UDC 577.164.11

152. Akira Takamizawa, Kentaro Hirai, and Yoshio Hamashima : Studies on the Pyrimidine Derivatives. XXII.[1] The Syntheses of New Thiamine Derivatives.

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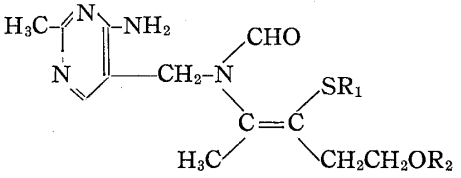
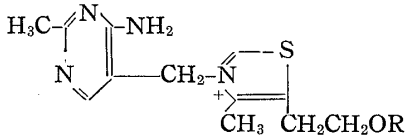
In our previous papers,[1,2] the syntheses of many S-alkoxycarbonylthiamine (I) and O,S-bis(alkoxycarbonyl)thiamine (II) derivatives which showed excellent thiamine activity were reported.

In an attempt to investigate related compounds with high thiamine activity, new thiamine derivatives were synthesized. Various phosphoryl groups have been introduced into the hydroxyl group of thiamine (III),[3] but direct introduction of substituents of organic groups to the hydroxyl group of III has not so far been reported. The O-acylthiamine (IV) was obtained either by the method of thiamine syntheses[4] or by rearrangement of S-acylthiamine (V).[5]



I : R_1=COOR, R_2=H
II : R_1=R_2=COOR or R_1=COOR, R_2=COOR′
V : R_1=COR, R_2=H



III : R=H
IV : R=COR

Direct introduction of the substituents of organic groups to the hydroxyl group of thiamine (III) was attempted by the use of phosgene. A suspension of thiamine hydrochloride in chloroform, when heated with phosgene in a sealed tube, gave O-chlorocarbonylthiamine (VI). This substance, which was unstable and difficult to purify, was assigned structure (VI) since it showed absorption bands at 1765 (C=O) and 1159 (C-O) cm^{-1} in its infrared spectrum.

When VI was treated with ethanol, there was obtained O-ethoxycarbonylthiamine hydrochloride (VII), which was found to be identical with the product obtained from S-ethoxycarbonylthiamine (VIII) by rearrangement.[1] VI, on treatment with dimethylamine, yielded a free base, m.p. 106~108° (decomp.), and on treatment with piperidine it gave a free base, m.p. 138° (decomp.). These substances exhibited absorption maxima at 235 and 267 mμ in the ultraviolet region and were proved by conversion into their hydrochlorides to be identical with O-dimethylcarbamoylthiamine hydrochloride (IX) and O-piperidinocarbonylthiamine hydrochloride (X), respectively, which were synthesized by the method mentioned later.

The above free bases were at first tentatively assigned structures of 4-aminopyrimidine systems (XI, XII). However, the elemental analyses showed values corresponding to $C_{15}H_{21}O_2N_5S$ and $C_{18}H_{25}O_2N_5S$, which have 1 mole of water less than the

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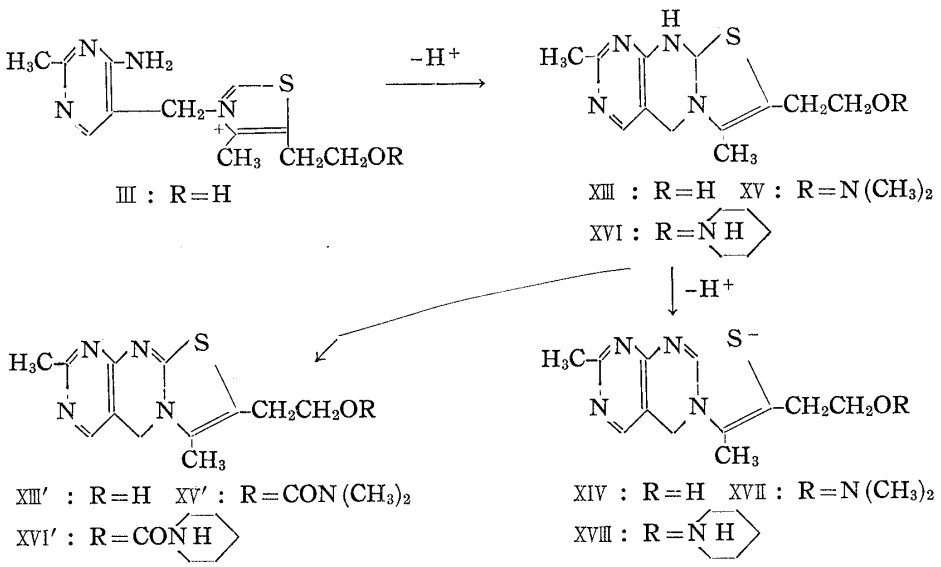
1) Part XXI. This Bulletin, **10**, 1107 (1962).
2) A. Takamizawa, K. Hirai : *Ibid.*, **10**, 1102 (1962).
3) P. Karrer, M. Viscontini : Helv. Chim. Acta, **29**, 711 (1946), etc.
4) *a*) T. Sano : Bull. Chem. Soc. Japan, **19**, 185 (1944). *b*) T. Matsukawa, S. Yurugi : Yakugaku Zasshi, **71**, 69 (1951), etc.
5) *a*) S. Yoshida : Yakugaku Zasshi, **74**, 993 (1954). *b*) H. Kawasaki : *Ibid.*, **74**, 789 (1954).

formulas (XI) and (XII). The infrared spectra of these compounds did not show any absorption of a N-formyl group around 1650 cm^{-1}. Moreover, in the nuclear magnetic resonance spectra, no signal*[2] of N-formyl proton was observed. These results suggest that these free bases of O-carbamoylthiamine may be formulated as XV and XVI, respectively.

Maier, *et al.*[6] showed that in alcoholic sodium ethoxide solution the amino group of thiamine adds, with simultaneous loss of a proton, to the thiazolium ring to yield the intermediate, *viz.*, tricyclic, dihydrothiochrome form (XIII); opening of the thiazole



ring and loss of a second proton produces the cyclic yellow thiol form (XIV). Kasahara[7] reported that substances having the 5,6-dihydropyrimido [4,5-*d*] pyrimidine system exhibit an absorption peak near 334 mμ. Our compounds also showed an absorption maximum at 334 mμ in alcoholic sodium ethoxide solntion. Th these facts support the assumption that the free bases of O-carbamoylthiamine take the tricyclic(dihydrothiochrome) forms (XV) and (XVI).

The alcoholic solutions of XV and XVI, upon standing, gradually exhibited a blue fluorescence, perhaps due to the oxidations to the thiochrome forms (XV') and (XVI'), respectively. Reaction of carbamoylchloride with thiamine sodium salt (XIX) produced the corresponding S-carbamoylthiamine, and S-dimethylcarbamoylthiamine (XX), S-piperidinocarbonylthiamine (XXI) and S-morpholinocarbonylthiamine (XXII) were obtained.

In Part XXI[1] of this series we reported the conversion of S-alkoxycarbonylthiamine into O-alkoxycarbonylthiamine by S→O rearrangement of the alkoxycarbonyl group. When S-dimethylcarbamoylthiamine (XX) and S-piperidinocarbonylthiamine (XXI) were treated with alkali or acid, they were converted into O-dimethylcarbamoylthiamine hydrochloride (IX) and O-piperidinocarbonylthiamine hydrochloride (X), which were found to be identical with the respective compounds obtained from VI. Therefore, it became evident that S→O rearrangement of the carbamoyl group takes place in the same manner as in the case of alkoxycarbonyl group. Application of this rearrangement afforded O,S-bis substituted thiamine derivatives.

*[2] The NMR spectra, measured with a Varian A-60 spectrometer at 60 Mc. in $CDCl_3$ *vs.* tetramethyl silane as internal reference, showed no signal at about 1.94 τ. (cf. Y. Asahi : The 16th Annual Meeting of the Pharmaceutical Society of Japan, November, 1962 at Shizuoka).

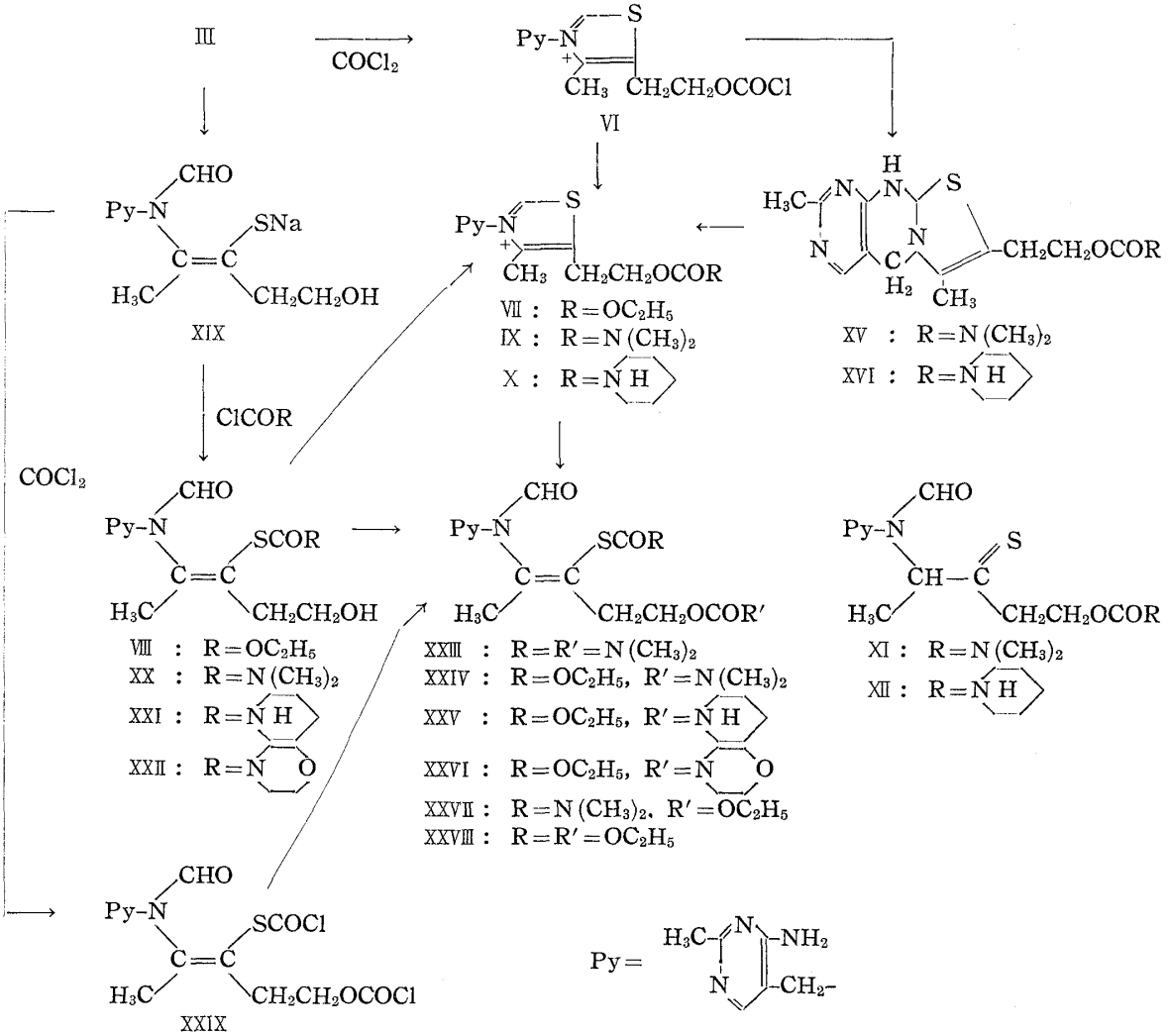
6) G.D. Maier, D.E. Metzler : J. Am. Chem. Soc., **79**, 4386 (1957).
7) S. Kasahara : This Bulletin, 8, 340 (1960).

O,S-Bis(dimethylcarbamoyl)thiamine (XXIII) was prepared by the reaction of dimethylcarbamoyl chloride in alcoholic sodium ethoxide solution with either thiamine sodium salt (XIX), S-dimethylcarbamoylthiamine (XX) or O-dimethylcarbamoylthiamine (IX).

Treatment of S-dimethylcarbamoylthiamine (XX) with ethylchloroformate in alcoholic sodium ethoxide solution yielded O-dimethylcarbamoyl-S-ethoxycarbonylthiamine (XXIV), which was found to be identical with the compound obtained from the reaction of O-dimethylcarbamoylthiamine (IX) with ethylchloroformate. In a similar manner, O-piperidinocarbonyl-S-ethoxycarbonylthiamine (XXV) and O-morpholinocarbonyl-S-ethoxycarbonylthiamine (XXVI) were obtained from S-piperidinocarbonylthiamine (XXI) and S-morpholinocarbonylthiamine (XXII), respectively.

O-Ethoxycarbonyl-S-dimethylcarbamoylthiamine (XXVII) was obtained by the action of dimethylcarbamoyl chloride on S-ethoxycarbonylthiamine (VIII) in alcoholic sodium ethoxide solution, and its identity was confirmed by direct comparison with the compound obtained from O-ethoxycarbonylthiamine (VII) by the action of dimethylcarbamoyl chloride in alcoholic sodium ethoxide solution.

A suspension of thiamine sodium salt (XIX) in chloroform, when heated with phosgene in a sealed tube, gave an oily substance which showed infrared absorption bands at 1761 (OCOO) and 1716 (SCOO) cm^{-1}. The assignment of structure of O,S-bis(chlorocarbonyl)thiamine (XXIX) to this substance was supported by the following facts. This compound, on treatment with dimethylamine, afforded O,S-bis(dimethylcarbamoyl)-thiamine (XXIII), and with ethanol, O,S-bis(ethoxycarbonyl)thiamine (XXVIII).



All of these S-carbamoylthiamines and O,S-bis substituted thiamines gave a negative, but after treatment with alkali or acid a positive thiochrome reaction. S-Carbamoylthiamine derivatives were absorbed only slightly in the intestine. O-Dimethylcarbamoyl-S-ethoxycarbonylthiamine (XXIV) showed a high intestinal absorption, but in this case, the dimethylcarbamoyl group of this compound appeared to have hardly been split off in the organism. Therefore, it is understood that this compound is difficult to be phosphorated to cocarboxylase and as a result it exhibits only a slight thiamine activity.*3

Experimental

O-Chlorocarbonylthiamine Hydrochloride (VI)——A suspension of thiamine hydrochloride (2 g.) and $COCl_2$ (4 cc.) in $CHCl_3$ (15 cc.) was heated for 4 hr. at 100° in a sealed tube, and evaporated to dryness under reduced pressure. The product was unstable and difficult to purify. IR ν_{max}^{Nujol} cm^{-1} : 1765 (C=O), 1159 (C-O).

Action of Ethanol on VI——To the foregoing product (VI), EtOH (20 cc.) was added and the solution was heated for 5 hr. at 50~60°. After standing overnight at room temperature, the reaction mixture was concentrated to dryness. The product was separated by recrystallization from EtOH into colorless needles (1.6 g.) of m.p. 208~210° (decomp.) and colorless needles (0.3 g.) of m.p. 230~232° (decomp.). These crystals were proved to be identical with O-ethoxycarbonylthiamine hydrochloride[1] (VII) and thiamine hydrochloride (III), respectively, by IR spectra and paper chromatography.*4

O-Dimethylcarbamoyldihydrothiochrome (XV)——A suspension of VI (from thiamine 2 g.) in $CHCl_3$ (30 cc.) was cooled to −5°, and a solution of dimethylamine in $CHCl_3$ was added dropwise under cooling until the solution became alkaline. After standing for 1 hr. at −5°, the reaction mixture was washed with a small amount of H_2O and then cold $NaHCO_3$ solution, dried over anhyd. $MgSO_4$ and concentrated *in vacuo* below 40°. The syrup obtained was induced to crystallize by addition of AcOEt. A minimum amount of EtOH was then added to dissolve the resulting crystals and the solution treated with charcoal. This solution, when allowed to stand for 2 days in a refrigerator at −20°, deposited pale yellow scales (0.3 g.), m.p. 106~108° (decomp.), which did not exhibit any fluorescence, but gave a positive thiochrome reaction. IR ν_{max}^{Nujol} cm^{-1} : 1700 (C=O), 1190 (C-O). UV $\lambda_{max}^{95\%\ EtOH}$ mμ (log ε) : 234 (3.99), 266 (3.74). Rf*4 : 0.43. *Anal.* Calcd. for $C_{15}H_{21}O_2N_5S$: C, 53.80; H, 6.31; N, 20.90. Found : C, 53.16; H, 6.54; N, 20.29.

O-Dimethylcarbamoylthoylthiamine hydrochloride (IX)——XV was treated with MeOH-HCl and the solution was concentrated *in vacuo* to dryness. The product was recrystallized from MeOH-AcOEt to give colorless needles, m.p. 230° (decomp.), Rf : 0.46. Thiochrome test is positive. IR ν_{max}^{Nujol} cm^{-1}: 1697 (C=O), 1185 (C-O). *Anal.* Calcd. for $C_{15}H_{22}O_2N_5SCl \cdot HCl$: C, 44.11; H, 5.68; N, 17.15. Found : C, 44.18; H, 5.95; N, 16.88.

O-Piperidinocarbonyldihydrothiochorome (XVI)——To a suspension of VI (from thiamine 4 g.) in $CHCl_3$, a solution of piperidine (4 g.) in $CHCl_3$ was added under cooling with a freezing mixture (ice and NaCl). After standing for 2 hr. under cooling, the solution was washed with cold $NaHCO_3$ and dried over anhyd. $MgSO_4$. The $CHCl_3$ was removed under reduced pressure below 40°, and the residue was induced to crystallize by the addition of small amounts of Me_2CO and AcOEt. The crystalline product was washed with Me_2CO, dissolved in a minimum amount of $CHCl_3$, and treated with charcoal. After filtration Me_2CO was added and the solution allowed to stand at −20° in a refrigerator, whereupon colorless crystals precipitated. Yield, 1.8 g. Recrystallization from Me_2CO and $CHCl_3$ gave colorless rhombics, m.p. 138° (decomp.), Rf : 0.51. IR ν_{max}^{Nujol} cm^{-1} : 1685 (C=O), 1150 (C-O). UV $\lambda_{max}^{95\%\ EtOH}$ mμ (log ε): 235 (4.12), 269 (3.88). *Anal.* Calcd. for $C_{18}H_{25}O_2N_5S$: C, 57.58; H, 6.71; N, 18.66; S, 8.54. Found : C, 57.48; H, 6.85; N, 18.28; S, 8.82.

O-Piperidinocarbonylthiamine Hydrochloride (X)——XVI was treated with MeOH-HCl and resulting crystals were recrystallized from MeOH-AcOEt. Colorless needles, m.p. 229° (decomp.). *Anal.* Calcd. for $C_{18}H_{26}O_2N_5SCl \cdot HCl \cdot H_2O$: C, 46.35; H, 6.27; N, 15.02. Found : C, 46.75; H, 6.28; N, 15.40.

S-Dimethylcarbamoylthiamine (XX)——Thiamine sodium salt (XIX) (8.5 g.) was suspended in 100 cc. of 99% EtOH and 3 g. of dimethylcarbamoyl chloride was added. The mixture was warmed at 50° for 1.5 hr. under stirring, filtered, and evaporated *in vacuo* to dryness. The residue was extracted with $CHCl_3$, washed with cold $NaHCO_3$ solution, and dried over anhyd. $MgSO_4$. Evaporation of the solvent

*3 Biological tests were undertaken by Dr. T. Mineshita, *et al.* of this Laboratory. A more detailed report was presented in Ann. Rep. Shionogi Research Lab., **12**, 6 (1962). In this report, alkoxycarbonylthiamine derivatives are described conventionally as carbalkoxythiamine

*4 AcOH-H_2O-BuOH = 1:5:4.

left a solid, which, after recrystallization, formed colorless prisms, m.p. 171~172°; yield 4.8 g. They gave a negative, but, after treatment with alkali, a positive thiochrome reaction. *Anal.* Calcd. for $C_{15}H_{23}O_3N_5S$: C, 51.00; H, 6.52; N, 19.82. Found : C, 51.10; H, 6.67; N, 19.60.

S-Piperidinocarbonylthiamine (XXI)——Piperidinocarbonyl chloride (1.5 g.) was added to a suspension of thiamine sodium salt (XIX) (4.2 g.) in EtOH and the mixture was warmed at 40~45° for 2 hr. under stirring. The precipitate was removed by filtration, the filtrate was cooled in an ice bath, and the separating crystals were collected and recrystallized from Me_2CO to colorless prisms, m.p. 156~157° (decomp.). Yield. 2.4 g. *Anal.* Calcd. for $C_{18}H_{27}O_3N_5S$: C, 55.00; H, 6.87; N, 17.80. Found : C, 54.80; H, 7.18; N. 17.79.

R-Morphorinocarbonylthiamine (XXII)——Morpholinocarbonyl chloride (3.0 g.) was added to a suspension of thiamine sodium salt (XIX) (8.4 g.) in 70 cc. of EtOH and the mixture was warmed at 40~45° for 2 hr. under stirring. After the same treatment as described above, the crude crystals were recrystallized from EtOH to colorless prisms, m.p. 170~171° (decomp.). Yield, 5.7 g. *Anal.* Calcd. for $C_{17}H_{25}O_4N_5S$: C, 51.70; H, 6.33; N, 17.70. Found: C, 51.49; H, 6.45; N, 17.71.

earrangements of S-Carbamoylthiamine into O-Carbamoylthiamine——XX (1 g.) was added to a solution of sodium ethoxide (prepared from 0.13 g. of Na and 10 cc. of abs. EtOH) under cooling and the solution was stirred for 20 min. at 25°. HCl was added and the solution was concentrated *in vacuo*. The residue was recrystallized from MeOH-AcOEt to colorless needles, m.p. 230° (decomp.), which was identical in all respects with O-dimethylcarbamoylthiamine hydrochloride (IX) obtained from the reaction of O-chlorocarbonylthiamine hydrochloride (VI) with dimethylamine.

In a simillar manner, XXI was converted into X.

O,S-Bis(dimethylcarbamoyl)thiamine (XXIII)——A) From XX : XX (1 g.) was added to a solution of sodium ethoxide (prepared from 0.13 g. of Na and 10 cc. of abs. EtOH) under cooling and the solution was stirred for 20 min. at 25°. Dimethylcarbamoyl chloride (0.61 g.) was then added and the solution was warmed at 50° for 30 min. under stirring. After removal of the separated NaCl, the filtrate was concentrated *in vacuo*, and the residue was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with $NaHCO_3$ solution, dried over anhyd. $MgSO_4$, and evaporated to give a solid, which, upon recrystallization from Me_2CO, formed colorless scales, m.p. 172°. IR ν_{max}^{Nujol} cm^{-1} : 1705 (C=O), 1180 (C-O). *Anal.* Calcd. for $C_{18}H_{28}O_4N_6S$: C, 50.93; H, 6.65; N, 19.80. Found : C, 50.90; H, 6.82; N, 19.52.

B) From IX : IX (1.6 g.) was dissolved in a solution of sodium ethoxide (prepared from 0.2 g. of Na and 40 cc. of EtOH), and 0.94 g. of dimethylcarbamoyl chloride was added. After warming at 50° for 60 min., followed by the same treatment as described above, this solution gave XXIII as colorless scales (0.45 g.), m.p. 171~172°, which was identified with the compound obtained from XX by IR spectral comparison.

C) From XXIX : Thoroughly dried thiamine sodium salt (XIX) (10 g.) and 5 g. of $NaHCO_3$ were suspended in 100 cc. of $CHCl_3$, and excess liquid $COCl_2$ was added under vigorous stirring at −30°. After standing at room temperature, the solution was filtered from the separated solid and the filtrate was concentrated *in vacuo*, leaving an oily product, which showed IR absorption bands at 1761 (OCOCl) and 1716 (SCOCl) cm^{-1}. Dimethylamine (2.5 g.) in $CHCl_3$ (30 cc.) was added to a solution of the oily product (XXIX) in $CHCl_3$ After stirring for 20 min. at room temperature, the $CHCl_3$ solution was washed with cold H_2O and dried over anhyd. $MgSO_4$. The solvent was evaporated to give a solid, which, upon recrystallization from Me_2CO, afforded XXIII as colorless scales, m.p. 171~172°. Yield, 0.35 g.

O,S-Bis(ethoxycarbonyl)thiamine (XXVIII)——A suspension of 3 g. of the crude compound (XXIX) in 20 cc. of EtOH was stirred for 5 hr. at room temperature. Treatment by the ordinary method gave 0.74 g. of XXVIII, which was shown to be identical with the compound reported in a previous paper.[1]

O-Dimethylcarbamoyl-S-ethoxycarbonylthiamine (XXIV)——A) From XX : Na (0.13 g.) was dissolved in EtOH (20 cc.) and 1 g. of XX was added. After stirring for 20 min. at 25°, $ClCOOC_2H_6$ (0.61 g.) was added and the solution was stirred for 20 min. at 45~50°. The precipitates were filtered off and the filtrate was concentrated *in vacuo*. The residue was extracted with $CHCl_3$ and washed with $NaHCO_3$ soluton and H_2O successively. The $CHCl_3$ solution, after shaking with 15% HCl, was dried over anhyd. $MgSO_4$ and evaporated. The crystals obtained were recrystallized from Me_2CO-AcOEt-H_2O to colorless rhombics, m.p. 113°. Yield, 0.5 g. IR ν_{max}^{Nujol} cm^{-1} : 1719 (C=O, at OCON), 1699 (C=O, at SCON), 1182 (C-O), 1153 (C-O). *Anal.* Calcd. for $C_{18}H_{27}O_5N_5S \cdot HCl \cdot H_2O$: C, 45.05; H, 6.30; N, 14.59. Found : C, 45.14; H, 6.48; N, 14.03.

B) From IX : The crude compound (IX) (prepared from 2 g. of III *via* VI) was dissolved in a solution of Na (0.2 g.) in EtOH (40 cc.). After addition of $ClCOOC_2H_5$ (0.92 g.) and stirring for 20 min. at 45~50°, the solution was treated as described above to give XXIV as colorless rhombics (0.62 g.), m.p. 108~109°, which was identified by IR spectra with the crystals described in A.

O-Piperidinocarbonyl-S-ethoxycarbonylthiamine (XXV)——Na (0.35 g.) was dissolved in EtOH (60 cc.) and a solution of XXI (3 g.) in EtOH (60 cc.) was added under stirring and cooling. After stirring for a further 20 min. at 25°, $ClCOOC_2H_5$ (1.65 g.) was added and the solution was stirred for 1 hr. at 45~50°. The precipitates were removed by filtration and the filtrate was concentrated *in vacuo*. The residue was extracted with $CHCl_3$ and the extract, after washing, drying and evaporating, yielded 1.5 g. of

crystals, which were recrystallized from Me_2CO-Et_2O to colorless prisms, m.p. 110°. IR ν_{max}^{Nujol} cm^{-1}: 1722 (C=O, at SCOO), 1693 (C=O, at NCOO). *Anal.* Calcd. for $C_{21}H_{31}O_5N_5S$: C, 54.20; H, 6.67; N, 15.05. Found : C, 54.27; H, 7.17; N, 14.71.

O-Morphorinocarbonyl-S-ethoxycarbonylthiamine (XXVI)——XXII (3 g.) was treated with $ClCOOC_2H_5$ (1.65 g.) in a alcoholic sodium ethoxide solution as described above. Colorless prisms, m.p. 116~118° (from Me_2CO) were obtained as the hydrochloride. Yield, 2 g. IR ν_{max}^{Nujol} cm^{-1} : 1718 (C=O, at SCOO), 1691 (C=O, at NCOO). *Anal.* Calcd. for $C_{20}H_{29}O_6N_5S \cdot HCl \cdot H_2O$: C, 46.10; H, 6.14; N, 13.42; H_2O, 3.45. Found : C, 46.45; H, 6.48; N, 13.37; H_2O, 3.49.

O-Ethoxycarbonyl-S-dimethylcarbamoylthiamine (XXVII)——A) From VIII : Na (0.33 g) was dissolved in EtOH (50 cc.) and VIII (5 g.) was added. After stirring for 5 min. at 17~20° to effect a clear solution, dimethylcarbamoyl chloride (1.52 g.) was added and the mixture was stirred for 4 hr. at 50°. On treatment as described above, colorless prisms, m.p. 76° (from Me_2CO-AcOEt) were obtained. Yield, 4.2 g. IR ν_{max}^{Nujol} cm^{-1} : 1741 (C=O, at OCOO), 1668 (C=O, at SCON). *Anal.* Calcd. for $C_{18}H_{27}O_5N_5S \cdot HCl \cdot H_2O$: C, 45.05; H, 6.11; O, 20.03. Found : C, 44.94; H, 6.40; O, 20.27.

B) From VII : The hydrochloride (5 g.) of VII was dissolved at room temperature in EtOH (50 cc.) containing 0.81 g. of Na. After stirring for 30 min., 1.26 g. of dimethylcarbamoyl chloride was added dropwise and the mixture was warmed at 50° for 2 hr. under stirring. Treatment by the ordinary method yielded 2.3 g. of colorless prisms, m.p. 76°, identical with the sample obtained as described above.

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Summary

Direct introduction of substituents of organic groups to the hydroxyl group of thiamine was achieved by the use of phosgene. O-Chlorocarbonylthiamine (VI) thus prepared was converted into O-carbamoylthiamine and O-alkoxycarbonylthiamine. The free bases of O-carbamoyl derivatives were assigned dihydrothiochrome structures (XV) and (XVI). S-Carbamoylthiamine derivatives were also prepared. Application of S→O rearrangement of the carbamoyl group gave various O,S-bis substituted thiamine derivatives.

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