to VI from IIc is shown in Chart 6.

The cleavage of the $-CH_2-N$ bond is assumed to start by nucleophilic attack of the ethoxide anion on the methylene group to form IIc. IIc is transformed into the ammonium-type structure (II'c) in an acidic solution, and in such a condition cleavage of the C–N bond must

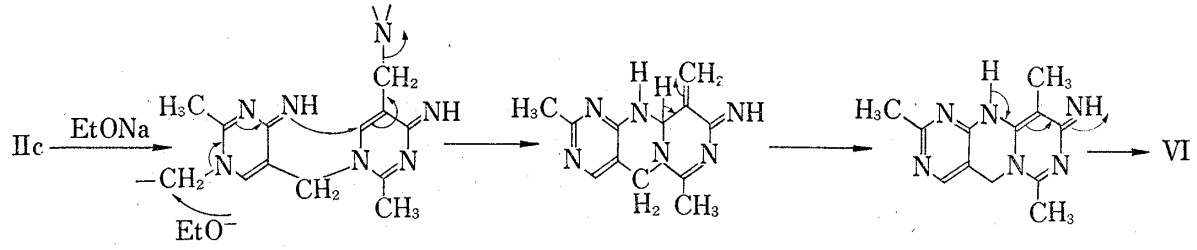


Chart 6

be easier. Therefore, the aqueous solution of IIc was reacted with acidic sodium sulfite to cause the above cleavage reaction, giving 4-amino-2-methylpyrimidinyl-5-methanesulfonic acid (IX), as was expected (Chart 7). This reaction is similar to the reaction of thiamine and sulfurous acid. It is known that the thiazole part of thiamine is substituted by the amine in the presence of an amine and sulfurous acid.[5] When III as amine was added to an aqueous solution of IIc, amine substitution occurred even in the absence of sulfurous acid to give thiamine. This shows that the decomposition of thiamine mono-acid salt in alcohol is a reversible reaction.

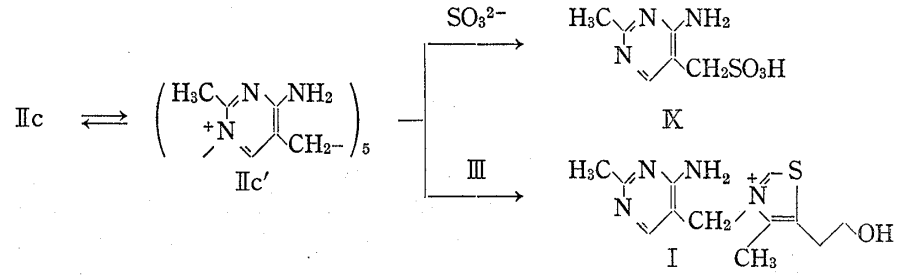


Chart 7

Experimental[6]

Separation of Product (Compound (X')) Obtained by Decomposition of Thiamine Mononitrate (Ib) in MeOH——A suspension of 3.3 g of Ib in 100 ml of MeOH was heated under reflux on a water bath for 2 hr.

5) T. Matsukawa and S. Yurugi, *Yakugaku Zasshi*, **71**, 1423, 1450 (1951); *idem*, *ibid.*, **72**, 33, 990 (1952); M. Sameshima, I. Sugimoto, and I. Utsumi, *ibid.*, **86**, 900 (1966); K. Inazu and R. Yamamoto, *Vitamin*, **34**, 328 (1966); K. Inazu, S. Nakanishi, and R. Yamamoto, *ibid.*, **37**, 170 (1968).

6) All melting points in this paper are uncorrected values. When not specifically indicated, NMR was measured with a Varian A-60 and tetramethyl silane (TMS) was used as the internal standard.

Ib dissolved gradually and a colorless precipitate formed. The precipitate was collected by filtration, washed with MeOH and dried to give 0.8 g of colorless crystals. The crystals were dissolved in warm water, acidified with dilute nitric acid and cooled. The resulting crystals were recrystallized twice from water to obtain colorless prism crystals. When the crystals were dried for 2 days over P_2O_5 under reduced pressure, compound (X'), *i.e.*, pentamethi(1,4-dihydro-4-imino-2-methyl)pyrimidinylen-1,5-amer (IIc), having a melting point of 205—208° (decomp.), was obtained. *Anal.* Calcd. for $(C_6H_7N_3 \cdot HNO_3)_5 \cdot 2H_2O$: C, 37.66; H, 4.63; N, 29.28. Found: C, 37.73; H, 4.54; N, 29.25. NMR (DMSO-d_6) δ: 2.64 (3H×5, s, pyrimidine-2-CH_3), 5.24 (2H×5, s, pyrimidine-5-CH_2-N), 7.90 (1H×5, s, pyrimidine-6-H). IR ν_{max}^{KBr} cm^{-1}: 1665 (C=N), 1380 (NO_2), 1230 (C–N).

The mother liquor was concentrated under reduced pressure. The residue was extracted with acetone, and acetone solution dried with anhydrous Na_2SO_4, then the solvent was distilled off under reduced pressure. When the residual oily substance was distilled under reduced pressure, 0.91 g (yield 73%) of light yellow oil with bp_6 133—135° was obtained. It was confirmed to be 5-(2-hydroxyethyl)-4-methylthiazole (III) by IR and NMR spectra.

Transformation of Compound (X) to Compound (X') (IIc)——In 30 ml of water, 0.6 g of the decomposition product of compound (X),[1] obtained by heating Ia with 95% MeOH, was dissolved by heating. Ten ml of 7% $AgNO_3$ was mixed in. The precipitated AgCl was removed by centrifugation. The resulting solution was mixed with a small amount of dilute nitric acid and the resulting crystals which precipitated were filtered off, washed with water and dried. Yield: 0.4 g. The product was recrystallized from water to give colorless crystals, mp 207—209°, which were identified a compound (X') obtained from Ib, by a mixed melting point and comparison of IR and NMR spectra.

1,4-Dihydro-4-imino-5-hydroxymethyl-1,2-dimethylpyrimidine Nitrate (IV)——A solution of 7 g of 4-amino-5-hydroxymethyl-2-methylpyrimidine and 4 ml of methyl iodide in 100 ml of MeOH was heated under reflux for 4 hr on a water bath. This was allowed to stand overnight at room temperature and then concentrated to about 20 ml under reduced pressure. The precipitate formed by cooling was collected by filtration. The product was recrystallized from MeOH to give yellow needles, 1,4-dihydro-4-imino-5-hydroxymethyl-1,2-dimethylpyrimidine hydroiodide, mp 208—211° (decomp.).

To a solution of 1.4 g of this compound in 10 ml of water, 0.85 g of $AgNO_3$ dissolved in 10 ml of water was mixed in. The resulting AgI was filtered off. The filtrate was concentrated under reduced pressure and dried to obtain 0.95 g of crude IV. The crude product was recrystallized from EtOH to give yellow prism crystals, mp 132—134°. *Anal.* Calcd. for $C_7H_{11}ON_3 \cdot HNO_3$: C, 38.89; H, 5.59; N, 25.91. Found: C, 38.80; H, 5.54; N, 25.52. NMR (DMSO-d_6) δ: 2.60 (3H, s, pyrimidine-2-CH_3), 3.84 (3H, s, pyrimidine-1-CH_3), 4.40 (2H, s, $-CH_2-O-$). IR ν_{max}^{KBr} cm^{-1}: 3300 (–OH), 1645 (–C=NH).

5-Hydroxymethyl-2-methyl-4-methylaminopyrimidine Nitrate (V)——To a solution of 5-hydroxymethyl-2-methyl-4-methylaminopyrimidine hydrochloride in 5 ml of water, 0.28 g of $AgNO_3$ dissolved in 5 ml of water was added at room temperature. The resulting AgCl was filtered off and the filtrate was concentrated under reduced pressure to give 0.32 g of crude V. The crude product was recrystallized from iso-PrOH to give colorless, prism crystals, mp 146—148°. *Anal.* Calcd. for $C_7H_{11}ON_3 \cdot HNO_3$: C, 38.89; H, 5.59; N, 25.91. Found: C, 38.87; H, 5.51; N, 25.79. NMR (DMSO-d_6) δ: 2.54 (3H, s, pyrimidine-2-CH_3), 3.02 (3H, t, $-NH_2^+-CH_3$), 4.40 (2H, s, $-CH_2-O$), 8.02 (1H, s, pyrimidine-5-H). IR ν_{max}^{KBr} cm^{-1}: 3340 (–O–H), 1680 (–NH–CH_3), 1600 (pyrimidine C=C).

Formation of 9-Amino-2,7,10-trimethyl-5H-dipyrimido[1,6-*a*: 4',5'-*b*]pyrimidine (VI)——i) A mixture of 1.2 g of 2-methyl-4-amino-5-bromomethylpyrimidine 2HBr (VII)[7] and 1.6 g of 4-amino-6-chloro-2,5-dimethylpyrimidine (VIII)[8] was fused by heating at 200—210° for 20 min on an oil bath and heated further for 2—3 min over a weak flame to liquify the sample. This was cooled and dissolved in 20 ml of water made to pH 6 with a dilute aqueous solution of NaOH. The precipitate formed was filtered off. To the filtrate, dilute aqueous NaOH solution was added then the mixture was cooled to give 0.8 g of powder. From this powder, 0.1 g of VI was isolated by preparative thin-layer chromatography (Merck's glass plate silica gel, 20×20 cm, thickness 2 mm, 150 mg of powder chromatography using MeOH as the development solvent). It was recrystallized from a mixed solution of MeOH and EtOH to give crystals, mp 291—293°. Mixed melting point on the admixture with the product obtained in ii) showed no depression, and NMR, IR and mass spectra of both were identical in every apesct.

ii) To a solution of EtONa, prepared from 0.5 g of Na and 50 ml of EtOH, 2.4 g of IIc was added and the mixture was heated under reflux for 2 hr on a water bath. This was allowed to stand for 2 hr at room temperature. The crystals which precipitated were filtered, washed with water and dried. This precipitate was crystallized from a mixed solution of MeOH and water to give 0.45 g of yellow crystals (VI), mp 290—293°. *Anal.* Calcd. for $C_{12}H_{14}N_6 \cdot H_2O$: C, 55.37; H, 6.19; N, 32.28. Found: C, 55.51; H, 5.97; N, 32.33. NMR (Varian HA-100) (CF_3COOH) δ: 2.37 (3H, s, 10-CH_3), 2.84 (3H, s, 7-CH_3), 2.91 (3H, s, 2-CH_3), 5.64 (2H, s,

7) J.K. Cline, R.R. Williams, and J.J. Funkelstein, *J. Am. Chem. Soc.*, **59**, 1052 (1937).
8) H.R. Henze, W.J. Clegg, and C.W. Smart, *J. Org. Chem.*, **17**, 1320 (1952).

5-methylene), 8.72 (1H, s, pyrimidine-4-H). IR ν_{max}^{KBr} cm^{-1}: 3300, 3200 ($-NH_2$); 1640 ($-C-NH_2$); 1600 (pyrimidine C=C).

Reaction between IIc and $NaHSO_3$——A solution of 0.96 g of IIc and 1.56 g of $NaHSO_3$ in 50 ml of water was allowed to stand, and the resulting crystals were filtered off. The filtrate was concentrated under reduced pressure and the resulting crystals were filtered. The crystals were combined, washed with water and dried to obtain 0.8 g of 4-amino-2-methylpyrimidinyl-5-methanesulfonic acid (IX). This substance was purified by the conventional method with aqueous NH_4OH and AcOH. mp 320°. Its IR spectrum completely agreed with that of the standard substance.

Reaction between IIc and III——A solution of 0.96 g of IIc and 2.56 g of the thiazole (III) in 30 ml of water was heated for 4 hr at 80° on a water bath. The solvent was distilled off under reduced pressure. The residue was washed with 100 ml of acetone and dried to give 1 g of crystalline substance. This substance was washed with about 5 ml of cold water to obtain 0.3 g of Ib as crude sample. This was recrystallized from water to give crystals, mp 185—187°. Mixed melting point on the admixture with the authentic sample of Ib showed no depression, and IR spectra of the two samples were completely identical.

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