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Kinetics of Reversible Cleavage of Thiazolium Salts[1]

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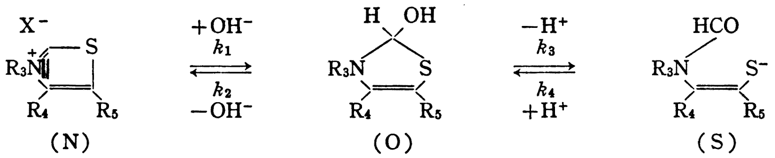
Reversible cleavages of thiamine mononitrate (I), dimethialium mononitrate (II), 3,4-dimethyl-5-(2′-hydroxyethyl)thiazolium iodide (III), thiamic acid (IV) and N-methylbenzothiazolium iodide (V) were automatically followed by anodic waves of the thiol forms. Pseudo-first-order rate constants for the cleavage, (k_N) or the recyclization (k_S), are proportional to (OH^-) or (H^+), respectively. The pH where k_N is equal to k_S, corresponds to pK_{av}: 9.3 (I), 9.4 (II), 10.3 (III), 11.2 (IV) and 6.5 (V). Electron donating groups seem to stabilize the thiazolium rings for OH^- and to decrease the acidities.

It has been well known[3] that thiazolium (N) in thiamine (I) cleaves reversibly to the thiol form (S) *via* the pseudobase (O) in alkaline solution. The present authors have previously reported the rates of thiol formations from I,[4a] 3,4-dimethyl-5-(2′-hydroxyethyl)thiazolium iodide (II),[4b] thiamine diphosphate,[4c] and thiamic acid (IV)[4d] observed by polarography and potentiometry. Maier, *et al.*[5] have also studied equilibrium between I_N, I_O, I_S, and yellow thiol form of thiamine. Recently Nogami, *et al.*[6] are studying the kinetics of reaction of I. These reactions are concerned with synthesis and biochemistry of thiamine derivatives. This paper deals with the kinetics of the reversible cleavages of thiazolium salts such as I, III, IV, dimethialium mononitrate (II) and N-methylbenzothiazolium iodide (V) in detail.



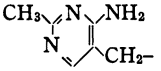
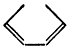
	R_3	R_4	R_5	X^-
I		CH_3	CH_2CH_2OH	NO_3^-
Ⅱ		CH_3	CH_3	NO_3^-
Ⅲ	CH_3	CH_3	CH_2CH_2OH	I^-
Ⅳ	$HOOCCH_2$ $^-OOCCH-$	CH_3	CH_2CH_2OH	—
V	CH_3	 (benzene ring)		I^-

Chart 1

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[1] Presented at the 17th Annual Meeting of Kinki Branch, Pharmaceutical Society of Japan, Nishinomiya, Nov. 1967.

[2] Location: *Juso, Higashiyodogawa-ku, Osaka.*

[3] R.R. Williams, *J. Am. Chem. Soc.*, **57**, 1856 (1935).

[4] *a*) A. Watanabe and Y. Asahi, *Yakugaku Zasshi*, **75**, 1046, 1050 (1955); Y. Asahi, *ibid.*, **80**, 1226 (1960); *b*) Y. Asahi, *ibid.*, **80**, 1222 (1960); *c*) A. Watanabe, Y. Asahi, and M. Hori, *ibid.*, **77**, 157 (1957); *d*) T. Fushimi, Y. Hara, S. Yurugi, and Y. Asahi, *Takeda Kenkyusho Nempo*, **26**, 15 (1967).

[5] G.D. Maier and D.E. Metzler, *J. Am. Chem. Soc.*, **79**, 4386 (1957).

[6] H. Nogami, J. Hasegawa, and T. Rikihisa, Annual Meeting of Pharmaceutical Society of Japan, Kyoto, April 1967; Tokyo, April 1968.

Experimental

Chemicals and Instruments——Samples (I—V) were offered from Dr. Hirano and his collaborators in our laboratories. Reagents were supplied from Wakō Pure Chemicals. Yanaco's PA2 type polarograph, a dropping mercury electrode with the constants: $m=1.465$ mg sec^{-1}, $\tau=4.35$ sec, $k_C=1.65$ and $h=90$ cm, and a N–calomel electrode as an external reference were used. pH was measured by a Beckman G type pH meter or by Radiometer's Titrigraph.

Kinetic Procedure——Buffer solutions were 0.1 M NaOAc–HCl for pH 4—6, 0.1 M KH_2PO_4–Na_2HPO_4–NaOH for pH 6—8 and 10.3—12, 0.1 M $Na_2B_2O_7$–NaOH–HCl for pH 8—10.3, and NaOH for pH 12—13.

One ml of aqueous or alkaline (2 eq) solution of 0.01 M thiazolium salts (I—V) degassed previously was added to 9 ml of the buffer solution under bubbling N_2. Current–time curve at -0.25 V was recorded automatically to follow the rapid reaction after stop of the bubble (Fig. 1). To trace the slow reaction, current–potential curves (polarograms) were recorded at appropriate intervals (Fig. 2).

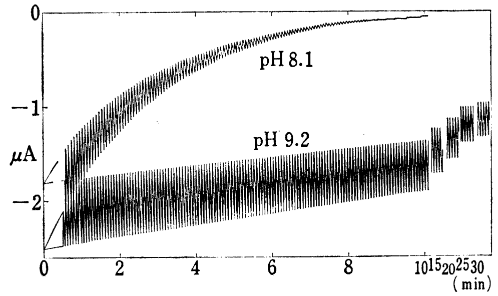

Fig. 1. Current–time Curves at -0.25 V in the Course of Reaction of Thiamine Thiol (1 mM) to the Thiazolium at 25°

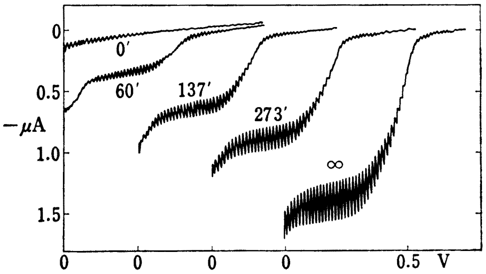

Fig. 2. Polarograms of Thiamine Thiol Developing from Thiamine Nitrate at pH 10.2 and at 0°

It has been noted[4)] previously that the heights of anodic waves due to reactions of the thiols with mercurous ion formed from the electrode are proportional to the concentrations of thiols. The half-wave potentials ($E_{1/2}$) were -0.39 (V), -0.43 (I, III), -0.47 V (II,IV), and the diffusion current constants (k_D) were 1.3 (I, II, IV) and 1.6 (III, V) μA mM^{-1} mg$^{-2/3}$ sec$^{1/2}$ at 25°.

Potentiometric Titration——Aqueous solution of II (0.01 M, 10 ml) with 1 eq HCl was titrated with 1 N NaOH at 25° in a N_2 atmosphere. The pyrimidine hydrochloride consumed immediately 1 eq NaOH to give pK_1 4.92. The thiazolium (II_N) consumed slowly (within 5 min) 2 eq NaOH to form thiolate (II_S) showing an inflection at pK_{av} 9.36. By slow titration of V_N with NaOH in the same manner, p$K_{av}$was estimated to be 6.5 for V_N. The pKs of other thiazoliums have been measured as following. I[4a)] pK_1 4.76, pK_{av} 9.33; III[4b)] pK_{av} 10.28; IV[4d)] pK_1 (β-CO_2H) 3.7, pK_{av} 11.

Result and Discussion

Formations of the thiols (S) from the thiazolium salts (N) and its reverse reactions for I—V were followed by the anodic wave heights of the thiols. Linear plots of log (i_e—i) *vs.* t were obtained in every kinetic runs (Fig. 3), where i_e and i are the wave heights at equilibrium and at t sec, respectively. The reactions are assumed to be reversible pseudo-first-order reactions regarding to the concentrations of N or S. The rate constants (k_N and k_S) for the reaction (Eq. 1) can be calculated by Eq. 2.

$$\mathrm{N} \underset{k_S}{\overset{k_N}{\rightleftharpoons}} \mathrm{S} \qquad \text{(Eq. 1)}$$

$$k_N+k_S=\frac{2.303}{t}\log\frac{i_e-i_0}{i_e-i} \qquad \text{(Eq. 2)}$$

The equilibrium constants (K) are, of course, given by Eq. 3, where i_0 and i_{100} are the heights at initial and at final in pH$\gg$pK, respectively.

$$K=\frac{k_N}{k_S}=\frac{i_e}{i_{100}-i} \qquad \text{(Eq. 3)}$$

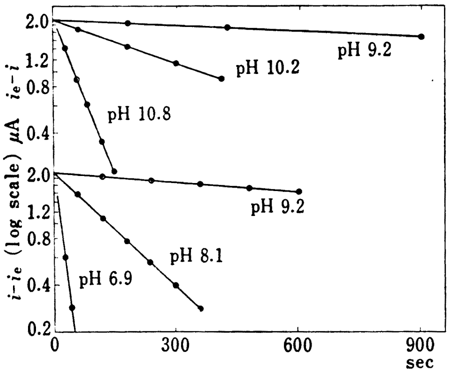

Fig. 3. Semi-log Plots of Anodic Currents of Thiamine Thiol in the Formation (Upper Part) and the Extinction (Lower Part) at 25°

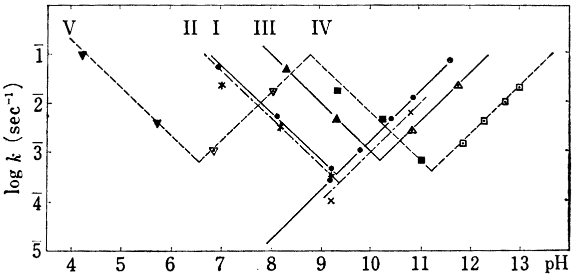

Fig. 4. pH Profiles of Rate Constants (log k_N, log k_S) of Reversible Cleavages of Thiazolium Salts (I—V) at 25°

The pH profiles of log k_N and log k_S estimated by Eq. 2 and Eq. 3 were shown by lines with slope of 1 and −1, respectively (Fig. 4). Namely k_N and k_S are proportional to activities of hydroxyl ion (OH^-) and hydrogen ion (H^+), respectively (Eq. 4).

$$k_N=k_{OH}\,(OH^-)$$
$$k_S=k_H\,(H^+) \qquad \text{(Eq. 4)}$$

The catalytic constants (k_{OH} and k_H) are given in Table I.

Table I. Second-order Rate Constants for Cleavages of Thiazolium Salts (I—V) (k_{OH}: M^{-1} sec^{-1}) and for the Reverse Reactions (k_H), and Dissociation Constants Estimated by Potentiometry (pK_{av}) and Kinetics (pK') at 25°

Subs.	log k_{OH}	log k_H	pK_{av}	pK'
V	4.2	3.4	6.5	6.6
I	1.3	5.9	9.3	9.3
II	1.0	5.8	9.4	9.4
III	0.6	7.0	10.3	10.2
IV	$\bar{1}$.4	7.8	11	11.2

One equivalent (eq) of OH^- or H^+ is concerned with the rate-determining step although 2 eq of OH^- or H^+ are consumed by the over-all reactions. The pH at $k_N=k_S$ (pK') coincides with the average dissociation constant (pK_{av}) obtained by potentiometric titration (Table I). This coincidence is expected from the relation as shown by Eq. 5.

$$pK_{av}=pH-\frac{1}{2}\log\frac{(S)}{(N)}=pH-\frac{1}{2}\log\frac{k_N}{k_S} \qquad \text{(Eq. 5)}$$

No detectable difference was observed among the rates in different buffer solutions with close concentration and pH. The log k_{OH} for I in a 0.1 M borate buffer (pOH 3.6) at 25° decreased with increasing ionic strength (μ) by potassium chloride: log k_{OH} 1.18 (μ 0.3), 1.05 (0.6) and 0.90 (1.3), which fulfiled Eq. 6.

$$\log k_{OH} = 1.46 - 0.5\mu^{1/2} \qquad \text{(Eq. 6)}$$

This fact suggests[7] a reaction of cation and anion to a less charged transition state, that is the reaction of thiazolium cation and hydroxyl anion to the pseudo-base.

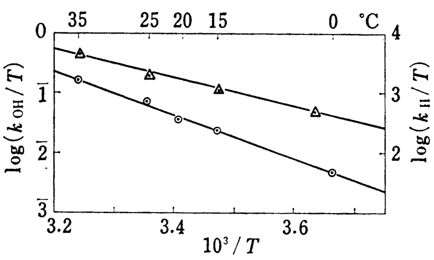

Fig. 5. Eyring's Plots of Rate Constants (k_{OH} at pOH 3.2 and k_H at pH 8.1) for Thiamine

The temperature dependence of k_{OH} for thiamine (I) was examined at pOH 3.2 and at 0—35°. Activation enthalpy $\Delta H^{\neq}$ 16.3 kcal mole^{-1} and activation entropy $\Delta S^{\neq}$ +2 e.u. for the cleavage of I were estimated from the Eyring's plots (Fig. 5). Linear plots of log (k_H/T) *vs.* $1/T$ were also obtained at pH 8.1 and at 2—35° to give $\Delta H^{\neq}$ 10.6 and $\Delta S^{\neq}$ +4 for the recyclization of I_S to I_N. Difference of enthalpy ΔH for the cleavage of I is, therefore, 5.7 kcal mole^{-1}, which is comparable to ΔH 7.3 estimated by temperature dependence of pK_{av} (9.75 at 0° and 8.85 at 50°).[4a] Unimolecular mechanism is predicted from the small positive activation entropy.[7]

The reaction processes are postulated as shown in Chart 2.

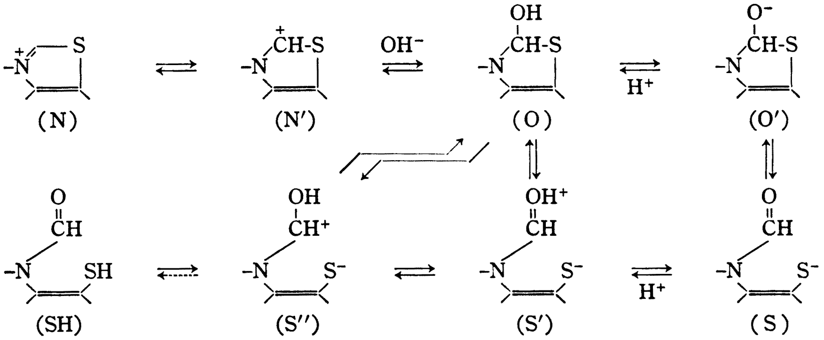

Chart 2

The initial step of the cleavage of thiazolium will be the attack of hydroxyl ion on the 2-position of thiazolium (N, N') with a partial positive charge to form the pseudo-base (O). The 1—2 bond in O will cleave to the thiolate anions with carbonium ion (S'') or protonated formyl (S'), which dissociates immediately to the anion (S). The thiol (SH) seems hardly to exist in an extremely alkaline solution. An alternative process will be the dissociation of hydroxyl in O to the anion (O') followed by cleavage of the 1—2 bond.

Since the intermediates (O, S', S'' and SH) were not detected with acid-base titration of the thiazoliums (I—V), the rate constants of initial steps (k_1 and k_4) seem to be smaller than those of second steps (k_3 and k_2), respectively. The concentration of intermediates (represented by O) in steady state is given by Eq. 7.

$$(\mathrm{O}) = \frac{k_1}{k_2+k_3}(\mathrm{N}) + \frac{k_4}{k_2+k_3}(\mathrm{S}) \qquad \text{(Eq. 7)}$$

In the successive reversible reactions, the rates for decrease of (N) at $k_1 \gg k_4$ in higher pH and of (S) at $k_1 \ll k_4$ in lower pH are treated as pseudo-first-order reactions with the rate constants as shown in Eq. 8.

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[7] E.S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt and Company, Inc., New York, 1960, pp. 159—198.

$$k_N = \frac{k_1 k_3}{k_2 + k_3}$$
$$k_S = \frac{k_2 k_4}{k_2 + k_3} \qquad \text{(Eq. 8)}$$

Nogami, *et al.*⁽⁶⁾ have estimated k_1 for I from the initial rate of alkaline consumption to be about twice of k_N and $k_2 \fallingdotseq k_3 > k_1$. Their values for k_N and k_S are close to those in the present paper measured with the anodic wave of thiols (S, including S′, S″ and SH).

Maier, *et al.*⁽⁵⁾ have obtained log $k_{OH}=0.9$ for the formation of colorless thiol of I from the increase of absorbance at 255 mμ in pH 10—10.8 at 19°. This value of log k_{OH} is close to our result (log $k_{OH}=1.0$ at 19°) interpolated from Fig. 5. They have also noted that a yellow thiol form in equilibrium with I_N goes to the colorless thiol (I_S) at pH 12—13 with the rate (log $k_H'=10.1$ at 19°). Since the yellow thiol of I in methanolic potassium hydroxide gave an anodic wave similar to that of I_S, both thiols can not be distinguished by polarography. The yellow thiol of I was also detected by flowing nuclear magnetic resonance whereas no other intermediate was observed.⁽⁸⁾

The structures of thiazolium (I—V) correlate with the rates (k_N and k_S) and pK_{av} (Table I). The larger pK_{av}, the larger k_N and the smaller k_S. Comparing I—IV, electron donating groups such as succinyl and methyl seem to protect for the attack of OH^- ion on the 2-position and to decrease the acidity. Extremely large k_N and small pK_{av} of V will be due to higher resonance stabilization of the thiophenol anion (V_S) than the enethiol (I_S—IV_S).

Acknowledgement The authors wish to express their thanks to Prof. Dr. H. Nogami, Tokyo University and his colleagues for their private communication and to Takeda Chemical Industries, Ltd. for permission of this study. Thanks are also due to Dr. Hirano, *et al.* for therir offering materials and to Mr. K. Shinozaki for technical assistance.

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8) Y. Asahi and E. Mizuta, *Talanta,* Submitted.