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189. Isamu Utsumi, Toshio Watanabe, Kiyoshi Harada, and Goro Tsukamoto*1 : Studies on Thiamine Disulfide. XIX.*2 On Formation of Hypothiaminic Acids and Related Reactions.*3

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Refluxing of monosulfoxide (Ia) of O-benzoylthiamine disulfide in aqueous ethanol or an alkaline decomposition of the compound Ia provided a new compound, O-benzoylhypothiaminic acid (Ⅱ) which is presumed to be an intermediate in the course of formation of O-benzoylthiaminic acid (Ⅵ). An alkaline hydrolysis of Ⅱ afforded hypothiaminic acid (V). These hypothiaminic acids were oxidized to the corresponding thiaminic acids by hydrogen peroxide.

From the fact that, in refluxing the compound Ia in aqueous ethanol, O-benzoylthiamine disulfide (Ⅲ) was formed besides the compound Ⅱ, it seemed reasonable to assume that the decomposition pathway is disproportionation and the alkaline decomposition is similar hydrolysis to that of carboxylic esters to give the sulfinic acid (Ⅱ) and thiol (Ⅳ).

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In the previous papers on the oxidation of thiol-type thiamines, it was reported that various thiol-type thiamine derivatives,*4 which are easily converted to thiamine *in vivo*, provide 2-(2-methyl-4-amino-5-pyrimidyl)methylformamido-5-hydroxy-2-pentene-3-sulfonic acid (Ⅶ, thiaminic acid).[1,2] Whereas, it was confirmed that thiamine alkyl sulfides which are hard to revert to thiamine *in vivo* afford the corresponding sulfoxides and sulfones.[3]

The considerations of oxidative pathway from these thiamine derivatives to thiaminic acid have let us suppose the formation of 2-(2-methyl-4-amino-5-pyrimidyl)methylformamido-5-hydroxy-2-pentene-3-sulfinic acids (hereinafter referred to V and called hypothiaminic acid.)

Meantime, cysteic acid and taurine are well known to be formed in the course of metabolism of biologically important cysteine and cystine through cysteine sulfinic acid (alanine sulfinic acid) and hypotaurine, respectively.[4]

In this connection, the production of V is of interest in the study of the metabolism of thiol-type thiamines.

The present paper deals with the synthesis of hypothiaminic acid (V) and related reactions.

Previously, the authors reported thiolsulfinate-type thiamine (S-monoxide of O-benzoylthiamine disulfide : Ia) which was considered to be an intermediate between thiamine disulfide and thiaminic acid.[5]

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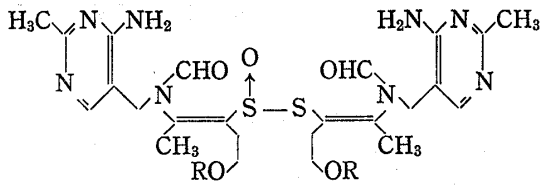
*3 Presented at the 23rd Annual Meeting of the Pharmaceutical Society of Japan, Sendai, October, 1966.

*4 *e.g.* thiamine disulfides, thiamine alkyl disulfides, S-acylated thiamines (containing S-calbalkoxythiamines), and cyanothiamine.

1) I. Utsumi, K. Harada, G. Tsukamoto : J. Vitaminol., **11**, 225 (1965).
2) I. Utsumi, K. Harada, G. Tsukamoto, I. Daira : *Ibid.*, **11**, 234 (1965).
3) G. Tsukamoto, T. Watanabe, I. Utsumi : Vitamins, **34**, 411 (1966).
4) L. Young, G.A. Maw : "Metabolism of Sulfur Compounds," Methuen Press, London (1958).
5) I. Utsumi, K. Harada, K. Kohno, G. Tsukamoto : Vitamins, **32**, 458 (1965).

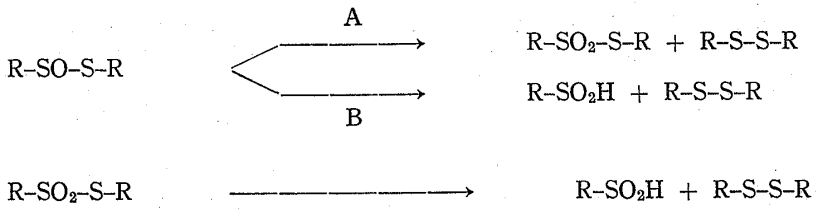


Ia : $R=COC_6H_5$ Ib : $R=H$

Chart 1.

Meanwhile, it has been reported that thiolsulfinates are unstable and easily disproportion to yield the corresponding thiolsulfonate and disulfide (A)[6,7] or sulfinic acid and disulfide (B).[8]

Now, if compound Ia passes the decomposition pathway of A, presumably the produced thiolsulfonate will be further decomposed to afford sulfinic acid and disulfide when it was allowed to react with sulfhydryl compounds or hydroxy anions.[9]



Meanwhile, sulfinic acid might be also produced through the B-course which W.E. Savige, *et al.*[8] have advocated recently in the report on the decomposition of S-monoxide of cystine.

Under these considerations, the disproportionation reaction of the compound Ia was investigated. The result was that thiolsulfonate could not be obtained, but sulfinic acid (II) was obtained.

The refluxing of monohydrate of Ia in 80% aqueous ethanol afforded colorless needles (II), m.p. 204~205° (decomp.). The ultraviolet absorption spectra were essentially identical with those of O-benzoylthiaminic acid (VI),[10] O-benzoylthiamine disulfide (III) or the corresponding thiolsulfinate (Ia).[5] On the other hand, the infrared spectrum (Fig. 1) was unexpectedly devoid of bands assignable to $-SO_2-S-$ of thiolsulfonate compounds and showed four bands at 1050~900 cm^{-1}, assignable to S=O of sulfinic acid group.[11]

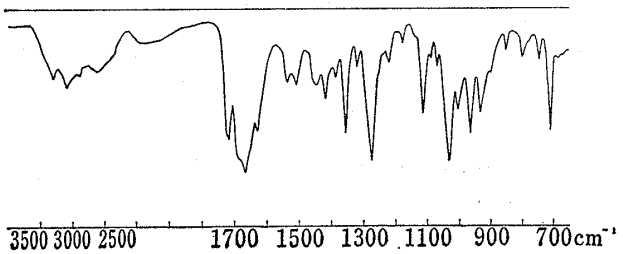


Fig. 1. Infrared Absorption Spectrum of O-Benzoylhypothiaminic Acid (II) in Potassium Bromide

In addition, the infrared spectrum exhibited characteristic absorption bands to benzoate, N-formyl, pyrimidine ring, and amino group linkaged to the ring. The elementary analytical date of II agreed with the formula $C_{19}H_{22}O_5N_4S$ which lacks one oxygen atom of O-benzoylthiaminic acid (VI). Compound II demonstrated closely similar behavior to those of VI toward paper partition chromatography, paper electorophoresis and titration curve.

All these observations suggest that compound II is 2-(2-methyl-4-amino-5-pyrimidyl)-methylformamido-5-benzoyloxy-2-pentene-3-sulfinic acid (hereinafter referred to O-benzoylhypothiaminic acid). Further, in view of the fact that the oxidation of II with

6) H.J. Backer, H. Kloosterziel : Rec. trav. chim., **73**, 129 (1954).
7) S. Oae, S. Kawamura : Bull. Chem. Soc. Japan, **35**, 1156 (1962).
8) W.E. Savige, J. Eagar, J.A. Maclaren, C.M. Boxburgh : Tetrahedron Letters, **1964**, 3289.
9) Houben-Weyl : "Methoden der Organischen Chemie" 4 Auflage, Georg Thieme Verlag, **9**, 317 (1955).
10) I. Utsumi, K. Harada, G. Tsukamoto : J. Vitaminol., **11**, 239 (1965).
11) S. Detoni, D. Hodzi : J. Chem. Soc., **1955**, 3163.

hydrogen peroxide[12] afforded almost quantitatively O-benzoylthiaminic acid (Ⅵ), the structure of Ⅱ was established as shown in Chart 2.

O-benzoylthiamine disulfide (Ⅲ) was obtained from the mother liquor which compound Ⅱ was filtered off, and the paper partition chromatography indicated that no products existed in the mother liquor except both the compounds Ⅱ and Ⅲ as eventual decomposition products.

The decomposition pathway of Ia thus could be shown in Chart 2.

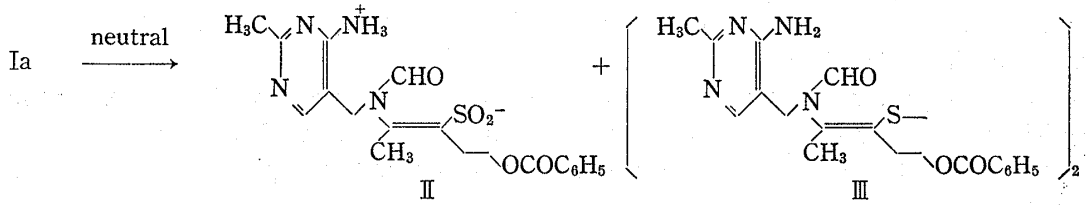


Chart 2.

With respect to the formation of sulfinic acid (Ⅱ) and disulfide (Ⅲ), the following two mechanisms are considered: (1) thiolsulfonate (Ⅲ′), of which formation is supposed from the disproportionation of Ia, might be also unstable and therefore hydrolized immediately to sulfinic acid (Ⅱ) and disulfide (Ⅲ) as shown in Chart 3, (2) the fission which W. E. Savige, *et al.* advocated would occur on thiolsulfinate itself and afford sulfinic acid and disulfide. It is not clear, however, by which way either (1) or (2) the compounds of Ⅱ and Ⅲ were formed.

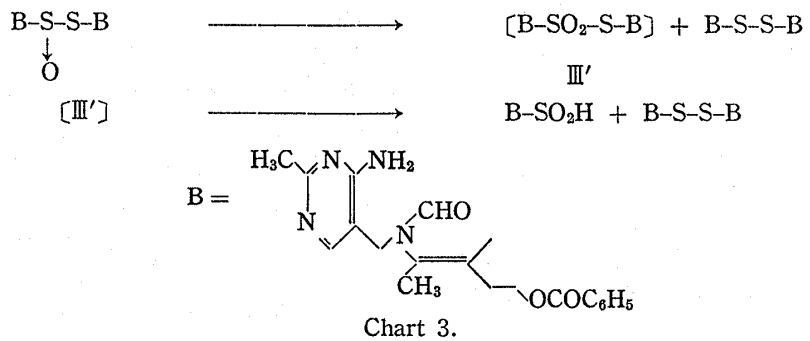


Chart 3.

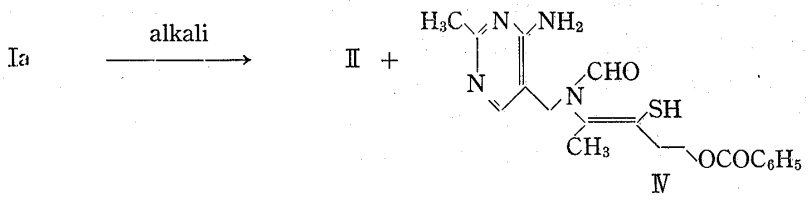
While, H. Kawasaki, *et al.* reported that the thiolsulfinate, monosulfoxide of thiamine benzyl disulfide, was hydrolized by alkali to give the corresponding thiol and sulfinic acid.[13]

The solution obtained by alkaline hydrolysis of Ia was applied to paper partition chromatography and paper electrophoresis, which revealed thiaminic acid-like product and thiamine. Therefore, the isolation of Ⅱ from alkaline hydrolized solution was tried.

The compound Ia was treated in 80% aqueous ethanol at room temperatures at pH 8~10, being considered the hydrolysis of benzoate moiety. Then, colorless needles, m.p. 204~205° (decomp.) was obtained, which was identical with Ⅱ in infrared spectrum.

Thus, it was certified that alkaline hydrolysis of Ia was decomposed corresponding thiol (Ⅳ) and sulfinic acid (Ⅱ) as represented as follows:

12) R. Otto, H. Ostrop: Ann., **141**, 370 (1867).
13) H. Kawasaki, H. Yonemoto: Yakugaku Zasshi, **77**, 640 (1957).



This reaction was presumed to be nucleophilic attack reaction of hydroxy anion to the sulfur atom of sulfinyl group.

Thus, O-benzoyl derivative of hypothiaminic acid (V) was obtained by the neutral or alkaline hydrolysis of Ia.

Similarly, hypothiaminic acid (V) will be prepared by the hydrolysis of the monosulfoxide of thiamine disulfide (Ib). However the formation of V from Ib was confirmed by only paper electrophoresis procedure and an attempt to prepare V by the debenzoylation of Ⅱ was made.

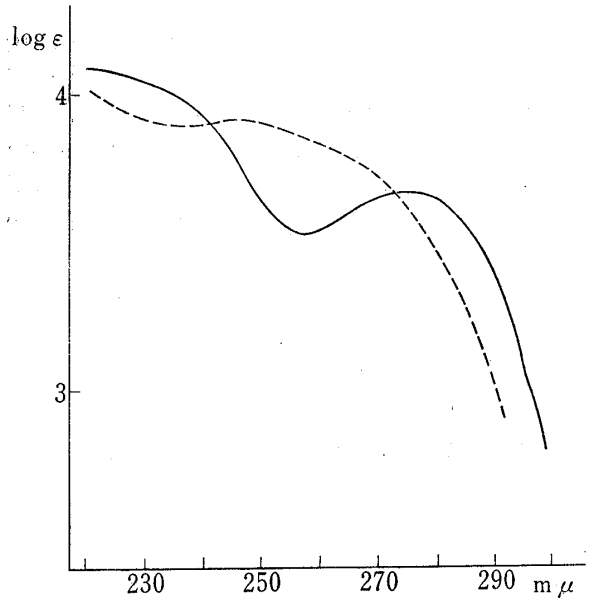


Fig. 2. Ultraviolet Absorption Spectra of Hypothiaminic Acid (V)
——— pH 7.3 - - - - - - pH 2.5

The compound Ⅱ was hydrolized in an alkaline solution under the same condition as described previously[14] and benzoic acid was obtained almost quantitatively. From the mother liquor, colorless prisms (V) m.p. 121~122° were obtained.

The elementary analysis of V showed empirical formula of $C_{12}H_{18}O_4N_4S \cdot 2\frac{1}{2}H_2O$. The compound V displayed major absorption peaks in ultraviolet spectra at 275 mμ in pH 7.3 solution and 247 mμ in pH 2.5 solution (Fig. 2). The absorption curves are different from those of thiaminic acid (Ⅶ) in opposition to the case of Ⅱ. Generally, it is known that sulfinic acids exhibit characteristic peak at near 247 mμ.[11] The characteristic absorption at 247 mμ, consequently, is presumed to be due to sulfinic acid group. But in the case of Ⅱ the characteristic peak of sulfinic acid group was not recognized. This would presumably be due to the benzoyl group absorption at near 240 mμ.

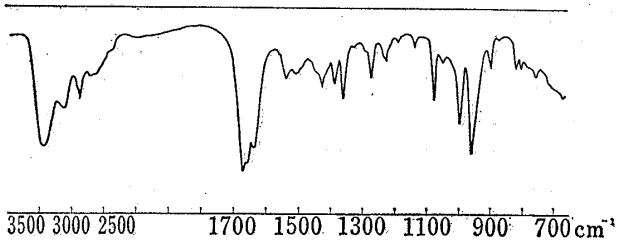


Fig. 3. Infrared Absorption Spectrum of Hypothiaminic Acid (V) in Potassium Bromide

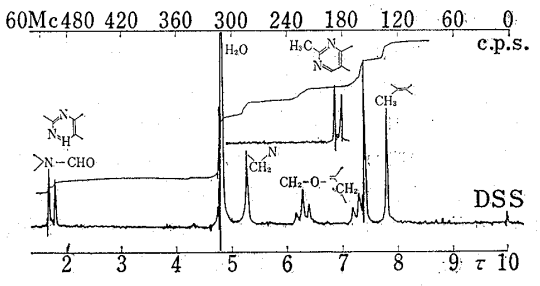


Fig. 4. Nuclear Magnetic Resonance Spectrum of Hypothiaminic Acid (V) in Deuterium Oxide (containing a little hydrochloric acid) at 60 Mc.p.s.

The infrared spectrum (Fig. 3) showed characteristic absorption bands at 990 and 995 cm^{-1}, which were presumed to be assignable to S=O of sulfinic acid group. The nuclear magnetic resonance spectrum, which was measured in deuterium oxide, showed all proton signals except labile protons (Fig. 4).

From these observations, the structure of V is presumed to be debenzoylated compound of II. In fact, the oxidation of V with hydrogen peroxide afforded thiaminic acid (VII) and the benzoylation of V gave II. Thus, the structure of V was confirmed.

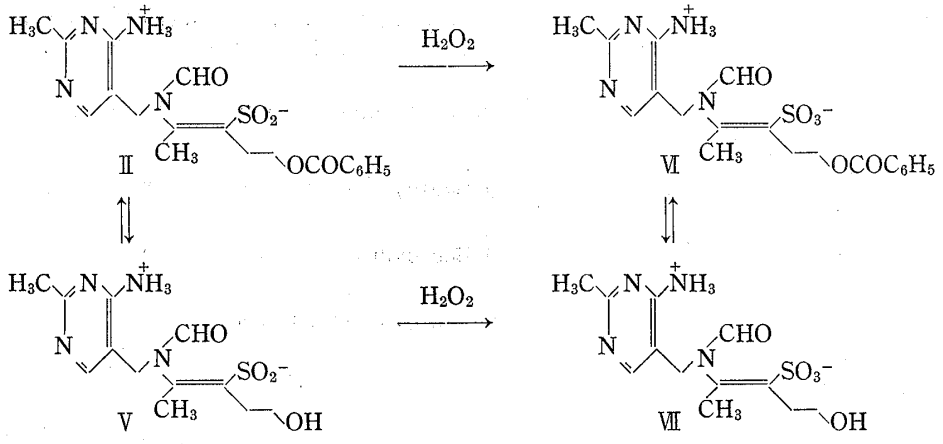


Chart 4.

In addition, from the absorption bands of NH_3^+ around 2600 cm^{-1} and 2000~1900 cm^{-1} in the infrared spectrum of O-benzoylhypothiaminic acid (II), and around 2650 cm^{-1} in that of hypothiaminic acid (V), it may be concluded that the compounds II and V behave themselve like zwitterion forms as thiaminic acids and their isomers do.[14] The titration curves of II and V were essentially identical with those of O-benzoylthiaminic acid (VI) and thiaminic acid (VII) described before.[10] The compounds II and V showed the same values in both paper partition chromatography and paper electrophoresis as the corresponding thiaminic acid, and were sparlingly soluble in most organic solvent. A little differences between the compounds II and VI is that the compound II is readily soluble in hot water while the compound VI is sparlingly soluble.

It has been well known that sulfinic acids are very unstable in acidic condition and easily disproportion to corresponding thiolsulfonate and sulfonic as follows : [15~18]

$$3R\text{-}SO_2H \longrightarrow R\text{-}SO_2\text{-}S\text{-}R + R\text{-}SO_3H + H_2O$$

Then, hypothiaminic acid (V) and O-benzoylhypothiaminic acid (II) were treated in acidic solution and thiaminic acid (VII) and O-benzoylthiaminic acid (VI) were obtained. From this result, the disproportionation of hypothiaminic acids is anticipated.

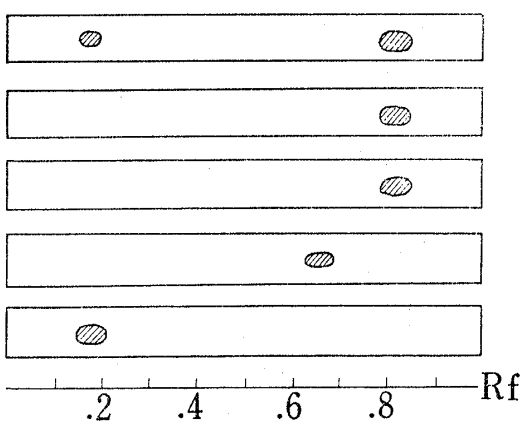
If the production of VI and VII from II and V is not due to oxidation reaction but to disproportionation reaction, it is presumed that either thiolsulfonate (III') or disulfide compounds, which would be formed by further decomposition of unstable compound (III'), should be produced.

In any case, if the sulfinic acids disproportion, they would produce thiochrome positive compound after the treatment of cysteine. We have studied the disproportiona-

14) G. Tsukamoto, K. Harada, I. Utsumi : This Bulletin, **14**, 823 (1966).
15) C.S. Marnel, R.S. Tohson : J. Org. Chem., **13**, 822 (1948).
16) R. Otto : Ann., **145**, 13, 317 (1868).
17) J.L. Kice, K.W. Bowers : J. Am. Chem. Soc., **84**, 605 (1962).
18) *Idem* : J. Org. Chem., **28**, 1162 (1963).

tion of O-benzoylhypothiaminic acid (Ⅱ) in aqueous methanol containing 0.5*M* hydrogenchloride at 70°.

The progress of the reaction was investigated by means of paper partition chromatography (BuOH-AcOH-H_2O=4:1:5). The chromatogram showed that the compound Ⅱ disappeared rapidly, and then a product having Rf 0.82 convertible to positive towards thiochrome reaction after treating with cysteine, appeared besides O-benzoylthiaminic acid (Ⅵ). In addition, nothing except the two products was detected in optimum



O-Benzoylhypothiaminic acid (Ⅱ) solution in MeOH containing 0.5 *M*HCl at 70° for 10～30 min.

Monosulfoxide of O-Benzoylthiamine disulfide (Ia)*

O-Benzoylthiamine disulfide (Ⅲ)*

O-Benzoylhypothiaminic acid (Ⅱ)*

O-Benzoylthiaminic acid (Ⅵ)*

Fig. 5. Chromatograms of Disproportionation Reaction Mixture of O-Benzoylhypothiaminic Acid (Ⅱ) and Related Compounds

* Hydrochloric acid solutions were spotted.

conditions. This observation would point out that the formation of Ⅵ and Ⅶ by the acidic treatment of Ⅱ and Ⅴ is clearly not due to oxidation but to disproportionation. However, it is in progress whether the product forming thiochrome is thiolsulfonate (Ⅲ′) or disulfide (Ⅲ) compounds.

The all above facts have proved the formation and structure of hypothiaminic acid which SH group of thiol-type thiamine was oxidized to SO_2H group, and a series of products in oxidation process of symmetrical disulfide-type thiamine was obtained.

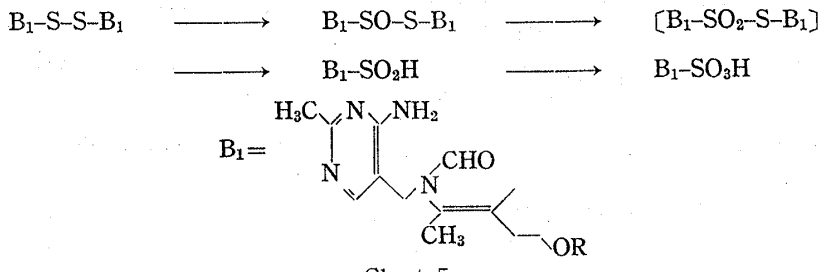


Chart 5.

It was presumed that hypothiaminic acids has no thiamine activity alike thiaminic acids,[19] but the production of positive compounds to thiochrome reaction *in vitro* let us think that hypothiaminic acids might have thiamine activity. The investigation on love-birds made it clear, however, that the compounds Ⅱ and Ⅴ have no thiamine activity. This observation is seemed to be analogous to the fact that cysteine sulfinic acid have no cysteine effect.[20] In addition, the toxicity of Ⅱ and Ⅴ was extremely low similary to that of thiaminic acids. This finding suggests that hypothiaminic acids are interesting

19) I. Utsumi, K. Harada, K. Kohno, G. Tsukamoto : J. Vitaminol., **11**, 248 (1965).
20) L. Young, G.A. Maw : " The Metabolism of Sulfur Compounds," p. 24 (1958), Methuen Press, London.

compounds which can be considered to occur in the body as intermediates of thiamine metabolism.

The behavior of sulfinyl group in the compound I when treated with sulfyhydryl compounds will be reported in near future.

Experimental

All melting points were uncorrected. Ultraviolet absorption spectra were taken with Shimadzu Recording Ultraviolet Spectrophotometer, SV-50A, and infrared absorption spectra were measured with Hitachi Infrared Spectrophotometer, EPI-S_2. Nuclear magnetic resonance spectrum was measured with Japan Electron Co. J.N.M-C60 spectrometer, using sodium salt of 2,2-dimethyl-2-silapentane-5-sulfonic acid as internal reference.

Hydroxylation of Monosulfoxide of O-benzoylthiamine Disulfide (Ia)——i) In neutral medium : A suspension of Ia (1.0 g.) in 80% aqueous EtOH (30 ml.) was refluxed on a boiling water bath for 30 minutes to afford an apparent solution. After refluxing for another 30 min., the solution was moderately condensed under reduced pressure and allowed to stand at room temperatures after the addition of ether until the solution became turbid to yield 0.25 g. of colorless needles. The crystals obtained wese recrystallized from 90% aqueous EtOH repeatedly to give colorless needles, m.p. 204~205° (decomp.). *Anal.* Calcd. for $C_{19}H_{22}O_5N_4S$: C, 54.54; H, 5.30; N, 13.39; S, 7.66. Found : C, 54.55; H, 4.92; N, 13.31; S, 7.60. UV $\lambda_{max}^{pH\ 7.3}$ mμ(log ε): 230(4.40), 275(3.81), $\lambda_{max}^{pH\ 2.5}$ mμ(log ε) : 230(4.35).

The mother liquor which Ⅱ was filtered off was condensed under reduced pressure, and the EtOH solution of the residue was allowed to stand at room temperatures to afford 0.62 g. of colorless prisms, m.p. 145~146° (decomp.), which were identified as O-benzoylthiamine disulfide by the comparison with infrared absorption spectra.

ii) In alkali medium : A suspension of Ia (0.50 g.) in 80% aqueous EtOH (10 ml.) was stirred at room temperatures adjusting its pH 7~9 by the dropwise addition of *N* NaOH. After one mole of *N* NaOH per mole of Ia was added, the mixture was adjusted its pH 7 by the addition of dil. HCl and the EtOH was removed under reduced pressure. To the residue was added a little water and extracted with $CHCl_3$. The aqueous layer, which was moderately concentrated under reduced pressure, was adjusted its pH *ca.* 1 by the addition of dil. HCl and allowed to stand at room temperatures for a night to give 30 mg. of crystals. The crystals obtaind were recrystallized from EtOH to afford colorless needles, m.p. 204~205° (decomp.), which were identified as O-benzoylhypothiaminic acid (Ⅱ) by the comparison with infrared absorption spectra.

Oxidation of 2-(2-Methyl-4-amino-5-pyrimidyl)methylformamido-5-benzoyloxy-2-pentene-3-sulfinic Acid (II)——A solution of Ⅱ (28 mg.), AcOH (6 ml.) and 30% hydrogen peroxide (0.1 ml.) was warmed at 40° for 8 hr. The reaction solution was allowed to stand at room temperatures for a night to separate colorless needles. It was condensed under reduced pressure to dryness without filtering off the separated crystals to yield 27 mg. of colorless needles, m.p. 237~238° (decomp.) which was not depressed by admixture with authentic O-benzoylthiaminic acid (Ⅵ) and identical with the authentic sample in infrared spectrum.

Hydrolysis of II——A solution of Ⅱ (700 mg.) in 80% EtOH (30 ml.) and *N*-NaOH (2.4 ml.) was heated at 70° adjusting its pH 9~10 by the dropwise addition of *N*-NaOH (2.4 ml.) for 3 hr. The reaction solution was condensed under reduced pressure, the residue was dissolved in H_2O. The solution was adjusted to pH 3.0 by the addition of dil. HCl and extracted with ether. The aqueous layer was condensed under reduced pressure. The residue was extracted with hot EtOH to remove inorganic salts and then the extract was evaporated to dryness under reduced pressure. The residue was recrystallized from 95% aqueous EtOH to give 580 mg. of colorless prisms, m.p. 121~122°. *Anal.* Calcd. for $C_{12}H_{18}O_4N_4S \cdot 2\frac{1}{2}H_2O$: C, 40.11; H, 6.45; N, 15.59; S, 8.92. Found : C, 40.11; H, 6.13; N, 15.37; S, 9.01.

The above ether extract was evaporated to dryness and the residue (195 mg.) was recrystallized from boiling water to give colorless plates, m.p. 122°, which showed no depression in the mixed melting point determination with benzoic acid.

Benzoylation of 2-(2-Methyl-4-amino-5-pyrimidyl)methylformamido-5-hydroxy-2-pentene-3-sulfinic Acid (V)——To a solution of Ⅴ (250 mg.) dissolved in H_2O (4 ml.) was added dropwise benzoyl chloride (140 mg.) while adjusting to pH 8~10 by addition of *N*-NaOH and cooling with ice water. After the addition was completed, the reaction mixture was stirred at room temperatures for 1hr., adjusted to pH 4.0 with dil. HCl, and then a crystalline product (95 mg.) separated. The crystals obtained were recrystallized from 95% EtOH to give colorless needles, m.p. 204~205° (decomp.) which were identified by infrared spectral comparison.

Oxidation of V——A solntion of Ⅴ (30 mg.) dissolved in AcOH (6 ml.) and 30% hydrogen peroxide (0.1 ml.) was allowed to stand at room temperatures for 4 days. The reaction mixture was condensed under reduced pressure, and the residue was recrystallized from 95% aqueous EtOH to yield 25 mg. of crystals. The crystals obtained were recrystallized from 95% aqueous EtOH repeatedly to afford colorless needles, m.p. 212~213° (decomp.) which was identical with authentic thiaminic acid (Ⅶ) monohydrate in infrared spectrum.

Disproportionation Reaction of II and V——The compound II (10 mg.) was dissolved in MeOH (special grade) containing 0.5 *M*HCl (1 ml.) and the flasks were immersed in a bath kept at 70±1°. One drop of samples was withdrawn at selected time and submitted to paper partition chromatography. The result was that the starting material (II) disappeared completely after 10 min., and the formation of products having Rf 0.18 and 0.82 was recognized as shown in Fig. 5. The chromatogram was unchanged on prolonged heating of the solution (30~60 min.). This conversion was depressed by decreasing HCl concentration.

i) Isolation of VI : The compound II (0.5 g.) was dissolved in MeOH containing 0.5 *M*HCl (10 ml.) and heated for 30 min. at 70±1°. The reaction mixture was condensed to dryness under reduced pressure. To the residue was added H_2O and allowed to stand for several days to separate colorless prisms, which were identical with authentic O-benzoylthiaminic acid (VI) monohydrate in infrared spectrum.

ii) Isolation of VII : To the residue obtained from V (0.5 g.) by the similar treatment described above was added EtOH and allowed to stand for several days to separate colorless needles, which were identical with authentic thiaminic acid (VII) monohydrate in infrared spectrum.

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