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Stability of Sulpyrine. I. Reversible Hydrolysis in Alkaline Solution[1]

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Hydrolysis of sulpyrine was investigated in neutral and alkaline solution.

4-Methylaminoantipyrine (MAA), 4,4′-[methylenebis(methylimino)]diantipyrine (Bis), and 4-(N-hydroxymethyl-N-methyl)aminoantipyrine (HMA) were identified as hydrolyzed products by nuclear magnetic resonance spectroscopy and spectrophotometry, and the determination methods of them were proposed.

Sulpyrine was in an equilibrium with these products and the percent composition of each species at equilibrium was affected by the initial concentration of sulpyrine. The ratios of the equilibrated amounts of Bis and HMA to that of MAA increased with an increase in the initial concentration of sulpyrine.

The apparent rate constants of hydrolysis and formation of sulpyrine were determined in the moderately diluted solution (below 4.5 mg/ml) where Bis was negligible. The results suggest that the apparent rate of hydrolysis is independent of pH, whereas that of formation apparently decreases with an increase in pH values, which is ascribed to an increase in the dissociation of hydroxymethanesulfonate into formaldehyde and bisulfite.

Keywords——stability in aqueous solution; sulpyrine; hydrolysis in alkaline solution; NMR; equilibrium

It is known that sulpyrine in aqueous solution is in an equilibrium with 4-methylaminoantipyrine (MAA) and hydroxymethanesulfonate (OMS).[3,4]

Ono, *et al.* have determined the equilibrated amounts of sulpyrine, MAA and OMS in aqueous solution and indicated that those amounts varied with pH in studying the mechanism of iodometric titration of sulpyrine.[4] However, the kinetics of hydrolysis of sulpyrine has not been studied in detail, and the relation between the hydrolysis rate and pH has not been investigated. Ikeda, *et al.* have studied the hydrolysis of methanesulfonic acid derivatives of substituted anilines, and reported that the hydrolysis is reversible between pH 4 and 8, but an equilibrium is not completely attained in the other pH range.[5,6]

In regard to the hydrolyzed products, the presence of 4,4′-[methylenebis(methylimino)]-diantipyrine (Bis) in addition to MAA in aqueous solution of sulpyrine has been reported,[7] but no quantitative study has been carried out.

The present investigation was undertaken to detect the hydrolyzed products quantitatively and to determine the kinetics of hydrolysis of sulpyrine in alkaline region. Since the kinetics of hydrolysis in acid region differs from that in alkaline region, the former will be described in a subsequent paper.

Experimental

Materials——Sulpyrine used was of J.P. VIII grade. MAA and Bis were synthesized referring to the literature.[4] MAA was crystallized as oxalate (mp 168°). Deuterium oxide had a purity of 99.9%. Other chemicals used were of reagent grade.

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[3] M. Samejima, I. Sugimoto, and I. Utsumi, *Yakuzaigaku*, **26**, 23 (1966).

[4] S. Ono, R. Onishi, and K. Kawamura, *Yakugaku Zasshi*, **86**, 11 (1966); *idem, ibid.*, **88**, 554 (1968).

[5] K. Ikeda, K. Miyata, T. Iwata, F. Kawata, and K. Kurome, *Chem. Pharm. Bull.* (Tokyo), **18**, 440 (1970).

[6] Y. Kurono, K. Ikeda, and K. Uekama, *Chem. Pharm. Bull.* (Tokyo), **23**, 409 (1975).

[7] M. Morita, *Yakugaku Zasshi*, **82**, 50 (1962).

Nuclear Magnetic Resonance (NMR) Studies——After sulpyrine was dissolved to give 10 mg/ml in deuterium oxide and 0.1N NaOD respectively, the NMR spectra were measured. The NMR spectra of the $CDCl_3$ extractive of the reaction solution were also measured in order to detect the small amount of products. A 20 ml of the reaction solution was shaken with a 2 ml of $CDCl_3$ and the NMR spectrum of the extractive was measured. Various singlets were found in the spectrum which represent the protons of $-CH_2-$ or $-CH_3$ of each product. The percent composition of the each product was determined by the ratio of the integral of these singlets to the total.

Determination of 4-(N-Hydroxymethyl-N-methyl)aminoantipyrine (HMA) in the Diluted Solution——It is impossible to determine the hydrolyzed products in the diluted solution (below 5.7×10^{-3}M) by means of NMR spectroscopy. Since Bis is negligible in this concentration, HMA can be determined by a following method. In alkaline range, HMA is extracted with chloroform. While in strong acid, 1 mol of HMA releases equimolar MAA and formaldehyde. Therefore the amount of HMA can be estimated by the determination of formaldehyde released from the chloroform extractive. A 5 ml of the reaction solution was shaken with a 5 ml of $CHCl_3$, and then the $CHCl_3$ extractive was shaken with a 5 ml of 0.1N HCl. Formaldehyde released in the acidic solution was determined by means of the method established by Bricker and Vail.[8]

Determination of Dissociation Constant of Hydroxymethanesulfonate (OMS)——The dissociation constant of OMS was determined by means of the two methods based on the estimation of formaldehyde produced in the dissociation. In the first method, the equilibrated amount of formaldehyde in OMS solution was determined directly by iodometric titration. A 5 ml of 4.3×10^{-2}M OMS solution of each pH was pipetted into a 50 ml of 0.01N iodine solution with a 3 ml of 37% hydrochloric acid, and shaken vigorously. After three minutes the excessive amount of iodine was determined by titration of 0.01N $Na_2S_2O_3$ solution. The consumed amount of iodine represents the amount of formaldehyde, namely that of dissociated OMS. The other method involves the separation of formaldehyde and undissociated OMS with ion exchange resins, followed by the determination of formaldehyde by means of the method established by Bricker and Vail.[8] A 5 ml of 4.3×10^{-2}M OMS solution was pipetted into a column (1 cm diameter) with a 2 ml of ion exchange resins (Dowex 4×1 Cl) and the resins were elutriated with a 15 ml of distilled water, and then formaldehyde in the eluate was determined by means of the method described above.

Determination of Hydrolysis and Formation Rate of Sulpyrine——The kinetic study was done in buffers of 0.05M borate or 0.05M phosphate system at 40° unless otherwise stated. The ionic strength of the experimental solution was maintained at 0.2 with KCl unless otherwise stated. The sample solution was withdrawn at appropriate intervals, and shaken quickly with chloroform in order to remove the hydrolyzed products from the sample solution, and then remaining sulpyrine in aqueous phase was determined by means of the method established by Kato, *et al.*[9]

Results and Discussion

Detection of Hydrolyzed Products

It is known that MAA and Bis are present in aqueous solution of sulpyrine.[7] Ono, *et al.* detected 4-(N-hydroxymethyl-N-methyl)aminoantipyrine (HMA) only in acid solution by

TABLE I. τ Values[a] of Sulpyrine, MAA and OMS at pH 7 and 12.3 at 26°

	pH 7		pH 12.3	
	$-CH_2-$	$-CH_3$ ($N2-CH_3$, $4N-CH_3$, $C-CH_3$)	$-CH_2-$	$-CH_3$
Sulpyrine (2.85×10^{-2}M) immediately after dissolved	5.96	6.91 7.13 7.72	5.95 —	6.90 7.12 7.71
Sulpyrine (2.85×10^{-2}M) 2 hr after dissolved	5.96, 5.72	6.91, 6.95 7.13, 7.43 7.72, 7.77		6.90, 6.93 7.11, 7.40 7.70, 7.75
MAA		6.93 7.41 7.75		6.95 7.42 7.76
OMS	5.71		—[b]	

[a] The signals of phenylprotons are omitted.
[b] OMS dissociates mostly to formaldehyde and bisulfidte at this pH.

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[8] C.E. Bricker and W.A. Vail, *Anal. Chem.*, **22**, 720 (1950).

[9] K. Kato, M. Umeda, and S. Tsubota, *Yakuzaigaku*, **24**, 116 (1964).

means of NMR spectroscopy.[4] In neutral pH range, McMillan and Pattison have reported the formation of carbinolamine in the dissociation of *n*-butylaminomethanesulfonic acid.[10]

In this study, the NMR spectrum of the neutral and alkaline solution of sulpyrine was determined in order to detect the hydrolyzed products, and the τ values are summarized in Table I. MAA and OMS were detected, but HMA and Bis were not detected at both pH 7 and 12.3.

Furthermore, the NMR spectrum of $CDCl_3$ extractive of alkaline solution of sulpyrine was determined in order to detect the other hydrolyzed products whose concentrations were too small to detect directly by NMR, and Table II summarizes the τ values obtained. Bis and another product in addition to MAA and OMS are present as the hydrolyzed products of sulpyrine. The signals at 5.57, 7.03, and 7.85 τ, which can not be assigned to Bis or MAA, suggest the possible existence of HMA, because the singlet at 5.57 τ represents the protons of $-CH_2-$ between two heteroatoms, and formaldehyde or sulpyrine is not extracted in $CDCl_3$.

Table II. τ Values of the $CDCl_3$ Extractive after the Reaction at pH 13 at 40°

	$-CH_2-$	$-CH_3$
Sulpyrine[a]	5.57, 5.84	7.03, 7.19, 7.20 7.27, 7.90, 7.25 7.85, 7.81
MAA+HCHO[b]	5.56, 5.82	7.03, 7.19, 7.20 7.26, 7.89, 7.25 7.85, 7.82
MAA[c]		7.22 7.26 7.82
Bis[c]	5.83	7.19 7.88

[a] Extracted with $CDCl_3$ after sulpyrine is dissolved to be 2.85×10^{-2} M in 0.1 N NaOD and kept at 40° for 30 minutes.
[b] Extracted with $CDCl_3$ after MAA and formaldehyde are dissolved in the mole ratio 1:1 in 0.1 N NaOD and kept at 40° for 30 minutes.
[c] dissolved directly in $CDCl_3$

In order to elucidate the process of formation of MAA, Bis and HMA, the NMR spectrum of the $CDCl_3$ extractive of the reaction solution of MAA and formaldehyde was also determined and shown in Table II. The τ values observed in the spectrum agreed with those in the spectrum of the $CDCl_3$ extractive of the alkaline solution of sulpyrine, which suggested that the hydrolyzed products of sulpyrine included the reaction products of MAA and formaldehyde, namely HMA and Bis.

It has been found that Bis is negligible as a hydrolyzed product in the low concentration below 5.7×10^{-3} M as described later. In order to confirm that HMA is one of the hydrolyzed products as suggested from Tables I and II, the stoichiometry of the product in the reaction system of MAA and formaldehyde was investigated in the low concentration where Bis was negligible. Figure 1 shows the concentration of the product formed at various mole fractions of MAA and formaldehyde, which was estimated by the determination of formaldehyde released from the product. From the result it is confirmed that 1 mol of MAA reacts with 1 mol of formaldehyde and produces 1 mol of the product. This agrees with that the product is HMA.

In respect of OMS released when sulpyrine hydrolyzes, Table I shows that OMS is present at pH 7 but not at pH 13. Since it is known that OMS dissociates to formaldehyde and bi-

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[10] F.H. McMillan and I.C. Pattison, *J. Pharm. Sci.*, **58**, 730 (1969).

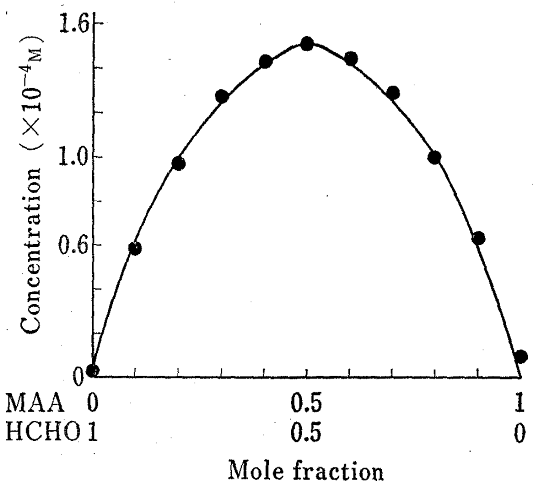

Fig. 1. A Plot of Concentration of HMA against Mole Fraction of MAA or HCHO

[HCHO]+[MAA]=3.4×10^{-3}M (corresponding to 1.2 mg/ml for sulpyrine)

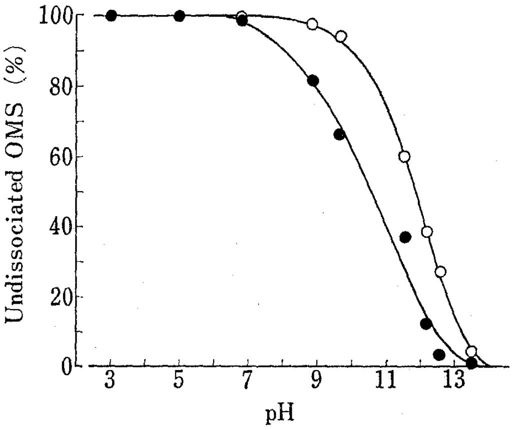

Fig. 2. Percentage of Undissociated OMS at Various pH

○: determined by iodometric titration
●: determined by spectrophotometry after separation with ion exchange resins

sulfite reversibly in aqueous solution,[11] the dissociation constant of OMS was determined by means of two methods. In the first method formaldehyde in OMS solution was determined by direct iodometric titration, and in the other method formaldehyde was determined by spectrophotometry after separated from undissociated OMS with ion exchange resins. As Fig. 2 shows, the amount of formaldehyde obtained by means of spectrophotometry after the separation with ion exchange resins was larger than that obtained by means of the direct titration. The equilibrated amount of formaldehyde in OMS solution may change in the column of ion exchange resins and the amount of formaldehyde may increase. On the other hand formaldehyde reacts with iodine immediately and no more OMS dissociates in strong acid. Therefore the results obtained by means of the direct titration seems to be more reliable than that by means of spectrophotometry. Furthermore the dissociation constant obtained above was found to be constant during 24 hours at room temperature, and this fact confirms that OMS does not decompose irreversibly.

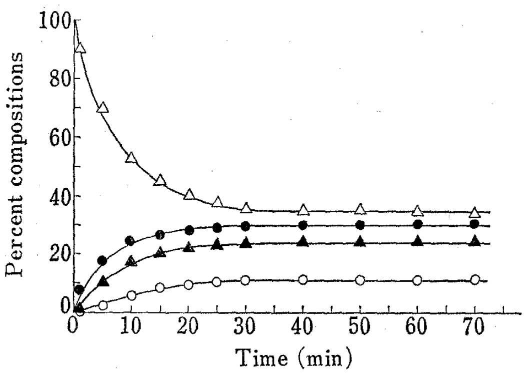

Fig. 3. Hydrolysis of Sulpyrine at pH 13 at 40°

initial concentration of sulpyrine: 15 mg/ml
△: sulpyrine, ●: MAA, ▲: Bis, ○: HMA

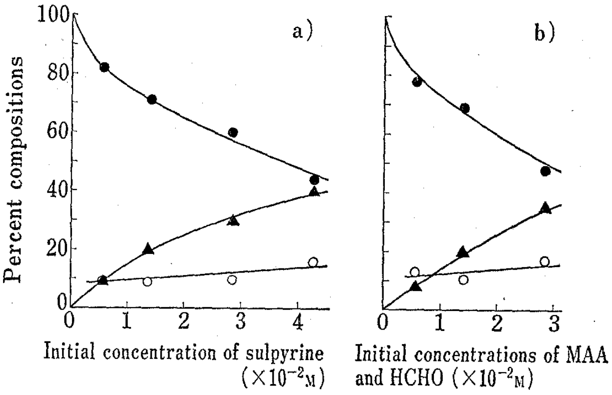

Fig. 4. Effect of Concentration on Equilibrium among Hydrolyzed Products of Sulpyrine at pH 13 at 40°

a) in the equilibrium attained in hydrolysis of sulpyrine
b) in the equilibrium attained in reaction of MAA and formaldehyde (1: 1 mole ratio)
○: HMA, ▲: Bis, ●: MAA

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[11] D.A. Blackaddar and Sir C. Hinshelwood, *J. Chem. Soc.*, **1958**, 2720.

Equilibrium among Hydrolyzed Products

Figure 3 shows the time course of hydrolysis of sulpyrine in the high concentration (15 mg/ml) enough to be determined by NMR spectroscopy. Sulpyrine and its hydrolyzed products, MAA, HMA, and Bis, reached to an equilibrium after about 40 minutes. In order to elucidate the stoichiometry of these products, the percent composition of each product at equilibrium was determined at various concentrations and shown in Fig. 4. The percentage of the amount of MAA decreased and that of Bis increased with an increase of the concentration in both the equilibrium after sulpyrine was dissolved and that after MAA and formaldehyde were added. The percent of composition of HMA was smaller and that of MAA was larger in the case of sulpyrine solution than in the case of the reaction of MAA and formaldehyde in Fig. 4. It seems to be due to the existence of the equilibrium as shown in Chart 1 in addition to the equilibrium among MAA, HMA, Bis, and formaldehyde in the case of sulpyrine solution. The effect of pH on the mole ratio of each hydrolyzed product at the equilibrium is shown in Table III. The percent compositions of MAA, Bis and HMA did not vary with pH between pH 8 and 13.

$$\text{sulpyrine} \rightleftharpoons \text{HMA} + NaHSO_3$$
$$\text{sulpyrine} \rightleftharpoons \text{MAA} + \text{OMS}$$
$$\text{OMS} \rightleftharpoons \text{HCHO} + NaHSO_3$$

Chart 1

In Fig. 4 when the initial concentration of sulpyrine was below 5.7×10^{-3} M, it was impossible to detect the signals of HMA and Bis by means of NMR spectroscopy. Figure 4 shows that MAA and HMA were formed mainly and Bis was negligible in the solution of sulpyrine whose initial concentration was below 5.7×10^{-3} M. In order to elucidate the equilibrium among

Table III. Effect of pH on Equilibrium[a] among Products at 40°

pH	Percent compositions		
	MAA	Bis	HMA
8.4	44	42	14
11.4	41	40	19
13.0	44	40	16

[a] equilibrium attained in the reaction of MAA and HCHO (2.85×10^{-2} M)

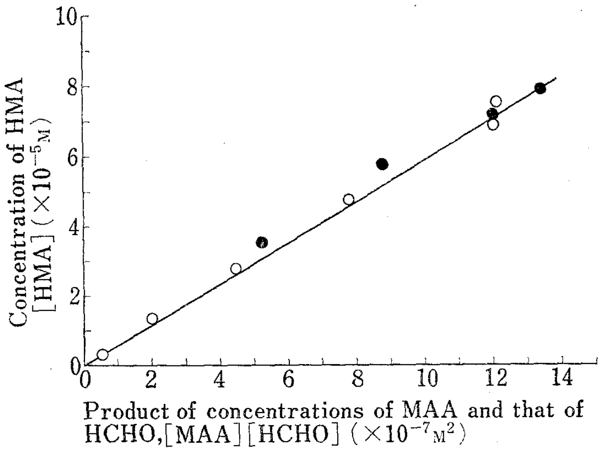


Fig. 5. Effect of Concentration on Equilibrium among MAA, HCHO and HMA at pH 13 at 40°

[MAA]+[HMA]: below 1.71×10^{-3}M (corresponding to 600 μg/ml for sulpyrine)
○: in the equilibrium attained in hydrolysis of Bis
●: in the equilibrium attained in reaction of MAA and formaldehyde

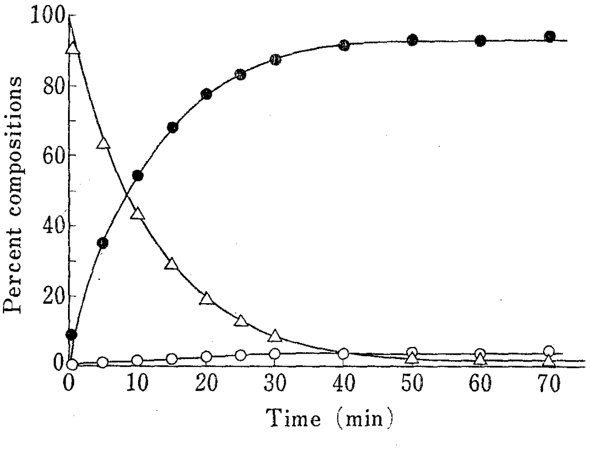


Fig. 6. Hydrolysis of Sulpyrine at pH 13 at 40°

initial concentration of sulpyrine: 300 μg/ml (8.55×10^{-4}M)
△: sulpyrine, ○: HMA, ●: MAA

MAA, HMA and formaldehyde, the concentration of HMA, [HMA], was plotted against the product of the concentration of MAA and that of formaldehyde, [MAA][HCHO], at equilibrium in Fig. 5. At the equilibrium attained after MAA and formaldehyde were dissolved, the linear correlation was observed. Furthermore the same linearity was observed at the equilibrium attained after Bis was dissolved. This indicates that Bis hydrolyzes to MAA, HMA and formaldehyde, and these products reach to an equilibrium. The slopes of the straight lines were in good agreement each other, and from those the formation constant of HMA ([HMA]/[MAA][HCHO]) was calculated to be 58.4 M^{-1}. The equilibrium among MAA, HMA and formaldehyde described above was studied in reaction of MAA and formaldehyde or in hydrolysis of Bis, and not in hydrolysis of sulpyrine. However it seems to be reasonable to consider that the same equilibrium may be established also in hydrolysis of sulpyrine. Figure 6 shows the time course of hydrolysis of sulpyrine and formation of MAA and HMA in the low concentration.

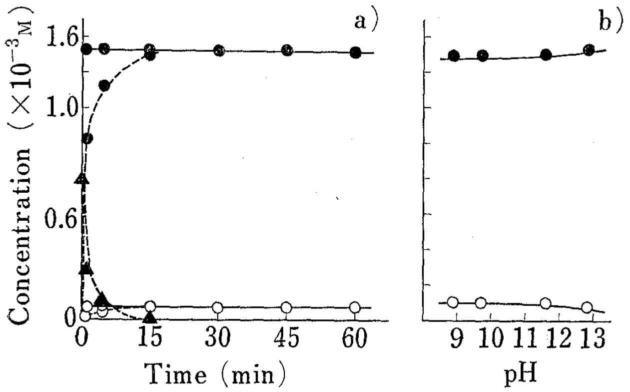


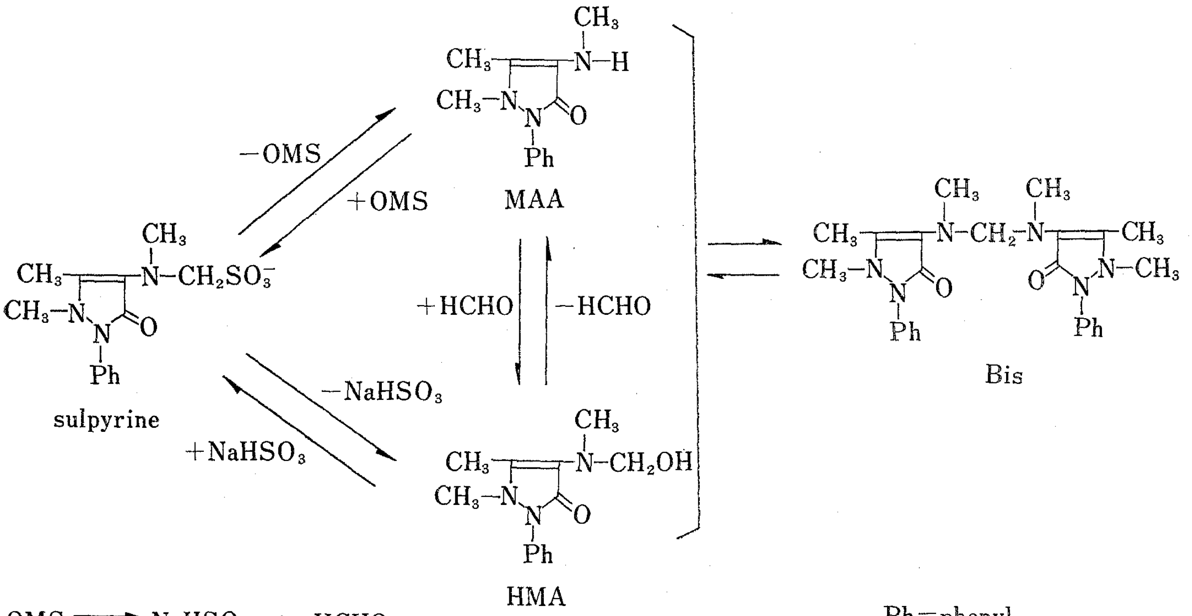
Fig. 7. Equilibrium of MAA and HMA at 40°

a) time course at pH 13
b) effect of pH
——: reaction of MAA and HCHO (initial concentration MAA: 1.6×10^{-3}, HCHO: $0.8\times10^{-3}\text{M}$)
------: hydrolysis of Bis (initial concentration $0.8\times10^{-3}\text{M}$)
▲: Bis, ●: MAA, ○: HMA

Possible Pathways in Hydrolysis of Sulpyrine

As shown in Fig. 3 and Fig. 6, which illustrate the time course of hydrolysis of sulpyrine, the hydrolysis process of sulpyrine seems to be a rate determining step and the equilibrium among MAA, HMA and Bis seems to be attained rapidly.

Figure 7-a) shows the time course of reaction of MAA and formaldehyde, and that of hydrolysis of Bis in the low concentration. An equilibrium was attained as soon as MAA and formaldehyde were dissolved, and this indicates that the reversible reaction from MAA to HMA is very fast. On the



OMS $\rightleftharpoons$ $NaHSO_3$ + HCHO

Ph=phenyl

Chart 2

other hand, when Bis was dissolved, an equilibrium was attained after about 20 minutes. In this case HMA and Bis could not be determined separately, so the amount of HMA was calculated from that of MAA on the basis of the fact that MAA and HMA were in an equilibrium from the start of the reaction. As shown in Fig. 7-a) the reversible reaction from MAA and HMA, to Bis was slower than that from MAA to HMA. The amounts of MAA and HMA at equilibrium were not affected in terms of pH as shown in Fig. 7-b).

The possible pathways in hydrolysis of sulpyrine are represented in Chart 2. In the higher concentration sulpyrine is in an equilibrium with the hydrolyzed products, MAA, HMA, and Bis. In the low concentration below 5.7×10^{-3} M Bis is negligible. In respect of hydrolysis of sulpyrine two pathways are possible—sulpyrine hydrolyzes directly to MAA and OMS in the first pathway, and directly to HMA and bisulfite in the other pathway. Since the reversible reaction from MAA to HMA is very fast, it can not be clarified which pathway is predominant. In respect of formation of Bis, Bis may be formed by reaction of 1 mol of MAA and 1 mol of HMA, by that of 2 mol of MAA and 1 mol of formaldehyde, or by that of 2 mol of HMA. However it seems to be reasonable to consider that MAA reacts with HMA and forms Bis in terms of simple dehydration. However it can not be clarified in which pathway Bis is formed because of the fastness of the reversible reaction from MAA to HMA.

The kinetics of hydrolysis of sulpyrine to MAA and/or HMA was studied in the low concentration as described later. On the other hand no more detailed studies on the kinetics of the reaction processes among MAA, HMA and Bis, and on the mechanism of the formation of Bis from MAA and HMA were carried out because HMA and Bis could be determined separately only by NMR spectroscopy which restricted experimental conditions.

Hydrolysis and Formation Rate of Sulpyrine

In the case of hydrolysis of methanesulfonic acid derivatives of substituted aniline, Ikeda, *et al.* reported that no equilibrium reaction occurs above pH 8 because of the irreversible decomposition of OMS.5) Furthermore they observed the decrease of the apparent formation rate constant with an increase in pH value, and interpreted it also in terms of the irreversible decomposition of OMS. However, in this study, as Fig. 3 and Fig. 6 show, an equilibrium was attained even at pH 13. Furthermore OMS did not decompose irreversibly as described above.

Since the equilibrium between MAA and HMA is attained rapidly as shown in Fig. 7 it can not be clarified whether sulpyrine hydrolyzes directly to MAA and OMS, hydrolyzes directly to HMA and bisulfite, or hydrolyzes on the both pathways. Therefore, if the sum of the rate constants of the two hydrolysis pathways is represented by the apparent rate constant of sulpyrine hydrolysis, k_1, and that of the two formation pathways by the apparent rate constant of sulpyrine formation, k_2, then k_1 and k_2 are represented by equations (1) and (2) respectively,

$$\ln \frac{S_o^2 - S_e S}{(S - S_e) S_o} = k_1 \frac{S_o + S_e}{S_o - S_e} t \qquad (1)$$

$$k_2 = k_1 \frac{S_e}{(S_o - S_e)^2} \qquad (2)$$

where S_o, S, and S_e represent the initial concentration of sulpyrine, the concentration of sulpyrine at time t, and that in the equilibrium state respectively. A plot of the left hand of equation (1) against time gave a straight line in alkaline region between pH 7 and 13 (Fig. 8). On the other hand, linearity was not observed below pH 5, and this fact indicates that the mechanism of hydrolysis of sulpyrine in acid region differs from that represented in terms of equations (1) and (2).

The apparent hydrolysis and formation rate constants in alkaline region were calculated in terms of equations (1) and (2), and the pH-rate constant profile is represented in Fig. 9. As Fig. 9 shows the apparent hydrolysis rate constant was pH-independent in the pH range between 7 and 13, whereas the apparent formation rate constant became small with an increase in pH value as observed in the case of methanesulfonic acid derivatives of substituted aniline by Ikeda, *et al.* However the apparent rate constant of formation of sulpyrine, k_2, being corrected by equation (3),

$$k_2' = \frac{1}{p} k_2 \tag{3}$$

where p stands for the ratio of the amount of undissociated OMS to the total amount, showed no significant pH-dependency (Fig. 10).

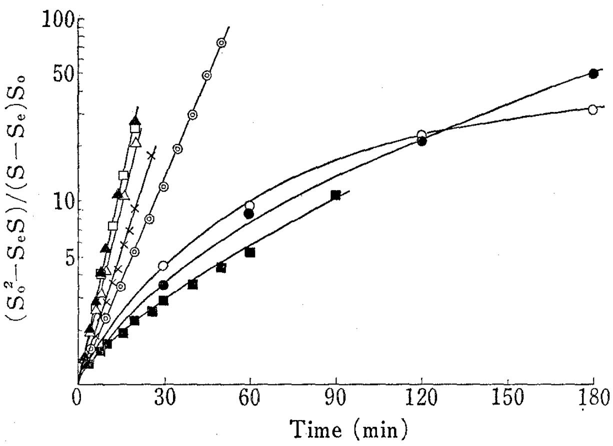


Fig. 8. Equilibrium Reaction between Sulpyrine and Hydrolyzed Products at Various pH

●: pH 1, 5°, 750 μg/ml ○: pH 3, 5°, 150 μg/ml
■: pH 5, 40°, 1500 μg/ml □: pH 7, 40°, 1500 μg/ml
▲: pH 9, 40°, 1500 μg/ml △: pH 10, 40°, 1500 μg/ml
×: pH 11, 40°, 1500 μg/ml ◎: pH 13, 40°, 1500 μg/ml

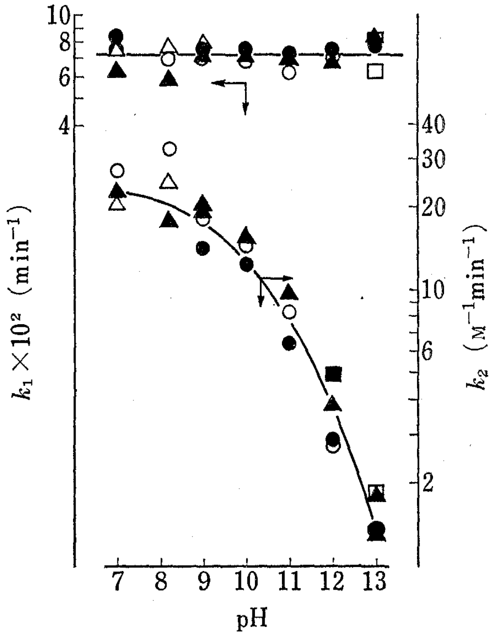


Fig. 9. pH-log k Profile for the Hydrolysis and Formation of Sulpyrine of the Various Initial Concentrations at 40°

●: 30, △: 150, ○: 750, ▲: 1500, □: 4500 μg/ml

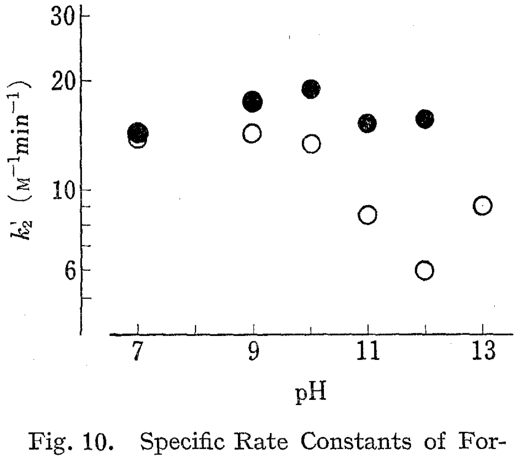


Fig. 10. Specific Rate Constants of Formation of Sulpyrine at Various pH

The specific rate constants were corrected in terms of the ratio of undissociated OMS to the total determined by iodometric titration (○) and by spectrophotometry after separation with ion exchange resins (●).

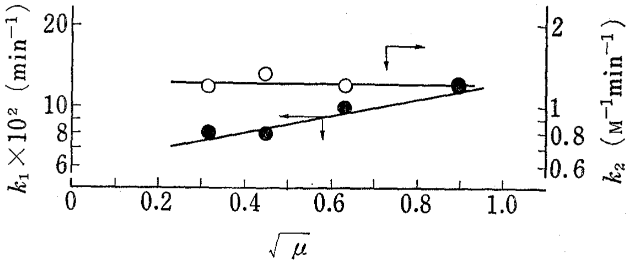


Fig. 11. Effect of Ionic Strength on the Hydrolysis and Formation of Sulpyrine at pH 13 at 40°

●: k_1 ○: k_2

The effect of ionic strength is shown in Fig. 11. For the apparent hydrolysis rate constant (k_1), the slope of the line was positive but much less than 1 (approximately 0.3), and this fact suggests that spontaneous hydrolysis of sulpyrine anion to MAA and/or HMA occurrs predominantly. This may support the consideration that hydroxy ion does not catalyze hydrolysis of sulpyrine as shown in Fig. 9. Furthermore, the apparent formation rate constant of sulpyrine (k_2) was not affected in terms of ionic strength, and this suggests that the neutral form of MAA reacts with OMS anion, or the neutral form of HMA reacts with bisulfite anion, and produces sulpyrine. This agrees with the pH-independency of k_2' shown in Fig. 10. Therefore the pH-dependency of the apparent formation rate constant of sulpyrine, k_2, is ascribable to the dissociation o OMS. This fact suggests that sulpyrine is formed directly by the reaction of MAA and OMS, and the pathway *via* HMA may be negligible.

The effect of temperature on the apparent rate constants (k_1 and k_2) was studied and the Arrhenius plots are shown in Fig. 12. The values of $\Delta H^{\neq}$ corresponding to k_1 and k_2 were 21.6 and 16.0 kcal/mol respectively.

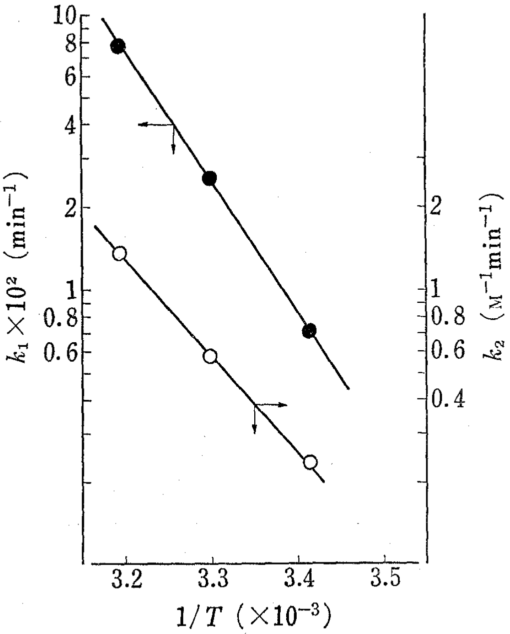


Fig. 12. Arrhenius Plots of Apparent Rate Constants for the Hydrolysis and Formation of Sulpyrine at pH 13

●: k_1 ○: k_2