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Thiamine Derivatives of Disulfide Type. IX.[1] Exchange Reactions between Symmetrical Disulfide and L-Cysteine[2]

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Kinetic studies were made about the exchange reactions between dibenzyl disulfide (I) and cysteine, and between diphenyl disulfide (III) and cysteine, in order to determine the dependence on the reaction rates of the structures of disulfides and of thiols. It was demonstrated that the greater the pK_a of the thiols or the smaller the pK_a of the leaving molecule from disulfides, the greater are the rate constants. It was also shown that these exchange reactions stood in equilibria.

Being compared this with the reactions between thiamine derivatives of disulfide type and thiols where reverse reaction does not proceed, it may be concluded that the absence of reverse reaction is due to the thiamine's behavior in aqueous solution that the thioanion-type structure readily turn to thiazolium-type one below neutral pH.

Exchange reaction between various thiamine derivatives of the disulfide type and thiols such as cysteine and glutathione had been studied kinetically in this series,[1,4-7] where it was proved that the main reaction could be presented by reactions (1) or (2) in which thiamine was formed through the exchange reactions between thiamine propyl disulfide (TPD) and L-cysteine and between thiamine disulfide (TDS) and L-cysteine.

$$\text{TPD} + \text{cysteine} \longrightarrow \text{thiamine} + \text{S-propylmercaptocysteine} \qquad (1)$$

$$\left.\begin{array}{l}\text{TDS} + \text{cysteine} \longrightarrow \text{thiamine} + \text{cysteine thiamine disulfide} \\ \text{cysteine thiamine disulfide} + \text{cysteine} \longrightarrow \text{thiamine} + \text{cystine}\end{array}\right\} \qquad (2)$$

Dependence of the reaction rate on the structures of the disulfides and the thiols was also studied. That was, in the disulfides, a tendency was observed that the stronger the electron-withdrawing effect of the alkyl groups the greater the rate constant, and in the thiols, the greater the pK_a of the SH group, the greater the specific rate constant in the reactions between TPD and thiols. The reverse reaction was not observable below pH 7 in the exchange reactions between thiamine derivatives of the disulfide type and thiols even though reactions between disulfide compounds and thiols are usually in equilibria. In the present paper, to clarify the reasons of these characteristic reactivities of thiamine derivatives of the disulfide type, a comparative study was made on the exchange reactions between the thiols and those disulfides which contain and do not contain the thiamine group, for example dibenzyl disulfide (I) and diphenyl disulfide (III).

1) Part VIII: H. Nogami, J. Hasegawa, N. Ikari and K. Takeuchi, *Chem. Pharm. Bull.* (Tokyo), **17**, 1541 (1969).
2) The 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, Apr. 1968.
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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 (1965).

Experimental

Thin-Layer Chromatographic Procedure——The same as the preceding paper.[1]

S-Benzylmercaptocysteine (II)——Dibenzyl disulfide (I) (5 g), 30% H_2O_2 aq. soln. (5 ml) and AcOH (100 ml) were mixed and allowed to stand for about 12 hr at room temperature. When the formation of dibenzyl disulfide oxides was observed distinctly on thin-layer chromatogram by I_2 vapor, water (500 ml) was added to the reaction mixture and a waxy precipitate appeared. The precipitate was extracted with ether, and the ether solution was washed with water until KI reagent did not react with the washed water. After the ether was distilled out, the residue was dissolved in acetone (100 ml) and cysteine aq. soln. (1.64 g in 30 ml) was added. A rosy substance precipitated immediately, and this was collected by filtration. The substance was washed with ether and water, and recrystallized from 0.25N HCl aq. soln. Colorless plate crystals were obtained. Yield 55%, mp 192° (decomp.). *Rf* value of this substance was 0.45 on thin-layer chromatogram. *Anal.* Calcd. for $C_{10}H_{13}O_2NS_2$: C, 49.42; H, 5.34; N, 5.74. Found: C, 49.25; H, 5.60; N, 5.59. Nuclear magnetic resonance (NMR) (in CF_3COOH) ppm; 2.74 (2H, doublet, $J=8.57$ cps, $-C\underline{H}_2CH(NH_2)COOH$, 2.98 (2H, singlet, $-C\underline{H}_2C_6H_5$), 4.50 (1H, broad, methin proton), 7.40 (5H, singlet, aromatic protons).

S-Phenylmercaptocysteine (IV)——Prepared by the same way as S-benzylmercaptocysteine except that the recrystallizing solvent was 0.5N HCl, mp 192° (decomp.).[8] The *Rf* value of this substance was 0.45 on thin-layer chromatogram. *Anal.* Calcd. for $C_9H_{11}O_2NS_2$: C, 47.14; H, 4.84; N, 6.12. Found: C, 47.60; H, 5.07; N, 6.02.

Apparatus——Determination of pH of the samples soln. was made with Toa-Electronics Ltd. pH meter model HM5A type equipped with glass electrode. Spectrophotometric measurements were made with a Hitachi 124 spectrophotometer equipped with a thermostated cell compartment and a Hitachi QPD34 recorder.

Buffer Solutions——The same as the preceding paper.[1]

50% Ethanolic Buffer Solutions——Equivolume mixtures of ethanol and buffer solutions were made and their apparent pH were determined with the pH meter.

Disproportionation of S-Benzylmercaptocysteine (II) and S-Phenylmercaptocysteine (IV)——II and IV were dissolved in 50% ethanolic buffer solutions and allowed to stand in nitrogen atmosphere on a boiling bath. Aliquots of these solutions were drawn at appropriate intervals and thin-layer chromatographed.

5,5′-Dithiobis(2-Nitrobenzoic Acid) (DTNB) Reagent——39.5 mg DTNB were dissolved in a phosphate buffer solution of pH 7.0 ($\mu=1$) to make 100 ml.[9]

Determination of Cysteine——1 ml of 1N HCl aq. soln. was added to 5 ml of the test solution and diluted with water to make 15 ml. After the solution was washed with 20 ml of *n*-hexane, 1 ml of DTNB reagent was added to a suitable aliquot of the solution adjusted to pH 8.0 with NaOH, and diluted with the phosphate buffer solution (pH 8.0) to make 25 ml. Absorbance of this solution mixture was measured at 412 mμ.[9]

Determination of Equilibrium Constant——Each 5×10^{-4} mole of cysteine, benzylmercaptan and S-benzylmercaptocysteine were dissolved together in 1 liter of 50% ethanolic buffer solution and bubbled with nitrogen to remove oxygen.

This solution was filled in ampules and bubbled with N_2 again. After the ampules were sealed, the solution was allowed to react at 40°, aliquots of this solution were drawn at predetermined intervales and the amount of cysteine was determined.

Determination of Apparent Dissociation Constants of the Thiols in a 50% Ethanolic Aq. Soln.——Spectrophotometric Method: 1.5×10^{-4} mole of cysteine was dissolved in 1 liter of 50% ethanolic buffer solutions of various pH and bubbled with N_2 to remove O_2. K_A values of cysteine in Chart 1 were determined by the procedure and Eq. (3) reported by Benesch, *et al.*[10] using these solutions at 16° and 30°.

$$\frac{(RS^-)}{(RS^-)_{max}}=\frac{[^-SCyNH_3^+]+[^-SCyNH_2]}{[CySH]_T}=\frac{K_A/K_B+K_D/[H^+]}{[H^+]/K_B+K_A/K_B\cdot K_D/[H^+]+1} \qquad (3)$$

$$\begin{array}{ccc} & \overset{K_A}{\rightleftharpoons} & \\ HSRNH_3^+ & & S^-RNH_3^+ \\ K_B\uparrow\downarrow & & \uparrow\downarrow K_C \\ HSRNH_2 & \underset{K_D}{\rightleftharpoons} & S^-RNH_2 \end{array}$$

Chart 1

Where K_A, K_B, K_C and K_D are the dissociation constants.

The pK_a values of benzylmercaptan and 5-mercapto-2-nitrobenzoic acid were determined by the same procedure as cysteine at a concentration of 0.8×10^{-4}M and 2.4×10^{-4}M respectively.

Titrimetric Method: 0.3635 g (3×10^{-3} mole) of cysteine was dissolved in 200 ml of a 50% ethanolic aq. soln. and this solution was potentiometrically titrated with 1N NaOH using pH meter, at 15° and 30°. pK_a was determined by the pH at the half-equivalent point graphically.

8) S. Sakakibara and H. Tani, *Bull. Chem. Soc. Japan*, **29**, 85 (1956).
9) G.L. Ellman, *Arch. Biopyem. Biophys.*, **82**, 70 (1959).
10) R.E. Benesch and R. Benesch, *J. Am. Chem. Soc.*, **77**, 5877 (1955).

The pK_a of phenylmercaptan was determined by the same method except using 0.022 g of phenylmercaptan and 0.1N NaOH.

Half-Equivalent Method: Since the equivalent point was not clear in the titration curve of benzylmercaptan with both 1N and 0.1N NaOH aq. soln., the pK_a of this compound was determined by measuring the pH at the stoichiometrically half neutralization point adding 2 ml of 0.1N NaOH into 50 ml of 0.01M phenylmercaptan. (50% ethanolic aq. soln.) at 15° and 30°.

Results and Discussion

Reaction Process

Since reactions between disulfides and thiols are usually in equilibria,[11] the reaction between I and cysteine was expected to be written by Eq. (4) and (5) (I-cysteine system).

$$\text{BzSSBz (I)} + \text{CySH} \rightleftharpoons \text{BzSH} + \text{CySSBz (II)} \quad (4)$$

$$\text{CySSBz (II)} + \text{CySH} \rightleftharpoons \text{BzSH} + \text{Cystine} \quad (5)$$

where BzSH and CySH are benzylmercaptan and cysteine respectively.

The results of the reactions starting from the either side of Eq. (4) or (5) were examined with thin-layer chromatography. As shown in Fig. 1, every reaction gave the same results which were expected from Eq. (4) and (5). Accordingly, it was found that the reaction between I and cysteine was described with two reversible equations as above.

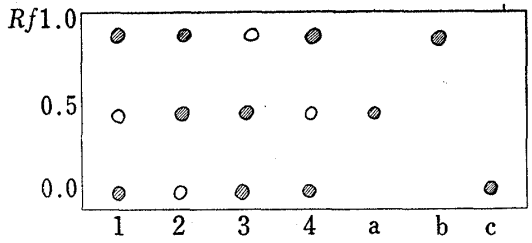


Fig. 1. Compositive Representation of Thin-Layer Chromatograms for the Reaction Mixtures of Dibenzyl Disulfide–Cysteine System at pH 6.0

plate: silica gel (Wakogel B-5)
developing solvent: 1N HCl
reference substance
a: S-benzylmercaptocysteine, b: cysteine, cystine, c: dibenzyl disulfide, benzylmercaptan
reaction between
1: dibenzyldisulfide and cysteine, 2: S-benzylmercaptocysteine and cysteine, 3: S-benzylmercaptocysteine and benzylmercaptan, 4: cystine and benzylmercaptan
●: reactant. ○: product

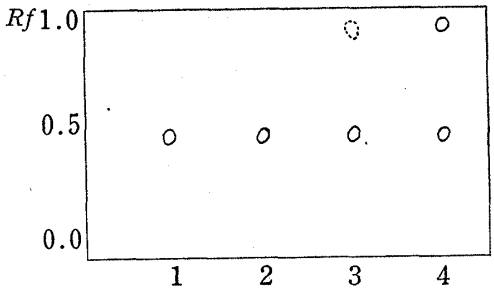


Fig. 2. Compositive Representation of Thin-Layer Chromatograms for Stability Test of S-Benzylmercaptocysteine

plate: silica gel (Wakogel B-5)
developing solvent: 1N HCl
conditions
1: pH 4.1 / pH 7.0 } after 10 min, 2: pH 8.0 immediately after the reaction started, 3: pH 8.0 after 10 min, 4: pH 8.0 after 30 min

Possibility of Disproportionation of S-Benzylmercaptocysteine

It was reported by Field, *et al.*[12,13] that disproportionation of unsymmetrical disulfides into the symmetrical disulfides such as shown by Eq. (6) was induced by either acid, base, trace thiols, heat or light. II was the unsymmetrical disulfide and disproportionation into I and cysteine may be occurred following to Eq. (6) beside Eq. (4) and (5).

$$\underset{\text{II}}{2\text{CySSBz}} \rightleftharpoons \text{Cystine} + \underset{\text{I}}{\text{BzSSBz}} \quad (6)$$

11) G. Dalman, J. McDermed and G. Gorin, *J. Org. Chem.*, **29**, 1480 (1964).
12) L. Field, T.F. Parsons and D.E. Parson, *J. Org. Chem.*, **31**, 3550 (1966).
13) M. Bellans, D.L. Tulleen and L. Field, *J. Org. Chem.*, **32**, 2591 (1967).

II in 50% ethanolic buffer solution were allowed to stand on a boiling water bath under an anaerobic condition and aliquots of these solution were taken at suitable intervals and were thin-layer chromatographed. As shown in Fig. 2, two spots were detected by ninhydrin reagent above pH 8.0 but only one spot was detected below pH 7.0 on chromatograms of these solutions within a half hour.

Thus, it can be concluded that Eq. (6) does not proceeded under these conditions between pH 7.0 and 4.1. The exchange reactions were studied at pH of below 7.0.

Apparent Dissociation Constants of Thiols in a 50% Ethanolic Buffer Solution

Since the present kinetic study was examined in 50% ethanolic buffer solutions, the dissociation constants in this solvent were necessary to calculate the kinetic parameters. It has been reported that the pK_a of thiols are greater in a mixture of water and an organic solvent than in water.[14,15]

In this report, apparent pK_a and apparent pH were used which were determined in 50% ethanolic buffer solutions, so that these might not always be corresponded to the true values.

But, since these were determined in the same condition, it may not come into question to use these values in order to calculate the kinetic parameters. In this experiment, the p*K*a values which determined from two different methods were same within experimental error, for example, pK_a values of benzylmercaptan were 10.70 by half-equivalent method and 10.87 by spectrophotometric method, and pK_a values of cysteine were 9.06 by spectrophotometric method and 8.85 by titration method. Apparent pK_a values in 50% ethanolic buffer solution were shown in Table I, and 10.70 as the pK_a of benzylmercaptan and 9.06 as that of cysteine were used.

Table I. Dissociation Constants of Various Thiols

Thiols	Solvents	pK_a	Method employed
BzSH	50% EtOH–aq. soln.	10.7 (16°)	half equivalent method
	50% EtOH–buffer soln.	10.87 (23°)	spectrophotometric method (at 250 mμ)
CySH	50% EtOH–buffer soln.	9.06 (16°)	spectrophotometric method
	50% EtOH–aq. soln.	8.85 (15°)	titration method
PhSH	50% EtOH–aq. soln.	7.9 (15°)	titration method
Thiamine	aq. buffer soln.	7.5	polarographic method[16]

Where PhSH is phenylmercaptan.

Rate Constants and Equilibrium Constants

The exchange reactions of I-cysteine system were carried out in equimolar concentrations of each reactants and the second order nature of the reactions were observed at the early stages of the runs at various pH, as shown in Fig. 3.

Then the second order rate constant was determined from observations of the initial stages of the reaction. Since the reactive species of thiol has been known as mercaptide anion,[5] Eq. (4) and (5) may be rewritten as Eq. (7) and (8)

$$\mathrm{BzSSBz} + \mathrm{Cys^-} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{BzS^-} + \mathrm{BzSSCy} \qquad (7)$$

$$\mathrm{CySSBz} + \mathrm{Cys^-} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \mathrm{BzS^-} + \mathrm{Cystine} \qquad (8)$$

14) M. Mizutani, *Z. Physik. Chem.* (Frankfurt), **118**, 318, 327 (1925).
15) N.F. Hall and M.R. Sprinkle, *J. Am. Chem. Soc.*, **54**, 3469 (1932).
16) I. Tachi and S. Koide, *J. Agri. Chem. Soc. Japan*, **25**, 330 (1961).

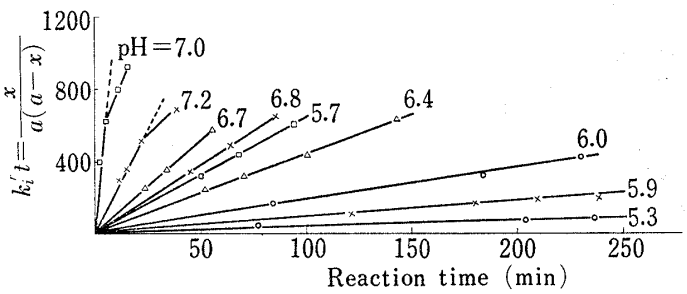


Fig. 3. Second Order Rate Equation Plots of Cysteine Formation or Decrease in the Reaction of I-Cysteine System at 40°

—○—: $k_1' t$, —×—: $k_2' t$,
—△—: $k_3' t$, —□—: $k_4' t$
a: initial concentration
x: the decrease in concentration of reactant in time t
Where k_i' is experimental rate constant represented in Eq. (7) and (8).

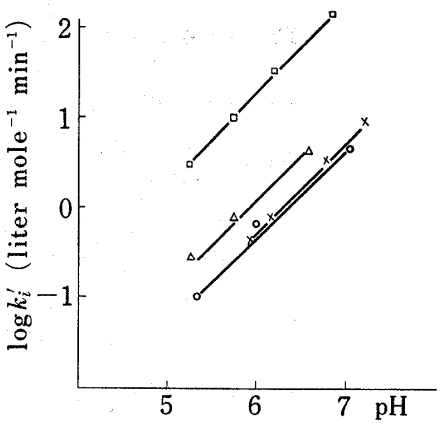


Fig. 4. pH *vs.* Rate Profile of the Reaction between Dibenzyl Disulfide and Cysteine at 40°

—○—: k_1', —×—: k_2',
—△—: k_3', —□—: k_4'
Where k' is experimental second order rate constant for Eq. (7) and (8).

where k_1—k_4 were rate constants of I-cysteine system. For example, in the reaction of Eq. (7) the initial decreasing rate of cysteine may be shown by Eq. (9) and (10)

$$\text{Reaction rate (V)} = \frac{-d[\text{CyS}^-]}{dt} = k_1'[\text{BzSSBz}_T]_0[\text{CySH}_T]_0$$
$$= k_1[\text{BzSSBz}_T]_0[\text{CyS}^-]_0 \qquad (9)$$

$$k_1' = \frac{k_1[\text{CyS}^-]_0}{[\text{CySH}_T]_0} = k_1 \cdot K_{\text{CySH}} \frac{1}{([\text{H}^+]+K_{\text{CySH}})} \qquad (10)$$

where k_1' is the experimental second order rate constant for the reaction between I and cysteine, the sign, $[\ ]_0$, is the initial total concentration of each component and K_{CySH} is the dissociation constant of the SH group of cysteine. So far as $[\text{H}^+] \gg K_{\text{CySH}}$ Eq. (10) may be simplified to Eq. (11). From Eq. (11) the plot of log k_1' versus pH can expected to be linear with a slope equal to 1.0.

$$\left.\begin{aligned} k_1' &= k_1 \cdot K_{\text{CySH}} \frac{1}{[\text{H}^+]} \\ \log k_1' &= \log k_1 \cdot K_{\text{CySH}} - \log [\text{H}^+] = C_1 + \text{pH} \\ C_1 &= \log k_1 \cdot K_{\text{CySH}} \end{aligned}\right\} \qquad (11)$$

The other experimental second order rate constants (k_2', k_3' and k_4') for Eq. (7) and (8) could be represented similarly as follows.
So far as

$$[\text{H}^+] \gg K_{\text{BzSH}},\ \log k_2' = \log k_2 \cdot K_{\text{BzSH}} - \log [H^+] = C_2 + \text{pH} \qquad (12)$$
$$[\text{H}^+] \gg K_{\text{CySH}},\ \log k_3' = \log k_3 \cdot K_{\text{CySH}} - \log [\text{H}^+] = C_3 + \text{pH} \qquad (13)$$
$$[\text{H}^+] \gg K_{\text{BzSH}},\ \log k_4' = \log k_4 \cdot K_{\text{BzSH}} - \log [\text{H}^+] = C_4 + \text{pH} \qquad (14)$$

where C_2, C_3 and C_4 are log $k_2 \cdot K_{\text{BzSH}}$, log $k_3 \cdot K_{\text{CySH}}$ and log $k_4 \cdot K_{\text{BzSH}}$ respectively. The relationship of Eq. (11)—(14) were observed for k_1', k_2', k_3' and k_4' below pH 7.0 and the specific rate constants were calculated from this pH range. Results were demonstrated in Fig. 4.

If the equilibrium constants of Eq. (7) and (8) are represented by K_1 and K_2 and those of Eq. (15) and (16) are K and K', we may give K as Eq. (19) and the experimental equilibrium constant K' as Eq. (20) as similar as Dalman, *et al.*[11]

$$\mathrm{BzSSBz} + 2\mathrm{CyS}^- \overset{K}{\rightleftharpoons} \text{cystine} + 2\mathrm{BzS}^- \tag{15}$$

$$\mathrm{BzSSBz} + 2\mathrm{CySH} \overset{K'}{\rightleftharpoons} \text{cystine} + 2\mathrm{BzSH} \tag{16}$$

$$K_1 = \frac{[\mathrm{CySSBz}][\mathrm{BzS}^-]}{[\mathrm{BzSSBz}][\mathrm{CyS}^-]} = \frac{[\mathrm{CySSBz}][\mathrm{BzSH}]K_{\mathrm{BzSH}}}{[\mathrm{BzSSBz}][\mathrm{CySH}]K_{\mathrm{CySH}}} \tag{17}$$

$$K_2 = \frac{[\mathrm{Cystine}][\mathrm{BzS}^-]}{[\mathrm{CySSBz}][\mathrm{CyS}^-]} = \frac{[\mathrm{Cystine}][\mathrm{BzSH}]K_{\mathrm{BzSH}}}{[\mathrm{CySSBz}][\mathrm{CySH}]K_{\mathrm{CySH}}} \tag{18}$$

$$K = K_1 \times K_2 = \frac{[\mathrm{Cystine}][\mathrm{BzSH}]^2(K_{\mathrm{BzSH}})^2}{[\mathrm{BzSSBz}][\mathrm{CySH}]^2(K_{\mathrm{CySH}})^2} \tag{19}$$

$$K' = K \times \frac{(K_{\mathrm{CySH}})^2}{(K_{\mathrm{BzSH}})^2} = \frac{k_1' \times k_3'}{k_2' \times k_4'} \tag{20}$$

where K_{BzSH} is the dissociation constant of benzylmercaptan. Experimental equilibrium constant K' was determined from the estimation of cysteine concentration where the reactions among II and cysteine and benzylmercaptan in the initial molar ratio of 1:1:1 reached equilibria. K' was consistent with K' calculated from the experimental rate constants for Eq. (7) and (8) as shown in Table II.

Table II. Equilibrium Constants in the Reaction between Dibenzyl Disulfide and Cysteine at 40°

pH	K' calculated from k'	K' estimated from cysteine concn. at eq.
4.0	0.037	
5.0	0.036	
5.5	0.036	
6.0	0.033	0.036
		0.037
		0.043
		0.034
6.5	0.035	0.033
		0.045
Average	0.035	0.038

Initial molar ratio of cysteine–benzylmercaptan–S-benzylmercaptocysteine is 1:1:1.

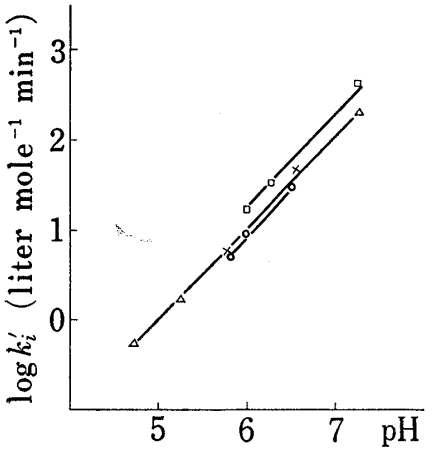


Fig. 5. pH *vs.* Rate Profile of the Reaction between Diphenyl Disulfide and Cysteine at 15°

—○—: k_1', —×—: $k_2'^{a)}$,
—△—: $k_3'^{a)}$, —□—: k_4'

a) Dotted lines of these two rates were happened to put into unity.

Reactions of III-cysteine system were examined in the same manner as the reaction of I-cysteine, and their rate constants and K' are shown in Table III and Fig. 5.

Activation Energies

The temperature dependence of rate constants was studied at pH 5.8 ranging from 10° to 50° and log k's were plotted by means of Arrhenius type plot as shown in Fig. 6 and Fig. 7. Activation energies were calculated from these plots as approximately 10 kcal·mole^{-1}. The results are summerized in Table IV. These were almost the same values as that on the reactions between thiamine derivatives of disulfide type and thiols in water.[1,5–7]

Table III. Equilibrium Constants in the Reaction between Diphenyl Disulfide and Cysteine at 15°

pH	K' calculated from k'	K' estimated from cysteine concn. at aq.
4.0	0.563	
5.0	0.389	
5.5	0.525	
6.0	0.489	0.394
6.5	0.491	0.507
		0.542
Average	0.491	0.481

Initial molar ratio of cysteine–phenyl mercaptan–S-benzylmercaptocysteine 1:1:1.

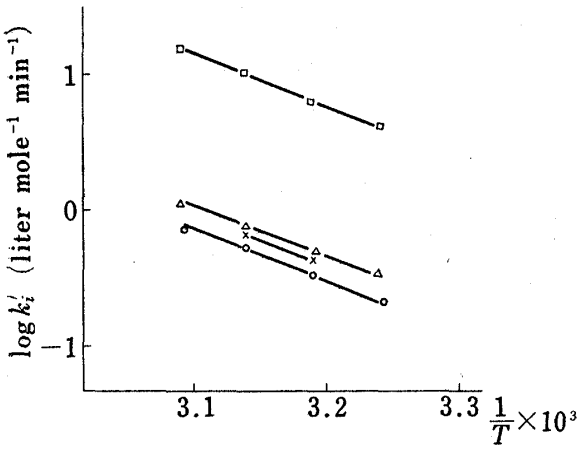


Fig. 6. Arrhenius Type Plots of log k_i' *vs.* the Reciprocal of Absolute Temperature of the Reaction between Dibenzyl Disulfide and Cysteine at pH 5.8

—○—: k_1', —×—: k_2', —△—: k_3', —□—: k_4'

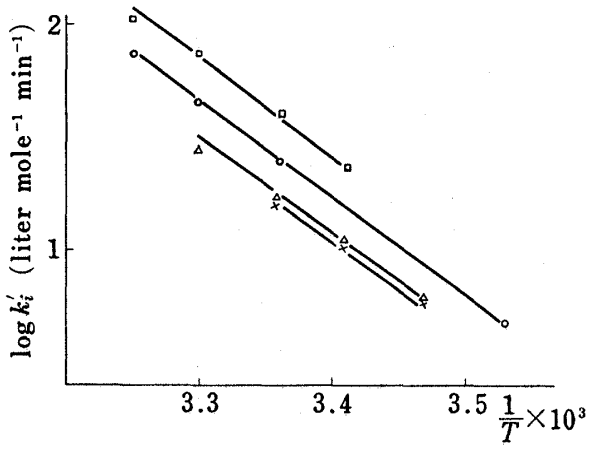


Fig. 7. Arrhenius Type Plots of log k' *vs.* the Reciprocal of Absolute Temperatures of the Reaction between Diphenyl Disulfide and Cysteine at pH 5.8

—○—: k_1', —×—: k_2', —△—: k_3', —□—: k_4'

Table IV. Parameters in the Reactions between Disulfides and Cysteine at 15°

System	k_1 L mole^{-1} min^{-1}	ΔE kcal mole^{-1}	k_2 L mole^{-1} min^{-1}	ΔE kcal mole^{-1}	k_3 L mole^{-1} min^{-1}	ΔE kcal mole^{-1}	k_4 L mole^{-1} min^{-1}	ΔE kcal mole^{-1}
I-Cysteine[a] (I=BzSSBz)	2.83×10	10.3	1.39×10^4	10.9	7.44×10^3	9.6	1.08×10^5	10.0
III-Cysteine (III=PhSSPh)	1.18×10^4	12.6	1.16×10^3	9.2	1.13×10^4	10.7	1.80×10^3	12.6
TDS-Cysteine	1.22×10^5				6.12×10^4			

a) 50% EtOH–aq. k'_s are defined in eq. (7) and (8).
L=liter

Correlation of Structure and Reactivity of Disulfide Compound

As for the rate constants k_1 of this experimental data, in Chart 2, it was observed that the greater the p$K_a$values of leaving group of disulfides, the smaller the rate constants in the reaction as shown in Table I and Table IV. For example, the order of the pK_a values were benzylmercaptan>phenylmercaptan>thiamine and the order the rate constants (k_1) were I-cysteine system<III-cysteine system<TDS-cysteine system. This was the same tendency as the reaction of unsymmetrical thiamine derivatives of disulfide type reported in previous

papers.[4,5] On the other hand, as for the thiol group, it was observed that the greater the pK_a values, the greater the rate constants. For example, in the reaction of I-cysteine system, k_2 was greater than k_3 as shown in Table IV. In the reaction of III-cysteine, on the other hand, k_3 was greater than k_2. Moreover, the rate constants of the reaction between cystine and benzylmercaptan was greater than that of the reaction between cysteine and phenylmercaptan.

$$RSSR + CyS^- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} RS^- + CySSR$$

$$CySSR + CyS^- \underset{k_4}{\overset{k_3}{\rightleftharpoons}} RS^- + Cystine$$

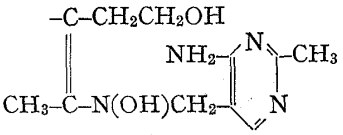
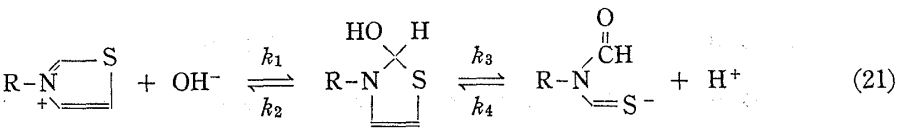
	R
I-cysteine system:	$-CH_2C_6H_5$
III-cysteine system:	$-C_6H_5$
TDS-cysteine system:	

Chart 2

A question is posed here that in the over-all reversible reaction, which reaction is predominant? that is, what is the main product? In the case of the reaction system of I-cysteine, cysteine was formed; but in the case of the reaction system of III-cysteine, phenylmercaptan was predominantly formed, it may be concluded that in the exchange reactions, the greater the anionic stability of the displaced mercaptide ion, the more susceptible to scission is the parent disulfide as reported by Kharasch, *et al.*[17]

Finally, reversible reactions were observed in the reactions between I and cysteine and between III and cysteine at the pH range of 5 to 7; but in the reaction between cysteine and DTNB the reagent used to determined thiols, reverse reaction was not observed and 5-mercapto-2-nitrobenzoic acid (V) was formed rapidly. The pK_a of the SH group of V ($4.65_{15}°$) was very much smaller than that of cysteine. In the reactions between the thiamine derivatives of the disulfide type and cysteine, the reverse reactions were not observed below neutral pH.[4] Reverse reactions were scarcely observed above pH 8.5 by thin-layer chromatogram. Comparing the rate constants of TDS with that of III, the k_1 was only about 10 times greater in the former reaction than the later and pK_a of thiamine was like to phenylmercaptan. Presuming from the correlation of pK_a and rate constant above mentioned, the reaction between the thiamine derivatives of disulfides type and cysteine were also expected to be reversible, but not observed at all experimentally.[1,5-7] Therefore it may be concluded that in the case of TDS-cysteine system the factors effecting on the reactivity were not only the pK_a of thiols and leaving groups of disulfide but other factor such as ring-opening reaction, for example, as reported by Nogami, *et al.*,[18] thioanion of thiamine in aqueous solution readily turned to a more stable thiazoliumtype below the neutral pH as shown in Eq. (21).



(21)

And this will be reported in the next paper in details.

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17) N. Kharasch and A.J. Parker, *J. Am. Chem. Soc.*, **82**, 307 (1966).
18) The 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, Apr. 1968.