Stability of Sulpyrine. II.[1] Hydrolysis in Acid Solution[2]

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Hydrolysis of sulpyrine was studied in acid region. Sulpyrine and the hydrolyzed products were directly determined by NMR spectroscopy.

In the pH range between 3 and 7, sulpyrine was found to be in an equilibrium with the hydrolyzed products, mainly 4-methylaminoantipyrine (MAA). A decreases in the rate of hydrolysis and formation of sulpyrine was observed with a decrease in pH value.

In the pH range below 3,4-(N-hydroxymethyl-N-methyl)aminoantipyrine (HMA) was detected as a hydrolyzed product in addition to MAA. An equilibrium was attained between sulpyrine and HMA immediately after sulpyrine was dissolved, and the ratio of the equilibrated amount of HMA to that of sulpyrine was found to increase with a decrease in pH value. Successively the first order hydrolysis of sulpyrine and/or HMA was observed and no equilibrium was attained between sulpyrine and the hydrolyzed products, which indicated that the reverse reaction was negligible.

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

In the previous study,[1] the hydrolysis of sulpyrine in alkaline region has been investigated and sulpyrine has been found to be in an equilibrium with the hydrolyzed products, 4-methyl-aminoantipyrine (MAA), 4,4'-[methylenebis(methylimino)]diantipyrine (Bis), and 4-(N-hy-droxymethyl-N-methyl)aminoantipyrine (HMA), in the range of pH 7 to 13.

In acid region, however, a rapid hydrolysis is observed at the early stage of the reaction and no equilibrium is attained between sulpyrine and the hydrolyzed products. These fact suggest the difference between the mechanism of hydrolysis in acid region and that in alkaline region. In studying the release rates of substituted aniline from its methanesulfonic acid derivative, Kurono, *et al.* have reported the same phenomena, and suggested that a consecutive reaction other than direct hydrolysis of the methanesulfonic acid derivative occurrs in acid region.[4]

However, no detailed studies of the kinetics of sulpyrine hydrolysis in acid region have been reported previously. The purpose of this study is to detect the hydrolyzed products quantitatively and determine the kinetics of hydrolysis of sulpyrine in acid region.

Experimental

Materials——Materials used were the same described previously.[1]

Kinetic Measurements by Means of Extraction Method——The kinetic study was made in buffers of 0.05M phthalate or 0.05M borate system at 40° and 5°. The ionic strength of the experimental solution was adjusted to 0.2M with KCl. A 1.0 ml of the experimental solution was pipetted into a 4.0 ml of pH 7.4 phosphate buffer in a 12 ml volumetric test tube, and shaken vigorously with a 5 ml of chloroform in order to extract the hydrolyzed products. Remaining sulpyrine in aqueous phase was determined by means of the method established by Kato, *et al.*, which was described in the earlier paper.[1]

1) Part I: S. Yoshioka, H. Ogata, T. Shibazaki, and T. Inoue, *Chem. Pharm. Bull.* (Tokyo), **25**, 475 (1977).
2) A part of this work was presented at the 95th Annual Meeting of the Pharmaceutical Society of Japan, Nishinomiya, April, 1975.
3) Location: *18-1, Kamiyoga 1-chome, Setagaya, Tokyo 158, Japan.*
4) Y. Kurono, K. Ikeda, and K. Uekama, *Chem. Pharm. Bull.* (Tokyo), **23**, 409 (1975).

Kinetic Measurements by Means of NMR Spectroscopy——The kinetic study by NMR spectroscopy was carried out at 26°. Sulpyrine was dissolved in deuterium oxide, and pH value was adjusted with deuterium chloride, and then the NMR spectra were measured at appropriate intervals.

The mole percent of each species in the experimental solution ([sulpyrine]: [MAA]: [HMA]) was determined on the basis of a following consideration.

Since the singlet at 6.94 τ is assigned to $-CH_3$ of sulpyrine and that at 7.02 τ is assigned to $-CH_3$ of MAA and HMA (Table I), the ratio of the amount of sulpyrine to the total amount of MAA and HMA is calculated by equation (1).

$$\frac{[\text{sulpyrine}]}{[\text{MAA}]+[\text{HMA}]} = \frac{I_{(6.94)}}{I_{(7.02)}} \tag{1}$$

where I represents the integral of the singlet at τ value shown in parentheses.

In the pH range below 7, hydroxymethanesulfonate (OMS) does not dissociate into bisulfite and formaldehyde as reported in the previous paper.[1] When sulpyrine hydrolyzes to MAA directly, equimolar OMS is formed. When sulpyrine hydrolyzes to MAA *via* HMA, bisulfite formed in the hydrolysis of sulpyrine to HMA reacts with formaldehyde formed in the hydrolysis of HMA to MAA, and forms OMS immediately. It is confirmed that no bisulfite eliminates from the system, because an equilibrium among sulpyrine, MAA and OMS is attained above pH 3, and an equilibrium among sulpyrine, HMA and bisulfite is attained below pH 3. Therefore the amount of OMS is equal to that of MAA and it is reasonable to determine MAA by the signals of OMS.

It is impossible to determine the integral of the singlet at 5.74 τ and that at 5.78 τ separately, which are assigned to $-CH_2-$ of OMS and sulpyrine respectively, so the total integral of these singlets is considered to represent the total amount of sulpyrine and MAA. Then the ratio of the amount of sulpyrine to the total amount of sulpyrine and MAA is calculated by equation (2),

$$\frac{[\text{sulpyrine}]}{[\text{sulpyrine}]+[\text{MAA}]} = \frac{\frac{1}{3}\times I_{(6.94)}}{\frac{1}{2}\times\{I_{(5.74)}+I_{(5.78)}\}} \tag{2}$$

where the integral is divided by 3 or 2 in order to obtain the integral value per proton, because the singlet at 6.94 τ represents three protons of $-CH_3$ of sulpyrine while those at 5.74 and 5.78 τ represent two protons of $-CH_2-$ of OMS and sulpyrine respectively.

Finally the mole percent of each species ([sulpyrine]: [MAA]: [HMA]) is calculated from equations (1) and (2).

Results and Discussion

Equilibrium between Sulpyrine and HMA

The hydrolysis and formation of sulpyrine in acid region was determined by means of the extraction procedure, and shown in Fig. 1. Figure. 1-a) shows the time course of the hydrolysis of sulpyrine at pH 1 and 3, and indicates that a part of sulpyrine hydrolyzes immediately after sulpyrine is dissolved and the percent of residual sulpyrine in the initial stage depends on the initial concentration of sulpyrine. For the formation of sulpyrine from MAA and hydroxymethanesulfonate (OMS), as shown in Fig. 1-b), the extent of the formation decreases with a decrease in pH value, and no sulpyrine is formed at pH 1.

In order to examine the rapid hydrolysis of sulpyrine at the early stage in detail, the kinetics of hydrolysis was studied at the lower temperature (5°), and shown in Fig. 2-a). The reaction seemed to reach apparently to an equilibrium in the higher initial concentration of sulpyrine. If sulpyrine hydrolyzes according to the reversible pattern described in the previous paper,[1] the apparent rate constants of hydrolysis and formation of sulpyrine, k_1 and k_2, can be calculated in terms of equations (3) and (4),

$$\ln\frac{S_o{}^2-S_eS}{(S-S_e)S_o} = k_1\frac{S_o+S_e}{S_o-S_e}t \tag{3}$$

$$k_2 = k_1\frac{S_e}{(S_o-S_e)^2} \tag{4}$$

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. As shown in Fig. 2-b), how-
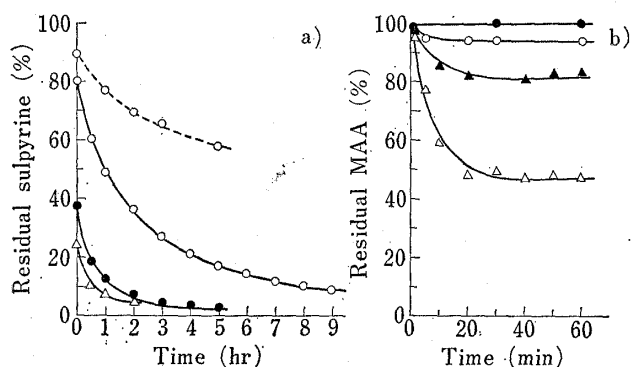
Fig. 1. Hydrolysis and Formation of Sulpyrine at 40° determined by the Extraction Procedure

a) hydrolysis of sulpyrine in various initial concentrations at pH 1 (——) and pH 3 (-----)
  ○: 15 mg/ml, ●: 1.5 mg/ml, △: 750 μg/ml
b) formation of sulpyrine from MAA and OMS ʼ1: 100 mole ratio) at various pH
  ●:pH 1, ○: pH 3, ▲: pH 4, △: pH5
initial concentration of MAA: 26 μg/ml

$$\text{aniline methanesulfonate} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{aniline} + HOCH_2SO_3^-$$

$$\downarrow k_2$$

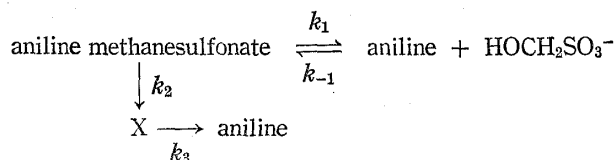$$X \xrightarrow[k_3]{} \text{aniline}$$

Chart 1

ever, the results did not satisfy equation (3), because of the rapid hydrolysis at the early stage and the extreme decrease of the rate in the latter stage. These facts suggest that the pattern of hydrolysis of sulpyrine in acid region is different from that in alkaline region.

Kurono, *et al.* have reported the same observation in the hydrolysis of methanesulfonic acid derivative of substituted aniline.[4] They determined the released substituted aniline in terms of the same extraction procedure as used in the present experiment, and obtained the same results as observed in the case of sulpyrine. They have explained these phenomena in terms of the concecutive process *via* an unknown product in addition to a direct hydrolysis and proposed the passways shown in Chart 1, where k_{-1} was considered negligible in strong acid. However, this chart may not offer any reasonable explanations for the concentration-dependency of the rate of hydrolysis which was observed in both cases of methanesulfonic acid derivative of substituted aniline and sulpyrine. If methanesulfonic derivative hydrolyzes according to Chart 1, the rate should be independent of the concentration,
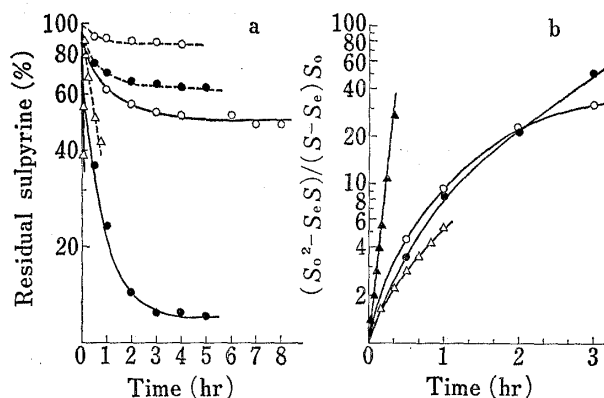

Fig. 2. Hydrolysis of Sulpyrine determined by the Extraction Procedure

a) hydrolysis of sulpyrine in various initial concentrations at pH 1 (——) and pH 3 (-----) at 5°
  ○: 750 μg/ml, ●: 150 μg/ml, △: 30 μg/m
b) a plot for equation (1) at various pH
  ●: pH 1, 5°, 750 μg/ml, ○: pH 3, 5°, 150 μg/ml,
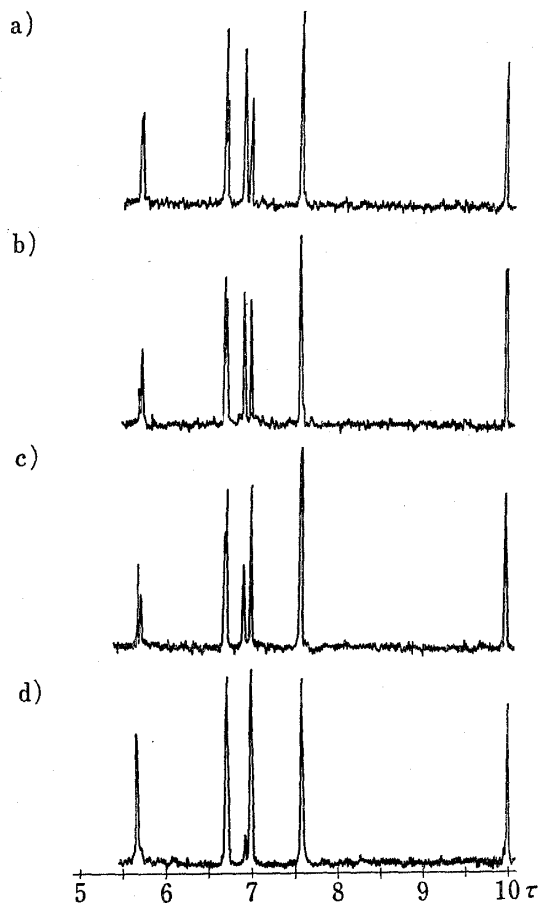 △: pH 5, 40°, 1500 μg/ml, ▲: pH9, 40°, 1500 μg/ml

Fig. 3. NMR Spectra of Aqueous Solution of Sulpyrine at pH 0.8

initial concentration of sulpyrine: 2.85×10^{-2}M
  a) 15 minutes after dissolved
  b) 100 minutes after dissolved
  c) 10 hours after dissolved
  d) 30 hours after dissolved

TABLE I. The Singlets in NMR Spectra[a] and Their Assignment

τ	Sulpyrine (pH 0.8)			MAA (pH 1)	Bis (pH 1)	OMS (pH 1)
	Change with time	Assignment				
5.74	increase[b]	OMS	$-CH_2-$			5.70
5.78	decrease	sulpyrine	$-CH_2-$			
6.73	decrease	sulpyrine	N_2-CH_3			
6.74	increase	MAA and HMA	N_2-CH_3	6.72	6.72	
6.94	decrease	sulpyrine	$4N-CH_3$			
7.02	increase	MAA and HMA	$4N-CH_3$	7.00	7.00	
7.60	no change	sulpyrine, MAA and HMA	C_3-CH_3	7.59	7.59	

a) measured in concentration 2.85×10^{-2} M at 26°
b) No peak was observed at initial stage

because Chart 1 involves a series of first order reactions and no reversible reaction occurs in strong acid. In regard to the separation method of the hydrolyzed products it has not been clarified in their study whether the unknown product X was extracted with chloroform as well as substituted aniline in the extraction procedure. Furthermore the extraction procedure which involves dilution of the reaction solution with pH 7.4 buffer seems to be inadequate to separate the hydrolyzed products from the solution, because the reaction is very rapid at the initial stage and the rate depends on the concentration.

Therefore a direct determination of sulpyrine was tried by NMR spectroscopy. Figure. 3 shows the change of NMR spectra of aqueous solution of sulpyrine at pH 0.8 with time. Various singlets were detected in the spectra, and are summarized in Table I. Whereas the singlets at 5.78, 6.73, and 6.94 τ decreased and those at 5.74, 6.74, and 7.02 τ increased with time, the singlet at 7.60 τ did not change with time. The assignment of each singlet shown in Table I is based on the consideration discussed later. The singlets observed in each spectrum of MAA, Bis and OMS are also summarized in Table I. The spectrum of Bis in 0.1 N DCl agrees with that of MAA in 0.1 N DCl, which indicates that Bis completely hydrolyzes to MAA and formaldehyde in strong acid, and is negligible as a hydrolyzed product of sulpyrine in this pH range. Since the ratios of the integrals of the singlets at 5.78, 6.73, and 6.94 τ to the total integrals of all singlets decrease with time as shown in Fig. 3, these singlets may be due to the protons of $-CH_2-$ and $-CH_3$ of sulpyrine. The singlets at 6.74 and 7.02 τ increasing with time may be assigned to the hydrolyzed products. The singlet at 7.60 may be due to both of sulpyrine and the hydrolyzed products because the ratio of its integral to the total does not change with time. The singlet at 5.74 τ is not detected at the initial stage, but appears and increases with time, which is able to be assigned to OMS because its position is compatible with that of OMS. Since the formation of OMS means that of MAA, the formation of MAA as a hydrolyzed product at pH 0.8 is confirmed. Furthermore the formation of the product other than MAA is also suggested on the basis of the fact that the singlets at 6.74 and 7.02 τ which represent the protons of the hydrolyzed products have appeared before the singlet of OMS appears.

In regard to the hydrolyzed products, the presence of MAA, HMA and Bis in alkaline region has been detected,[1] and Kawamura and Negoro have detected MAA and HMA in acid region.[5] From the NMR spectra it is possible to presume that the product other than MAA is HMA. It may be presumed that a part of sulpyrine hydrolyzes to HMA and bisulfite immediately after dissolved in 0.1 N DCl and gives the singlets at 6.74 and 7.02 τ. Then MAA is slowly formed and increases the ratio of the singlets at 6.74 and 7.02 τ. In order to confirm this presumption, the mole ratio of the unknown product was determined immediately after sulpyrine was dissolved to give various concentrations, and is shown in Table II.

5) K. Kawamura and Y. Negoro, Yakugaku Zasshi, 88, 554 (1968).

TABLE II. Effect of Concentration on Equilibrium
between Sulpyrine and HMA at pH 0.8

Total concentration ($\times 10^{-2}$M)	Mole ratio of sulpyrine	Mole ratio of HMA	[NaHSO$_3$][HMA]/ [sulpyrine] ($\times 10^{-3}$M)
2.85	0.64	0.36	5.7
4.27	0.76	0.24	6.4
5.69	0.83	0.17	5.2

TABLE III. Effect of Addition of NaHSO$_3$ on Equilibrium
between Sulpyrine and HMA at pH 0.8

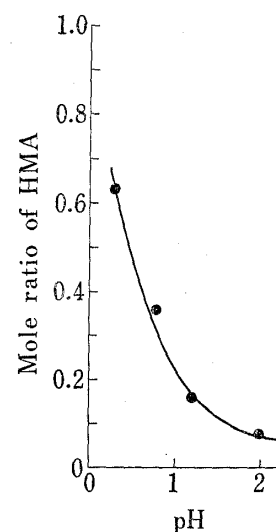| Concentration of NaHSO$_3$[a] ($\times 10^{-2}$M)	Mole ratio of sulpyrine	Mole ratio of HMA	[NaHSO$_3$][HMA]/ [sulpyrine] ($\times 10^{-3}$M)
0	0.64	0.36	5.7
1.42	0.76	0.24	6.6
2.85	0.83	0.17	6.8

a) added to 2.85×10^{-2} M sulpyrine solution

Fig. 4. Effect of pH on Equilibrium between Sulpyrine and HMA

initial concentration of sulpyrine: 2.85×10^{-2}M

As the concentration increases the mole ratio of the product decreases. Furthermore as shown in Table III, the addition of bisulfite to sulpyrine solution decreases the mole ratio of the product. The equilibrium constants were calculated on the basis of the presumption that the unknown product is HMA, and that sulpyrine and HMA reach immediately to an equilibrium. As shown in Tables II and III the equilibrium constants are almost same. These results may suggest that this presumption and the assignment of the singlets shown in Table I are valid. Kawamura and Negoro have detected the singlet which represents the protons of -CH$_2$- of HMA in the spectra of sulpyrine in 0.5 N D$_2$SO$_4$ including iodine.[5] In this study, however, the singlets of -CH$_2$- of HMA could not be detected. It might be covered with the signal of H$_2$O because the NMR spectra were measured in the lowest concentration to be detected by NMR, where sulpyrine hydrolyzes largely. Except the singlet of -CH$_2$- of HMA, the τ values assigned to sulpyrine and the hydrolyzed products are approximately in accord with those in the literature.[5]

Figure 4 shows the effect of pH on the equilibrium between sulpyrine and HMA. It is shown that the equilibrated amount of HMA increases with a decrease in pH value, and HMA is negligible above pH 3.

Hydrolysis and Formation Rate of Sulpyrine

Figure 5 shows the time course of hydrolysis of sulpyrine at pH 0.8 determined by NMR spectroscopy on the basis of the assignment shown in Table I.

Figure 6 shows the effect of pH on hydrolysis of sulpyrine. At pH 3.2, 5.7, and 6.9 an equilibrium seems to be attained between sulpyrine and MAA after 2 or 3 hours, whereas at pH 0.8 the apparent hydrolysis rate of sulpyrine and/or HMA is of first order until 10 hours and no equilibrium is attained, which indicates that formation of sulpyrine or HMA is negligible at pH 0.8. The amount of HMA shown in Fig. 6 indicated a large scatter due to the determination method, but the rate of the decrease of HMA almost agreed with that of sulpyrine at pH 0.8.

By means of the NMR spectroscopy, the rapid hydrolysis of sulpyrine at the initial stage at pH 1 was observed as well as by means of the extraction procedure, and the successive first order hydrolysis at pH 1 and the equilibrium between sulpyrine and MAA above pH 3 were also observed, which were not observed by means of the extraction procedure. It may be ex-
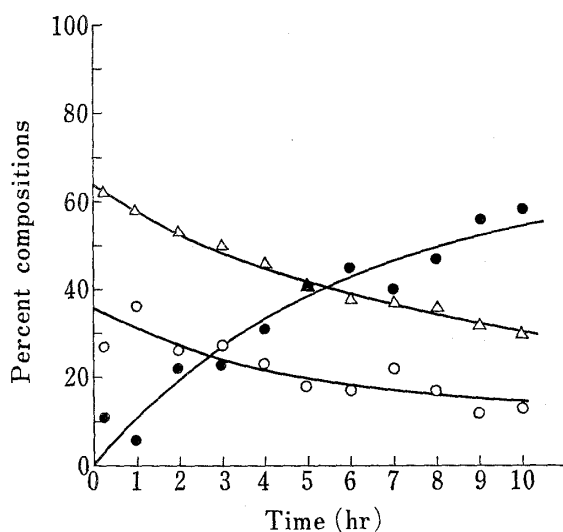
Fig. 5.   Hydrolysis of Sulpyrine at pH 0.8
determined by NMR

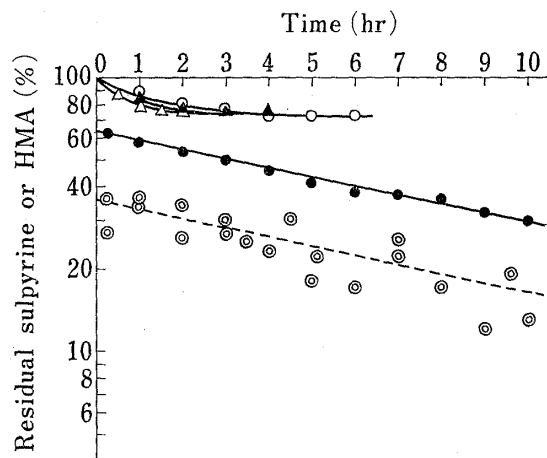initial concentration of sulpyrine: 2.85×10^{-2}M
△: sulpyrine, ○: HMA, ●: MAA

Fig. 6.   Hydrolysis of Sulpyrine at Various pH

initial concentration of sulpyrine: 2.85×10^{-2}M
sulpyrine ●: pH 0.8, ○: pH 3.2, ▲: pH 5.7, △: pH 6.9
HMA ◎: pH 0.8

plained by the assumption that the dilution of the experimental solution with pH 7.4 buffer in the extraction procedure shifts the equilibrium between sulpyrine and HMA, or that between sulpyrine and MAA.

The equilibrium observed at pH 3.2, 5.7, and 6.9 satisfies equation (3) and the apparent rate constants of hydrolysis and formation of sulpyrine, k_1 and k_2, can be calculated by equations (3) and (4) respectively.   Figure 7 shows the pH profiles of the rate constants.   The rate constants above pH 7 were estimated by extrapolating the specific rate constants in Arrhenius plots reported in the previous paper.[1]   In both cases the rate constants decrease remarkably below pH 7.

Possible Pathways of Hydrolysis of Sulpyrine

The possible pathways of hydrolysis of sulpyrine in acid region seems to be represented in terms of Chart. 2.   But it can not be clarified whether sulpyrine hydrolyzes to MAA
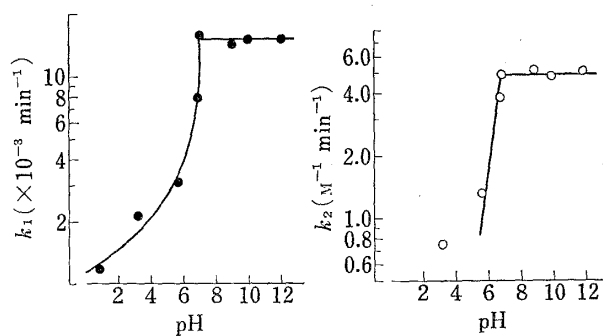

Fig. 7.   pH-log k Profile for Hydrolysis and
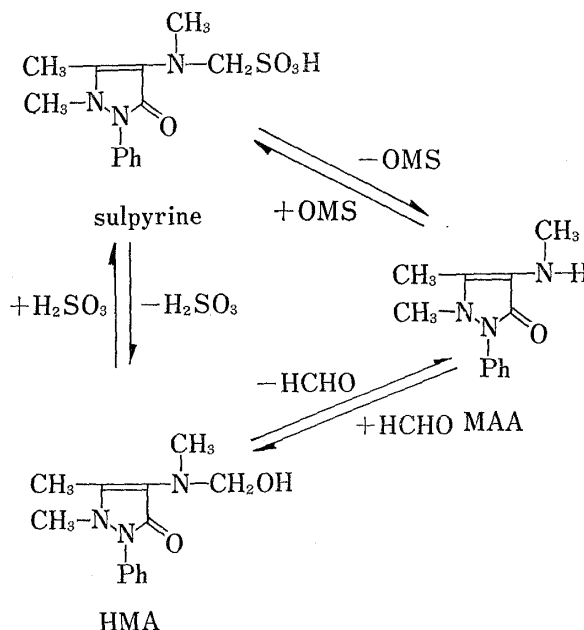Formation of Sulpyrine at 26°

●: k_1 ○: k_2

HMA

Chart 2

directly, hydrolyzes to MAA *via* HMA, or hydrolyzes on the both pathways, because the equilibrium between sulpyrine and HMA is attained very rapidly. In neutral and alkaline range, however, an equilibrium between MAA and HMA was found to be attained rapidly as described in the previous paper.[1] From this fact it is reasonable to consider that the equilibrium between MAA and HMA is possible even in acid range.

In the pH range below 3, HMA is formed immediately after sulpyrine is dissolved, and an equilibrium is attained between them. Then sulpyrine and/or HMA hydrolyze to MAA slowly. The rate is of first order, and reverse reaction is negligible.

Above pH 3, the amount of HMA is very small and negligible as a hydrolyzed product by means of the direct NMR spectroscopy, and sulpyrine may hydrolyze reversibly to MAA and OMS and an equilibrium may be attained between sulpyrine and MAA. Below pH 7 the apparent rate constants of hydrolysis and formation of sulpyrine remarkably decrease. In the previous paper,[1] it was suggested that sulpyrine hydrolyzes in terms of an unimolecular reaction of sulpyrine anion, and sulpyrine is produced in terms of the reaction of the neutral form of MAA and OMS anion. From the suggestion the decrease of k_1 below pH 7 may be attributed to the protonation of sulpyrine. The decrease of k_2 below pH 7 may be due to the protonation of OMS and not to that of MAA, because the pK_a of MAA is approximately 4.