[Chem. Pharm. Bull. 16(9)1773—1778(1968)] UDC 547.853.07 : 615.356.011.5 : 577.164.11

Studies on Pyrimidine Derivatives and Related Compounds. LIII.[1] The Reaction of Thiamine with Diethyl Acetylphosphonate (Supplement) and the Synthesis of Pseudothiamine

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(Received December 28, 1967)

The reaction of deuterioamino-O-acetylthiamine (Id) with dialkyl acetylphosphonate afforded 1-methyl-1-deuterio-3-(2-acetoxy) ethyl-4,9-dimethyl-1,6-dihydropyrimido-[4′,5′-4,5]pyrimido[2,3-*c*][1,4]thiazine (IIIf). Treatment of 2-methyl-4-(2-methyl-4-amino-5-pyrimidinyl) methyl-5-methyl-6-(2-acetoxy) ethyl-2 *H*-1,4-thiazin-3 (4 *H*)-one (IVa) with hydrogen peroxide in acetic acid gave 2-acetyl-2-hydroxy-3-(2-methyl-4-amino-5-pyrimidinyl)methyl-4-methyl-5-(2-acetoxy)ethylthiazoline (Va).

In previous papers,[3-5] it was reported that dialkyl acetyl(or benzoyl)phosphonate (IIa—b) reacted with thiamine after treatment with organic amines to give corresponding 1-methyl (or phenyl)-3-[2-hydroxy(or acyloxy)]ethyl-4,9-dimethyl-1,6-dihydropyrimido[4′,5′-4,5]-pyrimido[2,3-*c*][1,4]thiazine, and as one of the suggestions for clarifying the mechanism of this novel reaction, it has been also revealed that the reaction of deuterioamino-O-benzoylthiamine (Ie) with IIb gave the compound deuterated at position 1 (IIIe).[3] It was also reported that the compounds (IIIa—d) obtained there were readily hydrolyzed to give 2-methyl (or phenyl)-4-(2-methyl-4-amino-5-pyrimidinyl)methyl-5-methyl-6-[2-hydroxy (or acyloxy)]ethyl-2*H*-1,4-thiazin-3-(4*H*)-one (IVa—d),[3-5] and treatment of IVb with hydrogen peroxide in acetic acid gave 2-benzoyl-2-hydroxy-3-(2-methyl-4-amino-5-pyrimidinyl)-methyl-4-methyl-5-(2-benzoxy)ethylthiazoline (pseudo benzothiamine, Vb).[6]

In this paper, it was found that the reaction of deuterioamino-O-acetylthiamine (Id) with IIa afforded 1-deuterio compound (IIIf) as expected, and the treatment of IVa with hydrogen peroxide in acetic acid gave 2-acetyl-2-hydroxy-3-(2-methyl-4-amino-5-pyrimidinyl)methyl-4-methyl-5-(2-acetoxy)ethylthiazoline (pseudoacetothiamine, Va) analogously to the formation of Vb from IVb.

In a usual manner, the treatment of O-acetylthiamine hydrochloride (Ia) with deuterium oxide gave deuterioamino-O-acetylthiamine deuteriochlo-

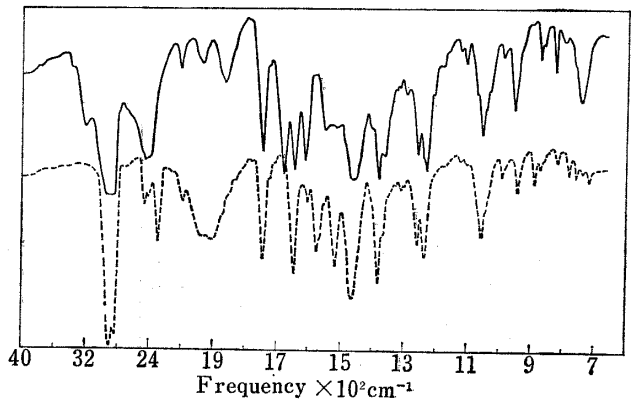

Fig. 1. Infrared Spectra of O-Acetylthiamine Hydrochloride (Ia) (——) and Deuterated O-Acetylthiamine Hydrochloride (Id) (------) (Nujol)

1) Part LII: A. Takamizawa, K. Hirai, Y. Hamashima, Y. Matsumoto, and S. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **16**, 1764, (1968).
2) Location: *Fukushima-ku, Osaka.*
3) A. Takamizawa, Y. Hamashima, H. Sato, S. Tanaka, H. Ito, and Y. Mori, *J. Org. Chem.*, **31**, 295 (1966).
4) A. Takamizawa, Y. Hamashima, Y. Sato, and H. Sato, *Chem. Pharm. Bull.* (Tokyo), **15**, 1178 (1967).
5) A. Takamizawa, Y. Sato, and H. Sato, *Chem. Pharm. Bull.* (Tokyo), **15**, 1183 (1967).
6) A. Takamizawa, Y. Sato, and S. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **14**, 588 (1966).

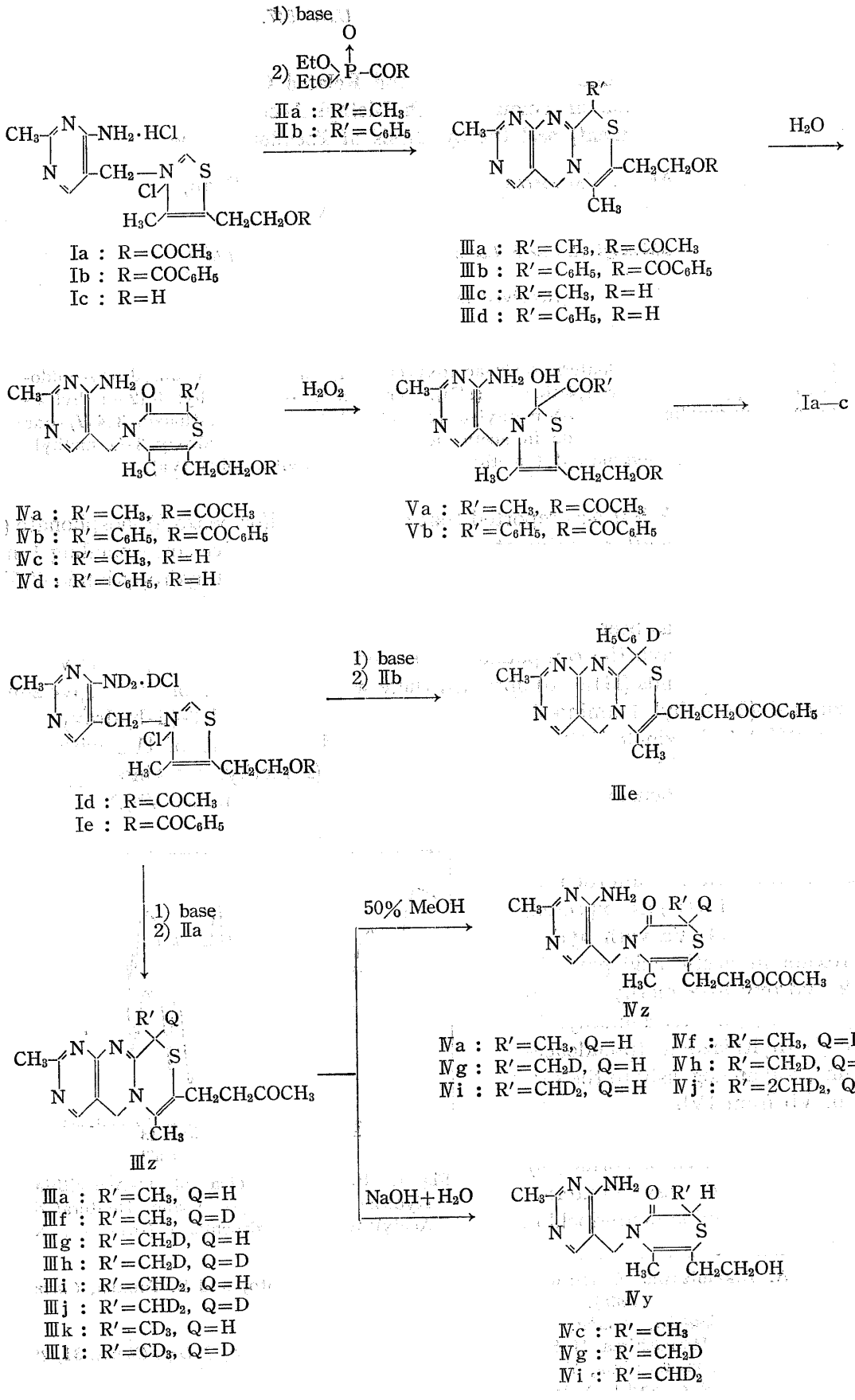

Chart 1

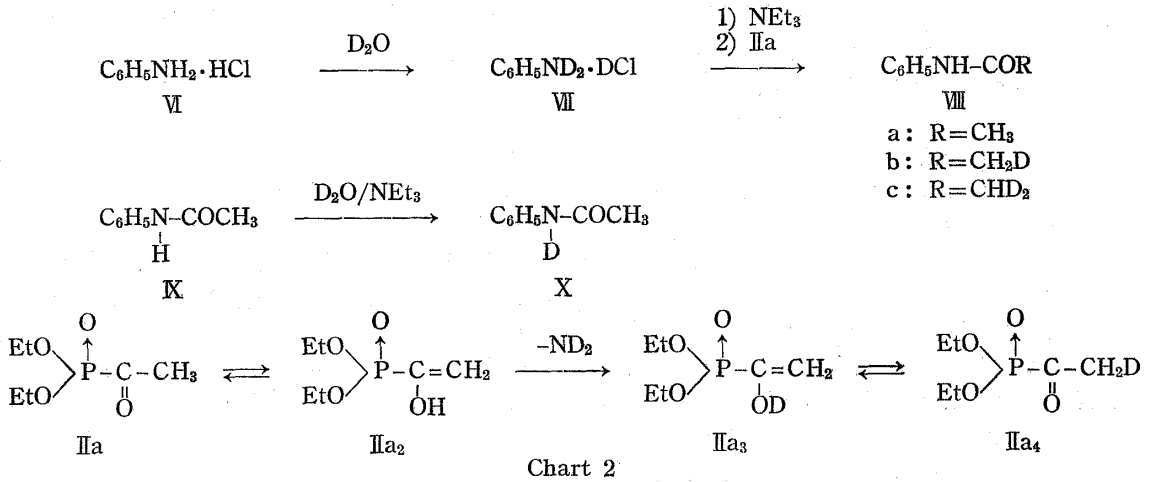

Chart 2

ride (Id) which was checked to be pure enough within the range of measurement error by infrared (IR) spectrum measurement (Fig. 1, Table I).

According to the previous papers,[3,4] in a dry box, Id was treated with triethylamine in DMF and reacted with IIa to give the similar product to one obtained previously by the reaction of thiamine (Ic) with IIa. This product was called tentatively IIIz. When nuclear magnetic resonance (NMR) spectrum[7] (Fig. 2) of IIIz was compared with that of IIIa, the proton signals correspond to those at 6.43 τ (quartet, 1H, J=7.0, C–CH–CH_3) and 8.52 τ (doublet, 3H, J=7.0, C–CH–CH_3) in IIIa appeared at 6.43 τ (multiplet, 0.5H) and 8.52 τ (triplet,1.7H), respectively, indicating 50% deuteration at C_1–position and 42.5% deuteration at C_1–CH_3 group of IIIz had occurred, and the rest part of both spectral pattern were identical. It was now revealed that IIIz was a mixture of >CH–CH_3 compound (IIIa), >CD–CH_3 compound (IIIf), >CH–CH_2D compound

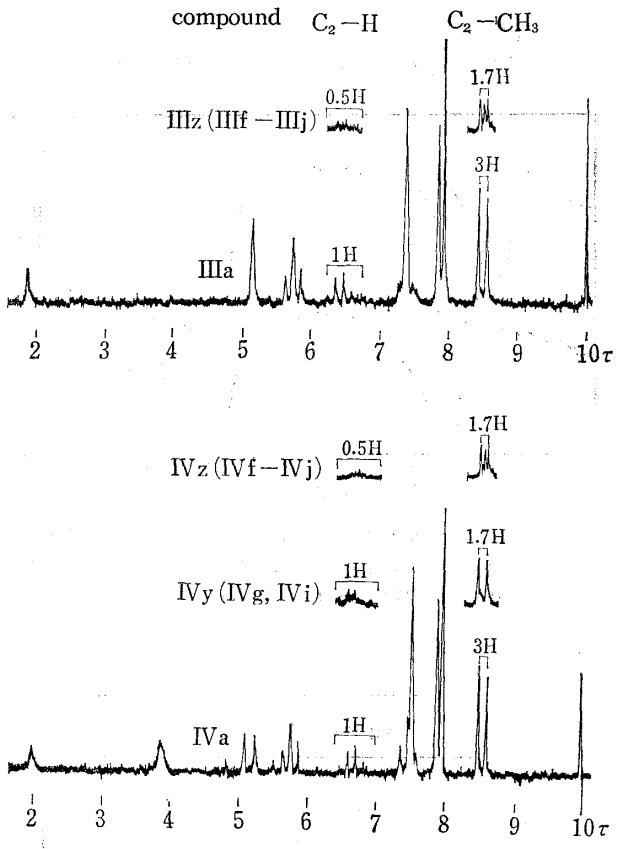

Fig. 2. NMR Spectra of IIIa, IIIz, IVa, IVy, and IVz

Table I. Exchange Data of NH to ND in O-Acetylthiamine Hydrochloride

ν_{NH} (cm^{-1})	ν_{ND} (cm^{-1})	ν_{NH}/ν_{ND}
3200	2455	1.31
3010	2305	1.31
2420	1920	1.26

7) NMR spectra were taken with a Varian A-60 spectrometer in deuteriochloroform containing TMS as an internal reference. Chemical shifts are presented in τ value, and coupling constants are in cps.

(IIIg), and >$CDCH_2D$ compound (IIIh). From the infromation of NMR spectra, the presence of >$CH–CHD_2$ compound (IIIi), >$CDCHD_2$ compound (IIIj), >$CHCD_3$ compound (IIIk), and >$CD–CD_3$ compound (IIIk) might be taken in consideration, but this consideration was negligible from the information including the mechanism of the product formation described below. Ring opening of IIIz by heating with 50% MeOH–H_2O resulted in the formation of 3–oxo–1,4–thiazine derivative (IVz), which showed same melting point as acetyl group holding compound IVa (mp 128—131°). As shown in NMR spectrum (Fig. 2), the proton signals at 6.69 τ (multiplet, 0.5H) and 8.57 τ (triplet, 1.7H) indicated that this compound was a mixture of >$CH–CH_3$ compound (IVa), –$CDCH_3$ compound (IVf), $CHCH_2D$ compound (IVg), and $CDCH_2D$ compound (IVh). On the other hand,IIIz, on treatment with NaOH–H_2O, underwent ring opening and deacetylation to give 3–oxo–1,4–thiazine derivative (IVy). The NMR spectrum (Fig. 2) of this compound showed the signals due to *keto–enol* form at 6.80 τ (multiplet, 1H), and 8.59 τ (doublet, 1.7H). Methyl signal was observed as same as in IIIz, but the deuterium attached to the same carbon atom was exchanged completely to the hydrogen, therefore, it was concluded that this compound was a mixture of >$CH–CH_3$ compound (IVa) and $CH–CH_2D$ compound (IVg). Accordingly, deuteration of above methyl group proceeded in earlier stage than the formation of IIIz, and the origin of the deuterium was considered to be due to the H–D exchange between enol form of IIa (IIa_2) and deuterioamino group. As a model experiment, aniline was treated with D_2O to afford deuterioaniline (Vb) which was checked to be pure by IR spectrum measurement (Fig. 3, Table II), and treated with triethylamine in DMF followed by the action of IIa to give acetanilide (VIIb) after purification using water. The NMR spectrum of this compuond exhibited that methyl protons of acetyl group were corresponded to 1.8H indicating 1.2H were deuterated.

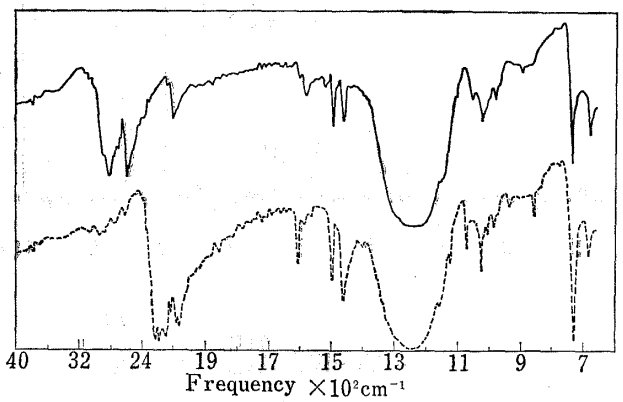

Fig. 3. Infrared Spectra of Aniline Hydrochloride (VIa) (——) and Deuterated Aniline Hydrochloride (VII) (------) (Perfluorocarbon)

Table II. Exchange Data of NH to ND in Aniline Hydrochloride

ν_{NH} (cm^{-1})	ν_{ND} (cm^{-1})	ν_{NH}/ν_{ND}
2800	2200	1.27
	2160	1.30
	2070	1.35
2580	1980	1.30

On the other hand, ND group was converted into NH group by treatment with water. Treatment of acetanilide with $D_2O–Et_3N$ afforded $ND–COCH_3$ compound (VIIc). From these results, D–H exchange of IIa through enol form (IIa_2) was confirmed.

Treatment of IVa with hydrogen peroxide in acetic acid gave the crystals of mp 176—178°. The elemental analysis of this compound agreed with the constitution having one more oxygen than IVa ($C_{16}H_{22}O_4N_4S$). IR spectrum showed NH bands at 3135 and 3325 cm^{-1}, C=O band at 1730 cm^{-1}, and broad band at 1657 cm^{-1} indicating the presence of NH_2 and C=O groups. NMR spectrum exhibited the following signals: 8.37 τ (singlet, 3H, Th–C_2–$COCH_3$), 8.05 τ (singlet, 3H, $OCOCH_3$), 7.70 τ (singlet, 3H, Pm–C_2–CH_3), 7.48 τ (triplet, 2H, J=6.0, Th–

C_5–CH_2–CH_2O–), 5.90 τ (triplet, 2H, J=6.0, Th–C_5–CH_2–CH_2–O), 5.33 τ (quartet, 2H, Pm–CH_2–Th), 3.33 τ (broad, 2H, Pm–C_4–NH_2), 2.45 τ (singlet, 1H, Pm–C_6–H). This spectrum was analogous to Vb obtained previously. Treatment of this compound with dil. HCl gave thiamine hydrochloride quantitatively as seen in Vb. From these results, this compound was confirmed to be pseudoacetothiamine–O–acetate (Va). Analogously, on treatment of IV with hydrogen peroxide, pseudoacetothiamine (Vc) was obtained as crystals of mp 176° in good yield.

Experimental[8]

Deuteration of O-Acetylthiamine Hydrochloride (Ia)——The solution of Ia (2.0 g) in 6 ml of D_2O was concentrated *in vacuo* at room temperature. The residue was dried over P_2O_5 overnight at room temperature then at 110° for 4 hr, repeated several time, to give deuterioamino–O–acetylthiamine deuteriochloride (Id). Sampling for IR measurement was carried out in dry box under N_2 stream. IR spectra data were shown in Fig. 1, Table I.

Reaction of Deuterioamino-O-acetylthiamine (Id) with Diethyl Acetylphosphonate (IIa)——In a dry box, 10 ml of triethylamine was added dropwise to 2.0 g of Id with stirring and in ice cooling during 10 min, then 0.86 ml of IIa was added. After 30 min, stirring was continued for 17 hr at room temperature. Triethylamine was removed *in vacuo* and the residue was added H_2O and 5% $NaHCO_3$ solution, then extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with H_2O, dried over Na_2SO_4, and concentrated *in vacuo* to give 1.65 g of the residue. After purification by alumina (activity II, neutral, 45 g) column chromatography using benzene, AcOEt (1:1), the product was recrystallized from AcOEt to give 0.83 g of yellow sticks, mp 120—123° (IIIz). $E^{1\%}_{1cm}$ mμ: 372 (317). The product (IIIa) obtained by the reaction of IIa with O–acetylthiamine showed mp 120—123°, $E^{1\%}_{1cm}$ mμ: 371 (321).

Neutral Hydrolysis of IIIz——The solution of 200 mg of IIIz in 6 ml of 50% MeOH was refluxed for 2 hr. The solution was evaporated *in vacuo*, and the residue was extracted with $CHCl_3$. The $CHCl_3$ extract was concentrated and the residue was recrystallized from AcOEt to give 180 mg of colorless neeldes (IVz), mp 128—131°, $E^{1\%}_{1cm}$ mμ: 232.5 (445), 279 (213). Undeuterated compound (IVa) showed mp 128—131°, $E^{1\%}_{1cm}$ mμ; 232 (442), 279 (211).

Alkaline Hydrolysis of IIIz——The solution of 450 mg of IIIz in 10 ml of 5%NaOH–EtOH was refluxed for 30 min. The solution was concentrated, the residue was added H_2O, and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with H_2O, dried over Na_2SO_4, evaporated *in vacuo*, and the residue was recrystallized from CH_3COCH_3 to give 300 mg of IVy, mp 159—161°, $E^{1\%}_{1cm}$ mμ: 232.5 (479), 279 (228). Undeuterated IVc showed mp 159—161°, $E^{1\%}_{1cm}$ mμ: 232.5 (490), 279 (235).

Deuteration of Aniline Hydrochloride (VI)——The solution of 500 mg of aniline hydrochloride in 15 ml of D_2O was concentrated *in vacuo* at 50°. The residue was added 15 ml of D_2O and concentrated *in vacuo* at 50°. The residue was dried over P_2O_5 overnight to give N–deuterioaminoaniline deuteriochloride (VII). Sampling for IR measurement was carried out in dry box under N_2 stream. IR spectral data were shown in Fig. 3, Table II.

The Reaction of N-Deuterioaniline Deuteriochloride (VII) with Diethyl Acetylphosphonate (IIa)——Two milliliters of Et_3N was added to VII obtained above, 0.64 g of IIa was added, and refluxed for 17 hr. Water was added to the reaction mixture, and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with H_2O, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was subjected to silica gel (50 g) column chromatography using $CHCl_3$–EtOH (1: 1). Recrystallization from benzene gave 80 mg of VIII. NMR: $NCOCH_3$ 7.9τ (1.8H).

Pseudoacetothiamine (Vc)——To the solution of 3 g of IVc in 17 g of AcOH, 1.1 g of 30% H_2O_2 was added. After standing in cooling for 1 days, AcOH was removed *in vacuo*, the oily residue was dissolved in 10% KOH, CO_2 was bubbled into the solution to adjust pH 8.6, and extracted with $CHCl_3$. The crystals separated from aqueous layer were collected to give 1.13 g (34%) of pseudoacetothiamine (Vc). Rec rystallization from EtOH gave the crystals of mp 154—156°. *Anal.* Calcd. for $C_{14}H_{20}O_3N_4S \cdot H_2O$: C, 49.10; H, 6.48; O, 18.69; N, 16.36; S, 9.37. Found: C, 49.34; H, 6.51; O, 18.32; N, 16.19; S, 9.52.

Pseudoacetothiamine Acetate (Va)——a) To the solution of 5 g of IVa in 25 g of AcOH, 1.56 g of 31.2% H_2O_2 was added in ice cooling. After standing at room temperature for 4 days, AcOH was removed *in vacuo*, the oily residue was added 5% Na_2CO_3, and extracted with $CHCl_3$. Separated crystals during extraction were collected to give 0.948 g of pseudoacetothiamine acetate (Va). From $CHCl_3$ extract, 250 mg of crude Va was obtained, yield 23.2%. Recrystallizaton from EtOH gave the crystals of mp 176°. *Anal.* Calcd. for $C_{16}H_{22}O_4N_4S$: C, 52.44; H, 6.05; O, 17.47; N, 15.29. Found: C, 52.51; H, 6.08; O, 16.99; N, 15.13.

8) Melting points are uncorrected.

b) To the suspension of 300 mg of Vc in 1.5 ml of pyridine, 0.4 g of Ac_2O was added and stirred for 4 hr at room temperature. After removal of pyridine *in vacuo*, the residual oil was washed with ligroin and ether, and treated with $CHCl_3$ to give 224 mg of crude crystals, yield 68.6%. Recrystallization from EtOH gave the crystals of mp 176°. These crystals were proved to be identical with Va obtained in a) by IR comparison.

Hydrolysis of Pseudoacetothiamine (Va)——The solution of 500 mg of Va in 14 ml of 1 N HCl was allowed to stand for 10 days at room temperature, and 310 mg of NH_4SCN was added to adjust pH 5.8. To the solution NaCl was added to separate out B_1–SCN salt quantitatively. Recrystallization from MeOH gave the crystals of mp 190° which showed identical IR spectrum with the authentic If.