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Reactions of Thiamine Disulfide Monosulfoxides with Mercaptans[1)]

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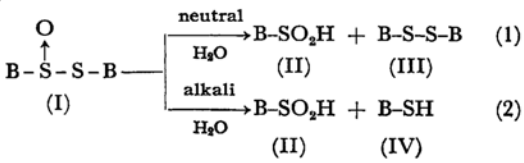
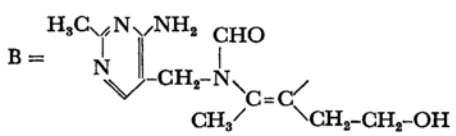
It was found that the reaction of thiamine disulfide monosulfoxide (I) with mercaptan is a competitive reaction between the so-called mercaptan-thiolsulfinate reaction and the apparent oxidation reduction reaction. This reaction offers an excellent method for the preparation of the mixed disulfides of thiamine and *t*-mercaptan or the unstable mixed disulfides, such as thiamine-glutathione disulfides.

In a previous paper,[2)] we showed that thiolsulfinate-type thiamine (I) gave 2-(2-methyl-4-amino-5-pyrimidyl)methylformamido-2-pentene-3-sulfinic acid (II, hereinafter called hypothiaminic acid) in alkaline or neutral hydrolysis as represented by



where



Yurugi *et al.* obtained thiamine butyl disulfide (VI, R=C_4H_7) in low yield on the oxidation of thiamine disulfide (III) in acetic acid with hydrogen peroxide, followed by reaction with butyl mercaptan in alkaline condition, and they suggested an intermediate in this reaction to be thiolsulfinate-type thiamine (I).[3)] In the meantime, we reported that

1) Part XXXII in a series on "Studies on Thiamine Disulfide."

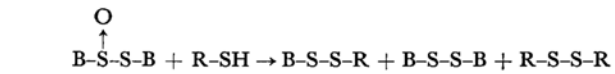
2) I. Utsumi, T. Watanabe, K. Harada and G. Tsukamoto, *Chem. Pharm. Bull.* (*Tokyo*), **15**, 1485 (1967).

3) S. Yurugi, H. Kawasaki and S. Noguchi, *Yakugaku Zasshi* (*J. Pharm. Soc. Japan*), **75**, 498 (1955).

the oxidation of III with hydrogen peroxide did not give the corresponding thiolsulfinate (I).[4,5] This result and the observation described in this paper suggest that thiamine butyl disulfide produced in Yurugi's experiment is formed not through thiolsulfinate (I) but through an other mechanistic route,[6] which will be reported in near future. In this paper, we would like to report the reaction of I with mercaptans.

Result and Discussion

When thiolsulfinate (I) is allowed to react with mercaptans as reported for the reaction of alkyl alkanethiolsulfinate with thiamine in thiol form (IV),[7] compound I is expected to produce the unsymmetrical disulfide-type thiamines (VI). In fact, compound I reacts smoothly and very rapidly with mercaptans at room temperature to give the unsymmetrical disulfides (VI) within a few minutes.



(I) (V) (VI) (III) (VII)

However, it was also found that symmetrical disulfides (III, VII) are concomitantly formed even under a stream of nitrogen.

It has been proposed that one mole of thiolsulfinate reacts with two moles of mercaptan to give two moles of unsymmetrical disulfide.[8] Whereas, Schöberl and Grafje, and Carson and Wong have suggested that a part of sulfenic acid, which is presumably formed in the reaction of thiolsulfinate with mercaptan, gives rise to disproportionation to give mercaptan and sulfinic acid.[9] If a similar reaction takes place in this case, the formation of hypothiaminic acid (II) and thiamine in thiol form (IV) would be observed.[2] However, these compounds (II, IV) can not be detected by paper electrophoresis[2] in the reaction solution.

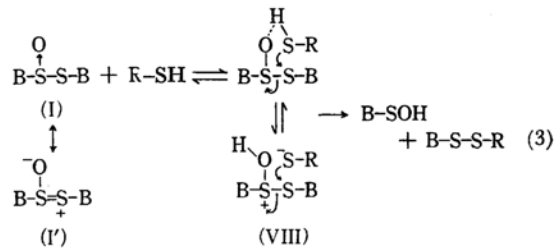
The product analyses were carried out in detail by means of paper partition and gas chromatography, and the formation of the following compounds were observed: (1) in the case of molar ratio, V/I=2, three compounds, *i. e.*, unsymmetrical disulfide (VI) and symmetrical disulfides (III, VII), (2) in the case of V/I <2, starting compound (I) in addition to these three compounds, (3) in the case of V/I $\gg 2$, thiamine (IV) and thiothiamine (3-(2-methyl-4-amino-5-pyrimidyl)methyl-4-methyl-5-β-hydroxyethylthiazoline-2-thione) as the secondary decomposed products.

The yield of thiamine propyl disulfide obtained from the reaction of I with propyl mercaptan in 80% ethanol are listed in Table 1.

Table 1. Effect of amount of PrSH on yield of BSSPr

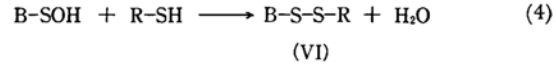
PrSH/I	1.0	1.2	1.4	1.6	1.8	2.0	2.1	3.0
Yield(%) of BSSPr	29	37	46	51	58	65	55	trace
PrSH(%) used in the formation of BSSPr	56	61	64	63	63	62	51	—

The reaction was carried out in 80% ethanol, and the main features of the reaction were the same even in absolute ethanol and acetic acid. Therefore, the predominant form of mercaptan should be undissociated acid which would either protonate or hydrogen-bond with sulfinate oxygen in the ground or transition state of the reaction, depending on the acidity of mercaptan.



(I) (I′) (VIII)

(3)



(VI)

(4)

Both protonation and hydrogen bonding would make the sulfinyl group a better leaving group which would favor the nucleophilic attack of mercaptan on sulfenyl sulfur resulting in the formation of sulfenic acid and unsymmetrical disulfide.

The symmetrical disulfides (III, VII) were produced in the same ratio as indicated in Table 2. The production of III suggests the possibility that III might be formed through oxidation-reduction reaction between I and mercaptan in accordance with the previous report in which the thiolsulfinate (I) and its *O*-benzoyl ester (IX, OH in structure I is $OCOC_6H_5$) were proved to behave as an

4) I. Utsumi, K. Harada, G. Tsukamoto and I. Daira, *J. Vitaminol. (Kyoto)*, **11**, 234 (1965).

5) I. Utsumi, K. Harada, K. Kohno and G. Tsukamoto, *ibid.*, **13**, 26 (1967).

6) The formation of VI would be due to mixed reactions between disproportionation reaction of disulfides with mercaptans and oxidation reactions of mercaptans in the presence of hydrogen peroxide.

7) T. Matsukawa and H. Kawasaki, *Yakugaku Zasshi (J. Pharm. Soc. Japan)*, **73**, 216 (1953).

8) L. D. Small, J. H. Bailey and C. J. Cavallito, *J. Am. Chem. Soc.*, **69**, 1710 (1947); *ibid.*, **71**, 3565 (1949); W. E. Savige, J. Eager, J. A. Maclen and C. M. Bocburgh, *Tetrahedron Letters*, **1964**, 3289.

9) A. Schöberl and H. Grafje, *Ann.*, **67**, 71 (1958); J. F. Carson and F. F. Wong, *J. Org. Chem.*, **26**, 3028 (1961).

TABLE 2. EFFECT OF ACIDITY OF MERCAPTANS ON THE REACTION OF I WITH RSH (RSH/I=2)

RSH	p-NO_2-phenyl	Phenyl	Benzyl	n-Pr
pK_a	5.11[10)]	6.7[11)]	9.43[11)]	10.63[11)]
Yield(%) of BSSR	trace[a)]	15	58	65
Yield(%) of RSSR	97	80	41	38[b)]
Yield(%) of BSSB	90	85	40	35

a) The unsymmetrical disulfide was detected only by paper partition chromatography ($BuOH \cdot AcOH \cdot H_2O = 4:1:5$).
b) Produced PrSSPr was measured by gas chromatography (Column: Diethylene Glycol Succinate (1.5 m), Carrier gas: He(60 ml/min), Column temp.: 100°C, (Retention time: 6 min)

TABLE 3. EFFECT OF EACH BuSH ON YIELD OF BSSBu (RSH/I=2)

RSH	n-Bu	s-Bu	t-Bu
Yield(%) of BSSR	75	86	90[a)]

a) t-BuSSBu-t (yield: 5%) produced in this case was detected by gas chromatography (Retention time under the same condition as abvove: 3.8 min).

oxidizing reagent.[5,12)]

In connection with the forgoing results, Tables 2 and 3 show that the mercaptans of higher acidity are oxidized more readily to the symmetrical disulfide (VII) than those of lower acidity, while the mercaptans of lower acidity yield a larger amount of unsymmetrical disulfide (VI) than the mercaptans of higher acidity. O-Benzoylthiamine disulfide monosulfoxide (IX) and thiamine benzyl disulfide monosulfoxide (ester of thiamine in thiol form (IV) and benzyl thiolsulfinic acid)[13)] also showed similar reactivities to those of I.

The formation of the symmetrical disulfides (III, VII) would be dependent on the following reaction initiated by the attack of mercaptan to protonated oxygen. However, the attacking site of mercaptan is thought to be protonated oxygen and sulfinyl sulfur in the transition state (VIII). Namely, mercaptan attacks protonated oxygen to give symmetrical disulfides (III) and the corresponding sulfenic acid of the attacking mercaptan.

$$R{-}S^{-} + \overset{H}{O}{-}\overset{S-B}{\underset{+}{S}}{-}B \longrightarrow R{-}SOH + \underset{(III)}{B{-}S{-}S{-}B} \qquad (5)$$

$$R{-}SOH + R{-}SH \longrightarrow \underset{(VII)}{R{-}S{-}S{-}R} + H_2O \qquad (6)$$

This apparent oxidation-reduction presumably depends on the acidity of attacking mercaptan, *i. e.* the more acidic mercaptan can protonate oxygen more strongly and the resulting mercapto anion is more strongly nucleophile than the undissociated mercaptan. Similar trends have also been observed in the oxidation of mercaptan by sulfoxide to give disulfide, in which the order of reactivity of mercaptan is $ArSH > ArCH_2SH > alkylSH$.[14)]

An important question remains as to the possible disproportionation reaction of the disulfides (VII, III) as follows:

$$B{-}S{-}S{-}R + R{-}SH \longrightarrow B{-}SH + R{-}S{-}S{-}R \qquad (7)$$

$$B{-}\overset{O}{\overset{\uparrow}{S}}{-}S{-}B + B{-}SH \longrightarrow B{-}SOH + B{-}S{-}S{-}B \qquad (8)$$

This must certainly be important when mercaptan is used more than two moles as indicated in Table 1.

Therefore, it can be concluded that the reaction of thiolsulfinate (I) with mercaptans is competitive reactions between the so-called mercaptan-thiolsulfinate reaction (Eq. (9)) and the apparent oxidation reduction (Eq. (10)).

$$\underset{(I)}{B{-}\overset{O}{\overset{\uparrow}{S}}{-}S{-}B} + \underset{(V)}{2\,R{-}SH} \begin{cases} \rightarrow 2\,\underset{(VI)}{B{-}S{-}S{-}R} + H_2O & (9) \\ \rightarrow \underset{(III)}{B{-}S{-}S{-}B} + \underset{(VII)}{R{-}S{-}S{-}R} + H_2O & (10) \end{cases}$$

It is of interest to note that these thiolsulfinate remained unchanged after four years at room temperatures. This pronounced stability which seems unusual for this type of compound would be due to the conjugation of $-S(O^-){=}S^+-$ double bond with both sides of olefinic double bonds.

Up to the present time, a number of thiamine alkyl disulfides have been prepared by a variety of methods,[15)] but hardly any are suitable for the preparation of the mixed disulfides of thiamine and t-mercaptan. In contrast, the reaction of I with t-mercaptan offers an excellent method for the preparation of the mixed disulfides.

Reduction of the mixed disulfides of thiamine and t-mercaptan by sulfhydryl compounds to

10) L. Lumper and H. Zahn, "Advances in Enzymology," Vol. 27, ed. by F. F. Nord, John Wiley and Sons, New York (1965), p. 202.

11) M. M. Kreevoy, E. T. Harper, R. E. Duvall, H. S. Wilgus, III, and L. T. Ditsch, *J. Am. Chem. Soc.*, **82**, 4899 (1960).

12) We also knew that the thiolsulfinates (I, IX) were immediately reduced with triphenylphosphine in chloroform at room temperatures to give quantitatively the corresponding disulfides and triphenylphosphine oxide respectively. See J. F. Carson and F. F. Wong, *J. Org. Chem.*, **26**, 1467 (1961).

13) H. Kawasaki and H. Yonemoto, *Yakugaku Zasshi* (*J. Pharm. Soc. Japan*), **77**, 640 (1957).

14) T. J. Wallace, *J. Am. Chem. Soc.*, **86**, 2018 (1964).

15) T. Matsukawa and S. Yurugi, "Review of Japanese Literature on Beriberi and Thiamine," ed. by N. Shimazono and E. Katsura, Vitamin B Research Committee of Japan, Kyoto (1965), p. 107—114.

thiamine in thiol form proceeds with some difficulty in comparison with thiamine *n*-alkyl disulfides. This observation of biological interest will be reported elsewhere.

In addition to the preparation of the mixed disulfides, this reaction is also suitable for preparing unstable unsymmetrical thiamine disulfides such as thiamine-glutathione disulfide[16] and thiamine pyridylmethyl disulfide derivatives.[17]

Experimental

Melting points are not corrected. Infrared absorption spectra were measured with a Hitachi Infrared Spectrophotometer, EPI-S_2. Gas chromatographic analyses were carried out with a Shimadzu Model GC-1C.

Since thiamine disulfide (III), which was formed when thiolsulfinate (I) was allowed to react with mercaptans, could not be isolated quantitatively as crystals, it was measured as follows: Amounts of thiamine disulfide in the filtrates were determined by thiochrome method after the filtrates, from which unsymmetrical disulfides of thiamine (VII) were filtered off, were proved to contain only thiamine disulfide (III).

An Alternative Method for Preparation of Thiolsulfinates (I, VIII). The following procedure for the preparation of thiolsulfinates is favorable in comparison with that described previously.[5]

To a stirred solution of thiamine disulfide or its O-benzoyl ester (0.10 mol) dissolved in 2N H_2SO_4 (500 m*l*) was added peracetic acid (0.12 mol) under cooling with ice-water. The reaction mixture was allowed to stand overnight at room temperature. The adjustment of the reaction solution to pH 6—7 with Na_2CO_3 or $NaHCO_3$ gave the corresponding thiolsulfinates (I, IX), free from other oxidation product contaminants, as pale yellow crystals in satisfactory yield (80—85%).

Reaction of Thiolsulfinate (I) with Propyl and Butyl Mercaptans. *i) With Propyl Mercaptan.* To a suspension of I (580 mg) in 80% ethanol (10 m*l*) was added molar equivalent of propyl mercaptan (Table 1) with stirring under a stream of nitrogen at room temperature, and the mixture was allowed to stand for 10 min. The reaction solution, in which the mole ratio of the thiolsulfinate to the mercaptan was 1 : 1—1.8, was filtered and the filtrates were condensed under reduced pressure. The resulting residues to which water (5 m*l*) was added were allowed to stand for a day at room temperature to give colorless prisms in yields shown in Table 1. The crystals obtained were confirmed to be nearly pure by thin layer chromatography. Recrystallization of the crystals from benzene gave colorless prisms, mp 128—129°C, which were identical in infrared spectrum with the authentic thiamine propyl disulfide prepared by the known method.[6]

The reaction solutions, in which the mole ratio of the thiolsulfinate to the mercaptan was 1 : 2.0, 2.1, were similarly treated without having been filtered.

In a case where the mole ratio of the thiolsulfinate to the mercaptan was 1 : 3.0, a similar treatment of the reaction solution to the above-mentioned method afforded colorless needles (50 mg), mp 233—234°C (decomp.). Recrystallization from water gave colorless needles, mp 237—238°C (decomp.), which were identical in infrared spectrum with authentic 3-(2-methyl-4-amino-5-pyrimidyl)methyl-4-methyl-5-β-hydroxyethylthiazoline-2-thione. The mother liquor was condensed under reduced pressure to remove water and the resulting residue was dissolved in ethanolic HCl to afford colorless needles (560 mg), mp 246—247°C (decomp.), which were found to be identical with authentic thiamine hydrochloride by infrared spectrum.

ii) With n-Butyl Mercaptan. The procedure was the same as above. The reaction of I (580 mg) with *n*-butyl mercaptan (180 mg) gave 0.55 g (75%) of colorless prisms, mp 139—140°C. Recrystallization of the crystals from benzene gave colorless prisms, mp 135—136°C, which were found by infrared spectrum to be identical with authentic thiamine butyl disulfide prepared by the known method.[7]

iii) With s-Butyl Mercaptan. The procedure was the same as above except that the reaction time was 30 min. The reaction of I (580 mg) with *s*-butyl mercaptan (180 mg) yielded 640 mg (86%) of colorless prisms, mp 121—123°C. Recrystallization of the crystals from ethyl acetate gave thiamine *s*-butyl disulfide as colorless prisms, mp 121—123°C.

Found: C, 51.99; H, 7.11; N, 15.11; S, 17.41%. Calcd for $C_{16}H_{26}O_2N_4S_2$: C, 51.88; H, 7.08; N, 15.13; S, 17.28%.

IR ν_{max}^{Nujol} cm^{-1}: 3300, 3100, 1670, 1645, 1065.

Hydrochloride: Colorless needles (ethanol-ether), mp 160—161° C(decomp.).

Found: C, 47.44; H, 6.73; N, 13.75%. Calcd for $C_{16}H_{27}O_2N_4S_2Cl$: C, 47.22; H, 6.69; N, 13.77%.

iv) With t-Butyl Mercaptan. The procedure was the same as above except that the reaction time was 1 hr. The reaction of I (580 mg) with *t*-butyl mercaptan (180 mg) gave 660 mg (90%) of colorless prisms, mp 151—152°C. Recrystallization from ethyl acetate gave thiamine *t*-butyl disulfide as colorless prisms, mp 151—152°C.

Found: C, 51.84; H, 7.06; N, 15.09; S, 16.79%. Calcd for $C_{16}H_{26}O_2N_4S_2$: C, 51.88; H, 7.08; N, 15.13; S, 17.28%.

IR ν_{max}^{Nujol} cm^{-1}: 3310, 3150, 1670, 1060.

Hydrochloride: Colorless needles (ethanol-ether), mp 182—184°C.

Found: C, 47.48; H, 6.80; N, 13.87%. Calcd for $C_{16}H_{27}O_2N_4S_2Cl$: C, 47.22; H, 6.69; N, 13.77%.

Reaction of Thiamine Benzyl Disulfide Monosulfoxide with Trityl Mercaptan. To a solution of thiamine benzyl disulfide monosulfoxide (420 mg) in 80% ethanol (10 m*l*) was added trityl mercaptan (550 mg) and the mixture was stirred at 50°C for 30 min. After the reaction, the reaction solution, in which oily precipitate separated, was condensed to dryness under reduced pressure and the resulting residue which was washed with petroleum ether was crystallized from ethanol to give crystals, 400 mg, mp 171—173°C. Recrystallization from ethanol gave thiamine trityl disulfide as colorless prisms, mp 171—173°C.

Found: C, 67.15; H, 5.93; N, 10.13; S, 11.40%. Calcd for $C_{31}H_{32}O_2N_4S_2$: C, 66.88; H, 5.79; N, 10.06; S, 11.52%.

16) K. Kohno, G. Tsukamoto, Y. Kakie and I. Utsumi, *Vitamins (Kyoto)*, **36**, 336 (1967).

17) I. Utsumi, T. Watanabe and G. Tsukamoto, *ibid.*, **37**, 276 (1968).

IR ν_{max}^{Nujol} cm^{-1}: 3275, 3175, 1660, 1065, 730, 700.

This compound was found by infrared spectrum to be identical with authentic thiamine trityl disulfide prepared by the reaction of I with trityl mercaptan.

Reaction of I with Benzyl Mercaptan. To a stirred suspension of I (580 mg) in 80% ethanol (10 m*l*) was added benzyl mercaptan (248 mg) in nitrogen at room temperature, and a clear solution was immediately obtained. The reaction solution was condensed under reduced pressure. Water (5 m*l*) was added to the resulting residue and left to stand for a day to give crystals, 570 mg. After the crystals were dried under reduced pressure, they were washed with ether to give crystals, 470 mg (58%). Recrystallization from ethyl acetate gave 450 mg of thiamine benzyl disulfide as colorless prisms, mp 152°C, which were found to be identical with authentic samples by infrared spectrum.

Evaporation of the washed ether solution gave crystals, 100 mg (41%), mp 71—72°C, undepressed on admixture with authentic dibenzyl disulfide.

Reaction of I with Phenyl Mercaptan. The reaction between I (580 mg) and phenyl mercaptan (220 mg) was carried out with a similar procedure to that described in the reaction of I with benzyl mercaptan.

Ethanol as solvent of the reaction mixture was removed under reduced pressure and to the resulting residue was added water (5 m*l*) to separate crystals, which were extracted with chloroform. The chloroform solution was dried over sodium sulfate and chromatographed over alumina (10 g). The column was eluted with chloroform-ethanol (99 : 1) tracing with Dragendorff's reagent. The first fraction which is negative toward the reagent was condensed to dryness under reduced pressure to yield crystals (178 mg, 80%), mp 61—62°C, which were found to be identical with authentic diphenyl disulfide by infrared spectrum. The second fraction which is positive toward the reagent was treated similarly. The residue thus obtained was crystallized from benzene to yield colorless crystals (115 mg, 15%), mp 137°C (decomp.), which were found to be identical with authentic thiamine phenyl disulfide by infrared spectrum.

Reaction of I with *p*-Nitrophenyl Mercaptan. To a stirred suspension of I (580 mg) in 80% ethanol (10 m*l*) was added *p*-nitrophenyl mercaptan (310 mg). Separated colorless needles, 300 mg, mp 181—182°C, were filtered. Recrystallization from ethanol gave colorless needles, mp 182°C, which were found to be identical with authentic di-*p*-nitrophenyl disulfide by infrared spectrum.

The mother liquor from which di-*p*-nitrophenyl disulfide was filtered off was submitted to paper partition chromatography, which showed two spots, one R_f 0.37 assignable to thiamine disulfide and the other R_f 0.72 presumed to be thiamine *p*-nitrophenyl disulfide.

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