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Kinetics and Mechanism of Transamination of Thiamine in the Presence of Bisulfite[1)]

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The transamination reaction of thiazole moiety of thiamine (PyTh) by aromatic amine (A) in the presence of bisulfite (S) was studied kinetically. The reaction between PyTh and A was found to be second order with respect to PyTh and A, whereas the concentration of S was independent to the reaction rate. The reaction rate was linearly correlated to the pK_a value of A. The transamination product (PyA) was also degradated by S in second order as well as PyTh. The complexation between PyTh and A was also investigated as a possible proceeding process of the transamination reaction.

Bisulfite is widely used as antioxidant for pharmaceuticals,[3)] but it causes various undesirable reactions in various pharmaceutical preparations.[4)] Among those incompatibilities the cleavage of methylene bridge of thiamine (PyTh) has been studied by many investigators.[5,6)] However, on the replacement of thiazole moiety of thiamine by amine (A)[7–9)] in the presence of bisulfite (S) detailed mechanism has not yet been known. This paper is concerned with the kinetic study on the transamination of PyTh in the presence of S. This peculiar reaction includes reversible and consecutive processes and accompanies the well recognized degradation of PyTh by S. Although the reaction mechanisms were not fully elucidated in this study, some interesting results were obtained. In connection with the reaction mechanism some studies were carried out on the complexation between PyTh and various aromatic amines.

Experimental

Materials——PyTh hydrochloride obtained commercially was recrystallized from 80% ethanol. Aniline derivatives were recrystallized from water and dried *in vacuo*. N-(2-Methyl-4-aminopyrimidyl-(5))-methylaniline derivatives (PyA) and 2-methyl-4-amino-5-pyrimidylmethane sulfonic acid (PyS) are the product of the transamination, were synthesized refering to Matsukawa's report.[7b)]

Kinetics Procedures——The concentration of PhTh was 2.96×10^{-3} M generally and those of aniline derivatives and sodium bisulfite were from 1.48×10^{-2} to 2.96×10^{-3} M and 1.48×10^{-2} to 2.96×10^{-3} M respectively. Reaction media were 0.2 M acetate buffer (from pH 3.0 to pH 6.0) and 0.1 M sodium phosphate buffer (from pH 6.0 to pH 7.5), which were maintained at appropriate temperature in thermostat. The air in vessel was replaced by N_2 to prevent consumption of bisulfite. The concentration of remaining PyTh was determined by the colorimetry of thiochrome produced by bromocyan. The production and the following decomposition of PyA were pursued by the determination of A by the diazo-coupling with Tsuda's reagent or H acid (1-amino-8-naphthol-3,6-disulfonic acid monosodium salt). It was confirmed that PyTh, PyA, and PyS were not colored by the diazo-coupling.

1) Main part of this study was presented at the 93rd Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1973.
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3) L.C. Schroeter, "Sulfur Dioxide," 1st. ed., Pergamon, New York, 1966.
4) D.D. Perrin and I.H. Pitman, *J. Chem. Soc.*, **1965**, 7071.
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Determination of pK_a——Potentiometric titration was carried out with pH-stat (TOA, type HS-2A) at 50° and ionic strength was adjusted to 0.2 at the neutralization point with NaCl. The pK_a values of sulfanilic acid and metanilic acid could not be obtained by this method and the literature values at 25° were used.[10]

NMR Spectra in D_2O——JEOL PS-100 spectrometer was used and the internal standard was Na-2,2-dimethyl-2-silapentane-5-sulfonic acid.

Results and Discussion

Preliminary Observations

Refering to the previous literatures, possible products of the reaction in the condition studied are PyA, PyS, 4-methyl-5β-hydroxyethylthiazole (Th), and 2-methyl-4-amino-5-hydroxymethylpyrimidine (PyOH).[6-9] Other minor products may be negligible according to the previous paper-chromatographic study.[11] The production of PyOH due to simple hydrolysis of PyTh can be disregarded in the present experimental condition from the results of Windheuser, *et al.*[12] Major and assumingly exclusive pathways to be considered are as follows.

$$\text{PyTh} + \text{A} \underset{k_{-1}}{\overset{k_1(\text{S})}{\rightleftharpoons}} \text{PyA} + \text{Th} \quad (1)$$

$$\text{PyTh} + \text{S} \xrightarrow[k_2]{} \text{PyS} + \text{Th} \quad (2)$$

$$\text{PyA} + \text{S} \xrightarrow[k_3]{} \text{PyS} + \text{A} \quad (3)$$

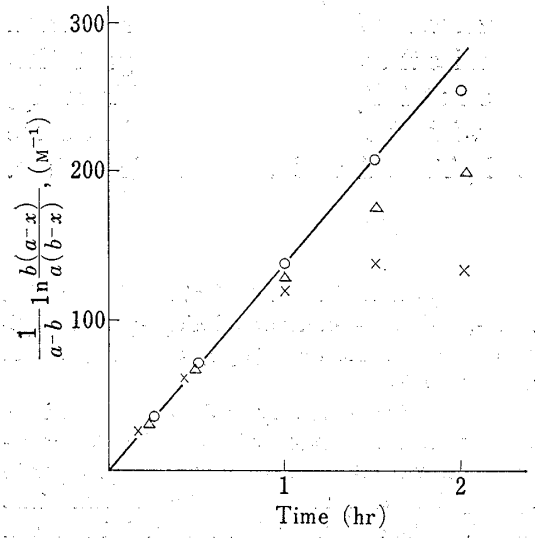

Fig. 1. Second Order Reaction Plots for the Reaction between PyTh and Aniline in the Presence of S

Initial concentration of PyTh (a) and that of S are constantly 2.96×10^{-3} and 5.92×10^{-3} M, respectively, and the concentration of aniline (b) was varied as 2.96×10^{-3} —×—: 5.92×10^{-3} —△—: and 1.48×10^{-2} M —○—. When $a=b$, the ordinate plots are $1/(b-x)-1/a$.

The reaction (1) was found to be second order with respect to the concentrations of PyTh and A at the initial stage of the reaction, and this reaction does not occur in the absence of S. The reaction was followed by the disappearances of aniline and PyTh. The concentration of S was found to be independent on the rate of reaction (1) in the observed concentration range (1.48×10^{-2}—2.96×10^{-3} M). The typical second order plots are shown in Fig. 1, where three experimental runs gave constant reaction rate at the initial stage independently of aniline concentration. The deviation from the linearity of the second order plots at the advanced stage may be due to the reversibility of the reaction and the successive reappearance of A due to the degradation of PyA by S. The concurrently occuring simple degradation of PyTh by S may affect the transamination, but as will be shown later, the reaction (2) is competetively inhibited as the concentration of A increases.

pH-Rate Profile of Reaction

Figure 2 shows the pH-rate (second order) profile of the reaction between PyTh and *p*-chloroaniline in the presence of S. The effects of buffer systems and their concentration

10) D.D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solution," Butterworths, London, 1965.
11) W.J. Lhoest, L.W. Busse, and C.A. Bauman, *J. Pharm. Sci.*, **47**, 254 (1958).
12) J.J. Windheuser and T. Higuchi, *J. Pharm. Sci.*, **51**, 354 (1962).

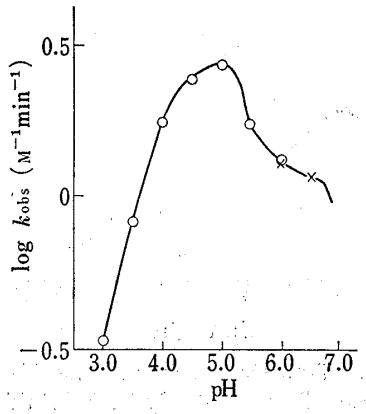

Fig. 2. The pH-Rate Profile for the Production of PyA and at 50°

concentration of PyTh; 2.96×10^{-3}M, concentration of *p*-chloroaniline; 2.96×10^{-3}M, concentration of S; 5.92×10^{-3}M. ○: 0.2M acetate buffer, ×: 0.1M sodium phosphate buffer. At pH 7.0 the reaction did not occur.

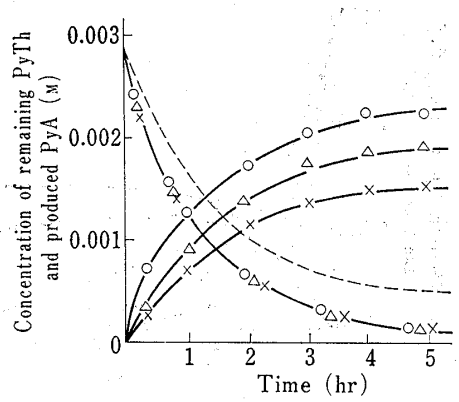

Fig. 3. Effects of Aniline Concentrations on the Cleavage of PyTh and the Production of PyA in the Presence of S at pH 5.0 and 50°

concentration of PyTh: 2.96×10^{-3}M, concentration of aniline:------, no aniline; —×—, 2.96×10^{-3}M; —△—, 5.92×10^{-3}M; —○—, 1.48×10^{-2}M, concentration of S: 5.92×10^{-3}M

were found to be negligible. The decrease of rate in acid range may be attributted to the ionization of *p*-chloroaniline whose pK_a is 3.44. The decrease of the rate above pH 5.0 may be ascribed to the dissociation of HSO_3^- to $SO_3^=$ (pK_{a2}=6.3) and in further alkaline range the transamination reaction does not take place probably because of the transition of the monocationic PyTh (pK_a=8.8) to thiol form.[12] From these results further studies were carried out at pH 5.0.

Effects of Concentrations of A and S

Figure 3 shows the effects of aniline concentration on the transamination and on the disappearance of PyTh. The concentrations of PyTh and S were held at 2.96×10^{-3}M and 5.92×10^{-3}M, respectively and that of aniline was changed from 2.96×10^{-3} to 1.48×10^{-2}M. The decrease of PyTh concentration is not influenced by amine concentration in the experimental error while the appearance of PyA estimated from the disappearance of aniline is facilitated with the increase of amine concentration. The dotted line shows the decay of PyTh in the absence of aniline, which was ascertained to be second order with respect to PyTh and S as had been reported.[6] If reaction (1) occur parallel to reaction (2), the disappearance rate of PyTh must be increase with A concentration. But apparently consistent decay of PyTh in spite of increase of A concentration indicates that reaction (2) is diminished as A increases. This may be interpreted by the assumption that an interaction occurs between PyTh and A, on which some investigation was carried out as will be described below.

When the concentrations of PyTh and A were held constant and that of S was changed, the higher the concentration of S the faster the degradation of PyTh as is shown in Fig. 4. But the initial rate of PyA formation is independent on S concentration. The following decrease of PyA is dependent on S concentration. That the degradation of PyA by S is second order with respect to the concentrations of PyA and S as that of PyTh due to S was ascertained. Typical increase and succeeding disappearance of PyA are shown in Fig. 5, which is the change of absorbance at 345 nm where only PyA has absorbance. In this instance the concentrations of PyTh, A (*p*-toluidine) and S were relatively high, *i.e.* 0.03, 0.03, and 0.1M, respectively and the reaction temperature was 25°.

Basicity of A and Transamination Rate

Table I shows the second order reaction rates between PyTh and various aromatic amines at 50°. Because the reactions were carried out at pH 5.0 and only unionized amine seemed

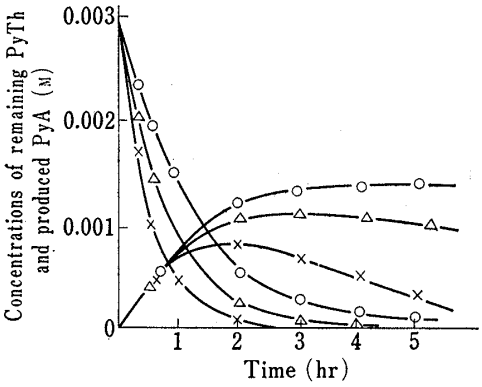

Fig. 4. Effects of S Concentrations on the Cleavage of PyTh and the Production of PyA in the Presence of Aniline at pH 5.0 and 50°

concentration of PyTh: 2.96×10^{-3}M, concentration of S: —×—, 1.48×10^{-2}M, —△—, 8.88×10^{-3}M, —○—, 5.92×10^{-3}M, concentration of aniline: 2.96×10^{-3}M. In the absence of S no transamination nor appreciable PyTh decay occured.

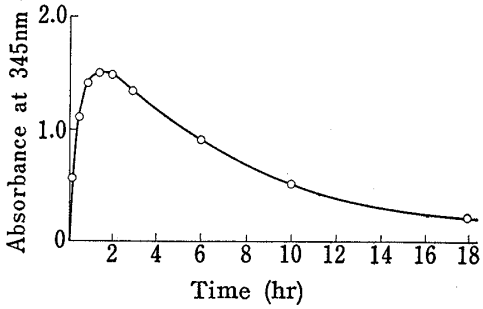

Fig. 5. Time Course showing the Absorbance Change of PyTh-*p*-Toluidine due to the Production of PyA in the Presence of S at 25°

concentrations: PyTh, 0.03M; *p*-toluidine, 0.03M; S, 0.1M

TABLE I. Second Order Reaction Rates between Thiamine and Aniline Derivatives in the Presence of Sodium Bisulfite at 50° and Ionic Strength 0.2

Aniline derivative		pK_a	k_1 (M^{-1} min^{-1})
Number	Substituent		
1	H	4.36	4.40
2	*m*-OCH_3	4.12	2.95
3	*p*-OCH_3	5.18	9.90
4	*m*-CH_3	4.61	4.96
5	*p*-CH_3	5.18	8.06
6	*p*-Br	3.75	3.10
7	*m*-Cl	3.44	2.72
8	*p*-Cl	3.75	2.94
9	*m*-COOH	3.19	3.06
10	*p*-COOH	2.46	1.96
11	*m*-SO_3H	3.69(25°)	2.65
12	*p*-SO_3H	3.21(25°)	1.85
13	*m*-$COCH_3$	3.46	2.89
14	*p*-$COCH_3$	1.87	0.64
15	*m*-NO_2	2.47	1.36

to be concern with the transamination (Fig. 2) the reaction rate between PyTh and free amine, k_1, was calculated with following equation,

$$k_1 = k_{obs}\frac{K_a + 10^{-5}}{K_a} \qquad (4)$$

where K_a is dissociation constant of anilinium ion and k_{obs} is the observed rate constant. Figure 6 is the plots of log k_1 *vs.* pK_a, which has fairly good linear relationship (slope=0.33). These results show that electron donating groups facillitate and electron withdrowing groups retard the reaction.

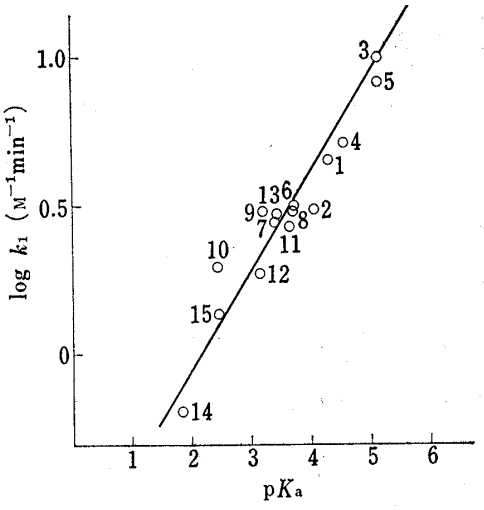

Fig. 6. Relationship between log k_1 and pK_a of Aniline Derivatives at 50°

Numbers refer to the compounds listed in Table I.

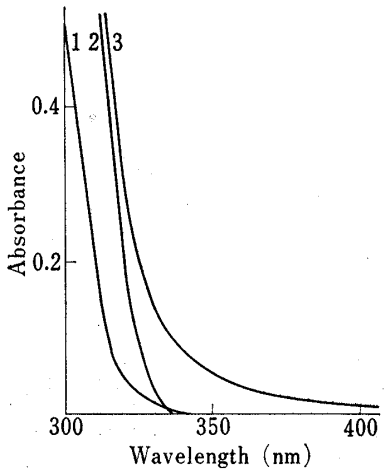

Fig. 7. UV Spectra of PyTh, *p*-Toluidine, and Their Mixture at pH 5.0 and Ionic Strength 0.2

curve 1, 0.03M PyTh; curve 2, 0.03M *p*-toluidine; curve 3, 0.03M PyTh+0.03M *p*-toluidine

Complexation between PyTh and A

The complexation between PyTh and some amine compounds has been studied spectrophotometrically by some authors.[13,14] Such complexation may possibly have a role in the transamination reaction. As is shown in Fig. 7 for example, the absorbance of PyTh+A (*p*-toluidine) is remarkably different from the sum of the individual absorbances. Figure 8 is the continuous variation diagram of the change of absorbance at 345 nm, which indicates 1:1 complexation. Table II summarizes the stability constants of 1:1 complexes between PyTh and various aromatic amine, K_c, and molar absorbancy of complex, ε_c, which were estimated by Scott's equation[15] at the wavelength shown. The relation between log K_c and log k_1 is shown in Fig. 9, which indicates, although roughly, that there is relationship between those two values. The complexation studies could be carried out only at considerably higher concentrations of PyTh and A and absence of buffer (in H_2O). The use of buffer solution as the medium of complexation or the adjustment of pH could not be carried out because of the low solubilities of amine. These difficulties hindered to correlate the complexation and kinetic data at similar conditions.

Table II. Stability Constant of Complexes of Thiamine and Aniline Derivatives in Water at 25°

No.	Substituent	K_c	Wavelength (nm)	ε_c
1	H	3.95	340	15.3
3	*p*-OCH_3	60.3	345	2.08
4	*m*-CH_3	17.9	345	3.49
5	*p*-CH_3	27.2	345	7.89
6	*p*-Br	12.0	345	14.4
7	*m*-Cl	16.2	340	9.09
8	*p*-Cl	2.41	340	29.2
14	*p*-$COCH_3$	11.4	345	9.87

13) Th. Eckert, *Arzneimittel-Forsch.*, **12**, 8 (1962).
14) J.E. Biaglow, J.J. Mieyal, J. Suchy, and H.Z. Sable, *J. Biol. Chem.*, **244**, 4054 (1969).
15) R.L. Scott, *Rec. Trav. Chim.*, **75**, 787 (1956).

Table III. Chemical Shifts of Nucleus Protons of PyTh, *p*-Toluidine, and Their Mixtures

	2′-CH_3	4-CH_3	$HOCH_2CH_2$-	$HOCH_2CH_2$-	-CH_2-	6′-H	2-H
Thiamine (0.1M)	2.59	2.68	3.23	3.94	5.62	8.08	9.72
Thiamine (0.1M)+ *p*-Toluidine (0.05M)	2.59 (0.00)	2.64 (+0.04)	3.22 (+0.01)	3.93 (+0.01)	5.57 (+0.05)	8.08 (0.00)	9.63 (+0.09)
Thiamine (0.1M)+ *p*-Toluidine (0.1M)	2.59 (0.00)	2.59 (+0.09)	3.22 (+0.01)	3.92 (+0.02)	5.53 (+0.09)	8.08 (0.00)	—a)
Thiamine (0.1M)+ *p*-Toluidine (0.15M)	2.59 (0.00)	2.59 (+0.09)	3.20 (+0.03)	3.91 (+0.03)	5.48 (+0.14)	8.07 (+0.01)	—a)

	CH_3	Phenyl H	
p-Toluidine (0.15M)	2.27	6.80	7.11
p-Toluidine (0.15M)+ Thiamine (0.1M)	2.32 (−0.05)	7.06 (−0.26)	7.24 (−0.13)

Values in parenthesis are differences of chemical shifts (δ, (ppm)).
a) Could not be observed since proton is exchanged with deuterium in D_2O.

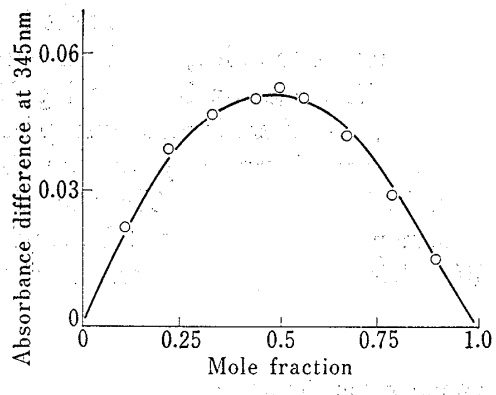

Fig. 8. Continuous Variation Plots for PyTh-*p*-Toluidine System

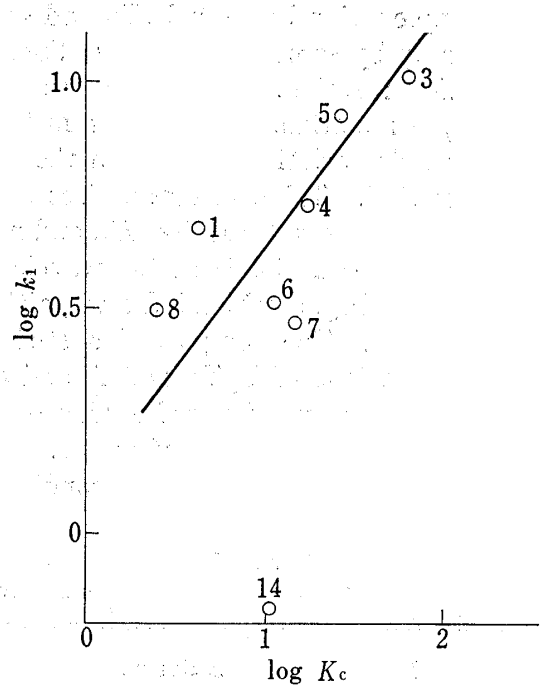

Fig. 9. Relationship between log K_c and log k_1

These complexations were further studied by NMR in D_2O. As is seen in Table III the chemical shifts of PyTh due to the presence of *p*-toluidine are shifted to higher magnetic field which indicate that PyTh behave as electron acceptor. Meanwhile the phenyl proton signals of *p*-toluidine in the presence of PyTh are shifted to lower magnetic field which shows that *p*-toluidine has π-electron donnor property. The significant shifts of PyTh are occured near the quarternary N, *i.e.* at bridge CH_2, 4-CH_3 and ring 2-H, and those of *p*-toluidine are at phenyl H, which indicate the participating moieties of the reactant molecules for the interaction. Similar phenomena were reported also for the interaction between PyTh and indole acetate.[14]

Conclusive Mechanism of Transamination

The transamination reaction is second order with respect to PyTh and A and independent of S concentration. Supposingly complexations occur competetively between PyTh and A and also PyTh and S. The PyTh-A complexation is dependent on the basicity of A and the reaction is governed by pK_a. The increase of A concentration suppress the simple degradation of PyTh by S. The role of S for transamination is not fully elucidated in the present study but S is inevitable. S decomposes PyA as is well recognized in PyTh and the reaction is second order with respect to S and PyTh or PyA. The transamination is reversible. The final product of the reactions occuring competetively, succeedingly and partly reversibly is PyS. For the convincing proof on the participation of this complexation further investigation may be necessary.