CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 19, No. 12 December 1971

Regular Articles

[Chem. Pharm. Bull. 19(12)2433—2441(1971)]

UDC 547.269.4-26.04 : 547.466.8.04 : 615.35.011.5

Thiamine Derivatives of Disulfide Type. XII.[1] Kinetic Studies on the Reaction between Propyl Propane-Thiolsulfinate or -Thiolsulfonate and Cysteine[2]

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(Received October 31, 1970)

The kinetic studies were conducted on the reaction between propyl propane-thiolsulfinate (PrSSOPr) or -thiolsulfonate ($PrSSO_2Pr$) and 1-cysteine. The reactions were shown by the following equations.

$$PrSSOPr + CySH \longrightarrow CySSPr + (PrSOH)$$
$$2(PrSOH) \longrightarrow PrSSOPr + H_2O \text{ and}$$
$$PrSSO_2Pr + CySH \longrightarrow CySSPr + PrSO_2H$$

The studies were carried out at an acidic pH because of the very fast rates of these reactions. The reaction species of cysteine was proved to be the protonated S-anion, $RN^+H_3 \cdot S^-$.

The values of the enthalpy of activation and the entropy of activation were calculated to be 8.4 kcal/mole, −15.1 *e.u.* for PrSSOPr and 2.9 kcal/mole, −19.2 *e.u.* for $PrSSO_2Pr$.

In the preceding paper,[1] the oxidation pathway of dipropyl disulfide (PrSSPr) and the properties of some oxidation products were studied. Among the oxidation products, thiolsulfinate (PrSSOPr) and thiolsulfonate ($PrSSO_2Pr$) were reactive with thiamine and thiamine propyl disulfide (TPD) was formed. The formation of thiamine derivatives was found to be rather complicated and to relate with opening and closing of thiazolium ring in thiamine molecule since the reactive species was estimated to be thiol-type thiamine.

The purpose of the present study is to investigate the reactivity of PrSSOPr and $PrSSO_2Pr$ with cysteine since the reaction is more simple than that with thiamine.

The product for the reaction between PrSSOPr and cysteine was reported to be S-propylmercaptocysteine (CySSPr).[4] The review articles on the reactivity of disulfide bond were published[5] and Nogami and his co-workers[6] conducted the kinetic study on several thiamine

1) Part XI: *Chem. Pharm. Bull.* (Tokyo), **19**, 2472 (1971).
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4) S. Yurugi, *Yakugaku Zasshi*, **74**, 511 (1954).
5) A.J. Parker and N. Kharasch, *Chem. Rev.*, **59**, 615 (1959); W.A. Pryor, "Mechanism of Sulfur Reactions," McGraw Hill, 1962, p. 59.
6) *a*) H. Nogami, J. Hasegawa, and N. Ikari, *Chem. Pharm. Bull.* (Tokyo), **15**, 685, 693 (1967); *b*) H. Nogami, J. Hasegawa, and K. Okazaki, *ibid.*, **17**, 1732 (1969); *c*) H. Nogami, J. Hasegawa, T. Suzuki, and K. Hirata, *ibid.*, **16**, 1273 (1968).

derivatives of disulfide type with cysteine. The kinetic studies on -SSO- and $-SSO_2-$ have not been conducted and the study was of interest in the comparison of reactivities of -SS-, -SSO- and $-SSO_2-$.

For the reaction of thiolsulfonate and thiol, the following route had been presented by Otto and Roessing (1887)[7] and Smiles and Gibson (1924),[8] but no other study has been reported.

$$RSSO_2R' + R''S^- \longrightarrow RSSR'' + R'SO_2^-$$

On the other hand, for the reaction of thiolsulfinate, Cavallito, *et al.*[9] had investigated on allicin, related with allithiamine, and suggested that the main reaction was

$$AlSSOAl + 2CySH \longrightarrow 2CySSAl + H_2O$$

where AlSSOAl (Al=$-CH_2CH=CH_2$) is allicin and CySH is cysteine and the side reaction was

$$AlSSOAl + CySH \longrightarrow AlSSOCy + AlSH$$

Utsumi, *et al.*[10] have presented the next scheme for the reaction between PrSSOPr and protein-SH

$$PrSSOPr + GSH \longrightarrow PrSSG + PrSOH$$

where GSH is protein SH and PrSOH is the sulfenic acid. Although the sulfenic acid was the compound suggested by Stoll and Seebeck[11] for the biosynthetic course of allicin but its further destiny was not explained.

One of reason why the reaction mechanism was not clear might be that -SSO- or $-SSO_2-$ compound was not separated in pure form by these investigators. It was possible to separate PrSSOPr and $PrSSO_2Pr$ in pure forms and the object of the present paper was to conduct kinetic study of the reaction between cysteine and PrSSOPr or $PrSSO_2Pr$, as mentioned previously.

Experimental

Materials——$PrSSO_2Pr$, PrSSOPr, PrSSPr and Sodium Propylsulfinate ($PrSO_2Na$): Prepared according to the procedure described in the preceding paper.[1]

1-Cysteine: Wako Pure Chemical Ind., Ltd. 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB): Tokyo Kasei Co. CySSPr: Prepared according to Yurugi.[4] All other chemicals were reagent grade.

Thin-Layer Chromatography——Samples were developed on silica gel (Wakogel B-5, Wako Pure Chemical Ind., Ltd.) plates of 250 μ thickness, using BuOH: AcOH: H_2O (8: 1: 1, v/v) solvent system. Cysteine and CySSPr were detected ninhydrin reagent. A little amount of cystine was produced from cysteine during the procedure of development. $PrSSO_2Pr$, PrSSOPr, PrSSPr and $PrSO_2H$ developed were detected by the method reported in the preceding paper.[1] Ether extract was spotted, if necessary.

Examination of Possible Related Reactions——Reactions were usually carried out in pH 4 or 5 acetate buffer (see Table I) containing 20—40% EtOH under nitrogen atomosphere. $CySSPr + PrSO_2H \xrightarrow[\text{(undetect.)}]{} PrSSO_2Pr + CySH$; pH 4 solution containing CySSPr (5×10^{-4}M) and $PrSO_2H$ ($PrSO_2Na$ was used, approximately 5×10^{-4}M) were stored at 37° for 30 minutes. $PrSSO_2Pr$ was not evident on the chromatogram of this solution. $2CySSPr + H_2O \xrightarrow[\text{(undetect.)}]{} PrSSOPr + 2CySH$; pH 4 solution containing CySSPr (5×10^{-4}M) was stored at 37° for 2 hours. PrSSPr was not detected on the chromatogram of this solution. PrSSPr

7) R. Otto and A. Roessing, *Chim. Ber.*, **20**, 2079, (1887).
8) S. Smiles and D.T. Gibson, *J. Chem. Soc.*, **125**, 176 (1924).
9) C.J. Cavallito, J.S. Buck, and C.M. Suter, *J. Am. Chem. Soc.*, **66**, 1952 (1944).
10) I. Utsumi, K. Harada, and K. Kōno, *Vitamin*, **27**, 294 (1963).
11) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **32**, 197 (1949).

$+CySH \xrightarrow[(slow)]{} CySSPr+PrSH$; pH 5 and pH 7 (0.04M phosphate buffer) solution containing 2.5×10^{-3}M PrSSPr, 5×10^{-3}M cysteine and 40% EtOH was stored at 37° for 2 hours. CySSPr was produced in small amounts in pH 5, although a considerable amount was formed in pH 7, accompanied by mercaptan odor.

Determination of Cysteine——The concentration of cysteine was determined according to Ellman[12] after the extraction of $PrSSO_2Pr$ (or PrSSOPr) from samples with ether to stop the reaction. An example of determination was as follows: A 5 ml of sample solution was mixed with a 10 ml of ether, shaked immediately and the ether was removed. The extraction procedure was repeated with a 5 ml of ether (the perfect removal of PrSSOPr by this procedure was ascertained spectrophotometrically). Then, a 0.5 ml portion of aqueous layer was mixed with a 10 ml of phosphate buffer (pH 8, $\mu=0.1$) and a 0.1 ml of DTNB reagent (1×10^{-2}M DTNB, pH 7, $\mu=0.1$ phosphate buffer solution). The absorbance at 412 mμ was measured immediately using a spectrophotometer, Hitachi Perkin Elmer model 139.

Determination of PrSSOPr——Absorbance at 240 mμ, λ_{max} characteristic for SO as described in the preceding paper,[1] was used. It was, however, corrected by the following equation, because the reaction product CySSPr also had rather strong absorbance. $C_{prssopr}=(7.271\ A_{app.}-2.510\ A_o)\times10^{-4}$ where $C_{prssopr}$ is molar concentration of PrSSOPr, $A_{app.}$ absorbance observed at time t, and A_o absorbance at $t=0$. The equation was obtained from that the absorbances of 2×10^{-4}M PrSSOPr and 4×10^{-4}M CySSPr in pH 4.0 were 0.420 and 0.145, respectively.

Table I. The Buffer Solutions Used ($\times10^{-3}$M)

pH[a]	CH_3COONa	CH_3COOH	HCl
5.0	18	7.5	
4.0	18	75	
3.3	18		17.7
2.7	18		20
2.1	18		26.5
1.7	18		42
1.4			48
1.1			100

a) in the presence of 4% of EtOH

Kinetic Run——The cysteine solution (usually 4×10^{-3}M aqueous solution freshly prepared was used), kept in a constant temperature water bath (±0.1°), was mixed to a pre-incubated buffer solution containing $PrSSO_2Pr$ or PrSSOPr ($1—5\times10^{-2}$M EtOH stock solution was used). The buffer solutions used are given in Table I. The buffer solutions were prepared using deionized and nitrogen-bubbled water. All the reaction solutions were made $\mu=0.1$ by addition of NaCl and 4% in EtOH concentration. The air in the flask was replaced by nitrogen, but when the reaction was fast, the replacement was not continued. Samples were withdrawn at different time intervals and the concentration of cysteine or PrSSOPr was determined. $PrSSO_2Pr$ and PrSSOPr were stable on chromatograms in these acidic conditions (pH 1—5, 37°, 2 hours).

Result and Discussion

Qualitative Studies by Thin-Layer Chromatography

The formation of TPD from thiamine and $PrSSO_2Pr$ or PrSSOPr was found in alkaline region.[13] However, the reaction between cysteine and $PrSSO_2Pr$ or PrSSOPr proceeded very rapidly at pH 8 and immediately finished. The experiment was, therefore, conducted in acidic pH.

The chromatograms of the reaction solutions at different molar ratio of $PrSSO_2Pr$ against cysteine are shown in Fig. 1. The formation of CySSPr at pH 4 and 37° has completed within 30 minutes. The stoichiometry of the reaction was assumed to be $PrSSO_2Pr$ and cysteine in 1:1 from the amounts of each reactant remained on the chromatogram. The $PrSO_2H$ formed

12) G.L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).
13) T. Matsukawa, S. Yurugi, H. Kawasaki, Y. Aramaki, and Z. Suzuoki, *Takeda Kenkyusho Nenpo*, **12**, 1 (1952).

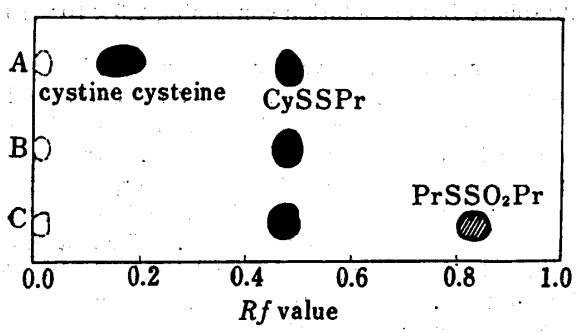

Fig. 1. Thin-Layer Chromatograms Illustrating the Reaction between Cysteine (5×10^{-3}M) and $PrSSO_2Pr$ ((A): 2.5×10^{-3}M, (B): 5×10^{-3}M, (C): 10×10^{-3}M) after 30 Minutes at pH 4 and 37°

plate: silica gel
developing solution: BuOH: AcOH: H_2O (8:1:1)

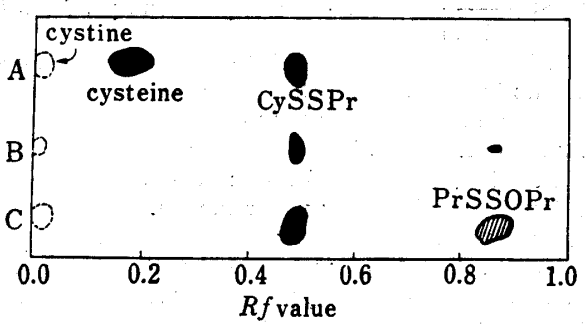

Fig. 2. Thin-Layer Chromatograms Illustrating the Reaction between Cysteine (5×10^{-3}M) and PrSSOPr ((A): 1.25×10^{-3}M, (B): 2.5×10^{-3}M, (C): 5×10^{-3}M) after 2 Hours at pH 5 and 37°

plate: silica gel
developing solution: BuOH: AcOH: H_2O (8:1:1)

was detected on the chromatogram.[1] Thus, the reaction can be described by the same equation as Otto, *et al.*[7] and Smiles, *et al.*[8] did. (Eq. 1). The reverse reaction was not observed on the chromatogram.

$$PrSSO_2Pr + CySH \longrightarrow CySSPr + PrSO_2H \quad (1)$$

The chromatograms of the reaction solutions at different molar ratio of PrSSOPr against cysteine are shown in Fig. 2. The reaction was slower than that of $PrSSO_2Pr$ and the molar ratio for the reaction was assumed from the remained amounts of each reactants on the chromatogram to be cysteine and PrSSOPr 2:1. The stoichiometry was also confirmed by the determination of both of cysteine and PrSSOPr in the reaction solution as described later (Fig. 4 and Fig. 5). Therefore, the reaction can be written as Eq. 2).

$$PrSSOPr + 2CySH \longrightarrow 2CySSPr + H_2O \quad (2)$$

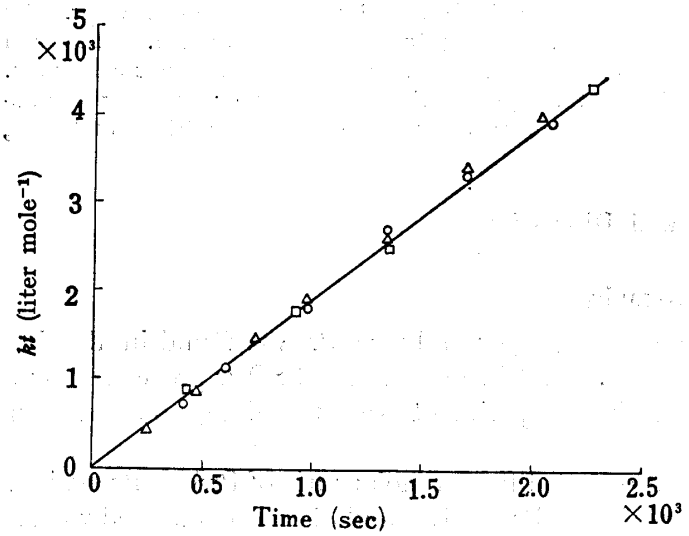

Fig. 3. Typical Second Order Rate Equation Plots for the Reaction between $PrSSO_2Pr$ and Cysteine at pH 2.1 and 37°

○: $PrSSO_2Pr$ 2×10^{-4}M, Cysteine 2×10^{-4}M
△: $PrSSO_2Pr$ 4×10^{-4}M, Cysteine 2×10^{-4}M
□: $PrSSO_2Pr$ 2×10^{-4}M, Cysteine 4×10^{-4}M

The reverse reaction was not proved on the thin-layer chromatogram. The reaction above mentioned is essentially the same as the main reaction suggested by Cavallito, *et al.*,[9] although they did not ascertaine the stoichiometry.

Reaction Rate

The time course of the reaction between $PrSSO_2Pr$ and cysteine determined by the decrease of cysteine was a typical second order as shown in Fig. 3.

The reaction rate, k, was obtained by the following calculation.

$$\underset{(a-x)}{PrSSO_2Pr} + \underset{(b-x)}{CySH} \xrightarrow{k} \underset{x}{CySSPr} + PrSO_2H$$

From $dx/dt = k(a-x)(b-x)$

$$kt = \frac{1}{(b-a)} \ln \frac{a(b-x)}{b(a-x)} \quad (a \neq b)$$

$$\text{or } kt = \frac{x}{a(a-x)} \quad (a=b)$$

In the case of the reaction between PrSSOPr and cysteine, the second order reaction plot was also obtained as shown in Fig. 4. The time course of the reaction was investigated from not only the decrease of cysteine, but also that of PrSSOPr, because the reaction was expected to be more complicated than that of $PrSSO_2Pr$. Fig. 5 represents

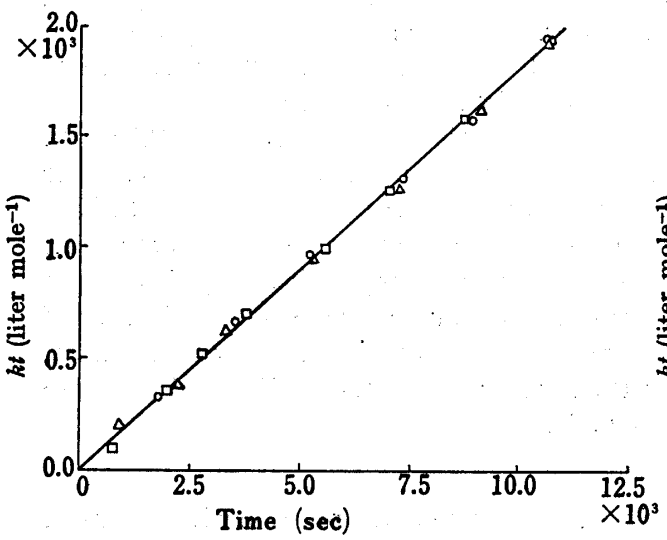

Fig. 4. Typical Second Order Rate Equation Plots for the Reaction between PrSSOPr and Cysteine at pH 4.0 and 37° (Measured from the Decrease of Cysteine)

○: PrSSOPr 2×10^{-4}M, Cysteine 4×10^{-4}M
△: PrSSOPr 4×10^{-4}M, Cysteine 4×10^{-4}M
□: PrSSOPr 4×10^{-4}M, Cysteine 8×10^{-4}M

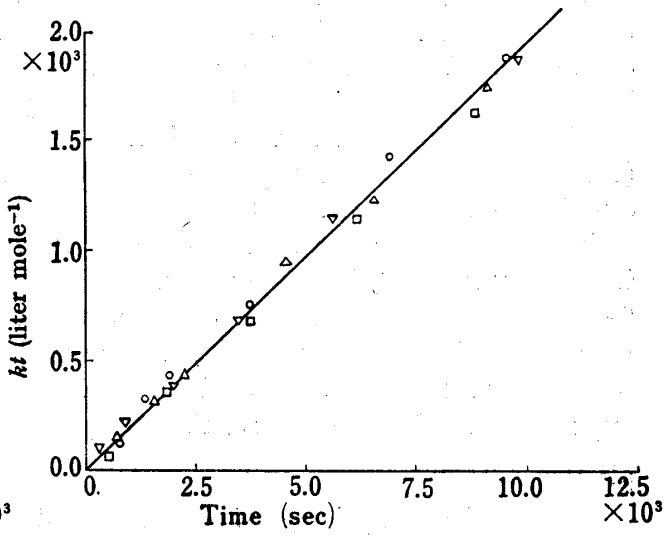

Fig. 5. Typical Second Order Rate Equation Plots for the Reaction between PrSSOPr and Cysteine at pH 4.0 and 37° (Measured from the Decrease of PrSSOPr)

○: PrSSOPr 2×10^{-4}M, Cysteine 1×10^{-4}M
△: PrSSOPr 2×10^{-4}M, Cysteine 4×10^{-4}M
□: PrSSOPr 2×10^{-4}M, Cysteine 9×10^{-4}M
▽: PrSSOPr 4×10^{-4}M, Cysteine 4×10^{-4}M

the time course obtained from the decrease of PrSSOPr. The results were in good agreement with those of Fig. 4. From these results, the reaction is suggested to proceed as follows

$$\text{PrSSOPr} + \text{CySH} \xrightarrow{k} \text{CySSPr} + (\text{PrSOH}) \qquad (3)$$

$$2(\text{PrSOH}) \xrightarrow{\text{fast}} \text{PrSSOPr} + H_2O \qquad (4)$$

This model reveals that the active species is PrSSOPr itself and PrSOH is intermediate. Such a view as sulfenic acid is intermediate to thiol sulfinate has been presented in the biosynthesis of allicin from allin[11] and the formation of S-monooxide of di-*tert*-butyl disulfide.[14] The explanation may be also supported by the fact that the reaction of $PrSSO_2Pr$ gives $PrSO_2H$.

The rate constant for this reaction was calculated as follows. When the concentration of CySSPr at time t is expressed as x, those of PrSSOPr and cysteine become $(a-x/2)$ and $(b-x)$, respectively. The rate, dx/dt, can be written by $dx/dt=k\ (a-x/2)\ (b-x)$. The integration gives

14) T. Colclough and J.I. Cunneen, *Chem. Ind.* (London), **1960**, 626.
$Me_3CSOCMe_3 \rightarrow Me_3CSOH + Me_2C{=}CH_2$ $2Me_3CSOH \rightarrow Me_3CSSOCMe_3 + H_2O$

$$kt = \frac{2}{(b-2a)} \ln \frac{2a(b-x)}{b(2a-x)} \quad (\text{at } b \neq 2a)$$

$$\text{or } kt = \frac{2x}{b(b-x)} \quad (\text{at } b=2a).$$

The reaction between the reaction product CySSPr and cysteine also observed as in the case of the reaction between TPD and cysteine, but it has been concluded that the side reaction could be ignored as far as the main reaction (TPD and cysteine) is proceeding.[6] When the reaction between PrSSOPr and cysteine is compared with that between TPD and cysteine, the rate of the former is larger than that of the latter. (The values of k_{obs} at pH 4.0 and 30° are 6.7×10^{-2} liter mole^{-1} sec^{-1} for TPD and 1.8×10^{-1} liter mole^{-1} sec^{-1} for PrSSOPr.) This means that the side reaction could safely be ignored in this experiment.

The comparison of Fig. 3 and Fig. 4 shows that $PrSSO_2Pr$ is more reactive than PrSSOPr. (The difference of reactivity is estimated to be about 10^3 times from the rate constants for cysteine S-anion as will be described later; The magnitude of the rate constants at 37° are estimated to be 10^6 liter mole^{-1} sec^{-1} for $PrSSO_2Pr$ and 10^3 liter mole^{-1} sec^{-1} for PrSSOPr.) On the other hand, it was found from the thin-layer chromatographic investigation in pH 5—7 that CySSPr was slower formed in the reaction of PrSSPr and cysteine than in that of PrSSOPr and cysteine, that is, PrSSPr was less reactive than PrSSOPr. It was, therefore, confirmed that the sequence of the reactivity for thiol was -SS-<-SSO-<-SSO_2- and the more oxygens in disulfide bond are, the larger reactivity is.

pH Dependency

The study in physiological pH was desirable in order to relate to the reaction with thiamine. But it was conducted in low pH, because the reaction between $PrSSO_2Pr$ and cysteine proceeded faster in neutral pH. As seen in Fig. 6, a linear relationship between log k_{obs} and pH with a slope of approximately 1 was observed. (The slight difference from the straight line may be caused by the effect of the dissociation of carboxyl group of cysteine, which pKa value is reported to be 1.86[15])

Although the reaction rate of PrSSOPr was not so fast as that with $PrSSO_2Pr$, it was also difficult to examine in neutral pH, thus it was studied in pH 3 to 5, as shown in Fig. 7.

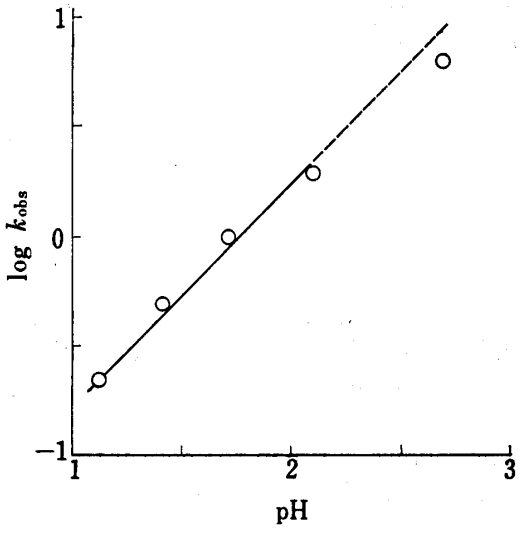

Fig. 6. The pH Profile for the Reaction between $PrSSO_2Pr$ and Cysteine at 37°

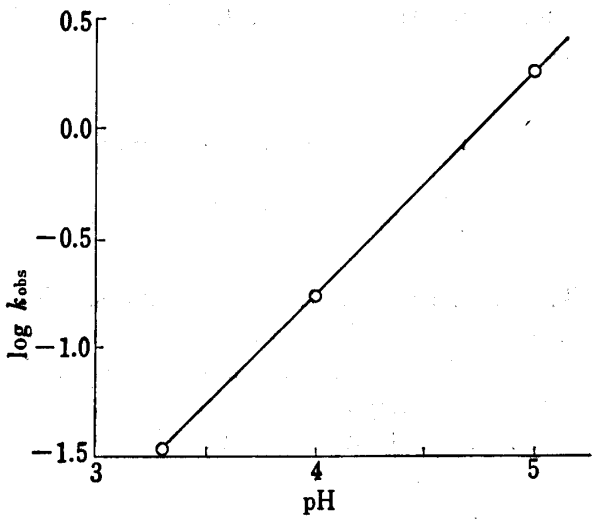

Fig. 7. The pH Profile for the Reaction of PrSSOPr and Cysteine at 37°

15) R.K. Cannan and B.C.J.G. Knight, *Biochem. J.*, **21**, 1384 (1927).

A linear relationship with a slop of 1 was observed, which was similar to the reaction between disulfide and thiol.[6]

Benesch and Benesch[16] reported that the dissociation of cysteine was shown as Chart 1 and presented Eq. 5).

$$\frac{[RN^+H_3\cdot S^-]+[RNH_2\cdot S^-]}{[CySH]_{Total}} = \frac{K_A/K_B+K_D/[H^+]}{[H^+]/K_B+K_A/K_B+K_D/[H^+]+1} \quad (5)$$

$$\begin{array}{ccc} HSRN^+H_3 & \overset{pK_A=8.53}{\rightleftharpoons} & {}^-SRN^+H_3 \\ pK_B=8.86 \uparrow\downarrow & & \uparrow\downarrow pK_C=10.36 \\ HSRNH_2 & \underset{pK_D=10.03}{\rightleftharpoons} & {}^-SRNH_2 \end{array}$$

Chart 1. Ionic Dissociation of Cysteine at 23.0°

The contribution of $RNH_2\cdot S^-$ to the reaction was neglected since the concentration of $RNH_2\cdot S^-$ is negligible small in the pH range of present study, and the reaction rate constant of $RNH_2\cdot S^-$ might be nearly the same magnitude as that of $RN^+H_3\cdot S^-$ from the result reported in the paper of this series.[6a] From these two reasons, Eq. 5) may be simplified to Eq. 6).

$$\frac{[RN^+H_3\cdot S^-]}{[CySH]_{Total}} = \frac{K_A/K_B}{[H^+]/K_B+K_A/K_B+1} \quad (6)$$

Eq. 6) may be simplified further to Eq. 7) since $[H^+]/K_B \gg K_A/K_B > 1$ holds for the pH range of present study.

$$\frac{[RN^+H_3\cdot S^-]}{[CySH]_{Total}} = K_A/[H^+] \quad (7)$$

Therefore, the relationship between apparent rate constant, k_{obs}, and the specific rate constant, k, concerned with $RN^+H_3\cdot S^-$, is shown as Eq. 8).

$$k_{obs} = k\cdot K_A/[H^+],\ \log k_{obs} = \log k\cdot K_A + pH \quad (8)$$

The pH-rate profiles given in Figs. 6 and 7 are explained by the relationship of Eq. 8), where cysteine S-anion is assumed to be the reactive species. The effects of buffer concentration and ionic strength were negligible, but the higher concentration of ethanol was, the smaller k value was observed. (Table II)

Table II. The Effects of Buffer Concentration, Ionic Strength and Ethanol Concentration on the Reaction between $PrSSO_2Pr$ or PrSSOPr and Cysteine S-anion ($RN^+H_3\ S^-$) at 37°

Substrate	Buffer Conc.[a]	μ	Ethanol (%)	pH	k (liter mole^{-1} sec^{-1})
$PrSSO_2Pr$	a	0.1	4	1.40	3.8×10^6
	a	0.3	4	1.36	4.3×10^6
	a	0.1	20	1.41	2.7×10^6
	b	0.2	4	1.70	4.0×10^6
	3b	0.2	4	1.26	3.7×10^6
PeSSOPr	c	0.1	4	4.00	3.6×10^3
	3c	0.1	4	4.00	3.4×10^3
	c	0.3	4	3.95	3.4×10^3
	c	0.1	30	4.45	1.4×10^3

a) a: HCl=0.048M
b: CH_3COONa=0.018M, HCl=0.042M
c: CH_3COONa=0.018M, CH_3COOH=0.075M

Temperature Dependency

The Arrhenius plots of the specific rate constant calculated by Eq. 8) for the reaction between $PrSSO_2Pr$ or PrSSOPr and $RN^+H_3 \cdot S^-$ are presented in Figs. 8 (pH 1.4) and 9 (pH 4.0), respectively. The pK_a value of cysteine was corrected by 7 kcal/mole for the dissociation of thiol.[16] The values of thermodynamic parameters for each reaction were calculated as given in Table III.

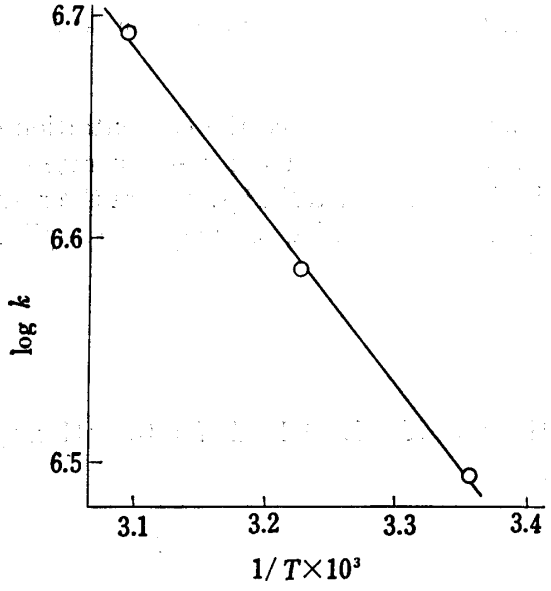

Fig. 8. Arrhenius Plots for the Reaction of $PrSSO_2Pr$ and Cysteine S-Anion ($RN^+H_3 \cdot S^-$) at pH 1.4

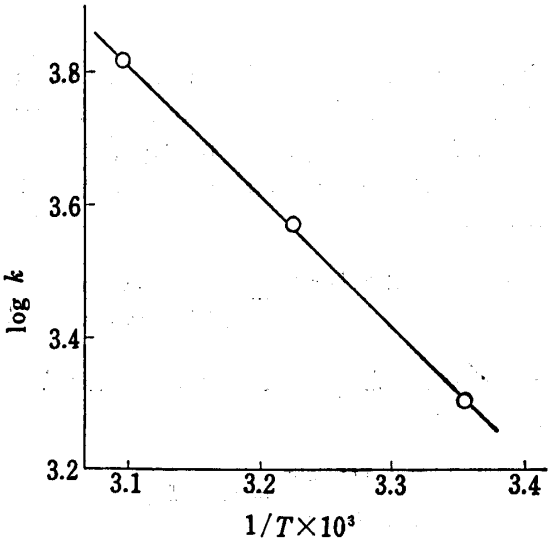

Fig. 9. Arrhenius Plots for the Reaction of PrSSOPr and Cysteine S-Anion ($RN^+H_3 \cdot S^-$) at pH 4.0

Table III. Thermodynamic Parameters for the Reaction between $PrSSO_2Pr$ or PrSSOPr and Cysteine S-Anion ($RN^+ H_3 \cdot S^-$)

Substrate	$\Delta H^{\neq}$ kcal/mole	$\Delta S^{\neq}$ *e.u.*	$\Delta F^{\neq}$ kcal/mole
$PrSSO_2Pr$[a]	2.9	−19.2	8.9
PrSSOPr[b]	8.4	−15.1	13.1
B_1SSPr[c]	11.7	−11.1	14.9
B_1SSB_1[d]	11.4	−3.7	12.5

a) measured at pH 1.4, 37°
b) measured at pH 4.0, 37°
c) B_1: protonated form of thiamine, 15°[6a]
d) B_1: protonated form of thiamine, 15°[6c]

It is noted that the value of the enthalpy of activation, $\Delta H^{\neq}$, for the reaction with $PrSSO_2Pr$ is extremely small. The magnitude of the entropy of activation, $\Delta S^{\neq}$, may be considered to be an usual one as shown in the bimolecular reaction between disulfide and sulfite.[17] The difference of the free energy of activation, $\Delta F^{\neq}$, between SSO and SSO_2 was obtained to be 4.2 kcal/mole.

The values of $\Delta H^{\neq}$, $\Delta S^{\neq}$ and $\Delta F^{\neq}$ of the reaction between cysteine S-anion ($RN^+H_3 \cdot S^-$) and TPD (B_1SSPr)[6a] or thiamine disulfide (B_1SSB_1)[6c] are also shown in Table III (B_1 shows

16) R.E. Benesch and R. Benesch, *J. Am. Chem. Soc.*, **77**, 5877 (1955).
17) R. Cecil and J.R. McPhee, *Biochem. J.*, **60**, 496 (1955).

the protonated form of thiamine). It was suggested from these data that $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ decrease with the increase of oxygen atoms in SS bond as SS>SSO>SSO_2.

The possible mechanisms of the reaction between $RN^{+}H_3 \cdot S^{-}$ and $PrSSO_2Pr$ (or PrSSOPr) are presented in Chart 2.

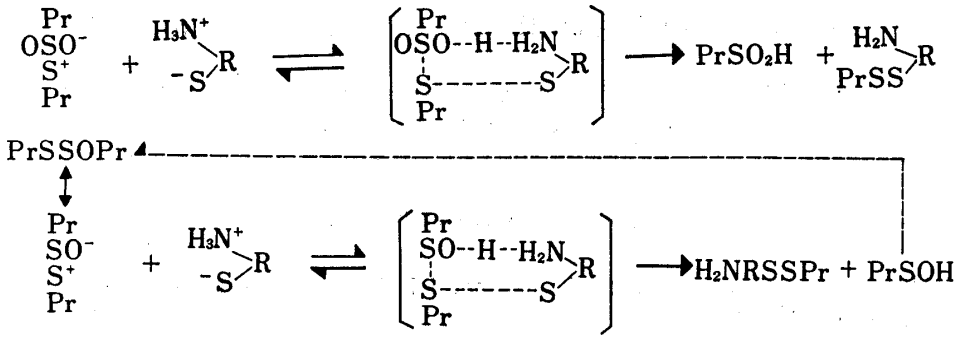

Chart 2. Proposed Mechanisms for the Reaction of $PrSSO_2Pr$ or PrSSOPr and Cysteine S-Anion

Acknowledgement The authors are grateful to Mr. J. Manaka, Director of Technological Research Laboratories of Takeda Chemical Industries, Ltd, for permitting one of us (K. A.) to participate in this series of study and to Mr. M. Murakami and Dr. H. Mima for kind encouragement.