of Nagoya and to Drs. Y. Mashiko and A. Kato of the Government Chemical and Industrial Research Institute, Tokyo, for their generous favor in taking the rotatory dispersion curves. Authors' thanks are due to Dr. K. Abe, the Director of this Laboratory, for his encouragement, to Mr. K. Kotera for infrared analysis, and to Mrs. F. Hisamichi, and Messrs. T. Yoda and T. Kōno for elementary analysis.

Summary

Physicochemical comparisons were made on Naramycin-B and isocycloheximide, which are stereoisomers of cycloheximide, and it was confirmed that the two antibiotics are similar but not quite equal to each other, and that, mainly from the interpretation of their rotatory dispersion curves, the absolute configurations of Naramycin-B and isocycloheximide respectively belong to (2S:4S:6R:αS)- and (2R:4S:6R:αS)-series.

(Received September 9, 1959)

UDC 577.164.11

62. Shizuka Kasahara: Action of Cyanogen Bromide on Thiamine. III.[1] Synthesis of 2-Methyl-6-(1-methyl-2-thiocyanato-4-hydroxy-1-butenyl)-5,6-dihydropyrimido[4,5-*d*]pyrimidine (Neocyanothiamine).

(*Osaka Branch, National Hygienic Laboratory*[*1])

The first paper of this series[2] reported that the sodium salt (II) of thiol-type thiamine was converted into N-(1-methyl-2-thiocyanato-4-hydroxy-1-butenyl)-N-[(2-methyl-4-amino-5-pyrimidinyl)methyl]formamide (cyanothiamine) (IV) by the action of cyanogen bromide.

Zima, *et al.*[3] obtained the sodium salt of thiamine, the so-called yellow salt, by the action of three equivalents of sodium ethoxide on thiamine hydrochloride (I) and presumed this salt to possess the chemical structure formulated as (III). However, presence of a cyclized thiol-type thiamine (III) has not been confirmed, nor has the derivative of (III) been obtained as yet. If thiamine changes into the sodium salt (III) as reported by Zima, *et al.*, thiocyanato derivative of (III) may be formed by the action of cyanogen bromide on the same salt.

Addition of three equivalents of sodium ethoxide to the dehydrated ethanol suspension of thiamine hydrochloride (I) afforded a yellow solution, the action of one equivalent of cyanogen bromide on which gave white needles, m.p. 152.5° (decomp.) (substance A). The elemental analyses of substance A gave the formula $C_{13}H_{15}ON_5S$.

The ultraviolet absorption spectrum of substance A in chloroform was quite similar to that of 2,8a-dimethyl-6,7,5a,8a-tetrahydrofuro[2,3-*h*]thiochromine (VIII),[4] as shown in Table I.

The infrared absorption spectrum of substance A showed absorption peaks at 2169 (SCN), 3356 (ν_{OH}), and 1051 cm^{-1} ($\nu_{C\text{-}O}$) and resembled that of thiochrome (VIII) except for the peak at 2169 cm^{-1} (SCN). Accordingly, substance A does not have a primary amino group

---

*1 6 Hōensaka-cho, Higashi-ku, Osaka (笠原 閑).

1) Part II : Yakugaku Zasshi, **79**, 963(1959).
2) Part I : *Ibid.*, **77**, 1133(1957).
3) O. Zima, R. R. Williams : Ber., **73**, 941(1940).
4) H. Hirano : Yakugaku Zasshi, **74**, 59(1954).

Table I.

	λ_{max} mμ (log ε)	λ_{min} mμ (log ε)
Substance A	240 (3.96), 325 (4.09)	279 (3.92)
Perhydrofurothiochrome	245 (3.93), 320 (4.08)	268 (3.37)

in the 4-position of the pyrimidine, but forms 5,6-dihydropyrimido[4,5-*d*]pyrimidine system. Substance A was hydrolyzed almost quantitatively with acid to cyanothiamine (IV).

From the foregoing experimental results, it can be concluded that the newly obtained substance A of m.p. 152.5° (decomp.) is 2-methyl-6-(1-methyl-2-thiocyanato-4-hydroxy-1-butenyl)-5,6-dihydropyrimido[4,5-*d*]pyrimidine (hereinafter designated as neocyanothiamine (V)).

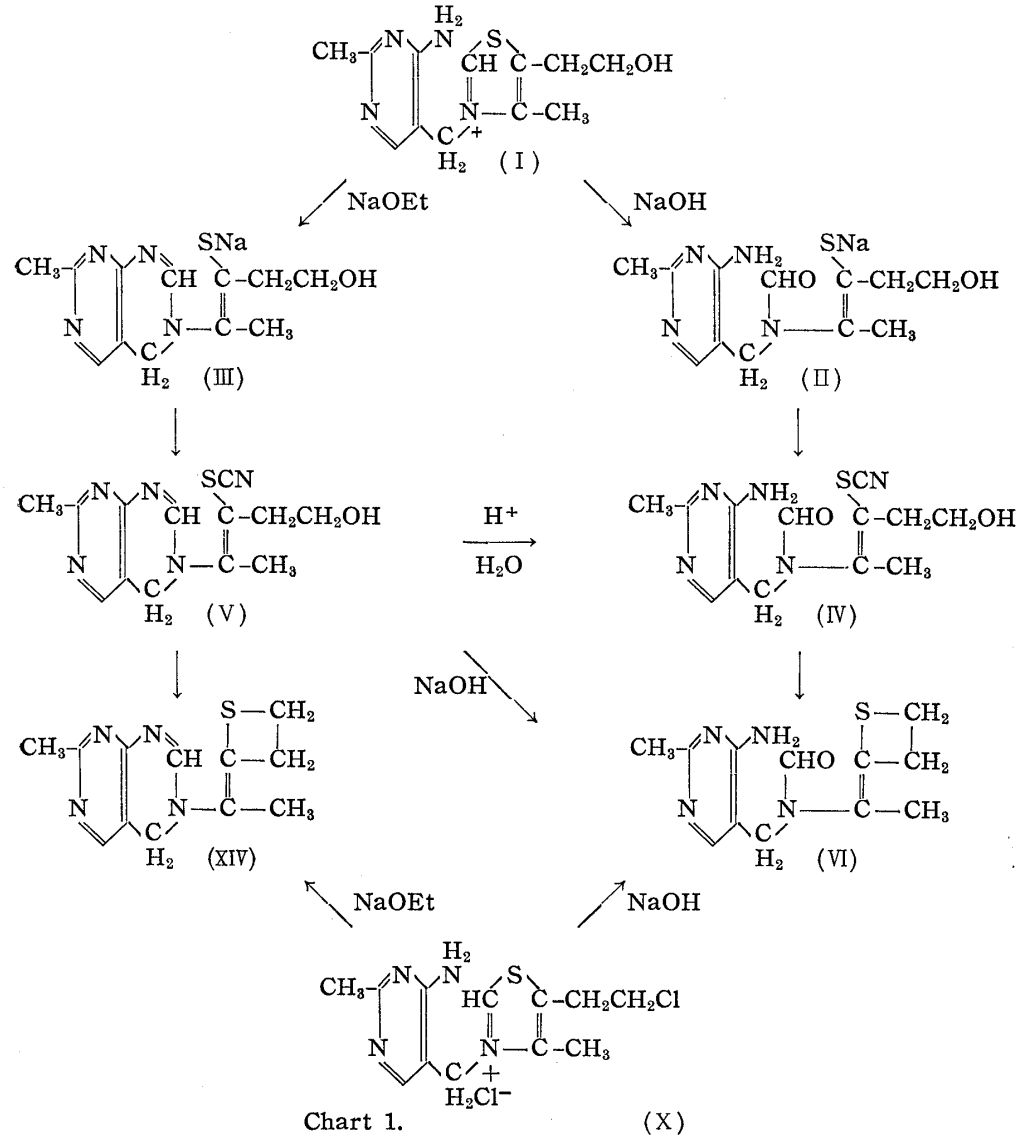


Chart 1.

On the other hand, Yamada, *et al.*[5] and Hirano, *et al.*[6] reported that reaction of the yellow salt (III) with alkyl halides did not give alkyl derivatives of (III).

Maier, *et al.*[7] reported that the ultraviolet absorption spectra and discoloration of the yellow salt in methanol or water were identical with those of the yellow form of

5) S. Yamada, *et al.* : Yakugaku Zasshi, **76**, 616(1956).
6) H. Hirano, *et al.* : Vitamins (Kyoto), **10**, 505(1956).
7) G. D. Maier, D. E. Metzler : J. Am. Chem. Soc., **79**, 4386(1957).

thiamine in methanolic potassium hydroxide.

From these literature, however, it is uncertain whether the substance in the yellow solution is the cyclic thiol-type thiamine (III) or some others. Therefore, attempt was made to investigate the behavior of thiamine in sodium ethoxide solution by ultraviolet absorption spectra.

The absorption spectrum of thiamine hydrochloride in 0.001*N* ethanolic sodium ethoxide showed a peak at 334 mμ, as shown in Fig. 1. The absorption band near 334 mμ did not change under perfectly anhydrous condition, even if the solution was allowed to stand at room temperature (17~20°) for 18 hours, but the absorbance at 334 mμ decreased not only by addition of a small amount of water to the solution, but also by the atmospheric moisture.

The absorption spectra of the solution, obtained by dissolving thiamine in 0.1*N* sodium ethoxide, diluting with water, and then adjusting to pH 3.0, 7.0, 9.6, or 10.3 with hydrochloric acid, were compared with those of aqueous thiamine solution at each of these pH, but there was found no difference in their spectra.

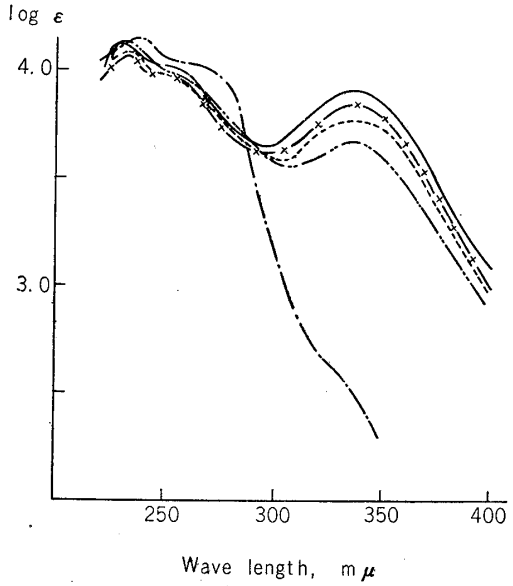


Fig. 1. Ultraviolet Absorption Spectra of Thiamine (in 0.001*N* EtONa)

——— In anhydrous state
—×—×— 1~5 min. after addition of water
---------- 20 min. after addition of water
—·—·— 4 hr. after addition of water
—·—·— 18 hr. after addition of water

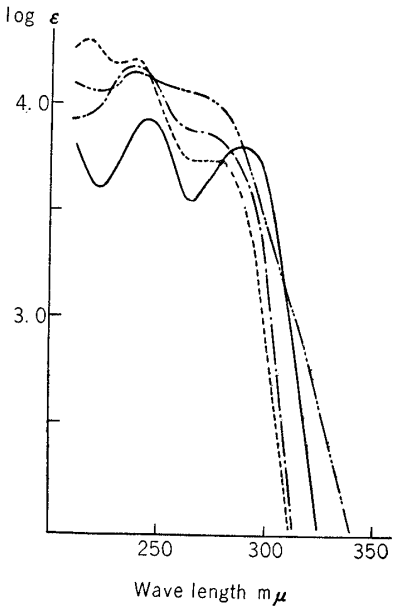


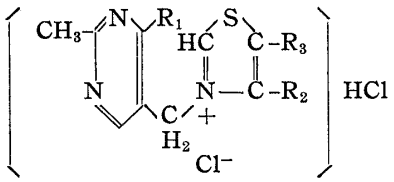
Fig. 2. Ultraviolet Absorption Spectra (in 0.001*N* NaOEt)

—·—·— Thiamine dissolved in 0.1*N* NaOH and diluted with EtOH after standing for 90 min.
—·—·— Cyanothiamine (IV)
---------- Thiamine anhydride (VI)
——— Pseudo-dihydrothiamine (IX)

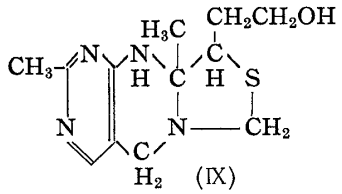
On the other hand, the absorption spectra of sodium ethoxide solution of sodium salt of thiol-type thiamine (II) and its derivatives, such as cyanothiamine (IV) and thiamine anhydride (VI), had no peak near 334 mμ (Fig. 2).

The absence of absorption peak near 334 mμ in the thiamine base, the so-called dihydrothiochrome (XVIII), may probably be due to the structural similarity of (XVIII) to pseudo-dihydrothiamine (IX) which has no peak over this wave-length region, as shown in Fig. 2.

The absorption spectra of following compounds were measured in the same way as in the case of thiamine : 3-(2-Methyl-4-amino-5-pyrimidinyl)methyl-4-methyl-5-(2-chloroethyl)thiazolium chloride hydrochloride (X), 3-(2-methyl-4-amino-5-pyrimidinyl)methyl-4,5-dimethylthiazolium chloride hydrochloride (XI), 3-(2-methyl-4-amino-5-pyrimidinyl)-methyl-4-methylthiazolium chloride hydrochloride (XII), and 3-(2-methyl-4-hydroxy-5-pyrimidinyl)methyl-4-methyl-5-(2-hydroxyethyl)thiazolium chloride hydrochloride (oxythiamine) (XIII) (Fig. 3). With the exception of oxythiamine, these compounds showed spectra similar to that of thiamine. These facts show that 4-amino group in the pyrimidine ring plays an important rôle for absorption near 334 mμ, but any substituent in 4- or 5-position of thiazolium ring does not.

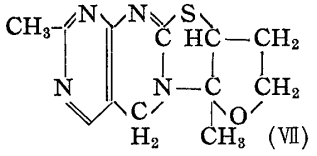


	R_1	R_2	R_3
X	$-NH_2$	$-CH_3$	$-CH_2CH_2Cl$
XI	$-NH_2$	$-CH_3$	$-CH_3$
XII	$-NH_2$	$-CH_3$	$-H$
XIII	$-OH$	$-CH_3$	$-CH_2CH_2OH$



(IX)

Chart 2.



(VII)

It was observed that the absorption spectrum of neocyanothiamine (V) or 2,8a-dimethyl-6,7,5a,8a-tetrahydrofuro[2,3-*h*]thiochromine (VII) in chloroform showed a peak near 330 mμ as that of 5,6-dihydropyrimido[4,5-*d*]pyrimidine system. Thiamine in sodium ethoxide medium also absorbs lights near 334 mμ and its absorption spectrum is similar

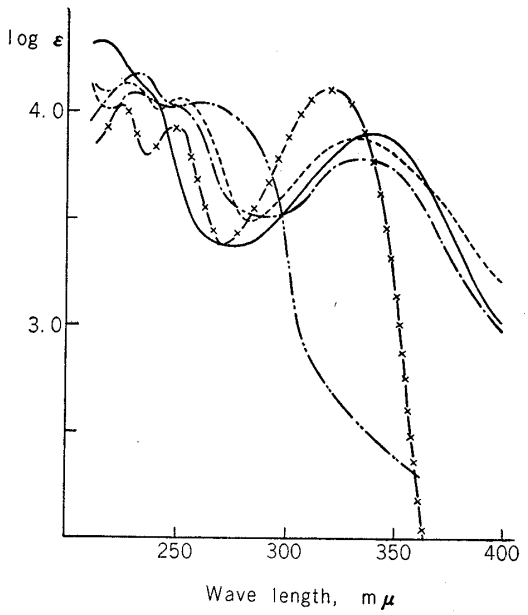


Fig. 3. Ultraviolet Absorption Spectra (in 0.001*N* NaOEt)

——— 5-Chloroethylthiamine (X)
—·—·— 4,5-Dimethylthiamine (XI)
---------- 4-Methylthiamine (XII)
——·——·— Oxythiamine (XIII)
—×—×— Perhydrofuro-thiochrome (VII)

to that of (VII) in the same medium (Fig. 3). These facts show that thiamine is converted in sodium ethoxide medium into the substance having the 5,6-dihydropyrimido[4,5-*d*]-pyrimidine system.

Additional support for the cyclized structure of thiamine comes from following experiments: By dissolving in sodium ethoxide, (V) decomposes into thiamine, producing a trace of thiamine anhydride (VI) and thiochrome (VIII) at the same time. However, it is impossible that a ring-opening of the 5,6-dihydropyrimido[4,5-*d*]pyrimidine in (V) occurs in sodium ethoxide solution. Therefore, thiamine, formed from (V) by the action of sodium ethoxide, has to possess the 5,6-dihydropyrimido[4,5-*d*]pyrimidine system, i.e. cyclized thiol-form (III). Thiamine so produced was fluorometrically determined and the yield was 91.5%. The solution obtained by dissolving (V) in sodium ethoxide also absorbs light near 336 mμ and its spectrum was similar to that of thiamine under the same condition, but shifted about 2 mμ toward a longer wave-length. Yonemoto[8] reported that 5-chloroethylthiamine (X) was converted into thiamine anhydride (VI) by the action of sodium hydroxide. From his report, it can be expected that (X) changes to (XIV) by the action of sodium ethoxide. The spectrum of (X) in sodium ethoxide also exhibits a peak at 340 mμ, as shown in Fig. 3 and shifted about 6 mμ toward a longer wave length than that of thiamine. These facts indicate that a trace of (XIV), produced together with thiamine from (V), causes such a bathochromic shift (2 mμ).

It is well known that dissolving of thiamine in alkaline media results in yellow coloration. A portion of aqueous thiamine solution was poured into 0.2*N* sodium hydroxide and the absorption curve of the resulting solution exhibited a peak at 333 mμ, similar to that of sodium ethoxide solution of thiamine.

From foregoing facts, it is believed that on dissolving in alkaline media, thiamine hydrochloride (I) is converted into cyclized thiol-form (III) with a removal of hydrochloric acid and the sodium salt of (III) changes further to neocyanothiamine (V) by the action of cyanogen bromide.

Mechanism of the Formation of Thiochrome

Neocyanothiamine (V) is sparingly soluble in dehydrated ethanol, but when heated with ethanol, thiochrome (VIII) was produced in a good yield. Hydrocyanic acid was not detected in the mother liquor. When the mother liquor was distilled, however, hydrocyanic acid as well as ethylamine was detected in the distillate.

Heating of (V) with various alcohols such as methanol, isopropanol, and isobutanol afforded thiochrome (VIII) and in these case, hydrocyanic acid as well as alkylamine was also detected in the distillate of the mother liquor.

Davies, *et al.*[9] obtained alkylbenzothiazole thiocarbamates by the reaction of alkyl thiocyanobenzothiazoles with alcohols. Riemschneider[10] reported that alkyl thiocyanate was converted into N-alkylthiocarbamate in the alcohols containing sulfuric acid.

From the present experimental results and literature available, it is presumed that when heated with alcohols, neocyanothiamine (V) is converted into the precursor, N-alkylthiocarbamate (XIX), by the addition of alcohol to its thiocyanato group and (XIX) thereby produced is converted further into thiochrome (VIII) as a result of intramolecular addition with a loss of N-alkylformamide between thiocarbamate group and hydrogen atom in 7-position of the 5,6-dihydropyrimido[4,5-*d*]pyrimidine system. Then N-alkylformamide removed from (V) is decomposed into hydrocyanic acid and alkylamine by pyrolysis.

8) H. Yonemoto: Yakugaku Zasshi, **77**, 1128(1957).
9) W. H. Davies, W. A. Sexton: J. Chem. Soc., **1944**, 11.
10) R. Riemschneider: J. Am. Chem. Soc., **78**, 844(1956).

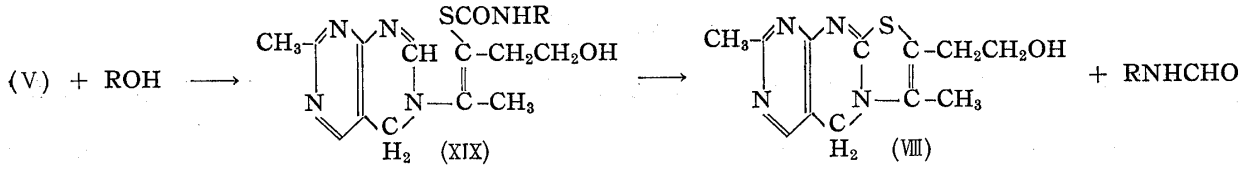


Chart 3.

Zima, *et al.*[3] reported that when heated in ethylene glycol, thiamine disulfide (XV) decomposes into thiochrome (VIII) and thiamine–thiazolone (XX). Sykes, *et al.*[11] suggested a free radical mechanism for the disproportionation of thiamine disulfide in hot, high-boiling hydroxylic solvents, thiochrome (VIII) and thiamine–thiazolone (XX) being produced.

They also stated that the oxidation of thiamine hydrochloride to thiochrome with potassium ferricyanide proceeded through the autoxidisable intermediate, dihydrothiochrome. The initial reaction was considered to be the one-electron change from the sodium salt of the open-chain form to the free thiol radical, followed by the formation of a cyclic radical.[12]

When heated in ethanol, however, the conversion of (V) to thiochrome also occurred in nitrogen atmosphere in a short period of time.

As reported in Part II of this series,[1] when thiochrome is formed from aqueous solution of thiamine by the action of cyanogen bromide and alkali, hydrocyanic acid equivalent to thiochrome is produced. In the case of the thiochrome formation from (V) by heating with ethanol, however, hydrocyanic acid is not produced. Therefore, it might be possible that by the action of sodium hydroxide solution, (V) is converted into thiochrome and hydrocyanic acid as a result of ring-closure between the thiocyanato group and hydrogen atom in 7-position of its 5,6-dihydropyrimido[4,5-*d*]pyrimidine system. However, the addition of sodium hydroxide solution to (V) did not give hydrocyanic acid and thiochrome, but afforded N-〔1-(2-thiacyclobutylidene)ethyl〕-N-〔2-methyl-4-amino-5-pyrimidinyl)methyl〕formamide (VI)[2,8,13] and thiamine.

Accordingly, neocyanothiamine (V) as well as cyanothiamine (IV) can not be intermediate to thiochrome by the action of cyanogen bromide and alkali upon the aqueous solution of thiamine.

Matsukawa, *et al.*[14] proved the fluorescent compound produced by the action of cyanogen bromide and alkali upon the aqueous solution of thiamine to be thiochrome and they suggested that dihydrothiochrome, formed from thiamine by the action of alkali, was oxidized to thiochrome (VIII) with the oxidants such as potassium ferricyanide or cyanogen bromide.[15]

Maier, *et al.*[7] obtained dihydrothiochrome by the addition of 2 equivalents of sodium ethoxide to thiamine in dehydrated ethanol. It was found, however that addition of 2 equivalents of sodium ethoxide to thiamine in dehydrated ethanol, followed by aqueous solution of cyanogen bromide and immediate basification with 30% sodium hydroxide also afforded (VI).

From foregoing experiments, the mechanism of the formation of thiochrome suggested by Sykes, *et al.*[11,12] and Matsukawa, *et al.*[15] leaves some room for reconsideration.

11) P. Sykes, A. R. Todd : J. Chem. Soc., **1951**, 534.
12) P. Nesbitt, P. Sykes : *Ibid.*, **1954**, 4585.
13) T. Kawasaki, T. Tomita, T. Motoyama : Vitamins (Kyoto), **13**, 57(1957).
14) T. Matsukawa, T. Iwatsu : Yakugaku Zasshi, **71**, 14(1951).
15) T. Matsukawa, H. Hirano : Vitamins (Kyoto), **13**, 362(1957).

Experimental

2-Methyl-6-(1-methyl-2-thiocyanato-4-hydroxy-1-butenyl)-5,6-dihydropyrimido[4,5-*d*]pyrimidine (Neocyanothiamine)—Metallic Na (6.9 g.) dissolved in dehyd. EtOH (300 cc.) was added to thiamine hydrochloride (34 g.) in dehyd. EtOH (100 cc.) under cooling with a freezing mixture and in N_2 current. NaCl separated out. To the yellow solution separated from NaCl, BrCN (11 g.) dissolved in dehyd. EtOH (50 cc.) was dropped slowly under cooling with the freezing mixture (ice and NaCl) and colorless needles (16 g.) separated out. This substance showed m.p. 145°(decomp.). Recrystallization from Me_2CO gave colorless needles of m.p. 152.5°(decomp.). *Anal.* Calcd. for $C_{13}H_{15}ON_5S$: C, 53.95; H, 5.23; N, 24.21; S, 11.08. Found : C, 53.93; H, 5.36; N, 24.39; S, 10.99.

To the mother liquor concentrated under a reduced pressure, H_2O was added and crystals (7.0 g.) that separated out were recrystallized from H_2O to crystals of m.p. 80°(decomp.), undepressed on admixture with the hydrate of (IV). Recrystallization from dehyd. EtOH gave (IV), m.p. and mixed m.p. 150°(decomp.).

Paper chromatography*2 of the mother liquor : Thiamine, Rf 0.26 (reddish by Dragendorff reagent and fluorescence by thiochrome reaction); thiamine disulfide, Rf 0.35 (reddish by Dragendorff reagent and fluorescence by thiochrome reaction after treatment with cysteine); thiochrome, Rf 0.45 (reagent and fluorescence); (IV), Rf 0.56 (reddish by Dragendorff reagent and fluorescence by thiochrome reaction after the treatment with cysteine).

Hydrolysis of Neocyanothiamine—a) (V) (1.5 g.) was mixed with *N* HCl (1.5 cc.) under cooling. Crystals (1.1 g.) that separated out were recrystallized from H_2O and melted at 79~80°, showing no depression on admixture with the hydrate of (IV). Recrystallization from dehyd. EtOH gave (IV), m.p. and mixed m.p. 150°(decomp.).

b) (V) (2.0 g.) was dissolved in *N* AcOH (5 cc.), the resultant yellow solution was neutralized with $NaHCO_3$, and crystals (1.97 g.) that separated out were recrystallized from dehyd. EtOH to (IV), m.p. and mixed m.p. 150°(decomp.).

Heating of Neocyanothiamine with Alcohols—a) (V) (2.0 g.) was heated in dehyd. EtOH (10 cc.) on a boiling water bath and allowed to stand in an ice-box. Yellow crystals (0.45 g.) that separated out showed m.p. 229°(decomp.), undepressed on admixture with thiochrome. To the mother liquor concentrated under a reduced pressure, H_2O was added and the crystals (0.9 g.) that separated out were recrystallized from dil. EtOH to thiochrome, m.p. and mixed m.p. 229°(decomp.).

Paper chromatography*2 of the mother liquor : Thiamine, Rf 0.31 (reddish by Dragendorff reagent and fluorescence by thiochrome reaction); thiochrome, Rf 0.45 (orange by Dragendorff reagent and fluorescence); (IV), Rf 0.54 (reddish by Dragendorff reagent and fluorescence by thiochrome reaction after treatment with cysteine); (VI), Rf 0.64 (reddish orange by Dragendorff reagent). CN^- was not detected in the mother liquor of thiochrome. The mother liquor was subjected to distillation and CN^- was found by the Prussian blue reaction in a portion of the distillate. The distillate, after addition of 10% NaOH and warmed on a boiling water bath for 30 min., was extracted with Et_2O. The extract, after adding HCl, was evaporated. The residue was tested by paper chromatography and $EtNH_2$ was detected at Rf 0.21 with ninhydrin.

b) (V) (500 mg.) was heated with MeOH (10 cc.) on a boiling water bath for 20 min. and the reaction mixture was distilled. When some AcOEt was added to the residue, crystals (300 mg.) separated out, which were recrystallized from H_2O to thiochrome, m.p. and mixed m.p. 229°(decomp.). In the distillate of the mother liquor of thiochrome, CN^- was detected by the Prussian blue reaction. The distillate, after warming with 10% NaOH, was extracted with Et_2O, the extract was concentrated after addition of HCl, and the residue was tested by paper chromatography and $MeNH_2$ was detected at Rf 0.14 with ninhydrin.

c) A mixture of (V) (500 mg.) and iso-PrOH (10 cc.) was heated on a boiling water bath in N_2. Thiochrome (200 mg.), m.p. and mixed m.p. 229°(decomp.), was obtained. In the distillate of the mother liquor of thiochrome, CN^- was detected by the Prussian blue reaction. The distillate, after warming with 10% NaOH, was extracted with Et_2O, the extract was concentrated after the addition of HCl, and the residue was tested by paper chromatography and iso-$PrNH_2$ (Rf 0.41) was detected.

d) A mixture of (V) (1.0 g.) and iso-BuOH (10 cc.) was heated on a boiling water bath under N_2. Thiochrome (400 mg.), m.p. and mixed m.p. 229°(decomp.), separated out.

Reaction of (V) with NaOEt—(V) (1.0 g.) was added to EtOH (10 cc.) solution of metallic Na (100 mg.). (V) dissolved assuming a yellow color. After addition of KSCN (1.0 g.), the reaction mixture was concentrated under a reduced pressure, H_2O was added to the residue, and left standing. Thiamine thiocyanate (250 mg.), m.p. and mixed m.p. 186°(decomp.), was obtained. Concentration of the mother liquor *in vacuo* and addition of H_2O gave (VI) hydrate, m.p. and mixed m.p. 109.5°. On drying, it formed crystals of 136°(decomp.), undepressed on admixture with (VI).

*2 $AcOH \cdot BuOH \cdot H_2O = 1 : 4 : 5$

Decomposition of Neocyanothiamine with NaOH—a) (V) (2.0 g.) was placed in ice-cooled 10% NaOH (10 cc.) and crystals (1.48 g.) that separated out were dissolved in a small amount of EtOH and decolorized. When the solution was allowed to stand after adding H_2O, (VI) hydrate, m.p. and mixed m.p. 109.5°, separated out. The crystals, after drying over P_2O_5 under a reduced pressure, melted at 136° (decomp.), undepressed on admixture with (VI). In the mother liquor, thiamine, Rf 0.38 (reddish by Dragendorff reagent and fluorescence by thiochrome reaction); thiochrome, Rf 0.44 (fluorescence), and (VI), Rf 0.71 (reddish orange by Dragendorff reagent), were detected.
b) (V) (0.6 g.) was placed in *N* NaOH (20 cc.), the resultant yellow-green solution was allowed to stand, and the yellow scaly crystals that separated out were recrystallized from 10% EtOH to (VI), m.p. and mixed m.p. 136° (decomp.).

Action of BrCN and NaOH on Thiamine Hydrochloride in EtOH—The solution of metallic Na (0.23 g.) in EtOH (10 cc.) was added to thiamine hydrochloride (1.7 g.) in dehyd. EtOH (10 cc.). NaCl separated out. After the filtrate was discolored by adding BrCN (0.6 g.), 10% NaOH (20 cc.) was added. The mixture was concentrated under a reduced pressure and left standing. Crystals (0.5 g.) that separated out were recrystallized from 10% EtOH to the crystals of m.p. 109.5°, undepressed on admixture with the hydrate of (VI). Drying over P_2O_5 under a reduced pressure gave (VI), m.p. and mixed m.p. 136° (decomp.).

Measurement of Absorption Spectra—Hitachi EPU Model-2A Spectrophotometer (quartz cell: 1 cm.) and Beckman Model G pH-meter were used. Thiamine hydrochloride of National Hygienic Laboratory Standard was dried at 105° for 2 hr. and used without treatment.

Absorption Spectra—a) Thiamine hydrochloride (3×10^{-6} mole) was placed in a flask of 100-cc. capacity, dissolved in 0.1*N* EtONa (1 cc.) in N_2, and made up to the mark with dehyd. EtOH. The absorption spectrum of this solution was measured.

Compounds (IV)~(VII) and (IX)~(XIII) were measured in the same way.
b) Thiamine hydrochloride (5.215 mg.) was dissolved in 0.1*N* EtONa (5 cc.) in N_2 and the volume was made up to 10 cc. with EtOH. This solution (2 cc.) was taken in 100-cc. flask which had been substituted with N_2 and diluted with H_2O, then 0.1*N* HCl was added to adjust pH. The content was made up to 100 cc. After making it certain that pH of the solution did not change, the absorption spectra were measured.
c) Aqueous solution of thiamine (100 γ in 2 cc.) was poured into 0.2*N* NaOH (8 cc.), where N_2 had been introduced, and the absorption spectrum was measured within 5 min. On the other hand, this solution was measured once more after standing for 90 min.

Determination of Thiamine—(V) and (IV) were dissolved in 0.1*N* EtONa (1 cc.) in N_2 and made up to 100 cc. with EtOH. The resulting solution (2 cc.) was diluted with KCl·HCl and then the determination of thiamine in these solutions was carried out fluorometrically using BrCN method.

Determination of Thiochrome—The above-mentioned 0.1*N* EtONa solution was diluted with KCl·HCl, strongly basified by addition of 30% NaOH, and then extracted with iso-BuOH. Thiochrome was determined by fluorometry and the results are shown in Table II.

Table II.

Sample	Thiamine (%)	Thiochrome (%)
Neocyanothiamine (V)	91.47	1.04
Cyanothiamine (IV)	62.87	0.44

The author is deeply grateful to Prof. T. Takahashi of the University of Kyoto for his kind guidance and wishes to express thanks to Dr. T. Kariyone, Chief of the National Hygienic Laboratory, Dr. S. Ogawa, Chief of Osaka Branch Laboratory, and Dr. T. Nakajima, Chief of the Department of this Laboratory, for their encouragements. He is also indebted to Dr. Y. Sawa, Research Laboratory of Shionogi & Co., Ltd., for supplying thiamine analogs, to Dr. T. Matsukawa, for the donation of pseudo-dihydrothiamine, to the members of Research Laboratory of Shionogi & Co., for the measurement of infrared absorption spectra, and to the members of Microanalytical Center of the University of Kyoto for elemental analyses.

Summary

From the measurement of absorption spectrum of thiamine dissolved in sodium ethoxide, it is considered that thiamine is converted into the cyclic thiol-form (III) as a result of intramolecular addition of 4-amino group in the pyrimidine ring and the thiazolium ring, losing hydrochloric acid and opening the thiazole ring. The reaction of 0.1 mole of cyanogen bromide with the cyclic thiol-type thiamine, produced from 0.1 mole of thi-