[Chem. Pharm. Bull. 14(4) 407~413 (1966)] UDC 547.789.04.07 : 577.16

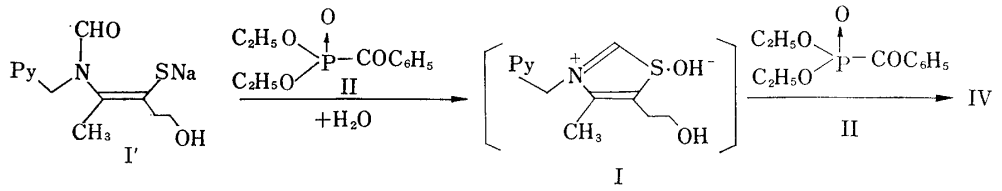
56. Akira Takamizawa, Yoshiro Sato, Sachiko Tanaka, and Hisako Itoh : Studies on the Pyrimidine Derivatives and Related Compounds. XXXV.*1 On the Reaction Product of Thiamine with Diethyl Benzoylphosphonate.*2

(*Shionogi Research Laboratory, Shionogi & Co., Ltd.**3)

It has previously been reported[1] that the reaction of thiamine sodium salt (I′) with diethyl benzoylphosphonate (II) gave a thiamine derivative (IV), m.p. 163~164° (decomp.), which did not seem to be an analogue of the known thiamine derivatives, but quite a new one having a benzoyl group into the thiazole ring of thiamine. In this paper, we shall present evidence for the structure of this compound and also a novel benzoylation reaction of the neutral form of thiamine (thiamine hydroxide) (I) which is accompanied by the rearrangement of thiazole to 1,4-thiazine.

Development of this reaction was checked by means of paper partition chromatography and also by the change of pH of the mixture (Table I). As the reaction proceeded, the initial pH (11.8) of the reaction mixture changed to 8.8, when the spot of IV began to appear on the chromatogram. Since the thiol-type sodium salt of thiamine can only exist above pH 9,[2] it is obvious that the neutral form of thiamine

Table I. Paper Partition Chromatography and the Change of pH of Reaction Mixture



Time	Temp. (°C)	pH	Spot of thiamine	Spot of IV	
10 min.	5	11.2	+++	−	
50	13	11.2	+++	−	
100	20	8.8	+++	−	
2.5 hr.	23	8.8	+++	+	
5	27	8.6	+++	++	
		8.4	++	++	allowed to stand overnight
10 min.	90	6.8	+	++	
1 hr.	100	6.4	−	++	
2	100	6.0	−	++	
5	100	5.8	−	++	

AcOH-BuOH-H_2O=1:4:5, Dragendorff reagent.

(thiamine hydroxide) (I), but not the corresponding thiol-type sodium salt, can participate in this reaction. When a suspension of thiamine hydrochloride in a small amount of water was treated with a saturated aqueous solution of three moles of sodium

*1 Part XXXIV. A. Takamizawa, K. Hirai, T. Ishiba, S. Hayakawa : Vitamins (Kyoto), **31**, 210 (1965).
*2 A preliminary communication of this work appeared in Tetrahedron Letters, **1964**, 2803, 3599.
*3 Fukushima-ku, Osaka, Japan (高見沢 映, 佐藤義朗, 田中幸子, 伊藤寿子).
1) A. Takamizawa, Y. Sato, S. Tanaka : Yakugaku Zasshi, **85**, 298 (1965).
2) O. Zima, R. R. Williams : Ber., **73**, 941 (1940).

hydroxide under 5°, and then acetone was added, a crystalline solid, whose infrared spectrum (Fig. 1) has only a weak carbonyl absorption at 1686 cm^{-1} (N-CHO), was obtained. A solution of this compound in water reacted with benzoyl chloride to form O,S-dibenzoylthiamine in 70% yield. Its ethanol solution, however, on treatment with benzoyl chloride, gave only 4% yield of S-benzoylthiamine. Thus, we regarded that this crystalline solid was not a thiol-type sodium salt of thiamine, but a mechanical mixture of I and an equimolar amount of sodium hydroxide. When this mixture was allowed to react with II, IV was obtained in 54% yield. On the other hand, when thiamine hydrochloride in a solution of three moles of sodium hydroxide was allowed to stand for thirty minutes, and then acetone was added, colorless crystals having a strong infrared carbonyl absorption at 1686 cm^{-1} (N-CHO) (Fig. 2) were obtained. This compound in ethanol, on treatment with benzoyl chloride, gave S-benzoylthiamine in 80% yield,[*4] whereas on reaction with II it gave only 6% yield of IV. Therefore, IV must have been formed from the neutral form of thiamine, but not the sodium salt of the thiol-type. For the preparation of I, the use of sodium carbonate or sodium bicarbonate was found to be less effective than that of sodium hydroxide (Table II). Comparison of the experiment No. 1 with No. 2 showed that the existence of three moles of base is essential for the formation of IV.

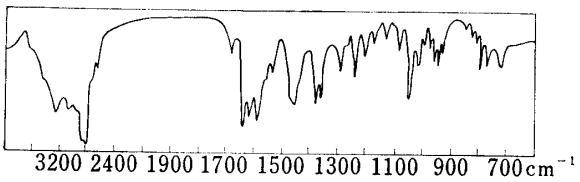


Fig. 1. Infrared Spectrum of Neutral Form Thiamine (I) (in Nujol)

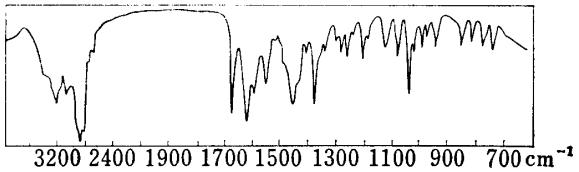


Fig. 2. Infrared Spectrum of Thiol Type Sodium Salt of Thiamine (I') (in Nujol)

Table II. Relations of the Preparation Method of I and Yield of IV

Exp. No.	Preparation of I	Yield of IV (%)
1	To an aqueous solution of thiamine hydrochloride, 3 moles of NaOH were added, and then the mixture was treated with acetone.	54.5
2	To an aqueous solution of thiamine hydrochloride, 2 moles of NaOH were added, and then the mixture was treated with acetone.	0
3	To an aqueous solution of thiamine hydrochloride, 3/2 moles of Na_2CO_3 were added, and then the mixture was treated with acetone.	28.5
4	To an aqueous solution of thiamine hydrochloride, 3 moles of $NaHCO_3$ were added, and then the mixture was treated with acetone.	5.8
5	To a product of Exp. No. 2, 1/2 mole of finely powdered Na_2CO_3 was mixed.	5.7
6	To crystals of thiamine hydrochloride, one mole of finely powdered Na_2CO_3 was mixed.	6.8

When IV was oxidized with chromium trioxide in acetic acid, thiamine thiazolone O-benzoate (XI) and benzoic acid were obtained. On hydrolysis IV gave V. Treatment of the acetate (VI) of V with potassium permanganate in 30% acetic acid afforded thi-

[*4] Since it was reported that I' reacted with benzoyl chloride to form only O,S-dibenzoylthiamine,[3] $NaS_2O_3COC_6H_5$ was employed for the preparation of S-benzoylthiamine. However, when the reaction of I' with benzoyl chloride was carried out in a cold ethanol solution, S-benzoylthiamine was obtained in good yield.

3) T. Matukawa, H. Kawasaki: Yakugaku Zasshi, **73**, 705 (1953).

amine thiazolone O-acetate (Ⅶ), benzoic acid and crystals (Ⅷ), m.p. 210~211° (decomp.). These results confirmed that Ⅳ maintains a fundamental structure of thiamine. On the other hand, Ⅷ contained no sulfur and was found to possess a molecular formula, $C_{14}H_{14}O_2N_4$, from the elementary analysis and the molecular weight determination. The ultraviolet spectrum of this substance in ethyl alcohol exhibited maxima at 237 mμ (ε 13,000) and 266 mμ (ε 13,700) due to the pyrimidine nucleus. Its infrared spectrum has absorption bands for amino (3335, 3125 cm^{-1}) and carbonyl (1673, 1649 cm^{-1}) groups. The nuclear magnetic resonance (NMR) spectrum in dimethylsulfoxide (Fig. 4) contained the signals for five aromatic protons around 2.17 τ. Heating of Ⅷ with hydrochloric acid furnished a basic substance and an acid, $C_8H_6O_3$. The former was identified as 2-methyl-4-amino-5-aminomethylpyrimidine (Ⅸ), and the latter as phenylglyoxylic acid (Ⅹ). The identity of Ⅷ with 2-methyl-4-amino-5-phenylglyoxyloylaminomethylpyrimidine was confirmed by synthesizing it from Ⅸ and phenylglyoxaloyl chloride. Accordingly, a N-C-C-C_6H_5 bond is in fact present in the molecule of Ⅳ, and therefore, either (A) or (B) may be proposed for the partial structure of Ⅳ. From the fact that thiamine thiazolone and benzoic acid were obtained in the above oxidation reaction, the structure (A) may be regarded more appropriate than (B). However,

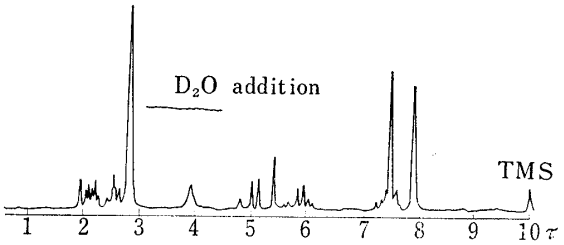
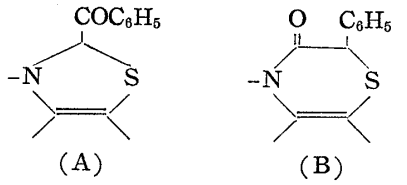


Fig. 3. Nuclear Magnetic Resonance Spectrum of 2-Phenyl-3-oxo-4-(2-methyl-4-amino-5-pyrimidyl)methyl-5-methyl-6-(2-benzoyloxy)-ethyl-2,3-dihydro-4*H*-1,4-thiazine (Ⅳ) (in $CDCl_3$)



the NMR spectrum (Fig. 3) of Ⅳ (or Ⅵ) showed a sharp singlet signal pattern at 2.78 τ for the phenyl group introduced into the thiazole ring, and we therefore prefer the structure (B).

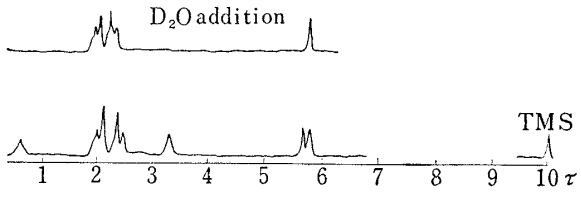


Fig. 4. Nuclear Magnetic Resonance Spectrum of 2-Methyl-4-amino-5-phenylglyoxyloylaminomethylpyrimidine (Ⅷ) (in Dimethylsulfoxide)

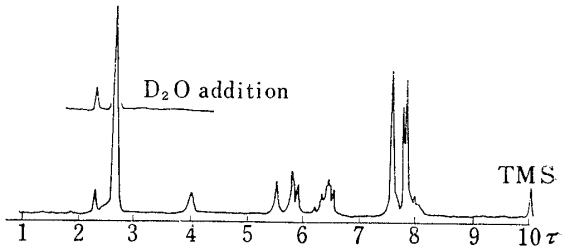


Fig. 5. Nuclear Magnetic Resonance Spectrum of S-(1-Acetyl-3-chloropropyl)-N-(2-methyl-4-amino-5-pyrimidyl)methylthiomandelamide (Ⅻ) (in $CDCl_3$)

Treatment of either Ⅳ or Ⅴ with concentrated hydrochloric acid at room temperature gave the same product (Ⅻ). This product Ⅻ, $C_{19}H_{23}O_2N_4ClS$, m.p. 163~164° (decomp.), has a new carbonyl band at 1710 cm^{-1} in the infrared spectrum. Its NMR spectrum (Fig. 5) exhibited the signals for the NH proton near 2.3 τ and the two methylene protons at 5.80 τ (doublet, J=6.2 c.p.s.), indicating the presence of the $-CH_2-NH-$ grouping. This compound must have been formed by the hydrolytic opening of the ring of Ⅳ or Ⅴ. Ⅻ, on treatment with alcoholic sodium hydroxide or on passing through an alumina column, gave XIII, $C_{19}H_{22}O_2N_4S$, m.p. 183~184° (decomp.), which was probably a dehydrochlorination product of Ⅻ. This compound showed the infrared carbonyl absorption bands at 1685 cm^{-1} and 1658 cm^{-1}. In its NMR spectrum (Fig. 6), four proton

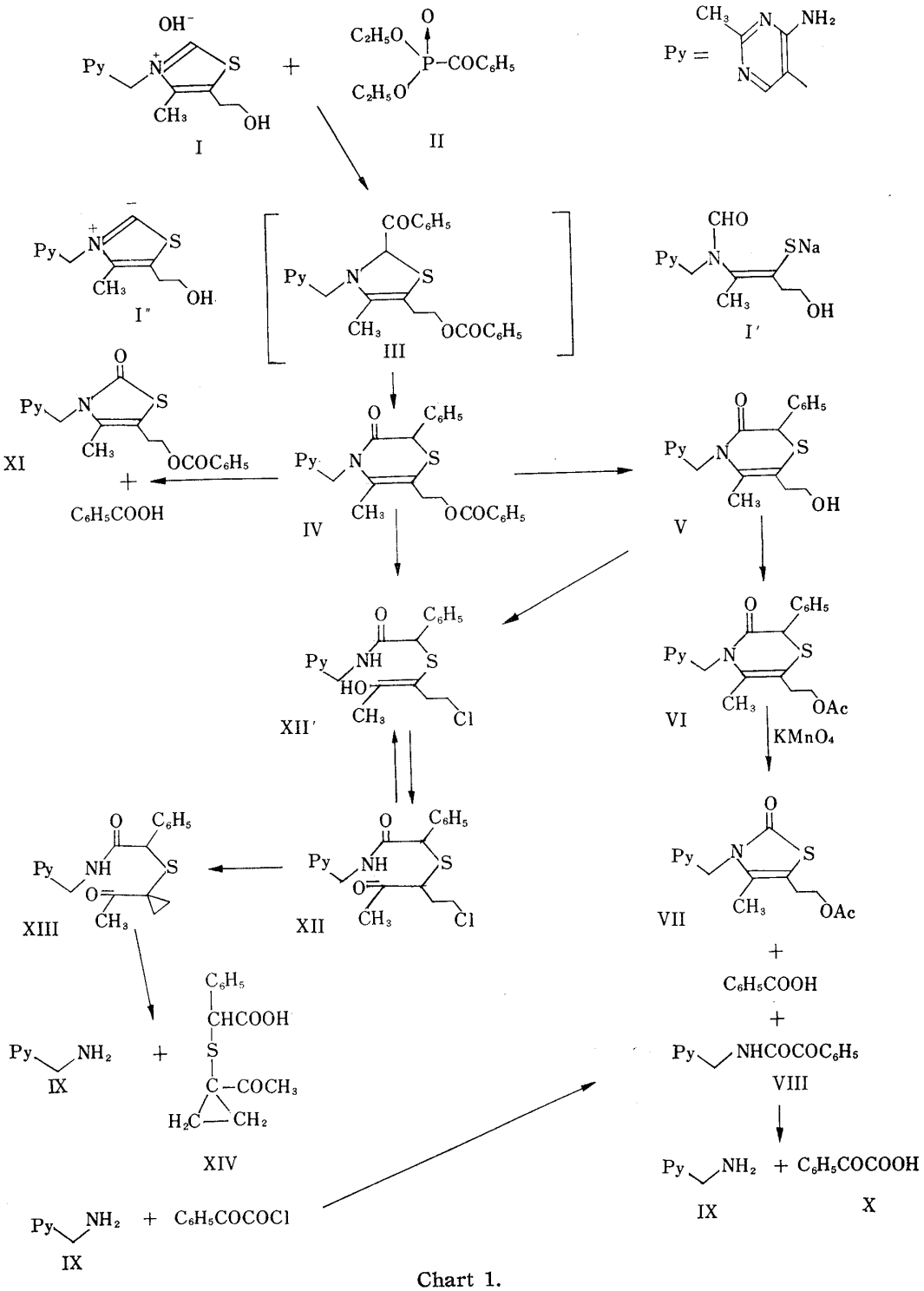


Chart 1.

signals of the A_2B_2 type were observed around 8.6 τ besides the signals for the -NH-CH_2- grouping and one singlet proton signal at 5.38 τ. These data indicate that XIII is S-(1-acetylcyclopropyl)-N-(2-methyl-4-amino-5-pyrimidyl)methylthiomandelamide. On hydrolysis with alcoholic sodium hydroxide, XIII yielded 2-methyl-4-amino-5-amino-methylpyrimidine (IX) and a carboxylic acid (XIV), $C_{13}H_{14}O_3S$, m.p. 123~124°, whose infrared spectrum has two strong carbonyl bands at 1715 cm^{-1} and 1660 cm^{-1}. The NMR spectrum (Fig. 7) of XIV showed signals for phenyl at 2.65 τ (singlet), $-\overset{|}{\underset{|}{C}}-H$ at

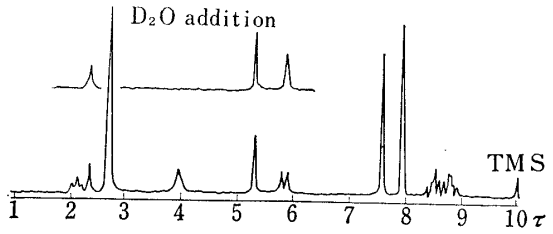


Fig. 6. Nuclear Magnetic Resonance Spectrum of S-(1-Acetylcyclopropyl)-N-(2-methyl-4-amino-5-pyrimidyl)methylthiomandelamide (XIII) (in $CDCl_3$)

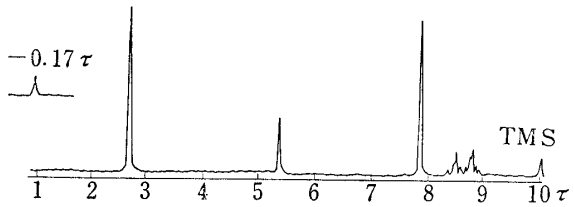


Fig. 7. Nuclear Magnetic Resonance Spectrum of S-(1-Acetylcyclopropyl)thiomandelic Acid (XIV) (in $CDCl_3$)

5.38 (singlet), acetyl at 7.85 τ (singlet), carboxylic acid at −0.17 τ, and four protons of the A_2B_2 type around 8.6 τ. This compound must represent an acid fragment of XIII and is S-(1-acetylcyclopropyl)thiomandelic acid. XII is thus S-(1-acetyl-3-chloro)propyl-N-(2-methyl-4-amino-5-pyrimidyl)methylthiomandelamide.

From these results, it was concluded that IV or V should be formulated as 2-phenyl-3-oxo-4-(2-methyl-4-amino-5-pyrimidyl)methyl-5-methyl-6-(2-benzoyloxy or hydroxy)-ethyl-2,3-dihydro-4*H*-1,4-thiazine.

The mechanisms for these reactions has been also investigating in our laboratory. The structure of isolated neutral form of thiamine are probably illustrated as I. Nevertheless thiamine may participate as a zwitterion (I″), suggested by Breslow,[4] in the reaction. Anyhow in this reaction, it seems probable that thiazoline derivative III whose benzoyl group was introduced into the 2-position of thiazole initially formed, which then rearranged to the thiazine form IV. The syntheses of VI and XII has already carried out by way of another route. It shall be reported on a next paper.

It is known that the nitrogen-carbon bond of unsaturated heterocyclic systems are cleaved under acidic condition, and Paquette[5] suggested that in 1*H*-azepin-2(3*H*)-one the N-CO bond was cleaved *via* Chart 2. However, it is worthy to note that the $-\overset{|}{N}-\overset{|}{C}=$ rather than the -N-CO bond was easily hydrolyzed, as illustrated in Chart 1, in our 1,4-thiazine system.

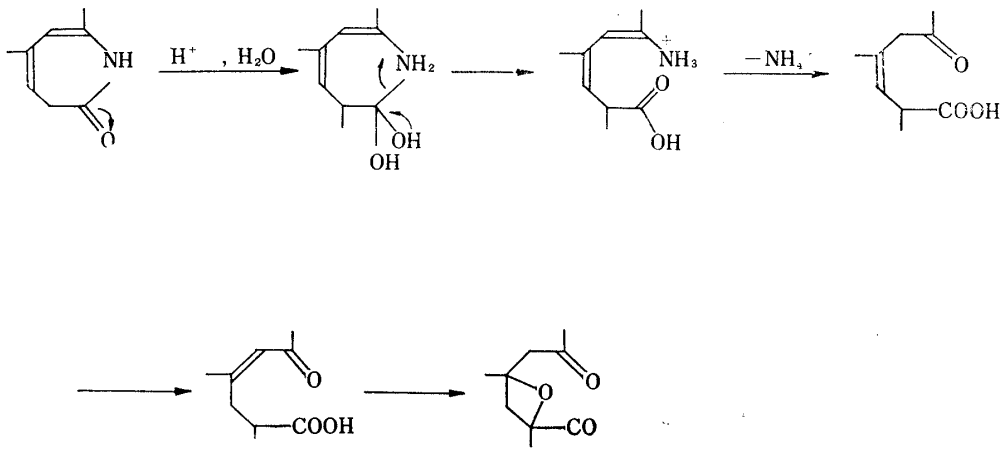


Chart 2.

4) R. Breslow : J. Am. Chem. Soc., **80**, 3719 (1958).
5) L. A. Paquette : *Ibid.*, **85**, 3288 (1963).

Experimental*5

Neutral Form of Thiamine (Thiamine Hydroxide) (I)——To a suspension of 33.7 g. of thiamine hydrochloride in 17 ml. of H_2O was added dropwise a cold solution of 12.0 g. of NaOH in 17 ml. of H_2O at 0~5° with stirring. Since yellow bulky crystals were precipitated, the stirrings became to troublesome. After all amount of alkali was added, 1 L. of acetone was added to give bulky crystalline precipitate comprehending the excess NaOH (1 mol. equiv.) solution. The precipitate was filtered with suction and dried over $CaCl_2$, and then P_2O_5 *in vacuo* at room temperature. Yield, 33.5 g.

2-Phenyl-3-oxo-4-(2-methyl-4-amino-5-pyrimidyl)methyl-5-methyl-6-(2-benzoyloxyethyl)-2,3-dihydro-4*H*-1,4-thiazine (IV)——To a suspension of 10.5 g. of above-mentioned product (I) in 50 ml. of dry toluene was slowly added 12.1 g. of diethyl benzoylphosphonate (II) under cooling with stirring. After the addition, the stirrings were continued at room temperature. The reaction mixture had a tendency to become warm, but the temperature should be controlled below 30° by cooling. When the temperature rised no longer, the reactant was heated on an oil bath for 5 hr. at 100°. The reaction mixture was extracted with 50 ml. of 2*N* HCl. The HCl extract was washed with ether and allowed to stand for several days under cooling. IV-HCl was precipitated, m.p. 146~148° (decomp.) (from H_2O). Yield, 7.2 g. (54.5%). *Anal.* Calcd. for $C_{26}H_{26}O_3N_4S \cdot H_2O \cdot HCl$: C, 63.39; H, 5.73; N, 11.38; S, 6.51. Found : C, 63.71; H, 5.92; N, 11.64; S, 6.14.

The HCl-salt was neutralized by aq. $KHCO_3$, and extracted with $CHCl_3$ to give IV, m.p. 163~164° (decomp.) (from aq. EtOH).

2-Phenyl-3-oxo-4-(2-methyl-4-amino-5-pyrimidyl)methyl-5-methyl-6-(2-hydroxyethyl)-2,3-dihydro-4*H*-1,4-thiazine (V)——A solution of 4.9 g. of IV in 20 ml. of 10% NaOH-aq. EtOH was heated with reflux for 30 min. After removal of the EtOH under reduced pressure the residue was extracted with $CHCl_3$ and washed with H_2O, dried over Na_2SO_4. V-$CHCl_3$ was precipitated from the $CHCl_3$ solution by means of concentration. It was recrystallized from aq. EtOH to give colorless needles of m.p. 195~197°(decomp.); from abs. EtOH to give m.p. 106~107°; and from $CHCl_3$ to give m.p. 102~104° (efferv.) having 1 mole of $CHCl_3$ as a crystal solvent. *Anal.* Calcd. for $C_{19}H_{22}O_2N_4S$: C, 61.59; H, 5.98; N, 15.12; S, 8.65. Found : C, 61.37; H, 6.13; N, 15.18; S, 8.40.

Oxidation of IV with Chromic Acid Anhydride——To a stirring solution of 1.11 g. of IV in 18 ml. of AcOH was gradually added a solution of 200 mg. of CrO_3 in 7.5 ml. of AcOH, and then allowed to stand overnight at room temperature. After evaporation of the AcOH *in vacuo* the residue was diluted with H_2O, and extracted with ether, washed with H_2O, dried over Na_2SO_4. Evaporation of the solvent afforded white crystals, m.p. 120~121°, which were identified with benzoic acid. Yield, 80 mg. The first water layer was neutralized with $NaHCO_3$, and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O and dried over Na_2SO_4. From this solution, white crystals, m.p. 233~234° (decomp.) (from AcOEt) were obtained, which were identified with thiamine thiazolone O-benzoate (XI). Yield, 120 mg. (14.5%).

Oxidation of Acetate (VI) of V with Potassium Permanganate——To a stirring solution of 3.0 g. of VI in 50 ml. of 30% AcOH was gradually added a solution of 1.80 g. of $KMnO_4$ in 80 ml. of 30% AcOH. After 10 min., the mixture was extracted with ether. The ether solution was washed with H_2O and dried over Na_2SO_4. Evaporation of the solvent gave white crystals, m.p. 121~122°, which were identified with benzoic acid. Yield, 136 mg. (14.7%).

The AcOH layer was then extracted with $CHCl_3$. The $CHCl_3$ solution was washed with 10% NaOH and dried over Na_2SO_4. The resulting residue from the $CHCl_3$ solution was recrystallized from EtOH to yield 376 mg. (16%) of colorless crystals, m.p. 170~171°(decomp.), which were identical with a specimen of thiamine thiazolone O-acetate (VII).

The remained AcOH fraction was neutralized with NaOH and MnO_2 which precipitated was filtered off, and the filtrate was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and dried over Na_2SO_4. Evaporation of the solvent gave colorless crystals, m.p. 210~211° (decomp.) (from EtOH). Yield, 320 mg. (17.1%). *Anal.* Calcd. for $C_{14}H_{14}O_2N_4$ (2-methyl-4-amino-5-phenylglyoxyloylaminomethylpyrimidine) (VIII): C, 62.21; H, 5.22; N, 20.73; O, 11.84; mol. wt., 270.3. Found : C, 62.21; H, 5.51; N, 20.61; O, 12.12; mol. wt., 278.

Hydrolysis of 2-Methyl-4-amino-5-phenylglyoxyloylaminomethylpyrimidine (VIII)——A solution of 287 mg. of VIII in 10 ml. of 10% HCl was heated at 70~75° for 5 hr. The mixture was extracted with ether. The ether was washed with H_2O, dried and evaporated. The residue was recrystallized from a mixture of ligroin and benzene to give 93 mg. (59%) of phenylglyoxylic acid, m.p. 62~63°, which was identical with an authentic specimen by mixed melting point determination and the IR spectra comparison. The HCl phase was concentrated *in vacuo*, neutralized and treated with picric acid. Picrate, m.p.

*5 All melting points are uncorrected, and IR spectra were measured in Nujol. All NMR spectra were taken on Varian Associates A-60 recording spectrometer with tetramethylsilane as an internal standard.

228~230°(decomp.), which was identical with an authentic specimen of 2-methyl-4-amino-5-aminomethylpyrimidine dipicrate, was obtained. Yield, 322 mg. (62%).

Synthesis of VIII——To a suspension of 5.24 g. of 2-methyl-4-amino-5-aminomethylpyrimidine (IX) in 50 ml. of pyridine was added dropwise 3.0 g. of phenylglyoxyloyl chloride ($b.p_{5.0}$ 82~86°) at room temperature. After 1.5 hr. the solvent was removed *in vacuo*. The residue was extracted with $CHCl_3$, and washed with H_2O, dried and evaporated. The resulting crystals were recrystallized from EtOH to give 1.85 g. (38.5%) of 2-methyl-4-amino-5-phenylglyoxyloylaminomethylpyrimidine (VIII), m.p. 210~211° (decomp.). *Anal.* Calcd. for $C_{14}H_{14}O_2N_4$: C, 62.21; H, 5.22; N, 20.73. Found : C, 62.11; H, 5.35; N, 20.69.

S-(1-Acetyl-3-chloropropyl)-N-(2-methyl-4-amino-5-pyrimidyl)methylthiomandelamide (XII)——To 112 ml. of 35% HCl was dissolved 7.0 g. of IV. The solution was allowed to stand for 24 hr. at room temperature. After dilution with 225 ml. of H_2O, the solution was washed with $CHCl_3$, and neutralized with $NaHCO_3$, and then extracted with $CHCl_3$. The extracts were washed with H_2O and dried over Na_2SO_4. The solvent was removed and the residue was recrystallized from AcOEt to give 2.1 g. (32.7%) of crystals, m.p. 163~164°(decomp.). *Anal.* Calcd. for $C_{19}H_{23}O_2N_4ClS$: C, 56.08; H, 5.70; N, 13.77; Cl, 8.71; S, 7.88. Found : C, 56.34; H, 5.89; N, 13.83; Cl, 8.68; S, 8.08.

S-(1-Acetylcyclopropyl)-N-(2-methyl-4-amino-5-pyrimidyl)methylthiomandelamide (XIII)——A solution of 3.1 g. of XII in 50 ml. of 5% KOH-EtOH was refluxed for 4 hr. After evaporation of the EtOH *in vacuo* the crystalline product which precipitated from the aqueous solution was collected and recrystallized from benzene to give crystals, m.p. 183~184°(decomp.). Yield, 2.17 g. (76.8%). *Anal.* Calcd. for $C_{19}H_{22}O_2N_4S$: C, 61.43; H, 6.78; N, 15.08; O, 8.62; S, 8.63. Found : C, 61.93; H, 6.30; N, 15.24; O, 8.42; S, 8.46.

Hydrolysis of S-(1-Acetylcyclopropyl)-N-(2-methyl-4-amino-5-pyrimidyl)methylthiomandelamide (XIII)——A solution of 3.0 g. of XIII in 20 ml. of 5% aq. NaOH-MeOH was refluxed for 24 hr. After removal of the MeOH the resulting aqueous solution was washed with $CHCl_3$ and neutralized with HCl, and then extracted with ether. From the ether solution, S-(1-acetylcyclopropyl)thiomandelic acid (XIV), m.p. 123~124°, was obtained. Yield, 224 mg. (11.1%). *Anal.* Calcd. for $C_{13}H_{14}O_3S$: C, 62.37; H, 5.63; O, 19.18; S, 12.81. Found : C, 62.44; H, 5.68; O, 18.76; S, 13.22. The aqueous layer was washed with $CHCl_3$ and concentrated *in vacuo*, and then treated with picric acid. 2-Methyl-4-amino-5-aminomethylpyrimidine (IX) dipicrate, which was identified with authentic sample, was obtained. Yield, 0.5 g. (10.3%).

The authors thank Professor Emeritus E. Ochiai and Professor S. Nagakura of Tokyo University for valuable discussions.

Summary

It was shown that a new thiamine derivative which was obtained from the reaction of the neutral form of thiamine (I) with diethyl benzoylphosphonate (II) should be formulated as 2-phenyl-3-oxo-4-(2-methyl-4-amino-5-pyrimidyl)methyl-5-methyl-6-(2-benzoyloxy)ethyl-2,3-dihydro-4*H*-1,4-thiazine (IV). A novel benzoylation reaction accompanied by the rearrangement from thiazole to 1,4-thiazine is also described.

(Received August 6, 1965)