UDC 547.853

180. Akira Takamizawa and Kentaro Hirai: Studies on the Pyrimidine Derivatives. XX.*1 On the Studies of S-Alkoxycarbonylthiamine. (1).

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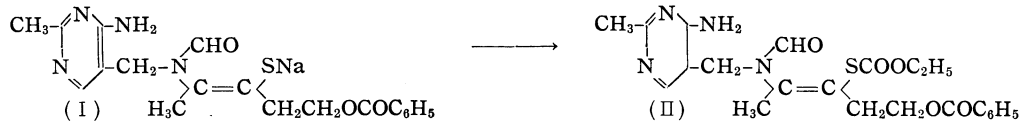
Since the discovery of the new thiamine derivative, allithiamine,[1] many reports have been published about the compounds which show thiamine-like activities and are absorbed from the intestine more readily than thiamine.

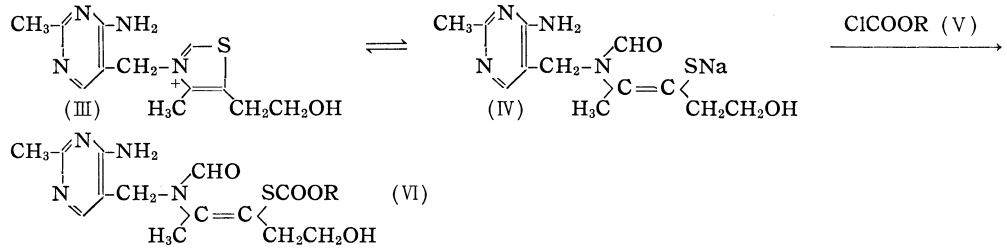
For the purpose of obtaining a compound possessing excellent thiamine activity, the authors prepared many S-alkoxycarbonylthiamine derivatives.

Formerly, Shirakawa[2] reported that the sodium salt of thiol-type O-benzoylthiamine (I) reacted with ethyl chloroformate to give O-benzoyl-S-ethoxycarbonylthiamine (II), which showed a slight thiamine activity, though its details have not been reported.



Since it seemed to be worthwhile to examine the biological activity of various S-alkoxycarbonylthiamines, synthesis of such compounds was undertaken.

When an aqueous solution of sodium salt of thiol-type thiamine (IV), which was prepared from thiamine (III) and sodium hydroxide, was reacted with an equivalent amount of alkyl chloroformate (V), the corresponding N-[(2-methyl-4-amino-5-pyrimidinyl)methyl]-N-(1-methyl-2-alkoxycarbonylthio-4-hydroxy-1-butenyl)formamide (S-alkoxycarbonylthiamine) (VI) was obtained.



(Va), (VIa) R=CH_3 (Vb), (VIb) R=C_2H_5 (Vc), (VIc) R=C_3H_7
(Vd), (VId) R=i-C_3H_7 (Ve), (VIe) R=C_4H_9 (Vf), (VIf) R=i-C_4H_9
(Vg), (VIg) R=i-C_5H_{11} (Vh), (VIh) R=C_6H_{11} (Vi), (VIi) R=C_8H_{17}
(Vj), (VIj) R=CH_2=CH-CH_2 (Vk), (VIk) R=$CH_3OCH_2CH_2$ (Vl), (VIl) R=$C_6H_5CH_2CH_2$

Various kind of alkyl, substituted alkyl and aralkyl chloroformate (Va~Vl) were used, and the corresponding S-alkoxycarbonylthiamine, S-substituted alkoxycarbonylthiamine and S-aralkoxycarbonylthiamine (VIa~VIl) were prepared, respectively.

They gave a negative thiochrome reaction but the reaction became positive after a treatment with either acid or alkali. The infrared spectra of these compounds showed

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*1 Part XIX: Yakugaku Zasshi, 82, 1202 (1962).

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1) *a*) M. Fujiwara, H. Watanabe: Proc. Japan Acad., **28**, 156 (1952). *b*) T. Matsukawa, S. Yurugi: *Ibid.*, **28**, 146 (1952).

2) K. Shirakawa: Yakugaku Zasshi **74**, 367 (1954).

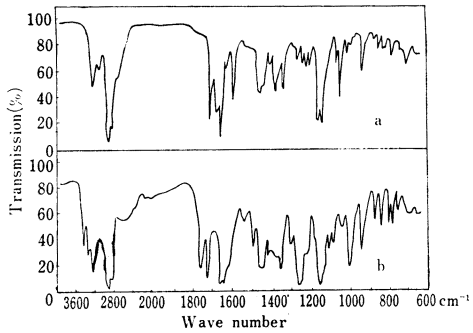
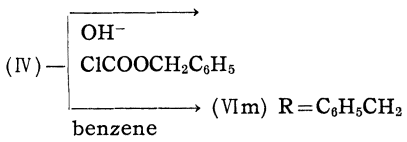


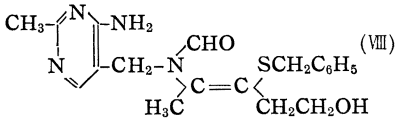
Fig. 1. Infrared Absorption Spectra (in Nujol)
a) S-Butoxycarbonylthiamine (VIe)-HCl
b) O,S-Bis(ethoxycarbonyl)thiamine (X)-HCl

absorption bands at 1710~1720 cm^{-1} ($\nu_{C=O}$), 1150 cm^{-1} (ν_{C-O}) based on SCOO and at about 1050 cm^{-1} (ν_{C-O}) based on primary OH. (Fig. 1).

It is known that an application of acid chloride to the thiol type of thiamine yields mostly O,S-diacylthiamine,[3] and only in a few cases S-acylthiamine[4] is obtained. However, interestingly, thiamine in alkaline solution was reacted with alkyl chloroformate to give S-alkoxycarbonylthiamine readily.

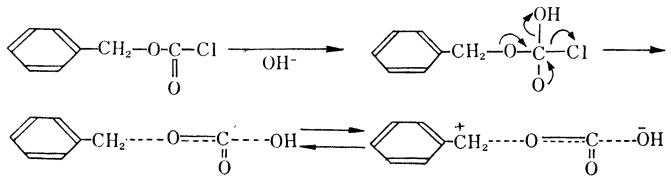
In this reaction, when thiamine in alkaline solution was reacted with benzyl chloroformate, it gave colorless prisms, m.p. 118°, and its infrared spectrum showed no C=O absorption. This compound was identified with thiamine benzyl sulfide (VIII), obtained by the reaction of thiamine and benzyl chloride in alcoholic solution.[5]





It was found that decarboxylation occured to give thioether type of thiamine. However, thiamine sodium salt (IV) was reacted with benzyl chloroformate in anhydrous benzene solution to give S-benzyloxycarbonylthiamine (VIm), it is considerably stable in cold alkaline solution. It has now been found that the thioether type of thiamine was yielded, accompanied with decarboxylation, in the presence of OH^- ion. It may be considered that this is caused by the stabilization of benzyl group through a transition state such as (IXa, b).

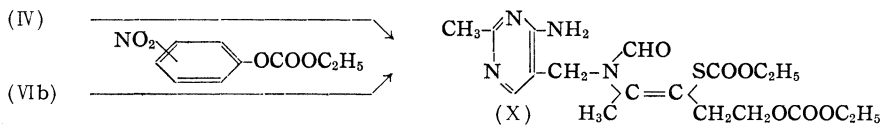
Further, the authors attempted to carbalkoxylate thiamine using reagents other than alkyl chloroformate. Recently, in the field of peptide synthesis, various reagents



(IXa) (IXb)

3) *a*) T. Matsukawa, H. Kawasaki : Yakugaku Zasshi, **73**, 705, 709 (1953). *b*) H. Kawasaki : *Ibid.*, **74**, 588, 1189 (1954). *c*) S. Yoshida : *Ibid.*, **74**, 993 (1954).
4) M. Matsui : Vitamins, **24**, 49 (1961).
5) H. Kawasaki, H. Yonemoto : Yakugaku Zasshi, **77**, 640 (1957). Reported as crystals containing benzene, m.p. 105° (decomp.).

have been used to protect the amino group of amino acids. Anderson *et al.*[6] synthesized many *tert*-butoxycarbonylamino acids by using *tert*-butyl *p*-nitrophenyl carbonate. The authors used ethyl *p*-nitrophenyl carbonate or ethyl *o*-nitrophenyl carbonate, and thiamine sodium salt was reacted with this reagent in NaOEt solution. From the reaction solution, crystals, m.p. 122~124°(decomp.), were obtained as a hydrochloride, which gave a negative thiochrome reaction but the reaction became positive after treatment with alkali. The ultraviolet spectrum was typical to aminopyrimidine (Fig. 2). Infrared spectrum of this compound showed absorptions at 1720, 1155(SCOO), 1750, 1260, 1010, 795(OCOO) cm^{-1} and the analytical value agreed with the molecular formula $C_{18}H_{26}O_6N_4S \cdot HCl \cdot H_2O$. These facts indicated that the compound was O,S-bis(ethoxycarbonyl)thiamine (X) hydrochloride hydrate.



Further, S-ethoxycarbonylthiamine (VIb) was reacted with ethyl *p*-nitrophenyl carbonate or ethyl *o*-nitrophenyl carbonate in alcoholate solution to give crystals, m.p. 122° (decomp.), after a treatment of reaction mixture as before. This compound was confirmed to be identical with O,S-bis(ethoxycarbonyl)thiamine hydrochloride obtained from thiamine sodium salt. It was found that the alcoholic OH group of (VIb) was substituted by ethoxycarbonyl group.

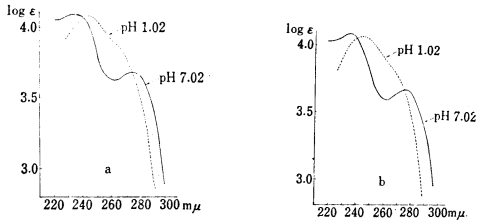


Fig. 2. Ultraviolet Absorption Spectra
a) S-Butoxycarbonylthiamine (VIe)-HCl
b) O,S-Bis(ethoxycarbonyl)thiamine (X)-HCl (in buffered solution)

All of these S-alkoxycarbonylthiamine derivatives and O,S-bis(ethoxycarbonyl)thiamine were found to have almost equal efficacy to thiamine and it was also found that absorption of these compounds through the intestinal canal was better than that compared with thiamine. Especially, S-ethoxycarbonylthiamine (VIb), S-butoxycarbonylthiamine (VIe) and O,S-bis(ethoxycarbonyl)thiamine (X), when orally administered, are excellent in regards to increase and duration in thiamine blood level as shown in Fig. 3 and Table I.[7]

Table I. Blood B_1 Levels and Urinary Excreted B_1 Following Oral Administration of 50 mg. of Alkoxycarbonylthiamine to Humans

Sample	No. of subject	Blood B_1 levels (μg./dl.)			μg. B_1 excreted (6 hr. urine)
		before	after 3 hr.	increase	
B_1-HCl	8	6.97	8.76	1.78±0.52	969± 67
S-Ethoxycarbonylthiamine (VIb)-HC	18	7.04	27.4	19.6 ±1.30	7587±385
S-Butoxycarbonylthiamine (VIe)-HCl	8	6.43	15.8	9.41±0.81	6681± 47
O,S-Bis(ethoxycarbonyl)thiamine(X)-HCl	8	7.20	29.7	22.5 ±1.93	7633±158

6) G. Anderson, A.C. McGregur : J. Am. Chem. Soc., **79**, 6180 (1957).
7) Biological tests were undertaken by Dr. T. Mineshita *et al.* of this laboratory. A detailed report will be presented elsewhere.

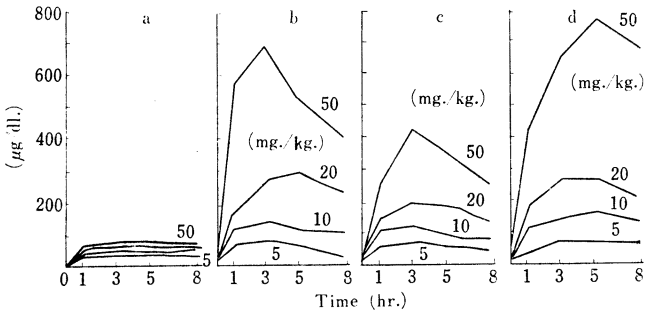


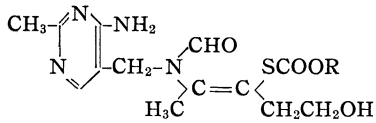
Fig. 3. Blood B_1 Levels Following Oral Administration of Various Doses of Alkoxycarbonylthiamine to Rabbits

a : B_1-HCl
b : S-Ethoxycarbonylthiamine (VIb)-HCl
c : S-Butoxycarbonylthiamine (VIe)-HCl
d : O, S-Bis(ethoxycarbonyl)thiamine (X)-HCl

Experimental

General Procedure for S-Alkoxycarbonylthiamine (VI)——a) To a solution of 0.01 mole of thiamine hydrochloride in 4 cc. of water, a cold solution of 0.03 mole of NaOH in 1.5 cc. of water was added with stirring in a cold bath. After standing for 30 min., 0.01 mole of alkyl chloroformate was added to this solution with stirring in a cold bath. A viscous oil separated solidified gradually. The product was filtered, washed with a small amount of water and recrystallized from a suitable solvent. When the oil separated did not solidify, it was extracted with either AcOEt or $CHCl_3$ and the extract, after dried over anhyd. $MgSO_4$, was evaporated to dryness *in vacuo*. The residue was covered with both Et_2O and petroleum ether and left standing to solidify, then purified by recrystallization from

Table II.



R		m.p. (°C) (decomp.)	Recryst. solvent	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Yield (%)
$-CH_3$ (VIa)		133～135	$(Me)_2CO$	$C_{14}H_{20}O_4N_4S$	47.19	5.66	15.72	47.49	6.00	15.94	23
$-C_2H_5$ (VIb)		144	AcOEt	$C_{15}H_{22}O_4N_4S$	50.84	6.26	15.81	50.92	6.45	15.79	85
〃	hydrochloride	175～176	EtOH-AcOEt	$C_{15}H_{22}O_4N_4S\cdot HCl$	46.09	5.93	14.33	46.44	6.21	14.00	
〃	nitrate	124～126	EtOH-AcOEt	$C_{15}H_{22}O_4N_4S\cdot HNO_3$	43.14	5.55	16.78	43.23	5.86	17.20	
〃	flavianate	192～194	EtOH	$C_{15}H_{22}O_4N_4S\cdot C_{10}H_6O_8N_2S$	44.90	4.22	12.57	45.15	4.69	12.57	
$-C_3H_7$ (VIc)		156～157	AcOEt	$C_{16}H_{24}O_4N_4S$	52.15	6.57	15.21	52.21	6.94	14.87	62
$-i-C_3H_7$ (VId)	hydrochloride	180	EtOH-AcOEt	$C_{16}H_{24}O_4N_4S\cdot HCl$	47.44	6.22	13.64	47.74	6.51	13.91	30
$-C_4H_9$ (VIe)		139～140	AcOEt	$C_{17}H_{26}O_4N_4S$	53.38	6.85	14.65	53.84	7.16	14.77	40
〃	hydrochloride	175	EtOH-AcOEt	$C_{17}H_{26}O_4N_4S\cdot HCl$	48.80	6.46	13.40	48.47	6.62	13.54	
$-i-C_4H_9$ (VIf)		142～143	benzene	$C_{17}H_{26}O_4N_4S$	53.38	6.85	14.65	53.58	6.96	14.29	23
$-i-C_5H_{11}$ (VIg)		134～136	benzene	$C_{18}H_{28}O_4N_4S$	54.78	7.33	14.17	54.52	7.12	14.13	10
$-C_6H_{11}$ (VIh)		152～154	AcOEt	$C_{19}H_{28}O_4N_4S$	55.86	6.91	13.71	56.07	6.90	13.61	10
$-C_8H_{17}$ (VIi)		165	AcOEt	$C_{21}H_{34}O_4N_4S\cdot 2H_2O$	53.14	7.86	11.81	53.03	7.56	12.01	5
$-CH_2CH{=}CH_2$ (VIj)		121～122	AcOEt	$C_{16}H_{22}O_4N_4S$	52.45	6.05	15.29	52.54	6.23	15.56	60
$-CH_2CH_2OCH_3$ (VIk)		127～128	AcOEt	$C_{16}H_{24}O_5N_4S$	49.98	6.29	14.57	50.15	6.43	14.55	55
$-CH_2CH_2C_6H_5$ (VII)	hydrochloride	172～173	EtOH-AcOEt	$C_{21}H_{26}O_4N_4S\cdot HCl$	54.01	5.61	12.00	54.12	6.04	11.67	7

a suitable solvent. b) To a suspension of 0.01 mole of thiamine sodium salt[8] in AcOEt, 0.01 mole of alkyl chloroformate was added with stirring. After stirring for 1 hr., precipitate was filtered and filtrate was evaporated *in vacuo*, the residue after solidifying, was recrystallized from a suitable solvent. (Table II). Thiochrome reaction was negative but the reaction turned to positive after a treatment of either acid or alkali.

S-Benzylthiamine (VIII)——a) To a solution of 1.1 g. of thiamine hydrochloride in 5.3 cc. of 10% NaOH, benzyl chloroformate was added with stirring in a cold bath. A viscous oil separated, which solidified gradually. The product was collected, washed with water, and dried to 0.4 g. of crude crystals. This was recrystallized from AcOEt to colorless prisms, m.p. 118°. *Anal.* Calcd. for $C_{19}H_{24}O_2N_4S$: C, 61.26; H, 6.50; N, 15.04. Found : C, 61.44; H, 6.70; N, 15.13. b) Thiamine in alcholic NaOH solution was reacted with benzyl chloride. After an evaporation of reaction mixture *in vacuo* crystals were obtained. The crude crystals were recrystallized from AcOEt to colorless prisms of m.p. 117~119°. The identity with a) crystals, m.p. 118°, was confirmed by IR in Nujol and mixed fusion tests.

S-Benzyloxycarbonylthiamine (VIm)——2.1 g. of sodium salt of thiamine was suspended in anhyd. benzene and the solution of benzyl chloroformate in benzene was added with stirring. After the reaction, undissolved materials were filtered and the filtrate was evaporated *in vacuo*. The residue solidified gradually, yield, 0.8 g. Recrystallization from AcOEt gave colorless prisms, m.p. 140° (decomp.), which were converted to the hydrochloride by the usual proceedure. The hydrochloride thus obtained was recrystallized from EtOH to colorless needles, m.p. 148~149° (decomp.). *Anal.* Calcd. for $C_{20}H_{24}O_4N_4S \cdot HCl \cdot H_2O$: C, 51.00; H, 5.78; N, 11.90. Found : C, 51.33; H, 6.05; N, 11.84. IR : $\nu_{C=O}$ 1712 cm^{-1} (Nujol).

O,S-Bis(ethoxycarbonyl)thiamine (X)——a) 12.8 g. of crude sodium salt of thiamine and 13.2 g. of ethyl *p*-nitrophenyl carbonate was suspended in 90 cc. of 2.3% NaOEt, and stirred for 2 hr. at 40~45°. The reaction mixture was filtered and the filtrate was evaporated *in vacuo*. Water was added to the residue and extracted with AcOEt. The AcOEt extract was washed with dil. Na_2CO_3 solution and H_2O, then was shaken with dil. HCl. The HCl layer was extracted with $CHCl_3$, and it was dried over anhyd. $MgSO_4$. The solvent was evaporated and the residual oil was solidified gradually, yield 6.0 g. Recrystallization from AcOEt gave colorless prisms of m.p. 120~122° (decomp.), showing positive Beilstein test, and negative thiochrome test, but later turned to positive after treatment with alkali. *Anal.* Calcd. for $C_{18}H_{26}O_6N_4S \cdot HCl \cdot H_2O$: C, 44.94; H, 6.08; N, 11.65; Cl, 7.37. Found : C, 45.05; H, 6.09; N, 11.61; Cl, 6.95. IR (Nujol) cm^{-1} : 1720, 1155 (SCOO); 1750, 1260, 1010, 795 (OCOO).

Aqueous layer of ACOEt extract was made acidic and extracted with AcOEt, dried and evaporated. The residue, needles of m.p. 114°, showed no depression of melting point with *p*-nitrophenol. b) 31.5 g. of S-ethoxycarbonylthiamine (VIb) and 18.9 g. of ethyl *p*-nitrophenyl carbonate was dissolved in 300 cc. of 1.2% NaOEt, and stirred for 1.5 hr. at 40~45°. The reaction mixture was treated as described above. 17 g. of product was obtained and recrystallized from AcOEt to colorless prisms of m.p. 122~124° (decomp.), which were shown to be identical with the compound obtained in a) by IR in Nujol. c) Instead of ethyl *p*-nitrophenyl carbonate, ethyl O-nitrophenylcarbonate was reacted with thiamine sodium salt or S-ethoxycarbonylthiamine as described above. O,S-Bis(ethoxycarbonyl)-thiamine was obtained in 33.5% and 35% yields respectively.

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Snmmary

Various kinds of S-alkoxycarbonylthiamine and O,S-bis(ethoxycarbonyl)thiamine were prepared. They showed a thiamine-like activity and were readily absorbed from the intestine. Some of them are excellent in regard to the increase and duration of thiamine blood levels when orally administered.

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8) O. Zima, R.R. Williams : Ber., **73**, 941 (1940).