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Thiamine Derivatives of Disulfide Type. XIII.[1] Kinetic Studies on the Degradation of Propyl Propanethiolsulfonate by Hydroxyl Ion[2]

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Alkaline degradation of propyl propanethiolsulfonate ($PrSSO_2Pr$) was investigated to study the reaction mechanism for the synthesis of thiamine derivatives of disulfide type.

It was postulated that $PrSSO_2Pr$ degrades to thiolsulfinate (PrSSOPr) and sulfinic acid ($PrSO_2H$) eventually as shown in the following equations.

$$PrSSO_2Pr + OH^- \longrightarrow PrSO_2^- + (PrSOH)$$

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

The values of the enthalpy of activation and the entropy of activation were calculated to be 8.2 kcal/mole and −21.7 e.u.

PrSSOPr underwent alkaline degradation as $PrSSO_2Pr$ did, but the rate was rather smaller than that of $PrSSO_2Pr$.

In order to study the mechanism of the production of thiamine propyl disulfide (TPD) from the reaction between thiamine and propyl propane-thiol sulfinate (PrSSOPr) or -thiol sulfonate ($PrSSO_2Pr$), the properties of PrSSOPr and $PrSSO_2Pr$[4] and these reactivities with thiol had been studied in the preceding paper.[1]

During the course of the investigation, it was found that $PrSSO_2Pr$ converted into PrSSOPr and sulfinic acid ($PrSO_2H$) in alkaline solution.[4] Although thiamine alkyl disulfide is produced from alkyl alkanethiol sulfonate and thiamine, the previous report[5] did not mention this breakdown reaction. It was estimated that PrSSOPr, produced by alkaline degradation from $PrSSO_2Pr$, reacts further with thiamine and undergoes also alkaline degradation but the rate is rather smaller than that of $PrSSO_2Pr$. Therefore, it was concluded that the alkaline degradation of $PrSSO_2Pr$ should be investigated to clarify the reaction of TPD production.

Although a number of reports for the hydrolysis of disulfide were published[6] in the relation with SS bond of protein, the reports on the S-oxides of disulfide were few. The sulfenic acid was an intermediate which suggested[7] ionic scission by hydroxyl ion, but the various final products of sulfenic acid were reported such as sulfinic acid and thiol,[6a,6b,8] sulfonic acid and disulfide[6c] or thiol only.[6d]

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1) Part XII: H. Nogami, J. Hasegawa and K. Aoki, *Chem. Pharm. Bull.* (Tokyo), **19**, 2433 (1971).

2) Presented to the Meeting of Kanto Branch Pharmaceutical Society of Japan, Tokyo, February 1967.

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4) H. Nogami, J. Hasegawa, and K. Aoki, *Chem. Pharm. Bull.* (Tokyo), **19**, 2472 (1971)

5) T. Matsukawa and H. Kawasaki, *Yakugaku Zasshi*, **73**, 216 (1953).

6) *a)* T.T. Lavine, *J. Biol. Chem.*, **117**, 309 (1937); *b)* H. Hirai, T. Nakao and M. Shimao, *Seikagaku*, **29**, 446 (1957); *c)* A.J. Parker and N. Kharasch, *J. Am. Chem. Soc.*, **82**, 3071 (1960); *d)* M. Senda, K. Mukoyama and K. Nakatsuji, The Annual Meeting of Polarography of Japan (1964).

7) O. Foss, "Organic Sulfur Compounds," Vol. 1, ed. by N. Kharasch, Pergamon Press, New York, N.Y., 1961, p. 83.

8) N. Kharasch and T.C. Bruice, *J. Am. Chem. Soc.*, **73**, 3240 (1950).

The fate of sulfenic acid was noted, since the alkaline decomposition of $-SSO_2-$ type was reported by Leandri and Tundo[9] that sulfenic acid was the product by the reaction $RSSO_2R+OH^- \rightarrow RSOH+RSO_2^-$, but the fate of sulfenic acid was not given.

Savige, *et al.*[10] have reported that sulfenic acid converts to SS monooxide in the hydrolysis of cystine SS dioxide as follows.

$$CySSO_2Cy + OH^- \longrightarrow CySO_2^- + (CySOH)$$
$$2(CySOH) \longrightarrow CySSOCy + H_2O$$

The alkaline degradation of $PrSSO_2Pr$ is reported in the present paper for the basic research on the mechanism of the reaction between thiamine and $PrSSO_2Pr$ or PrSSOPr. The study is also interesting in the view of the nucleophilic reaction for disulfide bond.

Experimental

Materials——$PrSSO_2Pr$, PrSSOPr and sodium propylsulfinate ($PrSO_2Na$): Prepared as reported in the preceding paper.[4]

Other chemicals and solvents were reagent grade.

Thin-Layer Chromatography——Silica gel (Wako gel B-5, Wako Pure Chemical Ind., Ltd.) plate, developing solutions and detection reagents used were all the same to the preceding paper.[4]

Kinetic Run——$PrSSO_2Pr$ in ethanolic stock solution was added to a pre-incubated phosphate buffer solution in a constant temperature water bath adjusted at specified temperature ±0.2°. The final reaction solutions were made pH 8.05, 0.06M in phosphate ion, $\mu=0.18$ with NaCl and 2% in EtOH concentration. At different time intervals, samples were withdrawn and poured into one-tenth volume of 1N HCl to stop the reaction.

Assay——Degradation rate was calculated by the increase of absorption at 240 mμ (λ_{max} of PrSSOPr), since $PrSSO_2Pr$ has no characteristic ultraviolet(UV) spectrum.[4] However, $PrSO_2^-$ has some absorption at this wave length. The absolute absorption of $PrSO_2Na$ could not be measured because of its strong hydroscopicity. The absorption was measured after the extraction of PrSSOPr from the almost completely degraded $PrSSO_2Pr$ solution to be 10% of equivalent PrSSPOr (see Fig 2). *n*-Hexane (Spectrophotosol: Wako Pure chemical Ind., Ltd.) was used for the extraction. Therefore, the following correction was made: $A_{corrected.} = 0.9\ A_{observed}$. Spectrophotometers used were Hitachi–Perkin Elmer model 139 and Hitachi model 124.

Stability of PrSSOPr in alkaline solution——The stability was evaluated from the change of UV spectrum (see Fig 6).

Polarographic investigation——The degradation of $PrSSO_2Pr$ and PrSSOPr was conducted in 0.06 M phosphate buffer (pH 8.05 $\mu=0.18$, EtOH 2%) at 37°. The sample solutions were poured into acetate-HCl buffer to make pH 4 to stop the reaction. The apparatus made by Yanagimoto Seisakusho, TA-1, was used.

Result and Discussion

Thin-Layer Chromatographic and Spectrophotometric Investigation

The chromatograms for the degradation of $PrSSO_2Pr$ were illustrated in Fig. 1. The formation of PrSSOPr and $PrSO_2H$ was observed with the decrease of $PrSSO_2Pr$. The degradation products were, therefore, regarded as PrSSOPr and $PrSO_2H$. The degradation was faster in higher pH. Fig. 2 illustrates the spectral change of $PrSSO_2Pr$ (3.8×10^{-4}M, pH 8). where (A) corresponds to the spectrum after 50 hours at 25° and (B) represents that after *n*-hexane extraction of solution (A). The shape of (B) was similar with that of $PrSO_2Na$. When spectrum (B) is substracted from (A), spectrum (C) is generated. (C) has a maximum at 240 mμ and is similar to that of 1.77×10^{-4}M PrSSOPr. On the other hand, PrSSOPr was more stable and the sample kept in the condition of Fig. 2 showed no remarkable change in the chromatogram and UV spectrum (Only *ca.* 5% decrease of the absorbance at 240 mμ was observed). It was, therefore, revealed from these experiments that 1/2M PrSSOPr was produced from 1M $PrSSO_2Pr$ ($PrSSO_2Pr+H_2O \rightarrow 1/2\ PrSSOPr+PrSO_2H$).

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[9] G. Leandri and A. Tundo, *Ann. Chim.* (Rome), **44**, 264, 340 (1954).

[10] W.E. Savege, J. Eagar, J.A. Maclaren and C.M. Roxburgh, *Tetrahedron Letters*, **1964**, 3289.

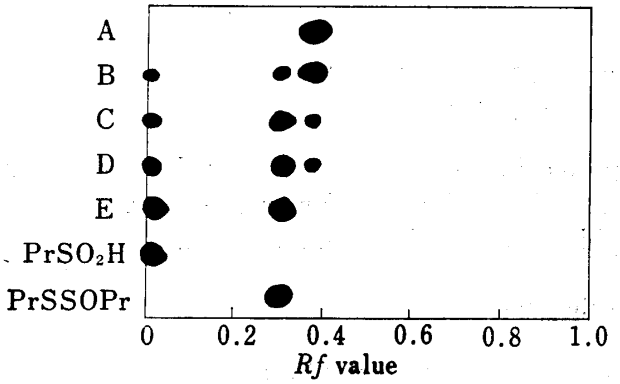

Fig. 1. Thin-Layer Chromatograms Illustrating the Degradation of $PrSSO_2Pr$

A: no heat ($PrSSO_2Pr$)
B: pH 7 after 4 hr at 37°
C: pH 7 after 2 hr at 60°
D: pH 8 after 4 hr at 37°
E: pH 8 after 2 hr at 60°
plate: silica gel
developing solution: heptane: dioxane (9: 1)
detection reagent: $KMnO_4$ or cysteine-brom phenol blue

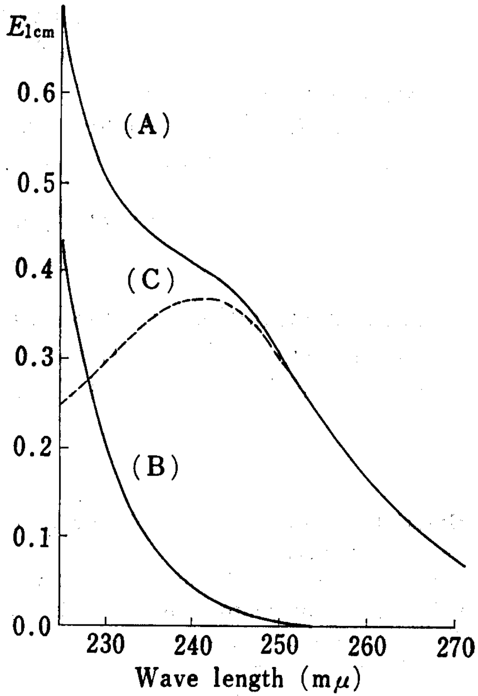

Fig. 2. Spectra of the Solution of 3.8×10^{-4} M $PrSSO_2Pr$ which was Degraded in pH 8 Phosphate Buffer at 25°

(A): spectrum after 50 hr (almost completely degraded)
(B): spectrum of solution (A) extracted by *n*-hexane
(C): spectrum substracted (B) from (A) (equal to 1.77×10^{-4} M PrSSOPr)

The production of 1/2M PrSSOPr can be explained from the fact that PrSSOPr is formed *via* PrSOH as shown by the following equations which is similar to the reaction between PrSSOPr and cysteine,[1]

$$PrSSO_2Pr + H_2O \longrightarrow PrSO_2H + (PrSOH) \tag{1}$$

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

Degradation Rate of $PrSSO_2Pr$ by Hydroxyl Ion

Fig. 3 illustrates a typical degradation curve of $PrSSO_2Pr$, in which the logarithm of $PrSSO_2Pr$ concentration was plotted as a function of time. At constant pH, the rate of the disappearance of $PrSSO_2Pr$ exhibits a first order dependency on $PrSSO_2Pr$ concentration.

Fig. 4 represents pH rate profile obtained from log k_{obs} at 37° in pH 6.4—8, where a straight line with a slope of 1.0 is shown. The nature of the pH profile indicates that reaction with hydroxyl ion expressed by Eq. (3) is responsible for the degradation.

$$PrSSO_2Pr + OH^- \xrightarrow{k} PrSO_2^- + (PrSOH) \tag{3}$$

The relationship between apparent rate constant, k_{obs} and bimolecular rate constant, k, is shown as Eq. (4)

$$k = k_{obs}/[OH^-] \tag{4}$$

The effect of buffer concentration was not evident as shown in Table I.

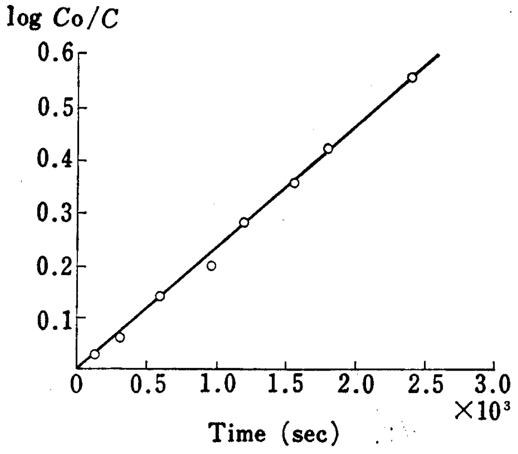

Fig. 3. A Plot Illustrating the First Order Disappearance of $PrSSO_2Pr$ in pH 8 Phosphate Buffer at 37°

Co: initial concentration

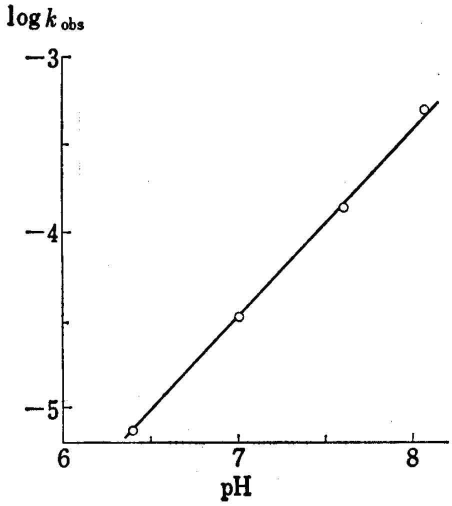

Fig. 4. The pH Profile for the degradation of $PrSSO_2Pr$ in 0.06M Phosphate Buffer ($\mu=0.18$, ethanol 2%) at 37°

TABLE I. The Effect of the Concentration of Phosphate Buffer on the Degradation of $PrSSO_2Pr$ at pH 8, $\mu=0.36$, 37°

Concentration of phosphate buffer	k_{obs} (sec^{-1})
0.12M	5.41×10^{-4}
0.06M	5.20×10^{-4}
0.03M	5.95×10^{-4}

Polarographic Investigation

Cavallito, *et al.*[11] conducted the polarographic investigation on allicin (SS monooxide of diallyl disulfide) and revealed that it easily underwent hydrolytic breakdown even at pH 6.5. Asahi[12] studied on β-lipoic acid (SS monooxide of α-lipoic acid) and indicated that it was more unstable than α-lipoic acid in alkaline medium. As referred to these studies, polarographic investigation was also made for the hydrolysis of $PrSSO_2Pr$.

An example of polarogram for $PrSSO_2Pr$ is presented in Fig. 5.

At the condition of pH 8 and 37°, a compound having E½ of approximately −0.85V increases with the elapse of time. This compound was nothing but PrSSOPr and the increase of the E½ height was equal to that of absorbance at 240 mμ above mentioned.

The degradation rate of $PrSSO_2Pr$ has been also confirmed from polarography. The values of k_{obs} were 5.8×10^{-4} sec^{-1} from polarography and 5.2×10^{-4} sec^{-1} from spectrophotometry, respectively.

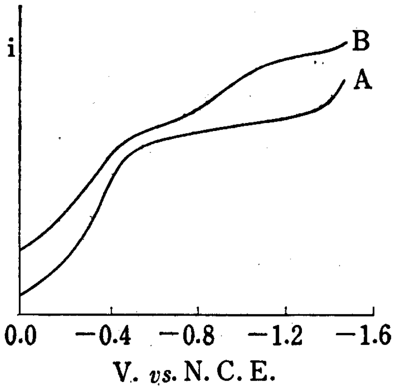

Fig. 5. Polarogram Illustrating the Degradation of $PrSSO_2Pr$ in pH 8 Phosphate Buffer at 37°

A: initial B: after 1 hr

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[11] C.J. Cavallito, J.S. Buck and C.M. Suter, *J. Am. Chem. Soc.*, **66**, 1952 (1944).

[12] Y. Asahi, *Yakugaku Zasshi*, **80**, 684 (1960).

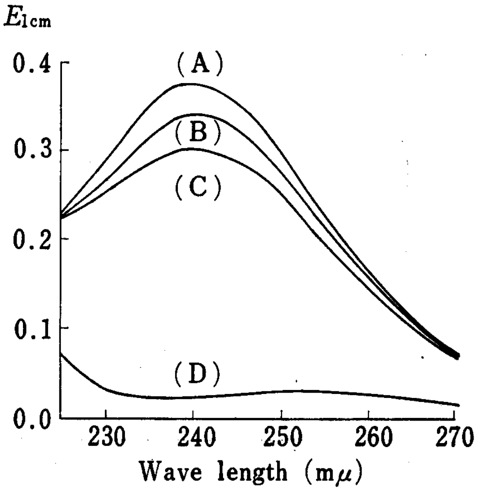

Fig. 6. UV Spectrum Change of 1.8×10^{-4}M PrSSOPr in pH 8 Phosphate Buffer at 50°

(A): no heat (B): after 2 hr
(C): after 5 hr (D): after 150 hr

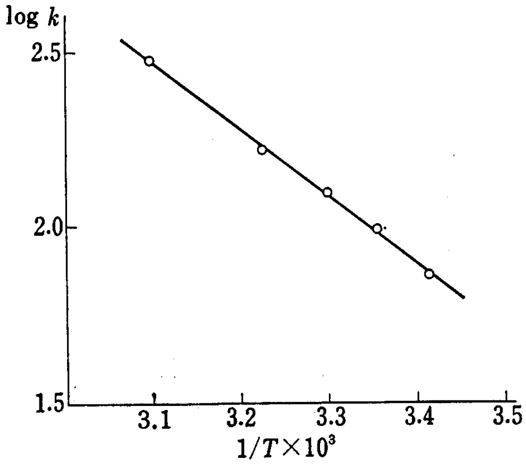

Fig. 7. Arrhenius Plot of the Bimolecular Reaction Rate Constant for the Reaction of $PrSSO_2Pr$ and Hydroxyl Ion

Stability of PrSSOPr in Alkaline Solution

Fig. 6 shows the spectrum change of PrSSOPr at pH 8 and 50°. It is apparent from Fig. 6 that the degradation rate was rather lower than that of $PrSSO_2Pr$. (Under the condition of (B) in Fig. 6, $PrSSO_2Pr$ decomposed more than 95%) From these results, it might be said that the alkaline degradation of $PrSSO_2Pr$ was a sequential reaction shown as $PrSSO_2Pr \rightarrow PrSSOPr \rightarrow$ decomposed products.

The alkaline degradation products of thiolsulfinate have been reported to be disulfide and sulfinic acid by Cavallito, *et al.*[11] (on allicin) and by Savige, *et al.*[10] (on cystine SS monoxide). Although the study for the products of PrSSOPr with their stoichiometry remained as one undissolved problem, it was noted that PrSSOPr was more stable than allicin. The polarogram of allicin showed the complete degradation after 18 hours at pH 6.5 and 30°,[11] while PrSSOPr stored at the same condition was quite stable in the chromatogram and UV spectrum.

Thermodynamic Parameters

Fig. 7 shows the Arrhenius plot of the bimolecular reaction rate constant for the reaction between $PrSSO_2Pr$ and hydroxyl ion. The thermodynamic parameters obtained from Fig. 7 are given in Table II, where those for the reaction between $PrSSO_2Pr$ and cysteine S-anion[1] are compared.

Table II. Thermodynamic Parameters for Nucleophilic Reactions between $PrSSO_2Pr$ and Hydroxyl Ion or Cysteine S-Anion (37°)

Nucleophile	ΔH^{*} kcal/mole	ΔS^{*} e.u.	ΔF^{*} kcal/mole
OH^-	8.2	−21.7	14.9
$Cy(N^+H_3)S^-$ [a]	2.9	−19.2	8.9

[a] measured at pH 1.4[1]

For the reaction with hydroxyl ion, the enthalpy of activation, ΔH^{*}, was rather higher than that for cysteine S-anion and the entropy of activation, ΔS^{*}, was almost the same value as that for cysteine S-anion. The difference of the free energy of activation, ΔF^{*}, between hydroxyl ion and cysteine S-anion was estimated to be approximately 6 kcal/mole.

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