amine hydrochloride in ethanol suspension by the action of 0.3 mole of sodium ethoxide, gives 2-methyl-6-(1-methyl-2-thiocyanato-4-hydroxy-1-butenyl)-5,6-dihydropyrimido[4,5-*d*]pyrimidine (V). This substance is hydrolyzed into N-(1-methyl-2-thiocyanato-4-hydroxy-1-butenyl)-N-〔(2-methyl-4-amino-5-pyrimidinyl)methyl〕formamide (IV) by dilute acid and converted into thiochrome through thiolcarbamic ester derivative (XIX) on heating with alcohols and also decomposed into N-〔1-(2-thiacyclobutylidene)ethyl〕-N-〔(2-methyl-4-amino-5-pyrimidinyl)methyl〕formamide (VI) and thiamine by the action of sodium hydroxide. On the basis of these experimental results, the mechanism of the formation of thiochrome from thiamine is discussed with that suggested by Sykes, *et al.* and by Matsukawa, *et al.*

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63. Shizuka Kasahara: Action of Cyanogen Bromide on Thiamine. IV.[1] Studies on 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])

In the previous paper of this series,[1] it was shown that addition of sodium ethoxide to the suspension of thiamine hydrochloride (I) followed by the application of cyanogen bromide converted (I) to the cyclized thiol-form (III) and then to 2-methyl-6-(1-methyl-2-thiocyanato-4-hydroxy-1-butenyl)-5,6-dihydropyrimido[4,5-*d*]pyrimidine (V). In this paper, results of further studies on various reactions of (V) will be described.

When aqueous suspension of (V) was kept at 2° overnight, (V) slowly dissolved to form a yellow solution, separating out N-(1-methyl-2-thiocyanato-4-hydroxy-1-butenyl)-N-〔(2-methyl-4-amino-5-pyrimidinyl)methyl〕formamide (cyanothiamine) (IV) (7.06%). Addition of potassium thiocyanate to the mother liquor gave thiamine thiocyanate and after concentration, N-〔1-(2-thiacyclobutylidene)ethyl〕-N-〔(2-methyl-4-amino-5-pyrimidinyl)-methyl〕formamide (thiamine anhydride) (VI) was obtained in a crystalline form. In hot water, however, (V) dissolved in a short time and when cooled, thiochrome (VIII) precipitated as crystals. From the mother liquor of (VIII), thiamine thiocyanate and (VI) were obtained in the same way.

In a cold place, the yield decreased in the order of thiamine, (IV), (VI), and (VIII), and the yield of (VIII) was so low as to be detected only by paper chromatography. In hot water, however, yield of (VIII) increased and it was obtained as crystals.

When (V) was slowly dissolved in 50% ethanol at 2°, a similar yellow solution was obtained. The solution was concentrated and (IV) separated out in 42.6% yield. Addition of potassium thiocyanate to the filtrate gave thiamine thiocyanate.

In Part III of this series, it was reported that the yellow color produced by the action of alkali on thiamine was due to formation of a cyclized thiol-form (III) of thiamine.[1] As (V) shows a similar yellow coloration when dissolved in water or 50% ethanol, it is believed that the reaction of (V) with water gives the cyclized thiol-form (III) of thiamine, which affords thiamine thiocyanate by addition of potassium thiocyanate.

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

1) Part III: This Bulletin, 8, 340(1960).

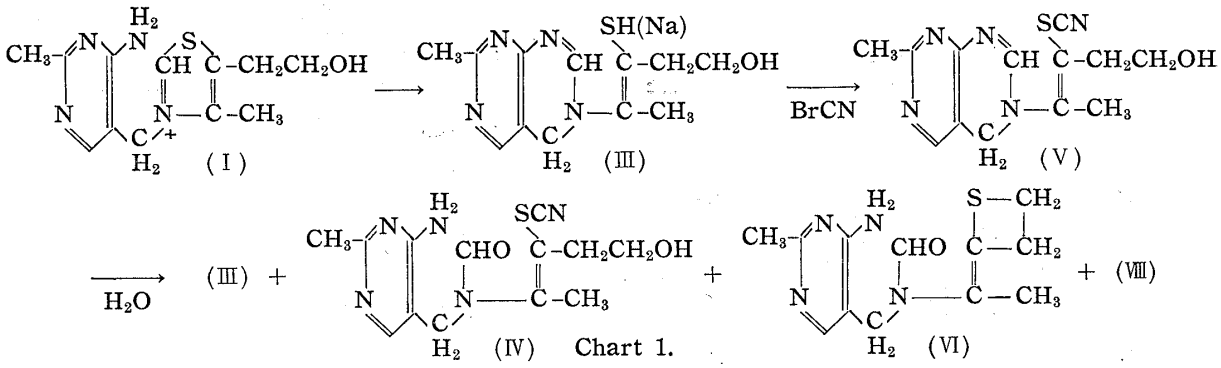

Chart 1.

It was shown that when heated with alcohols, (V) converted into N-alkylthiocarbamate (XIX) as a result of the addition of alcohol to thiocyanato group of (V) and that (XIX) was decomposed to thiochrome (VIII) and N-alkylformamide.[1] It has also been considered that 2-thiocyanoketones change to 2-hydroxythiazole through an intermediate produced as a result of the addition of water to thiocyanato group.[2] It may, therefore, be concluded that when dissolved in hot water, the thiocyanato group in (V) first changes to thiolcarbamate (XIX) by the addition of water and then cyclization takes place to give (VIII) in the same way as observed in the case of alcoholic solution of (V).

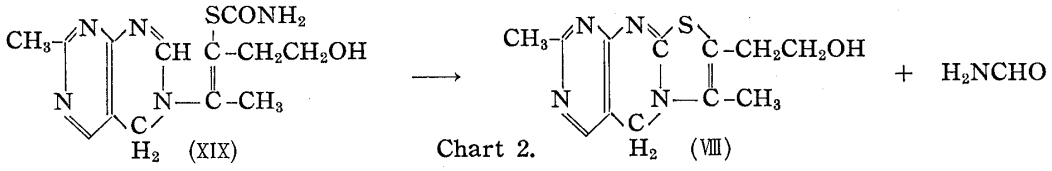

Chart 2.

Ross[3] obtained benzene disulfide, ethylthiobenzene, and thiophenol by the reaction of thiocyanobenzene with sodium ethoxide. Davies, *et al.*[4] similarly prepared 2-mercaptobenzothiazole and 2-ethylthiobenzothiazole from 2-thiocyanobenzothiazole, while Takahashi, *et al.*[5] reported that the reaction of 3-nitro-4-thiocyanopyridine with 2*N* sodium hydroxide or sodium ethoxide gave a mercapto compound and they interpreted the reaction mechanism as shown in Chart 3.

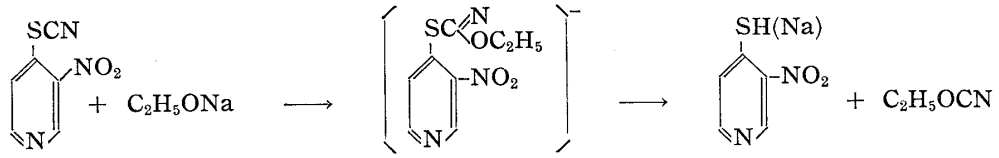

Chart 3.

It was found that thiochrome (VIII) was formed through the intermediate (XIX) from (V), that the decomposition of (V) to (III) was also effected more easily by use of sodium ethoxide or sodium hydroxide, and that the yield of thiamine from (V) by the action of water decreased in 50% ethanol.

From these facts, the mechanism of the formation of thiamine from (V) is assumed to be as follows : The action of OH^- on thiocyanato group in (V) gives an intermediate (IX) which is decomposed into thiamine (III) and cyanic acid. In order to prove the presence of cyanic acid which may have been produced by degradation of (IX) to (III), the solution

2) R. Adams : Org. Reactions, **4**, 377(1951).
3) J. Ross : J. Am. Chem. Soc., **56**, 727(1934).
4) W. H. Davies, W. A. Sexton : J. Chem. Soc., **1944**, 11.
5) T. Takahashi, K. Ueda : This Bulletin, **2**, 78, 196(1954).

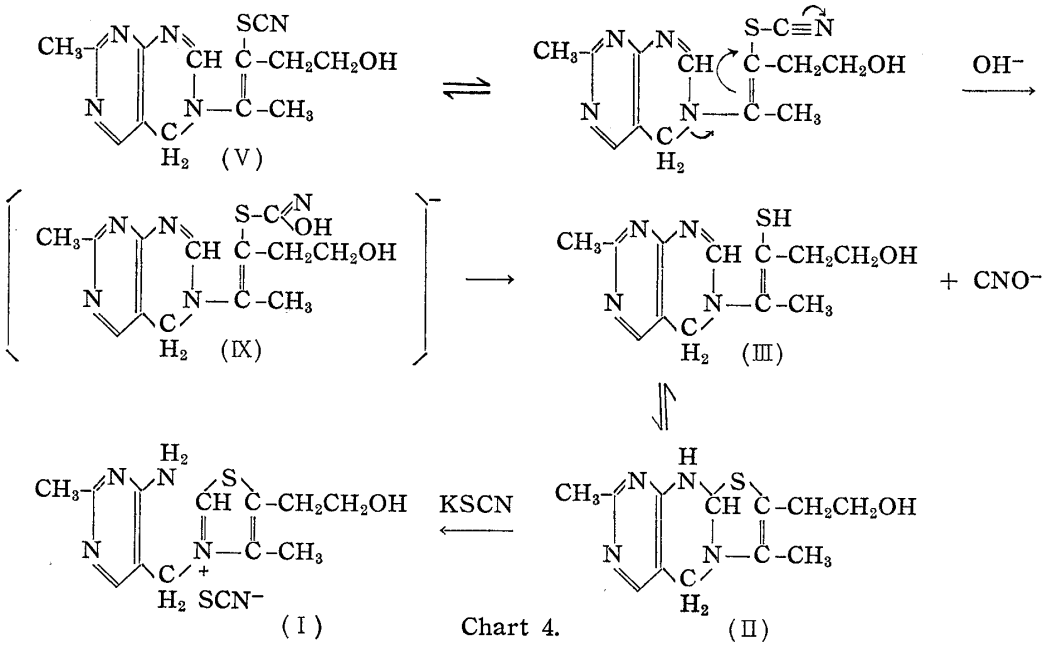

Chart 4.

was distilled and ammonia was detected in the distillate. By adding ammonia to the solution of (V) and then heating, cyanic acid was also detected as urea.

The yield of thiamine anhydride (VI) from (V) by the action of cold water is higher than that from (IV) and thiamine thereby obtained is of type (III). This fact shows that (VI) is formed from (V) without going through (IV).

The hydrolysis of neocyanothiamine (V) with a small amount of hydrochloric acid (ca. 0.1 equivalent) also gave (IV) in a good yield together with an unknown substance of m.p. 194°(decomp.) as a by-product. It is therefore considered that a proton attacks the nitrogen atom in 6-position of the pyrimidine ring in 5,6-dihydropyirmido[4,5-*d*]pyrimidine of (V) and the onium compound (X) so formed, is converted to the hydrochloride of (IV) by the action of water. The hydrochloric acid combined with (IV) is taken up by (V), since (IV) is weaker in basicity than (V), and (IV) separates out as a free base. Furthermore, when (V) was dissolved in strong acid like 10% hydrochloric acid and heated, the decomposition of (IV) occurred and 2-methyl-4-amino-5-aminomethylpyrimidine (XI), formic acid, and a small amount of 2-imino-3-(2-methyl-4-amino-5-pyrimidinyl)methyl-3a-methyl-3a,5,6,6a-tetrahydrofuro[2,3-*d*]thiazole (XII)[6] were produced.

On the other hand, new interesting results were observed concerning the reaction of (V) with acid under anhydrous condition.

Complete mixing of (V) with dehydrated crystalline formic acid yielded a yellow mixture. Addition of sodium hydrogencarbonate and water gave white crystals (substance B) of m.p. 194°(decomp.). In this case, the reaction of (V) with glacial acetic acid also gave substance B. The mother liquor of substance B, when made strongly alkaline with potassium hydroxide, afforded (XII) and thiamine anhydride (VI). When the above-stated formic acid solution of (V) was subjected to paper chromatography (AcOH-BuOH-H_2O= 1:4:5), (IV) was detected at Rf 0.57 but not (VI). It was thereby concluded that a part of (V) was converted by formic acid into (IV) and further to (VI) by alkali.[7] From the result of elemental analyses and molecular weight determination, substance B was represented as $C_{13}H_{17}O_2N_5S$, corresponding to (IV) in which one molecule of water was introduced into (V).

6) H. Yonemoto : Yakugaku Zasshi, **78**, 472(1958).
7) *Idem* : *Ibid.*, **77**, 1128(1957); Part I : *Ibid.*, **77**, 1133(1957).

The action of dilute hydrochloric acid or sodium hydroxide on substance B afforded (XIII) as colorless crystals and formic acid in a good yield. Substance B is not a formate but a formamide, since it was sparingly soluble in water, but easily soluble in dilute sodium hydroxide. The ultraviolet absorption spectra of substance B both in chloroform (Fig. 1) and ethanol solution (Fig. 2) were similar to that of (XII), though rather hypsochromic.

By comparing the ultraviolet absorption spectra of 2-methyl-4-amino-5-acetamidomethylpyrimidine (XX) with that of 2-methyl-4-acetamido-5-acetamidomethylpyrimidine (XXI), Takamizawa[8] reported that the difference between them was almost negligible, though the latter is somewhat hypsochromic.

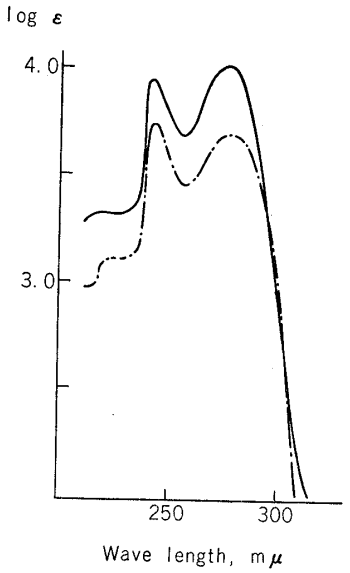

Fig. 1. Ultraviolet Absorption Spectra (in $CHCl_3$)
——— compound (XIII)
—·—·— compound (XII)

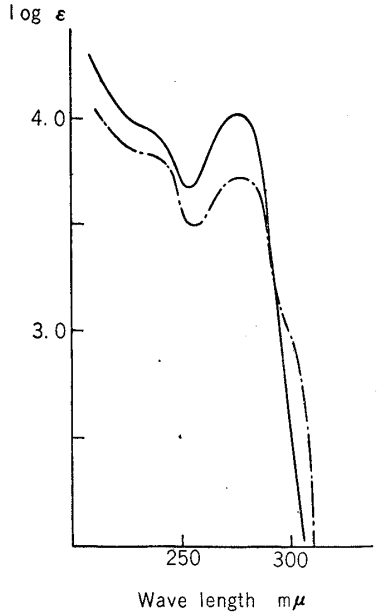

Fig. 2. Ultraviolet Absorption Spectra (in EtOH)
——— compound (XIII)
—·—·— compound (XII)

The infrared absorption spectrum of substance B did not exhibit absorption bands near 1654 cm^{-1} due to deformation frequency of primary amino group, and absorption near 1050 cm^{-1} due to stretching frequency for C–O of primary alcohol, but it showed one absorption band near 3370 ($CHCl_3$) and 3230 cm^{-1} (Nujol). Thus, it is assumed that substance B possesses a secondary amino group. Further, strong absorption bands were present at 1710 ($CHCl_3$) and 1701 cm^{-1} (Nujol).

Yoshida, *et al.*[9] reported that in the case of N-acyl derivatives (XXII and XXIII) of thiothiamine and its analogs, the absorption above 1700~1720 cm^{-1} was taken as the absorption of monosubstituted amide-I. Therefore absorption band at 1710 ($CHCl_3$) or 1701 cm^{-1} (Nujol) in substance B can be attributed to monosubstituted amide-I. Besides these bands of amino or amide group, the infrared absorption spectrum of substance B is in good agreement with that of (XII).

From the foregoing facts and literature available, substance B is presumed to be a formamide, namely, 2-imino-3-(2-methyl-4-formamido-5-pyrimidinyl)methyl-3a-methyl-3a,5,6,6a-tetrahydrofuro[2,3-*d*]thiazole (XIII).

8) A. Takamizawa : *Ibid.*, **74**, 748(1954).
9) S. Yoshida, R. Takasaki : This Bulletin, **6**, 527(1958).

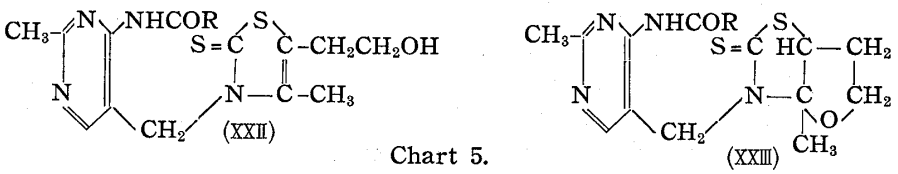

Chart 5.

The cleavage of (V) by mineral acid under anhydrous condition was examined. When ethanolic hydrochloric acid was added, (V) dissolved once and gave white crystals on standing. Dissolving of this crystal in water and neutralization with sodium hydrogencarbonate gave (XII) in 57.9% yield. On the other hand, (XIII) was obtained if pyridine was used instead of sodium hydrogencarbonate. This fact shows that under anhydrous conditon, (V) changes to its chloride (XV) by the action of hydrochloric acid and subsequently to hydrochloride of (XIII) after addition of water. Hydrochloride of (XIII) thus formed was hydrolyzed to (XII) and formic acid by the action of hydrochloric acid.

By hydrolysis with aqueous acid, (V) afforded crystals of m.p. 194°(decomp.) in 5.65% yield together with (IV). By mixed fusion, this crystal was identified with (XIII).

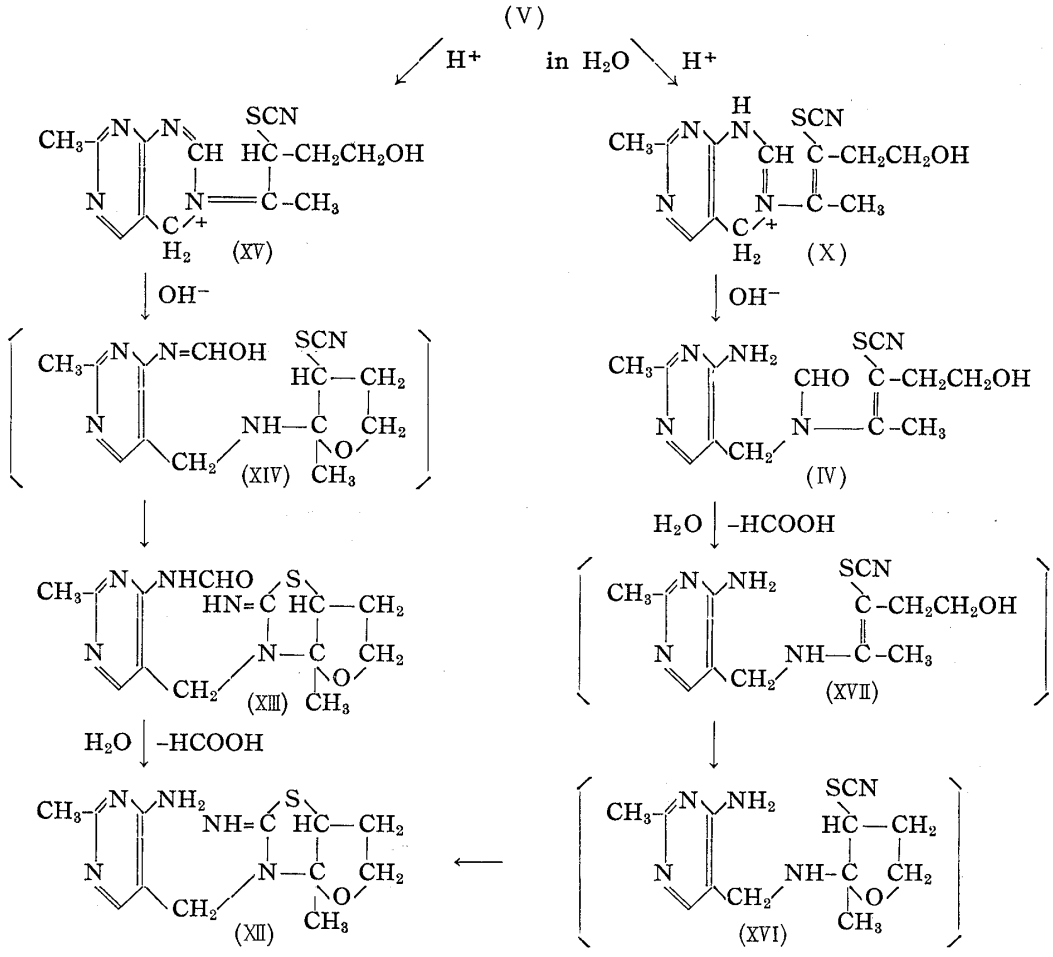

Chart 6.

From these experimental results, it is assumed that (V) may dissolve as (XV) or as (X) in acid medium and the equilibrium between the two forms shifts toward (XV) under anhydrous condition but toward (X) under hydrous condition. When these salts are neutralized, (IV) seems to be produced from (X), and (XIII) from (XV) through (XIV).

When (XIII) was dissolved in water with warming, crystals did not separate out even

after cooling. The paper chromatography*[2] of the resulting solution revealed two spots at Rf 0.38 (XII) and Rf 0.70. When exposed to the lights near 254 mμ, the spot at Rf 0.70 showed a strong violet fluorescence, but the fluorescence was rather weak by the lights near 350 mμ. From the coincidences both in Rf value and fluorescence, this spot was assumed to be that of 2,8a-dimethyl-6,7,5a,8a-tetrahydrofuro[2,3-*h*]thiochromine (VII).[10] When the solution was evaporated to dryness and ethyl acetate was added, colorless prisms of m.p. 150°(decomp.) were obtained. This was identified with (VII) from mixed fusion, elemental analyses, and the fact that it changes to thiochrome (VIII) by heating with dilute hydrochloric acid.

When (XIII) was warmed in 20% acetic acid and examined by paper chromatography, (XII) and (VII) were detected, but (VIII) gradually increased in amount. The solution, after heating for 90 minutes, was neutralized with sodium hydrogencarbonate and (VIII) was obtained. In a cold place, however, (XIII) was hydrolyzed with 20% acetic acid to (XII) and formic acid. Furthermore, when heated in nonaqueous solvents, such as ethanol, isobutanol, or acetone, hydrolysis of (XIII) to (XII) did not occur, (XIII) remained almost unchanged, and separated out on cooling.

Yonemoto[6] reported that (XII) was first converted to (VII) on heating with water or with aqueous solution of weak acid and then the tetrahydrofuran ring in (VII) was opened to give thiochrome (VIII).

From these results, it can be considered that when heated with water, (XIII) is hydrolyzed into (XII) and formic acid. The formate of (XII) thus produced is further converted into (VII) as a result of ring closure with a loss of ammonia between 4-amino group in pyrimidine and 2-imino group in thiazole ring. The presence of formic acid and ammonia which may be produced by the disproportionation of (XIII) to (VII) was proved with chromotropic acid and Nessler's reagent, respectively.

Conversion of (XIII) to (VII) occurs more easily than in the case of (XII), when heated with water. This is probably due to the fact that the solution becomes weakly acid by the presence of formic acid produced by hydrolysis of (XIII).

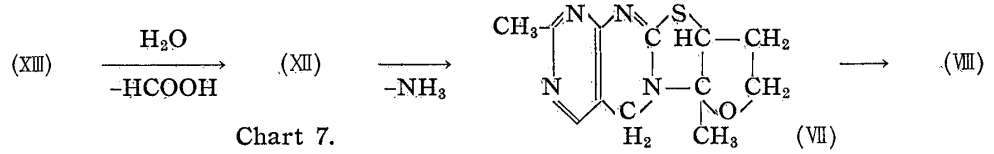

Chart 7.

By reduction with cysteine at room temperature, neocyanothiamine (V) was completely reduced into thiamine, while most of cyanothiamine (IV) remained unchanged.

Cyanothiamine (IV) is so stable in water or alcohol that it can be recrystallized from these solvents, but a prolonged heating with ethanol gives thiamine anhydride (VI) together with a small amount of thiamine-thiazolone (XVIII) and thiochrome (VIII). Thiamine anhydride (VI) can also be produced from (IV) by heating with acetone or water.

These facts indicate that the chemical properties of thiocyanato group in (V) are different from that in (IV). In other words, (V) has a strong electron-doner group such as tertiary amine in β-position of thiocyanato group, but (IV) has no such strong electron-donor group. It is therefore assumed that (V) readily takes electron-pair donor conjugation system and as a result, the reaction of water or alcohol with thiocyanato group becomes easy. When basified, however, the thiocyanato group in (IV) is also attacked by OH^- and then (IV) changes to (XXV) through (XXIV).

*[2] Developing agent : Water-saturated BuOH. When a mixture of AcOH-BuOH-H_2O was used as a developing agent, (XIII) was easily hydrolyzed into (XII) by the action of acetic acid during development.

10) H. Hirano : Yakugaku Zasshi, **74**, 59(1954).

It may therefore be concluded that activity of the thiocyanato group in (V) is higher than that in (IV). This is an interesting fact, considering the mechanism of the disproportionation of thiamine disulfide on heating with hydroxylic solvents[11] and biological action of thiamine.

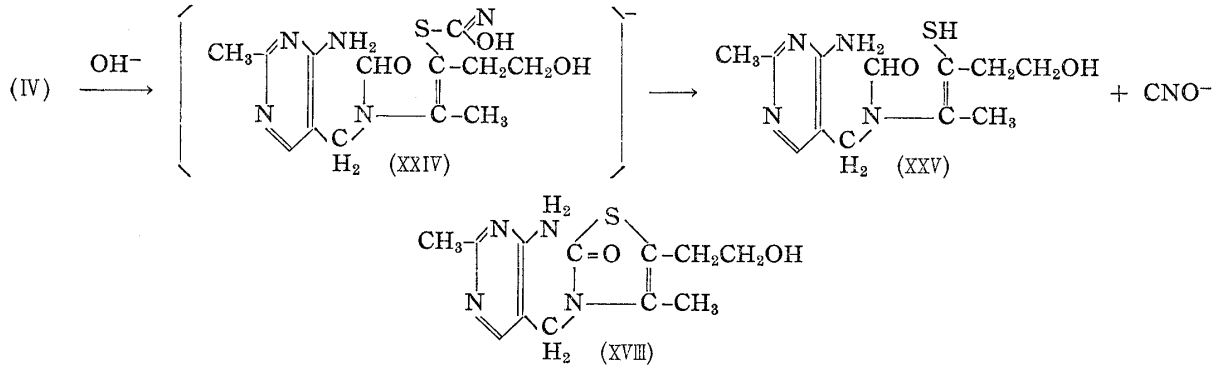

Chart 8.

Experimental

Reaction of Neocyanothiamine with Water—a) (V) (2.0 g.) was suspended in H_2O (50 cc.) under cooling with ice and allowed to stand overnight in a refrigerator (at 2°), when (V) slowly dissolved to form a yellow solution. After concentration under a reduced pressure, crystals (150 mg.) that separated out were recrystallized from H_2O to crystals of m.p. 78~80°(decomp.), undepressed on admixture with the hydrate of (IV). KSCN (2.0 g.) was added to the mother liquor and crystals (570 mg.) that separated out were recrystallized from H_2O to crystals of m.p. 186°(decomp.), undepressed on admixture with thiamine thiocyanate. The mother liquor was once more concentrated under a reduced pressure and crystals (400 mg.) separated out. Recrystallizatiom from H_2O gave crystals of m.p. 109.5°, undepressed on admixture with the hydrate of (VI).

b) A mixture of H_2O (40 cc.) and (V) (2.0 g.) was heated for 20 min. on a boiling water bath. The compound dissolved to form a red-brown solution which was allowed to stand in a refrigerator and (VIII), m.p. and mixed m.p. 229°(decomp.) (400 mg.), separated out. KSCN (2.0 g.) was added to the mother liquor and thiamine thiocyanate, m.p. and mixed m.p. 186°(decomp.), separated out. The mother liquor was concentrated once more, the crystals (400 mg.) that separated out were dissolved in EtOH and H_2O was added to the solution by which the hydrate of (VI), m.p. and mixed m.p. 109.5°, separated out. The crystals became (VI), m.p. and mixed m.p. 136°(decomp.), after drying over P_2O_5 under a reduced pressure.

c) (V) (2.5 g.) was suspended in 50% EtOH (20 cc.) under cooling with ice and allowed to stand overnight in a refrigerator. The compound slowly dissolved to form a yellowish green solution which was concentrated under a reduced pressure and the hydrate of (IV) (1.2 g.) separated out. KSCN (2.0 g.) was added to the filtrate and thiamine thiocyanate, m.p. and mixed m.p. 186°(decomp.), separated out.

Decomposition of (V) with HCl—a) (V) (5.0 g.) was suspended in 0.1*N* HCl and allowed to stand overnight in a refrigerator. The insoluble matter (4.0 g.) was collected. This substance showed m.p. 79°(decomp.), undepressed on admixture with the hydrate of (IV). Recrystallization from EtOH gave crystals of (IV), m.p. and mixed m.p. 150.5°(decomp.). When the filtrate of (IV) was neutralized with $NaHCO_3$, white crystals separated out, which were washed with hot H_2O. Recrystallization of the residue (0.3 g.) from Me_2CO gave needles of m.p. 194°(decomp.).

b) (V) (1.0 g.) was dissolved in 10% HCl (10 cc.) and heated on a boiling water bath for 10 min. A portion of the reaction mixture was subjected to steam distillation and HCOOH was detected in the distillate with chromotropic acid. Concentration of the reaction mixture under a reduced pressure, followed by addition of warm EtOH gave 2-methyl-4-amino-5-aminomethylpyrimidine dihydrochloride (120 mg.), m.p. and mixed m.p. 268°(decomp.). The mother liquor was concentrated under a reduced pressure, the residue was dissolved in H_2O, and addition of KOH to the solution afforded crystals (220 mg.). Recrystallization from 40% EtOH gave crystals of m.p. 215°(decomp.), undepressed on admixture with (XII).

11) P. Sykes, A. R. Todd: J. Chem. Soc., **1951**, 534; P. Nesbitt, P. Sykes: *Ibid.*, **1954**, 4581; P. Sykes: *Ibid.*, **1955**, 2390.

2-Imino-3-(2-methyl-4-formamido-5-pyrimidinyl)methyl-3a-methyl-3a,5,6,6a-tetrahydrofuro[2,3-*d*]thiazole (XIII)—a) (V) (10 g.) was mixed well with freeze-dried HCOOH (10 cc.). Resulting yellow solution was allowed to stand in an ice-box for 1 hr., then mixed well with $NaHCO_3$ (30 g.), H_2O was added gradually, and white crystals separated out with evolution of CO_2. The crystals were dissolved in hot Me_2CO, decolorized, and allowed to stand, by which colorless needles (6.5 g., yield, 61.18%), m.p. 187~190.5°(decomp.), separated out. Recrystallization from Me_2CO gave crystals of m.p. 194°(decomp.). *Anal.* Calcd. for $C_{13}H_{17}O_2N_5S$ (XIII): C, 50.80; H, 5.80; O, 10.41; N, 22.79; S, 10.43; mol. wt., 307. Found: C, 50.87; H, 5.77; O, 10.36; N, 22.91; S, 10.37; mol. wt., 300±10 (solvent, $CHCl_3$). UV λ_{max}^{EtOH}: 276 mμ(log ε 4.00). $\lambda_{max}^{CHCl_3}$ mμ(log ε): 242 (3.95), 277 (4.02).

On admixture of the above product with the crystals of m.p. 194°(decomp.) obtained from (V) by treatment with HCl, no depression of m.p. was recognized. The mother liquor was made strongly alkaline with KOH and crystals (3.0 g.) separated out. Yield, 28.97%. Recrystallization from 40% EtOH gave (XII), m.p. and mixed m.p. 215°(decomp.).

The filtrate separated from (XII) was shaken with iso-BuOH. The extract was dried over anhyd. Na_2SO_4 and concentrated under a reduced pressure. The residue was dissolved in a small amount of EtOH. Addition of hot H_2O to the solution and standing gave (VI) hydrate as colorless scaly crystals, m.p. and mixed m.p. 109.5°. When dried over P_2O_5, (VI) melted at 136°(decomp.).

b) (V) (2.0 g.) was added to frozen AcOH (3 cc.) and dissolved by mixing. After excess of AcOH was removed under a reduced pressure, the solution was neutralized with $NaHCO_3$ and crystals (0.98 g.) separated out. These crystals were washed with hot H_2O and the hydrate, m.p. and mixed m.p. 79~80°(decomp.), of (IV) separated out from the cooled filtrate. Recrystallization of the residue (850 mg.) from Me_2CO gave crystals of m.p. 194°(decomp.), undepressed on admixture with the crystals obtained from (V) by treatment with HCOOH.

Cleavage of (V) by EtOH·HCl—a) (V) (1.0 g.) was dissolved in 8% EtOH·HCl (5 cc.) and allowed to stand overnight. White crystals separated out which were dissolved in H_2O and neutralized with $NaHCO_3$. White crystals (0.6 g.) separated out. Recrystallization from 40% EtOH gave crystals of m.p. 215°(decomp.), undepressed on admixture with (XII) obtained from (IV). *Anal.* Calcd. for $C_{12}H_{17}ON_5S$: C, 51.59; H, 6.13; N, 25.07. Found: C, 51.78; H, 6.25; N, 24.95.

b) (V) (2.0 g.) was dissolved in 8% EtOH·HCl (8 cc.), left to stand overnight, and neutralized with pyridine. After being concentrated under a reduced pressure, $NaHCO_3$ was added, followed by H_2O, whereby crystals separated out. Recrystallization from Me_2CO gave crystals of m.p. 194°(decomp.), undepressed on admixture with (XIII) obtained from (V) by the action of HCOOH.

Decomposition of (XIII) by HCl—(XIII) (300 mg.) was dissolved in *N* HCl (2 cc.) and the solution was left to stand overnight. The solution was neutralized with $NaHCO_3$ and white crystals (290 mg.) separated out. Recrystallization from 40% EtOH gave crystals of m.p. 215°(decomp.), undepressed on admixture with (XII) obtained from (IV). *Anal.* Calcd. for $C_{12}H_{17}ON_5S$: C, 51.59; H, 6.13; N, 25.07. Found: C, 51.77; H, 6.20; N, 24.85. λ_{max}^{EtOH}: 277 mμ(log ε 3.72). $\lambda_{max}^{CHCl_3}$ mμ(log ε): 242 (3.74), 278 (3.69).

Decomposition of (XIII) by NaOH—(XIII) (300 mg.) dissolved easily in *N* NaOH (1 cc.) and white crystals (250 mg.) separated out when left to stand. Recrystallization from 40% EtOH, then from dehyd. EtOH, gave crystals of m.p. 215°(decomp.), undepressed on admixture with (XII). *Anal.* Calcd. for $C_{12}H_{17}ON_5S$: C, 51.59; H, 6.13; N, 25.07. Found: C, 51.85; H, 6.38; N, 24.85.

Heating of (XIII) with H_2O—a) A suspension of (XIII) (1.0 g.) in H_2O (20 cc.) was heated for 90 min. and the solution was evaporated to dryness. The residue was dissolved in AcOEt with heating and left to stand until cool. White prisms (6.0 g.) separated out, which were recrystallized from a mixture of AcOEt and EtOH. The product half melted at 120° and melted completely with decomposition at 150°. No depression of m.p. was shown on admixture with (VII). *Anal.* Calcd. for $C_{12}H_{14}ON_4S\cdot\frac{1}{2}H_2O$ (VII): C, 53.11; H, 5.57; N, 20.65. Found: C, 53.11; H, 5.98; N, 20.62.

b) Above-stated crystals (100 mg.) of m.p. 150°(decomp.) were dissolved in 10% HCl and heated for 10 min. After cool, KOH was added and crystals separated out. Recrystallization from H_2O gave thiochrome (VIII) as pale yellow prisms, m.p. and mixed m.p. 229°(decomp.).

c) (XIII) (100 mg.) was dissolved in H_2O (5 cc.) by heating. The solution was made weakly acid by addition of dil. H_2SO_4 and was subjected to steam distillation. In the distillate, formic acid was detected with chromotropic acid.

d) (XIII) (100 mg.) was dissolved in H_2O (5 cc.) with heating and the solution was placed in a well-stoppered container in which 0.1*N* HCl had been placed. After NaOH was added to the solution of (XIII), the container was left to stand overnight. When Nessler reagent was added to the above 0.1*N* HCl solution, brown precipitate was produced.

Decomposition of (XIII) with 20% AcOH—a) A solution of (XIII) (300 mg.) dissolved in 20% AcOH was heated. The resulting solution gradually colored yellow. The reaction mixture was examined by paper chromatography, changing the heating period of time. When heated for 10 min., (XII), Rf 0.33 (orange by Dragendorff reagent), and (VII), Rf 0.70 (fluorescence and orange-red by Dragendorff reagent), were detected. When heated for 40 min., (XII), Rf 0.33 (orange by Dragendorff reagent), (VIII),

Rf 0.44 (fluorescence and orange by Dragendorff reagent), and (VII), Rf 0.69 (fluorescence and orange-red by Dragendorff reagent), were detected. When the heating period was extended to 90 min., (XII), Rf 0.33 (orange by Dragendorff reagent, but in lower concentration than those in above cases), (VIII), Rf 0.45 (fluorescence and orange by Dragendorff reagent, but in higher concentration than those in above cases), and (VII), Rf 0.68 (fluorescence and orange-red by Dragendorff reagent), were detected. When the reaction mixture was neutralized with $NaHCO_3$, pale yellow crystals separated out. Recrystallization from 40% EtOH gave thiochrome (VIII), m.p. and mixed m.p. 229°(decomp.).

b) A solution of (XIII) (200 mg.) dissolved in 20% AcOH (5 cc.) was allowed to stand overnight, the solution was neutralized with $NaHCO_3$, and crystals (0.1 g.) separated out. Recrystallization from dehyd. EtOH gave (XII), m.p. and mixed m.p. 215°(decomp.).

Heating of (XIII) with iso-BuOH—A mixture of (XIII) (200 mg.) and iso-BuOH (6 cc.) was heated for 3 hr., cooled, and crystals (120 mg.) separated out. The crystals showed m.p. 194°(decomp.), undepressed on admixture with (XIII).

Reduction with Cysteine—a) (V) (5 mg.) was dissolved in 1% cysteine hydrochloride (0.5 cc.) and the solution was immediately tested by paper chromatography. Thiamine, Rf 0.23, thiochrome, Rf 0.36, and an unknown substance, Rf 0.57, were detected.

b) (IV) (5.0 mg.) was dissolved in 1% cysteine hydrochloride (0.5 cc.) and the solution was immediately tested by paper chromatography. Thiamine, Rf 0.22, (IV) hydrochloride, Rf 0.38, and (IV), Rf 0.52, were detected.

Decomposition of Cyanothiamine (IV) by Heating—a) A mixture of (IV) (2.0 g.) and EtOH (10 cc.) was heated on a boiling water bath for 8 hr. Colorless needles (200 mg.) separated out, which were recrystallized from dil. EtOH to crystals of m.p. 237°(decomp.), undepressed on admixture with thiamine-thiazolone (XVIII). Concentration of the mother liquor and addition of AcOEt gave thiochrome, m.p. and mixed m.p. 229°(decomp.). After the AcOEt solution was concentrated, H_2O was added to the residue and (VI) (500 mg.), m.p. and mixed m.p. 109.5°, separated out.

b) (IV) (0.5 g.) dissolved in Me_2CO (30 cc.) was heated on a boiling water bath for 6 hr. Concentration of the reaction mixture and addition of H_2O gave (VI) hydrate, m.p. and mixed m.p. 109.5°.

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Summary

2-Methyl-6-(1-methyl-2-thiocyanato-4-hydroxy-1-butenyl)-5,6-dihydropyrimido[4,5-*d*]-pyrimidine (V) is decomposed into thiamine (III), N-(1-methyl-2-thiocyanato-4-hydroxy-1-butenyl)-N-[(2-methyl-4-amino-5-pyrimidinyl)methyl]formamide (IV), thiamine anhydride (VI), thiochrome (VIII), and HCNO by the action of water. The yield decreases in the order of (III), (IV), (VI), and (VIII), while in hot water, the yield of (VIII) is increased.

The action of hydrous acid on (V) gives (IV). On the other hand, the reaction of (V) with acid under anhydrous condition gives its salt (XV), which is converted into 2-imino-3-(2-methyl-4-formamido-5-pyrimidinyl)methyl-3a-methyl-3a,5,6,6a-tetrahydrofuro[2,3-*d*]-thiazole (XIII) by the action of sodium hydrogencarbonate and water. (XIII) changes to 2-imino-3-(2-methyl-4-amino-5-pyrimidinyl)methyl-3a-methyl-3a,5,6,6a-tetrahydrofuro[2,3-*d*]thiazole (XII) with a loss of formic acid by the action of mineral and acetic acid or sodium hydroxide.

When heated with water, (XIII) also changes to 2,8a-dimethyl-5a,6,7,8a-tetrahydrofuro-[2,3-*h*]thiochromine (VII) through (XII) produced by hydrolysis liberating ammonia.

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