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Decomposition of Thiamine in Alcohol Solution. I

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Thiamine monochloride (Ia) was found to easily decompose in methanol solution to produce a crystalline compound X (II), which is believed to be derived from the pyrimidine part of thiamine, and 5-(2-hydroxyethyl)-3-methylthiazole (III). The ratio and rate of this decomposition are affected by the kind of alcohol, its dilution rate, reaction temperature, the acidity and basicity of the solution and concentration of Ia. Decomposition of Ia in methanol in the presence of pyridine gave N-[2-methyl-4-aminopyrimidyl-(5)]-methylpyridinium chloride (V) and III.

There are many reports[2] on the stability of thiamine in aqueous solution. However, no reports have been presented on the stability of its salts in alcohol because of their low solubility and the general consideration of high stability, except by Inazu, *et al.*[3]

This paper reports the decomposition of thiamine monochloride (Ia) in alcohols. When thiamine monosalt (I) was dissolved for recrystallization in methanol, it was found that thiamine monochloride (Ia) and mononitrate (Ib), which has been used as food enriching additives and medicine, decompose unexpectedly very rapidly, but thiamine monochloride hydrochloride (Ic) was stable. Therefore decomposition seemed to be specific for the monosalt of thiamine (Chart 1).

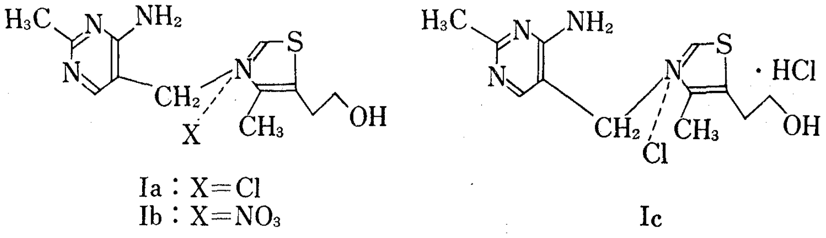

Chart 1

Inazu, *et al.*[3] determined the residual ratios of Ic after keeping its ethanolic solution at 100° for 10 hr in a sealed tube. Their reaction conditions were more extreme than ours, and they did not make clear the decomposition products and a reaction mechanism.

A two percent solution of Ia in 95% methanol was maintained at room temperature (23° ±1), 45° and 65°, respectively. The residual ratios of thiamine were determined by the thiochrome method at intervals as shown in Fig. 1.

At 65°, Ia began to decompose rapidly, and the residual ratio was about 40% after 15 min and 15.6% after 1 hr. At 45°, the induction period of decomposition was about 15 min. After 6 hr, the residual ratio decreased to 16.5%. At 23°, Ia was not decomposed after 7 hr, but its residual ratio was less than 20% after 22 hr.

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

[2] A. Watanabe, *Takeda Kenkyusho Nempo*, 7, 126 (1943); T. Matsukawa and H. Iwatsu, *Yakugaku Zasshi*, **70**, 28 (1950); H. Hirano, *ibid.*, **77**, 1004 (1957); J.J. Windhauser and T. Higuchi, *J. Pharm. Sci.*, **51**, 354 (1962).

[3] K. Inazu, S. Sunagawa and R. Yamamoto, *Vitamin*, **38**, 41 (1968).

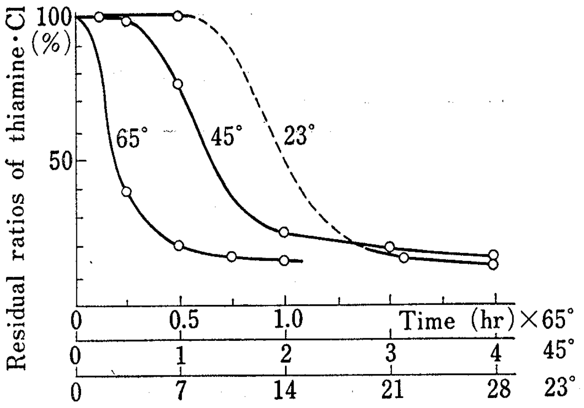

Fig. 1. Residual Ratios of Thiamine after 0.25—28 hr at Different Temperatures in 95% Methanol [Initial Concentration of Thiamine Monochloride (Ia)=2%]

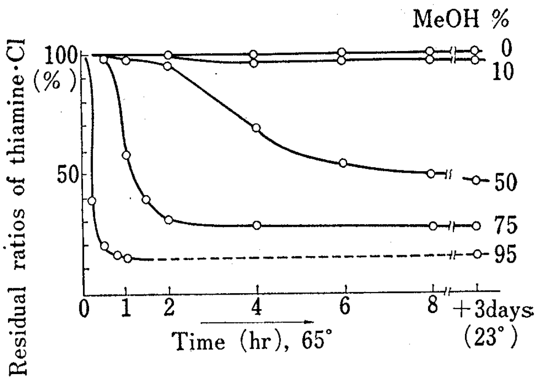

Fig. 2. Residual Ratios of Thiamine after 0.25—8 hr at 65° (following 3 days at 23°) and Different Concentrations of Methanol [Initial Concentration of Thiamine Monochloride (Ia)=2%]

Two percent aqueous solution of Ia and 2% solutions of Ia in 10, 50, and 75% methanol were maintained at 65°, and residual ratios of thiamine were determined at intervals as shown in Fig. 2.

Decomposition was dependent on methanol concentration. In aqueous solution, no decomposition was observed.

It is well known that the stability of thiamine in aqueous solution is largely affected by the pH of the solution and decomposition occurs especially in basic solution. To investigate the effect of the acidity and basicity of methanol solution on decomposition, potassium hydroxide equivalent to 1/2, 1, 3/2, and 2 moles of Ic was added to 2% solutions of Ic in 95% methanol, and then were maintained at 45°. The results showed that a solution containing 1 mole of potassium hydroxide alone caused decomposition similar to that described previously (Fig. 3). Decomposition in a solution containing more than 3/2 moles of the alkali was different from that seen in a pattern of the decomposition curve. In this case, the induction period of decomposition was not observed, and the residual ratio of thiamine was 30 to 40% higher compared with that of the former. This fact indicates that decomposition with more than 3/2 moles of the alkali depended on the stability of thiamine in basic solution and was almost independent of the solvent effect of methanol.

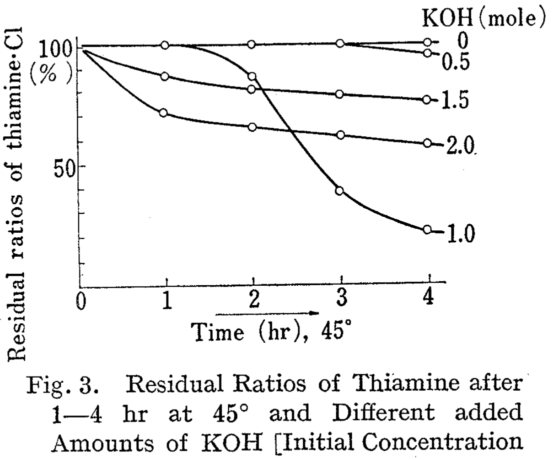

Fig. 3. Residual Ratios of Thiamine after 1—4 hr at 45° and Different added Amounts of KOH [Initial Concentration of Thiamine Chloride Hydrochloride (Ic) =2% in 95% Methanol]

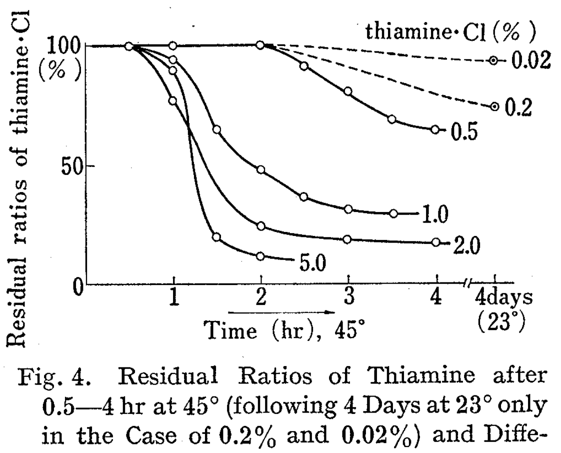

Fig. 4. Residual Ratios of Thiamine after 0.5—4 hr at 45° (following 4 Days at 23° only in the Case of 0.2% and 0.02%) and Different Initial Concentrations of Thiamine Monochloride (Ia) in 95% Methanol

To investigate the effect of thiamine concentration, methanol solutions of Ia at certain concentrations were kept at 45°, and the residual ratios of thiamine were determined at intervals of 30 min. Figure 4 shows that the decomposition rate and ratio depended on the initial concentration of Ia. A solution of the initial concentration of less than 0.5% hardly decomposed.

We examined the effect of ethanol and propanol in addition to methanol. Ia decomposed in ethanol and propanol, like in methanol. The decomposition rate fell in the order of methanol, ethanol and propanol (Fig. 5).

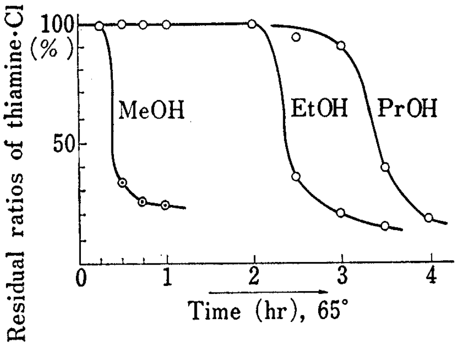

Fig. 5. Residual Ratios of Thiamine after 0.25—4 hr at 65° in Different 90% Alcohols [Initial Concentration of Thiamine Monochloride (Ia) = 2%]

The results indicate that the decomposition is attributable to the effect of the alcohols, particularly that of methanol, and is specific to the monosalt of thiamine, and then after an induction period, decomposition accelerated very rapidly.

Next, larger amounts of Ia were treated with 95% methanol by a method described in Experimental, to give a colorless crystalline substance (II), which will be referred to as compound X hereafter, and a light yellow oil in 70% yield. The oil was identified as 5-(2-hydroxyethyl)-4-methylthiazole (III) by nuclear magnetic resonance (NMR) and infrared (IR) spectra. II was assumed to be a compound derived from the pyrimidine part of thiamine, and its chemical structure and properties will be described in our next paper (Chart 2).

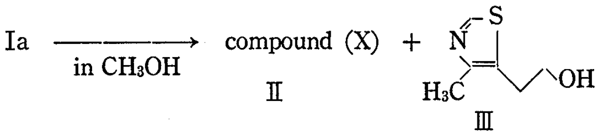

Chart 2

Thiamine has been reported to react with sulfurous acid in aqueous solution to give III and 4-amino-2-methylpyrimidinyl-5-methanesulfonic acid (IV) in good yield, and the optimum pH range for this reaction is 5 to 6.⁴⁾ If an amine such as pyridine is included in this decomposition, pyridine substitutes partly to the thiazole portion by nucleophilic attack to give a pyridinium body (V).⁵⁾ But these reactions are not common to all the thiamine homologs; for example, oxythiamine (VI) is hardly decomposed by sulfurous acid.⁶⁾ We found that

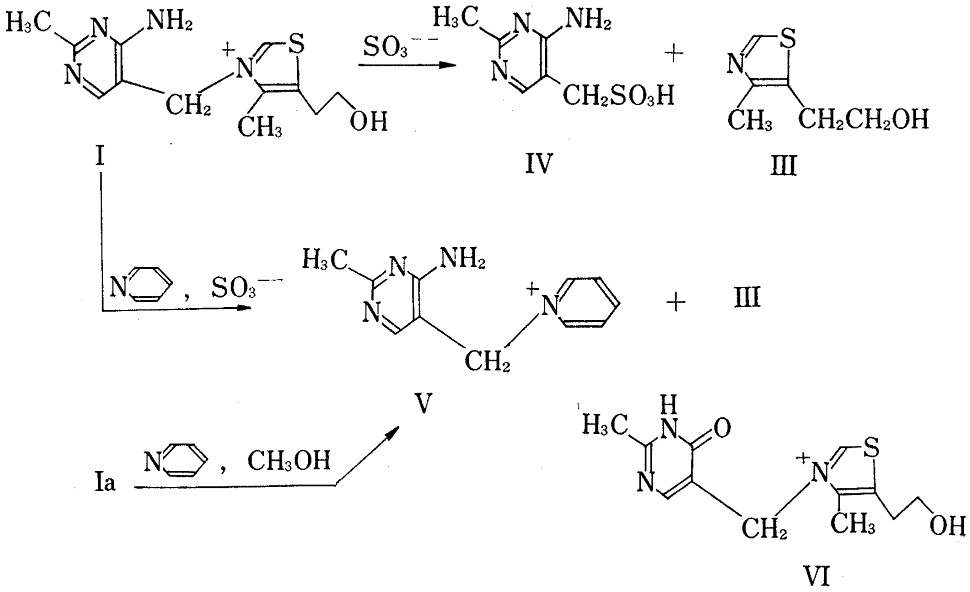

Chart 3

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4) R.R. Williams, R.E. Waterman, J.C. Keresztsy and E.R. Buchman, *J. Am. Chem. Soc.*, **57**, 536 (1935).
5) T. Matsukawa and S. Yurugi, *Yakugaku Zasshi*, **71**, 1423 (1951).
6) T. Matsukawa and S. Yurugi, *Yakugaku Zasshi.*, **72**, 990 (1952).

the decomposition seemed to be a reaction similar to the one above of thiamine with sulfurous acid, and attempted to study the decomposition in the presence of pyridine. Although Ia yielded scarcely any II, V was obtained in 50% yield. On the other hand, in the case of treatment of VI (monochloride) with methanol, VI was recovered. All of these series of reactions were presumed to be nucleophilic reactions to bridged methylene, in which the amino group at the 4-position in the pyrimidine nucleus might play an important role in the mechanism of these reactions (Chart 3).

Experimental[7]

Method of Preparing Thiamine Monochloride (Ia)——Thiamine chloride hydrochloride (Ic), 34 g, was suspended in 250 ml methanol, then 20 g NEt_3 was gradually added with shaking. Ic dissolved rapidly to make a homogeneous clear solution, which was concentrated to about one-half its volume under reduced pressure[8] and allowed to stand overnight at room temperature. The resultant crystals were filtered off, washed with a small quantity of cold methanol, and vacuum-dried over P_2O_5 at 70° for 5 hr to yield 15 g of Ia, mp 160—163° (decomp.). Purity: 99.6% (determined by the thiochrome method.) *Anal.* Calcd. for $C_{12}H_{16}ON_4S \cdot Cl$: C, 47.91; H, 5.70; N, 18.63. Found: C, 48.07; H, 5.63; N, 18.40.

Determination of Residual Thiamine in Alcohol Solutions——Method of Determination: A sample solution was diluted with 0.1N hydrochloric acid to give a concentration of 1.5 μg/ml of Ia, then was quantitatively analyzed by the thiochrome bromocyanate method with an Auto Analyzer. This method was used after it had been ascertained that compound X (II) and 5-(2-hydroxyethyl)-4-methylthiazole (III), which were the decomposition products of Ia, were independent of the determination.

1) Effect of Temperature: Samples of Ia, 500 mg, were weighed, transferred to 25 ml volumetric flasks, and dissolved in 95% methanol previously heated to 23° (room temperature), 45°, and 65°, to a volume of 25 ml. Sample solutions were maintained at each given temperature. Solution samples of 0.5 ml each were pipetted out at regular time intervals and, to arrest the reaction, poured into enough 0.1N hydrochloric acid to make up 100 ml. The residual amount of Ia in the sample was determined by the above method.

2) Effect of Methanol Concentration: Samples of 500 mg were weighed, transferred to 25 ml volumetric flasks, and dissolved in various concentrations of methanol previously maintained at 65°. The mixtures were immediately brought to a volume of 25 ml, thermostated at 65°±2°, and treated as in 1).

3) Effect of the Acidity and Basicity of the Solution: Samples of 500 mg each of Ic were weighed out in 25 ml volumetric flasks and dissolved in about 20 ml of 95% methanol pre-heated to 45°. Immediately, calculated amounts of 1N potassium hydroxide solution (in 95% methanol) and 95% methanol were added to make up 25 ml. The mixtures were then maintained at 45° and treated as in 1).

4) Effect of Ia Concentration: A constant amount of Ia was weighed and transferred to a 25 ml volumetric flask to give a desired concentration of Ia. It was dissolved in 95% methanol previously heated to 45° to make up 25 ml, maintained at 45°, and treated as in 1).

5) Effect of Alcohol Type: A sample of 500 mg of Ia was weighed out in a 25 ml volumetric flask, dissolved in a 90% alcohol pre-heated to a volume of 25 ml, thermostated at 65°, and treated as in 1).

Isolation of Decomposition Product of Ia——A sample of Ia, 3 g, was dissolved in 100 ml of 95% methanol, and heated on a water bath at 60° for 2 hr. Colorless, curdy crystals precipitated after 10 min heating, the amount increasing with time. After cooling, the crystals were filtered off, washed with methanol, and dried over P_2O_5 at 70° for 2 days under reduced pressure to give 1 g of wet, colorless crystals, mp 255—258° (decomp.). A variety of attempts to purify it were unsuccessful. The mother liquid after filtration of the crystals was concentrated under reduced pressure, the residue was extracted with $(CH_3)_2CO$, the $(CH_3)_2CO$ solution was dried with anhydrous sodium sulfate, then the solvent was distilled under reduced pressure. The remaining oil was distilled under reduced pressure to give 0.87 g of light-yellow oil, bp_6 133—134°, in 70% yield. The substance was identified as 5-(2-hydroxyethyl)-4-methylthiazole by IR and NMR spectroscopy.

An Attempt to Decompose Oxythiamine (VI)——Oxythiamine chloride hydrochloride, 3.4 g, was dissolved in 100 ml of 95% methanol containing 0.4 g of sodium hydroxide to prepare a methanol solution of oxythiamine monochloride. It was heated on a water bath at 60° for 2 hr, and the paper partition chromatography and thin-layer chromatography of the reacted solution were observed. III did not form. The solution was concentrated to dryness under reduced pressure with absolute methanol, and EtOH–HCl was added to the mother liquid, which was concentrated to dryness under reduced pressure. The residual crystals were identified as oxythiamine chloride hydrochloride, which was the starting substance, from NMR and IR spectroscopy. The yield was 3 g.

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[7] All the melting points in this report are uncorrected.

[8] Ia did not decompose entirely by heating in methanol in the presence of NEt_3.

Production of a Pyridinium Salt by Amine-Substitution of Ia in Methanol——A mixture of 3 g (Ia) and 2.4 g of pyridine was dissolved in 100 ml of 95% methanol and heated on a water bath at 60° for 2 hr. After filtration of a small amount of the precipitate, the filtrate was concentrated under reduced pressure, and the residue was extracted with ethyl ether. The crystals insoluble in ether were dissolved in ethanol–hydrochloric acid, and concentrated to dryness under reduced pressure. The residual crystals were washed with ethyl ether then ethanol and dried. The yield was 1.5 g. Recrystallization with hot ethanol resulted in scaly crystals, mp 258° (decomp.). The substance was identified as N-[2-methyl-4-aminopyrimidyl-(5)]-methylpyridinium chloride hydrochloride (V) by IR and NMR spectroscopy and by a mixed melting point test with crystals of authentic sample. On the other hand, drying of the ethyl ether-extract previously described, over anhydrous magnesium sulfate and distillation of the solvent under reduced pressure gave 0.8 g of a crude thiazole body (III), the structure of which was confirmed by IR and NMR spectroscopy after purification by distillation, bp_6 133—135°.

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