

# Thiamine Derivatives of Disulfide Type. VIII.<sup>1)</sup> The Exchange Reaction between Thiamine Propyl Disulfide and the Various Thiols<sup>2)</sup>

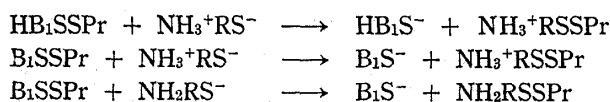
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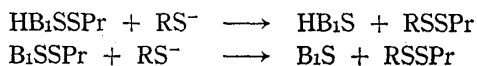
The exchange reactions between TPD and polyfunctional thiols such as glutathione, 2-aminoethylmercaptan, 2-hydroxyethylmercaptan and 2-carboxyethylmercaptan were examined under an anaerobic condition. The following conclusions were drawn from the results presented.

From an analysis of the pH-rate profile of the reactions between TPD and thiols containing the amino group in a molecule, the reaction was revealed as the combination of the following three reactions through the pH range of 3 to 12,



where  $\text{HB}_1\text{SSPr}$  is the protonated form of TPD,  $\text{NH}_3^+\text{RS}^-$  is the protonated amino-thiol anion,  $\text{HB}_1\text{S}^-$  is the protonated thiamine and  $\text{NH}_3^+\text{RSSPr}$  is the protonated amino-alkyl propyl disulfide.

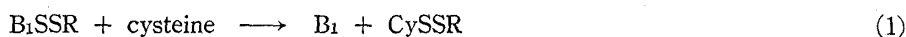
On the other hand, in the reaction between TPD and thiol, 2-hydroxyethylmercaptan, the following two reaction equations were presented through the experimental pH range of 3.5 to 12.0.



The second order rate constants of individual reactions were determined. The activation energies of these reactions obtained were about 10 kcal mole<sup>-1</sup>, and the values of activation entropies were 5 to 24 cal mole<sup>-1</sup> deg<sup>-1</sup>. These second order rate constants were correlated with the  $\text{p}K_a$  values of the thiol group with Brönsted equation,  $\log k = \alpha \text{p}K_a + C$ , with a resultant very large  $\alpha$  value. From this relationship, it may be concluded that these thiols are an extremely good nucleophile to the disulfide bond and especially to protonated TPD.

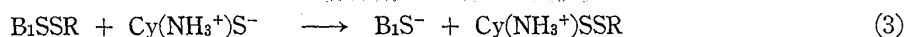
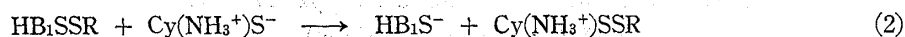
In previous reports of this series,<sup>4-7)</sup> the exchange reactions between the various thiamine derivatives of the disulfide type and cysteine, as an example of the most common thiol derivatives in the body, have been studying to understand the exchange reaction in the body.

It was proved that the main reaction was expressed by Eq. (1) following the second order kinetics, in the formation of thiamine and the decrease in cysteine.



- 1) Part VII: H. Nogami, J. Hasegawa and K. Okazaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1732 (1968).
- 2) The 87th Annual Meeting of Pharmaceutical Society of Japan, Kyoto, Apr. 1967.
- 3) Location: a) 7-chome, Hongo, Bunkyo-ku, Tokyo; b) 2-chome, Sakurashinmachi, Setagaya-ku, Tokyo.
- 4) H. Nogami, J. Hasegawa and N. Ikari, *Chem. Pharm. Bull.* (Tokyo), **15**, 685 (1967).
- 5) H. Nogami, J. Hasegawa and N. Ikari, *Chem. Pharm. Bull.* (Tokyo), **15**, 693 (1967).
- 6) H. Nogami, J. Hasegawa, T. Suzuki and K. Hirata, *Chem. Pharm. Bull.* (Tokyo), **16**, 1273 (1968).
- 7) H. Nogami, J. Hasegawa and K. Okazaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1732 (1968).

where  $B_1SSR$  is thiamine alkyl disulfide,  $B_1$  is thiamine and  $CySSR$  is cysteine alkyl disulfide. From an analysis of the pH rate profile of the exchange reaction, the above reaction was found to be a combination of the following three reactions presented by Eq. (2), (3) and (4) between the pH range of 3.5 and 13.0,



where  $HB_1SSR$  is protonated thiamine alkyl disulfide,  $Cy(NH_3^+)S^-$  is protonated cysteine,  $HB_1S^-$  is protonated thiamine and  $Cy(NH_3^+)SSR$  is protonated cysteine alkyl disulfide.

On comparing the reactivities of thiamine derivatives of disulfide type, the specific rate constants of the reactions between protonated thiamine derivatives of the disulfide type and cysteine anion were found to be five to fifteen times greater than that of nonprotonated ones. The tendency was found that the stronger the electronwithdrawing effect of the substituent of S-S bond, the greater the reactivity in the exchange reaction.

Past reports have referred only to the structure and reactivity of the disulfide aspect, and very little is written about the structure and reactivity of thiol aspect in the scission of the sulfur-sulfur bond. Especially as for the thiamine derivatives, only cysteine was studied as the thiol compound. The purpose of the present study is to investigate the structural influences of the thiols, especially the groups adjoining the thiol group, in the scission of sulfur-sulfur bond. Glutathione, 2-aminoethylmercaptan (I), 2-hydroxyethylmercaptan (II) and 2-carboxyethylmercaptan (III) were used in this study. Kinetic studies on the reaction between thiamine propyl disulfide (TPD) and these polyfunctional thiols were carried out; the second order rate constants were correlated with the  $pK_a$  values of the thiol group with the Brönsted equation.

### Experimental

**Materials**—TPD: reported in the previous paper and supplied by Takeda Chem. Ind., Ltd.

Glutathione: analytical grade, Wako Pure Chemical Ind., Ltd.

2-Aminoethylmercaptan (I), 2-Hydroxyethylmercaptan (II) and 2-Carboxyethylmercaptan (III): analytical grade, Tokyo Kasei Kogyo Co., Ltd.

**Thin-Layer Chromatographic Procedure**—After adding a few drops of 10% HCl aq. soln., the reaction mixtures were chromatographed using a development solvent of 1N HCl aq. soln. on a plate prepared with a 250  $\mu$  layer of Wako B silicagel. Ninhydrin reagent was used to detect thiamine, glutathione and I. Dragendorff reagent was used to detect thiamine, TPD and I. Sodium dichromate reagent in sulfuric acid and iodine vapor were used to detect all of the components.

**Kinetic Procedure**—Aq. solns. of TPD and thiol were prepared in a concentration of  $1 \times 10^{-2}$  mole-liter $^{-1}$  using dist. water bubbled with nitrogen to remove oxygen. The solns. were mixed in a buffer solution which were also bubbled with nitrogen at a concentration as shown in Table I in the molar ratio of 1:1 of disulfide and thiol and allowed to react at 15° in a nitrogen atmosphere. Aliquots of this mixture were drawn at a given interval and acidified with 1N HCl aq. soln. pH meter (TOA Electronics Ltd. model HM-5A) was used to determine the pH values of the solutions.

The concentrations of thiamine in these samples were determined by the thiochrome method<sup>8)</sup> with Hitachi Model 124 spectrophotometer, and the second order rate constants were calculated. No correction was applied in the calculation of the rate constants, since the oxidative decomposition of thiols was found to be less than 3 percent of the total thiol.

**Reaction I (Reaction between TPD and I)**—When a reaction mixture of pH 8 in which approx. 80% thiamine was formed was chromatographed, an unknown spot was detected by means of ninhydrin and dragendorff reagent at  $R_f$  0.60. The  $R_f$  value of this spot differed from that of I (0.92) and dipropyl disulfide (0.01). This part of the gel was collected and extracted with hot ethanol. The residue of this extract gave colorless and very hygroscopic crystals. IR  $\nu_{max}^{KBr}$  cm $^{-1}$ : 2000—3100 (a few bands  $NH_3^+$  broad), 1590 ( $NH_3^+$ ), 1340—1480 (a few bands  $-CH_2-$ ).

8) Y. Kochi and S. Kasahara, *Vitamin*, 7, 513 (1953).

TABLE I. List of Buffer Solutions and Initial Concentrations for the Reactions between TPD and Thiols

Glutathione	pH	3.80	4.00	4.35	5.90	6.90	7.50	8.05	8.90	9.10	9.85	10.25	10.95
	I.C. <sup>a)</sup> $\times 10^{-4}$ mole/liter	5.0	5.0	5.0	2.0	2.0	1.0	1.0	1.0	1.0	0.5	0.5	0.5
	buffer. soln. <sup>b)</sup>	1	1	1	2	2	2	2	3	3	4	4	4
I	pH	3.50	4.30	4.80	6.50	6.80	7.30	7.50	8.05	8.90	9.55	9.75	11.75
	I.C. <sup>a)</sup> $\times 10^{-4}$ mole/liter	5.0	5.0	2.0	2.0	2.0	1.0	1.0	1.0	1.0	0.5	0.5	0.5
	buffer. soln. <sup>b)</sup>	1	1	1	2	2	2	2	2	3	4	4	4
II	pH	3.10	3.30	3.80	4.50	5.90	6.90	7.50	8.05	8.25	9.65	10.50	11.50
	I.C. <sup>a)</sup> $\times 10^{-4}$ mole/liter	10.0	10.0	10.0	5.0	5.0	5.0	2.0	2.0	2.0	0.6	0.5	0.5
	buffer. soln. <sup>b)</sup>	1	1	1	1	2	2	2	2	3	4	4	4
III	pH	3.45	4.00	4.95	6.10	6.85	7.40	7.75	8.40	9.20	9.80	10.70	11.80
	I.C. <sup>a)</sup> $\times 10^{-4}$ mole/liter	10.0	10.0	5.0	5.0	2.0	2.0	1.0	1.0	1.0	0.5	0.5	0.5
	buffer. soln. <sup>b)</sup>	1	1	1	2	2	2	2	3	3	4	4	4

a) I.C. : initial concentration

b) buffer solution 1:  $\frac{1}{10}$ N AcOH- $\frac{1}{10}$ N AcONa 2:  $\frac{1}{10}$ M  $\text{KH}_2\text{PO}_4$ - $\frac{1}{10}$ M  $\text{Na}_2\text{HPO}_4$   
3:  $\frac{1}{10}$ M  $\text{KH}_2\text{PO}_4$ - $\frac{1}{10}$ M  $\text{Na}_2\text{B}_4\text{O}_7$  4:  $\frac{1}{10}$ M  $\text{Na}_2\text{B}_4\text{O}_7$ - $\frac{1}{10}$ N NaOH

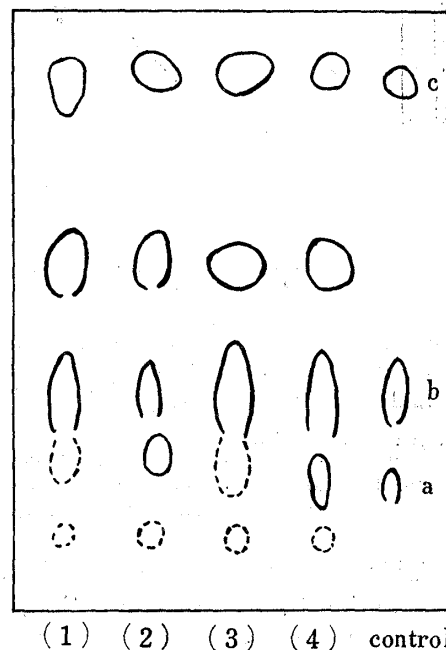
**Reaction II (Reaction between TPD and II)**—When a reaction mixture of pH 8 in which approx. 80% thiamine was formed was chromatographed, an unknown spot was detected by means of sodium dichromate and iodine vapor at  $R_f$  0.60. The  $R_f$  value of this spot differed from that of II (0.90) and di(2-hydroxyethyl)-disulfide (0.90). The fraction of this unknown spot was extracted with ether from the reaction mixture after adding HCl aq. soln. The residue of this extract was a colorless and oily substance. IR  $\nu_{\text{max}}^{\text{CHCl}_3}$   $\text{cm}^{-1}$ : 3550 ( $-\text{OH}$ ), 1050 ( $-\text{C}-\text{O}$ ), 2920, 2970, 1385 ( $-\text{CH}_2-$ ).

**Reaction III (Reaction between TPD and III)**—When a reaction mixture of pH 8 in which approx. 80% thiamine was formed was chromatographed. An unknown spot was detected by sodium dichromate and iodine vapor at  $R_f$  0.60. The  $R_f$  value of this spot differed from that of III (0.90) and di(2-carboxyethyl)disulfide (IV), (0.90). The fraction of this unknown spot was extracted with ether from the reaction mixture after adding HCl aq. soln. The residue of this extract was a colorless and waxy substance. IR  $\nu_{\text{max}}^{\text{CHCl}_3}$   $\text{cm}^{-1}$ : 3000—2500 ( $\text{C}=\text{O}$ ), 1700 ( $\text{C}=\text{O}$ ). When the extract was heated for a long time, colorless crystals precipitated which were identified as IV mp 153°.

## Results and Discussion

The reactions between TPD and each of the thiols glutathione, I, II, and III were carried out in a buffer solution of pH 8.0 under an anaerobic condition. The thin-layer chromatograms of the resultant reaction mixtures acidified by adding hydrochloric acid are given in Fig. 1.

Spots of an unknown compound and thiamine were detected on thin-layer chromatograms as well as the reactants TPD and thiols. The unknown spot detected by ninhydrin on the chromatogram of the reaction mixture between TPD and glutathione was identified as glutathione propyl disulfide



(1) (2) (3) (4) control

Fig. 1. Composite Representation of Thin-Layer Chromatograms for the Reaction Mixtures between TPD and Thiols at pH 8

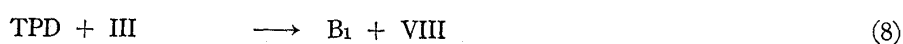
developing solvent: 1N HCl aq. soln.

control: a) TPD, b) thiamine, c) thiols

samples: (1) TPD+glutathione, (2) TPD+I, (3) TPD+II, (4) TPD+III.

(V) by Nogami, *et al.*<sup>9)</sup> in our previous paper. Since these previous results show that the unknown spots are the unsymmetrical alkyl disulfides, therefore the present unknown spots are also expected to be the corresponding alkyl disulfide in the reactions. And the infrared spectrum showed that the unknown spots were 2-aminoethyl propyl disulfide (VI), 2-hydroxyethyl propyl disulfide (VII) and 2-carboxyethyl propyl disulfide (VIII) for the reaction of I, II and III, respectively.

Therefore, the main reactions between TPD and thiols were the exchange reaction with the formation of thiamine and alkyl propyl disulfide represented by Eq. (5), (6), (7) and (8).



The reactions between TPD and thiols were examined in equal concentration at 15° in a nitrogen atmosphere and it was found that the formation of thiamine followed the second order kinetics.

The difference was found in pH-rate profiles for the reactions between TPD and the thiols containing the amino group in the molecule and those for the reactions between TPD and the other thiols. In the former reactions a plateau was observed between pH 8.0 and 10.5, on the other hand a slope of 1.0 was observed in the latter reactions in the same pH range, as shown in Fig. 2 and 3.

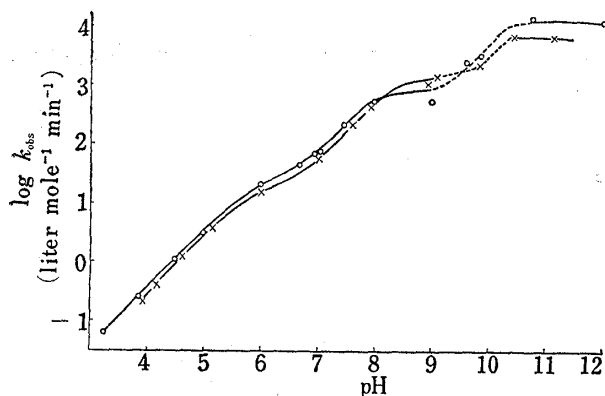


Fig. 2. The pH-Rate Profiles for the Reaction between TPD and I (O---O) and between TPD and Glutathione (x---x) at 15°

O, x : experimental values

smooth curve: calculated line as described in this report

dotted line: Calculation values were not obtained, because of the unknown parameters  $K_e$  and  $K_d$ .

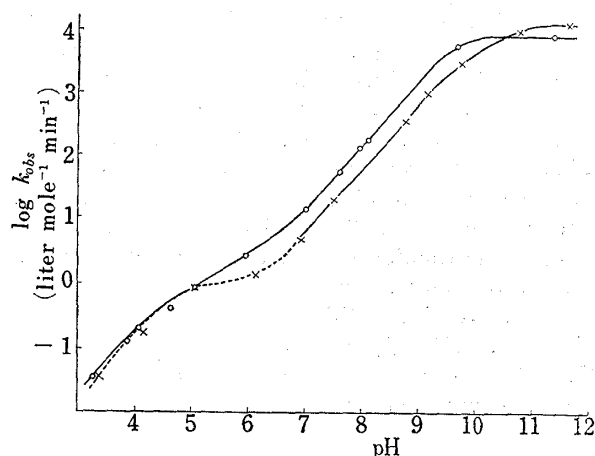


Fig. 3. The pH-Rate Profiles for the Reaction between TPD and II (O---O) and between TPD and III (x---x) at 15°

O, x : experimental values

smooth curve: calculated line as described in this report

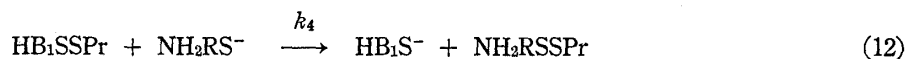
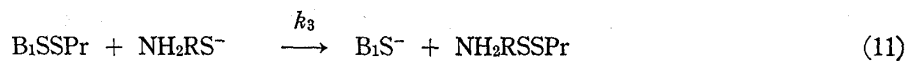
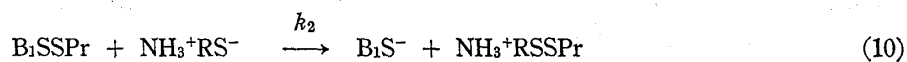
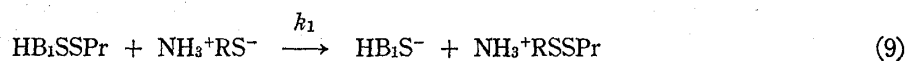
dotted line: Calculation values were not obtained.

This difference may be resulted from the effect of the dissociation of the amino group of the amino-thiols on the reaction.

The simple profiles were obtained in the reactions between TPD and II and between TPD and III which have no dissociable group in this pH range.

In the case of reactions between TPD and amino-thiols, the reaction species were assumed to be two mercaptide ions, *i.e.*  $\text{NH}_3^+\text{RS}^-$  and  $\text{NH}_2\text{RS}^-$ , and protonated TPD ( $\text{HB}_1\text{SSPr}$ ) and nonprotonated TPD ( $\text{B}_1\text{SSPr}$ ). Then the reactions between TPD and glutathione and between

TPD and I were expressed to be a mixture of the following four reactions in the experimental pH range as shown by Eq. (9), (10), (11) and (12), where  $\text{HB}_1\text{S}^-$  is protonated thiamine.



But the Eq. (12) may be ignored under the situation where the  $\text{p}K_a$  values of the species indicate that significant concentrations of  $\text{HB}_1\text{SSPr}$  and  $\text{NH}_2\text{RS}^-$  cannot exist simultaneously. Therefore, the reaction rate may be expressed by Eq. (13).

$$\begin{aligned} V &= \frac{-d[\text{TPD}]}{dt} = \frac{d[\text{B}_1]}{dt} = k_1[\text{HB}_1\text{SSPr}][\text{NH}_3^+\text{RS}^-] \\ &\quad + k_2[\text{B}_1\text{SSPr}][\text{NH}_3^+\text{RS}^-] + k_3[\text{B}_1\text{SSPr}][\text{NH}_2\text{RS}^-] \\ &= k_{\text{obs}}[\text{TPD}_T][\text{RSH}_T] \end{aligned}$$

where  $[\text{TPD}_T]$  and  $[\text{RSH}_T]$  represent the total concentrations of the individual components.

Eq. (14) was obtained with the similar procedure of Eq. (7) to Eq. (17) represented previously.<sup>5)</sup>

$$k_{\text{obs}} = \frac{K_a[\text{H}^+]^2 \left( k_1 + \frac{k_2 K_B}{[\text{H}^+]} + \frac{k_3 K_B K_c}{[\text{H}^+]^2} \right)}{([\text{H}^+] + K_B) \{ [\text{H}^+]^2 + [\text{H}^+](K_a + K_b) + K_a K_c \}} \quad (14)$$

The dissociation constants of amino-thiols are  $K_a$ ,  $K_b$ ,  $K_c$ , and  $K_d$  as given in Chart 1 and the dissociation constant of pyrimidine moiety in TPD is  $K_B$ .

When  $[\text{H}^+] \gg K_B$ , Eq. (14) can be simplified as Eq. (15), neglecting  $[\text{B}_1\text{SSPr}]$  and  $[\text{NH}_2\text{RS}^-]$ .

$$k_{\text{obs}} = \frac{K_a k_1}{[\text{H}^+]} \quad (15)$$

$$\begin{aligned} \log k_{\text{obs}} &= \log K_a k_1 - \log [\text{H}^+] \\ &= C + \text{pH} \end{aligned} \quad (16)$$

When  $K_B \gg [\text{H}^+] \gg K_c$ ,  $K_d$ , Eq. (14) can be simplified as Eq. (17), neglecting  $[\text{HB}_1\text{SSPr}]$  and  $[\text{NH}_2\text{RS}^-]$ .

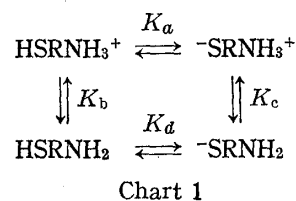
$$k_{\text{obs}} = \frac{K_a k_2}{[\text{H}^+] + K_a + K_b} \quad (17)$$

$$\begin{aligned} \log k_{\text{obs}} &= \log K_a k_2 - \log ([\text{H}^+] + K_a + K_b) \\ &= C + \log ([\text{H}^+] + K_a + K_b) \end{aligned} \quad (18)$$

When  $[\text{H}^+] \ll K_c$  and  $K_d$ , Eq. (14) can be simplified to Eq. (19), neglecting  $[\text{HB}_1\text{SSPr}]$  and  $[\text{NH}_3^+\text{RS}^-]$ .

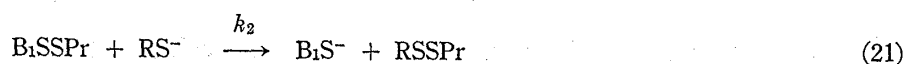
$$k_{\text{obs}} = k_3 \quad (19)$$

The plots for Eq. (16), (18), and (19) were found for pH values below 5, between 7 and 8 and above 10, respectively. The values for  $k_1$ ,  $k_2$  and  $k_3$  were calculated from the equations for each of these pH ranges.



The calculated values for  $k_1$ ,  $k_2$  and  $k_3$  were  $7.37 \times 10^4$ ,  $1.44 \times 10^4$  and  $0.62 \times 10^4$  liter mole<sup>-1</sup> min<sup>-1</sup> for glutathione using  $pK_a$  value of 9.2 for SH and  $NH_3^+$  groups at 23° and 2.19 for COOH group at 25°. The calculated values for  $k_1$ ,  $k_2$  and  $k_3$  were  $9.69 \times 10^3$ ,  $1.81 \times 10^4$  and  $1.11 \times 10^4$  liter mole<sup>-1</sup> min<sup>-1</sup> for I, using  $pK_a$  value of 8.35 at 23°. Only the SH group of II, which has a  $pK_a$  of 9.48 at 20°,<sup>11)</sup> dissociates in this experimental pH range. Therefore there is only one reactive species of II.

Then the reaction between TPD and II was expressed by Eq. (20) and (21).



The decrease rate of TPD and the formation rate of thiamine in the pH range of 3 to 12 can be shown by the following Eq. (22).

$$V = \frac{-d[TPD]}{dt} = \frac{d[B_1]}{dt} = k_1[HB_1SSPr][RS^-] + k_2[B_1SSPr][RS^-] = k_{obs}[TPD_T][RSH_T] \quad (22)$$

$$k_{obs} = \frac{K_a K_B}{(K_a + [H^+])(K_B + [H^+])} \left( k_1 \frac{[H^+]}{K_B} + k_2 \right) \quad (23)$$

Eq. (23) can be simplified to Eq. (24), (25) and (26) in each pH condition

at  $K_B \ll [H^+]$

$$k_{obs} = \frac{K_a k_1}{[H^+]}$$

$$\log k_{obs} = \log K_a k_1 - \log [H^+] = C + pH \quad (24)$$

at  $K_B \gg [H^+]$

$$k_{obs} = \frac{k_2 K_B K_a}{(K_B + [H^+])(K_a + [H^+])}$$

$$[H^+] \gg K_a$$

$$K_{obs} = \frac{K_a k_2}{[H^+]}$$

$$\log k_{obs} = \log K_a k_2 - \log [H^+] = C + pH \quad (25)$$

$[H^+] \ll K_a$

$$K_{obs} = k_3 \quad (26)$$

The plots for Eq. (24), (25) and (26) were found for pH values below 4.5, between 7 and 9.5 and above 10, respectively.

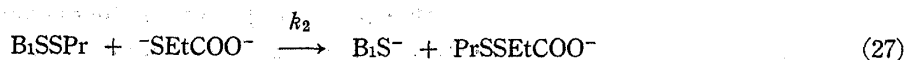
Similarly, the rate constant  $k_2$  for the reaction between TPD and III calculated from Eq. (25) and (26) were  $2.37 \times 10$  liter mole<sup>-1</sup> min<sup>-1</sup> at 15° using the  $pK_a$  value of 10.37<sup>12)</sup> at 25°. Since the  $pK_a$  of the carboxyl group of III is 4.38 at 25°, the reaction species of III were assumed to be two mercaptide ions, *i.e.*  $-SEtCOOH$  and  $-SEtCOO^-$ . Therefore, the three reactions between  $HB_1SSPr$  and  $-SEtCOOH$ , between  $HB_1SSPr$  and  $-SEtCOO^-$  and between  $B_1SSPr$  and  $-SEtCOOH$  were expected at the pH range between 3.2 and 6.5. However, these reac-

10) R.E. Benesch and R. Benesch, *J. Am. Chem. Soc.*, **77**, 5877 (1955).

11) J.P. Danehy and C.J. Noel, *J. Am. Chem. Soc.*, **82**, 2511 (1960).

12) C. Long, "Biochemists' Hand Book," ed., Richard Clay and Company Ltd., Bungay, Suffolk, Great Britain, 1961, p. 50.

tions could not be clearly distinguished, although a slope equal to I was observed below the pH 4.5.



The temperature dependence was studied at pH 4.0 and 7.5 where only one reaction would be expected at temperatures ranging from 15° to 37°. The rate constant was determined at 15°, 20°, 25°, 30° and 37°, respectively. The results are plotted in Fig. 4 and 5 by means of Arrhenius type plot. Activation energies and entropies were calculated and shown in Table II.

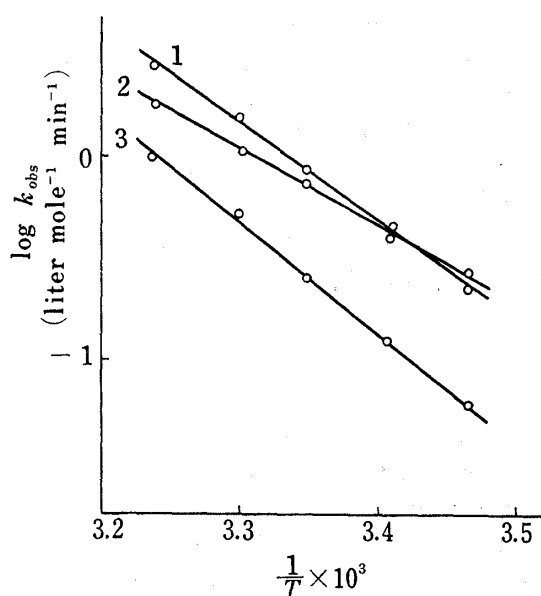


Fig. 4. Arrhenius Plots of  $\log k_{\text{obs}}$  versus the Reciprocal of Absolute Temperatures for the Reactions between TPD and Thiols at pH 4.0

1) glutathione 2) I 3) II

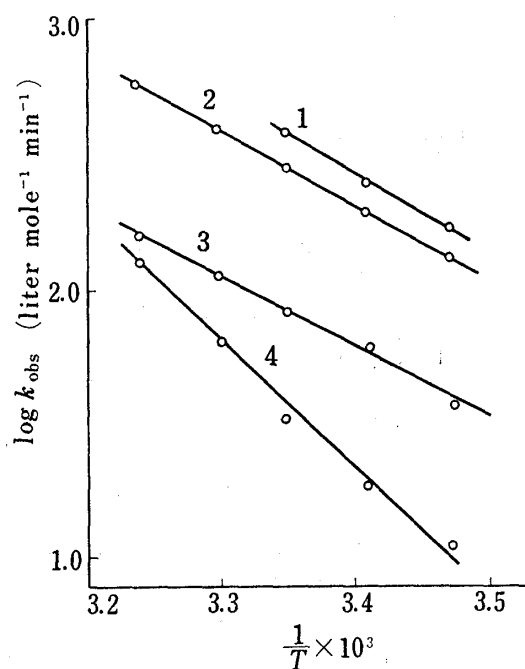


Fig. 5. Arrhenius Plots of  $\log k_{\text{obs}}$  versus the Reciprocal of Absolute Temperatures for the Reactions between TPD and Thiols at pH 7.5

1) glutathione 2) I 3) II 4) III

TABLE II. Summarized Parameters for the Reactions between TPD and Thiols

Thiols		$k$ at 15° liter mole <sup>-1</sup> min <sup>-1</sup>	$E_a$ kcal mole <sup>-1</sup>	A=frequency factor liter mole <sup>-1</sup> min <sup>-1</sup>	$\Delta S$ cal mole <sup>-1</sup> deg <sup>-1</sup>
L-Cysteine HSCH <sub>2</sub> CH(NH <sub>2</sub> )COOH	$k_1^a)$	$2.30 \times 10^4$	12.3	$3.64 \times 10^{12}$	- 9.2
	$k_2^b)$	$2.60 \times 10^3$	10.9	$5.13 \times 10^{11}$	-13.0
Glutathione	$k_1$	$7.37 \times 10^4$	9.1	$5.69 \times 10^{11}$	-14.8
	$k_2$	$1.44 \times 10^4$	9.0	$1.74 \times 10^{11}$	-14.5
I HSCH <sub>2</sub> CH <sub>2</sub> NH <sub>2</sub>	$k_1$	$9.69 \times 10^3$	8.1	$3.20 \times 10^{10}$	-20.5
	$k_2$	$1.81 \times 10^3$	8.4	$2.21 \times 10^9$	-23.9
II HSCH <sub>2</sub> CH <sub>2</sub> OH	$k_1$	$7.94 \times 10^4$	9.1	$2.24 \times 10^{11}$	-14.7
	$k_2$	$7.30 \times 10^3$	11.9	$1.39 \times 10^{12}$	-11.0
III HSCH <sub>2</sub> CH <sub>2</sub> COOH	$k_2$	$2.37 \times 10^4$	11.6	$7.04 \times 10^{12}$	- 7.8

a)  $k_1$ : HB<sub>1</sub>SSPr+RS<sup>-</sup> b)  $k_2$ : B<sub>1</sub>SSPr+RS<sup>-</sup>

The effect of ionic strength was examined at pH 4.0 in acetate buffer and no detectable effect was observed in the range of the strength 0.2 to 0.8. No detectable buffer concentration

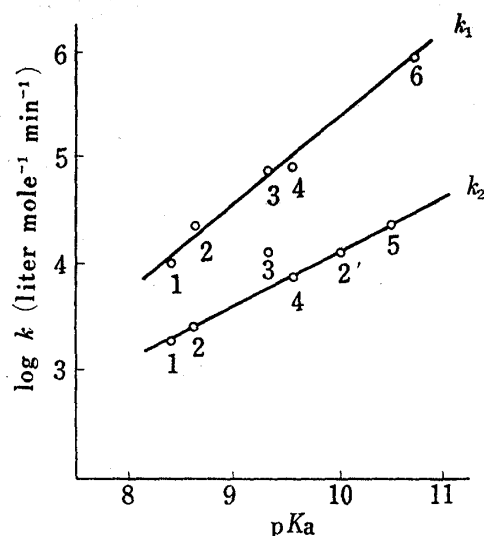


Fig. 6. Brönsted Type Plots of  $\log k$  versus  $pK_a$  for the Reactions between TPD and Thiols

1) I, 2) cysteine  $\text{NH}_3^+\text{CyS}^-$ , 2') cysteine  $\text{NH}_2\text{CyS}^-$ , 3) glutathione, 4) II, 5) III, 6) propyl mercaptan

$$\log k = \alpha pK_a + C$$

(28)

Comparing the scission of disulfide bond by mercaptide with the hydrolysis of *p*-nitrophenyl acetate catalyzed by mercaptide,<sup>14)</sup> it was found that  $\alpha$  values of the former reaction were 2.15 and 1.4 times greater than that of the latter and the specific rate constant of the former reaction with cysteine was 335 times greater than that of the latter.

Since, the  $\alpha$  value for  $k_1$  was equal to that for the hydrolysis of *p*-nitrophenyl acetate catalyzed imidazol<sup>14)</sup> derivatives which are known as a strong nucleophile, therefore mercaptide can be taken as a extremely good nucleophile to the disulfide bond. It can be thought that the nucleophilicity to the protonated TPD is greater than to the nonprotonated TPD on comparing the  $\alpha$  value for  $k_1$  and  $k_2$ .

The rate constants of the two ionic species of cysteine, *i.e.*  $\text{NH}_3^+\text{CyS}^-$ ,  $\text{NH}_2\text{CyS}^-$  were plotted on the same line of the Brönsted type plot, as shown in Fig. 6. Therefore it can be thought that the protonation of the amino group of cysteine affects only the dissociation constant of the SH group, *i.e.* nucleophilicity.

The thiols studied here possess a variety of other functional groups. Nevertheless they all conform to the single Brönsted plot shown in Fig. 6. Therefore it was concluded that these groups only affect the dissociation of the SH group in molecule and the nucleophilicity.

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effect on the reaction rate was observed in 0.05—0.15 mole/liter of acetate concentration at pH 4.0.

In a previous paper on the exchange reaction between disulfide compound and mercaptide,  $S_N2$  reaction were mentioned.<sup>5)</sup> And as for the reactivity of mercaptide, Ogilvice, *et al.*<sup>13)</sup> reported that the Brönsted equation could be applied to the hydrolysis of *p*-nitrophenyl acetate catalyzed by mercaptide, showing that mercaptide worked as a nucleophile. Although the transition state and other mechanism of the exchange reaction were not yet cleared, two Brönsted type plots for  $k_1$  and  $k_2$  were applied for the  $pK_a$  of SH group, assuming that an ionized mercaptide works as a nucleophile also in the exchange reaction. Two straight lines were obtained as shown in Fig. 6. The  $\alpha$  values calculated from Eq. (28) were 0.82 and 0.53, and the  $C$  values were found to be  $-2.84$  and  $-1.16$ . for  $k_1$  and  $k_2$ , respectively.

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