[Chem. Pharm. Bull. 23(2) 409—415 (1975)] UDC 547.551'269.3.03.04

Release Rates of Substituted Aniline from Its Methanesulfonic Acid Derivative in Acid Region[1)]

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(Received August 8, 1974)

Reaction of substituted aniline liberation from its methanesulfonic acid derivative (MSD) was studied in acid region. From the ultraviolet spectral changes, the extraction of liberated parental anilines by 1,2-dichloroethane, and thin-layer chromatography, it was suggested that a consecutive reaction other than direct hydrolysis of MSD occurs in acid range. Reaction scheme for the liberation of free amine was proposed and the analytical method of kinetic data was presented. The pH-profile of total decreasing rate of *p*-chloroaniline MSD had a slope of −1 below pH 3.5 and it was pH-independent above pH 5.0. The pH-profile of the direct hydrolysis around pH 2.0—1.0 had a slope steeper than −1 and it was −1 below pH 1.0, while the estimated profile of the indirect pathway had a slope of −1 and it became pH-independent below pH 1.0. For the explanation of these pH-profiles the protonation of MSD was considered and its dissociation constant was estimated.

In the previous studies[3—5)] the release of substituted aniline from its methanesulfonic acid derivatives (MSD) has been investigated at neutral region and found to be pH-independent in the range of pH 4.0—10.0. In the lower pH region, however, the reaction rate was found to increase in a complexed manner and the detailed study has not been carried out. From the therapeutic point of view the change in acidic conditions is of importance because MSD of drugs administered orally encounters with strongly acid gastric juice and this may affect the absorption, so that the availability of the parental drug. For the detailed study on the rapid change in lower pH region, MSD of *p*-chloroaniline (PCA) whose reaction rate is convenient to follow was used.

Experimental

Preparation of Substituted Aniline MSD——Synthesis was followed to the method reported by Neelakantan, *et al.*[6)] and the purity of the derivatives was determined by the method same to that in the previous paper.[4)]

Kinetic Procedure——Buffer systems used were as follows; 1/15M phosphate (pH 5.5—8.1), 1/5M acetate (pH 2.8—5.0), 1/5M HCl—1/5M KCl (pH 1.5—2.5) and 1/20M to 1/2M H_2SO_4 (below pH 1.5). The procedure to determine rate constant from the change in ultraviolet (UV) spectra at low concentration of MSD (about 5×10^{-5}M) was same to that in the previous studies.[4)]

The extraction procedure of released aniline with 1,2-dichloroethane (DCE) was also used for kinetic study in relatively higher concentration of MSD; *i.e.* from 1×10^{-3} to 2×10^{-3}M. At appropriate intervals, 1 ml of the sample solution was withdrawn and diluted to 25 ml with pH 7.4 phosphate buffer. 5 ml of the diluted solution was extracted with 5 ml of DCE and the DCE phase was subjected to UV spectrophotometry. That free aniline derivatives can be extracted quantitatively in this procedure was ascertained. All kinetic study was carried out at 25°.

1) This report constitutes Part VII of the studies entitled "Methanesulfonic Acid Derivative of Drug," where Part VI is in: Y. Kurono, K. Ikeda, and K. Uekama, *Chem.Pharm. Bull.* (Tokyo), **23**, 340 (1975).
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3) K. Ikeda, K. Miyata, T. Iwata, F. Kawata, and K. Kurome, *Chem. Pharm. Bull.* (Tokyo), **18**, 440 (1970).
4) K. Ikeda, Y. Kurono, and T. Tukamoto, *Chem. Pharm. Bull.* (Tokyo), **20**, 863 (1972).
5) K. Ikeda and Y. Kurono, *Chem. Pharm. Bull.* (Tokyo), **21**, 1198 (1973).
6) L. Neelakantan and W.H. Hartung, *J. Org. Chem.*, **24**, 1943 (1959).

Thin-Layer Chromatography——The acid sample solution of MSD (about 2×10^{-2}M) was diluted with pH 7.4 phosphate buffer and the diluted solution (about 1×10^{-3}M) was spotted on Spotfilm® (silicagel layer containing fluorescent agent) of Tokyo Kasei Co. Ltd. The developing solvents were DCE and *n*-BuOH–aceic acid (4: 1) and the spots were detected by UV light (2536 Å).

Results and Discussion

Release of PCA from Its MSD in Acid Region

The release of PCA from its MSD in acid region was found to be composed of two distinctly different rate processes by the extraction study. Figure 1 shows the time course of PCA liberation at several acid pH, where the abscissa time scale is changed at 60 minutes to show the whole change of the reaction. The ordinate is the concentration of the extracted PCA. It shows that the more acid the medium the faster the first and also the second processes. The apparent resess of PCA liberation at the initial stage can not be attributed to the reversible process of the hydrolysis of MSD. Since formation rate of MSD from substituted aniline and hydroxymethanesulfonate is proportional to free aniline concentration as has been reported in the previous study,[3] the hydrolysis rate is practically irreversible at this pH range considering pK_a value of *p*-chloroanilinium ion, 4.0.

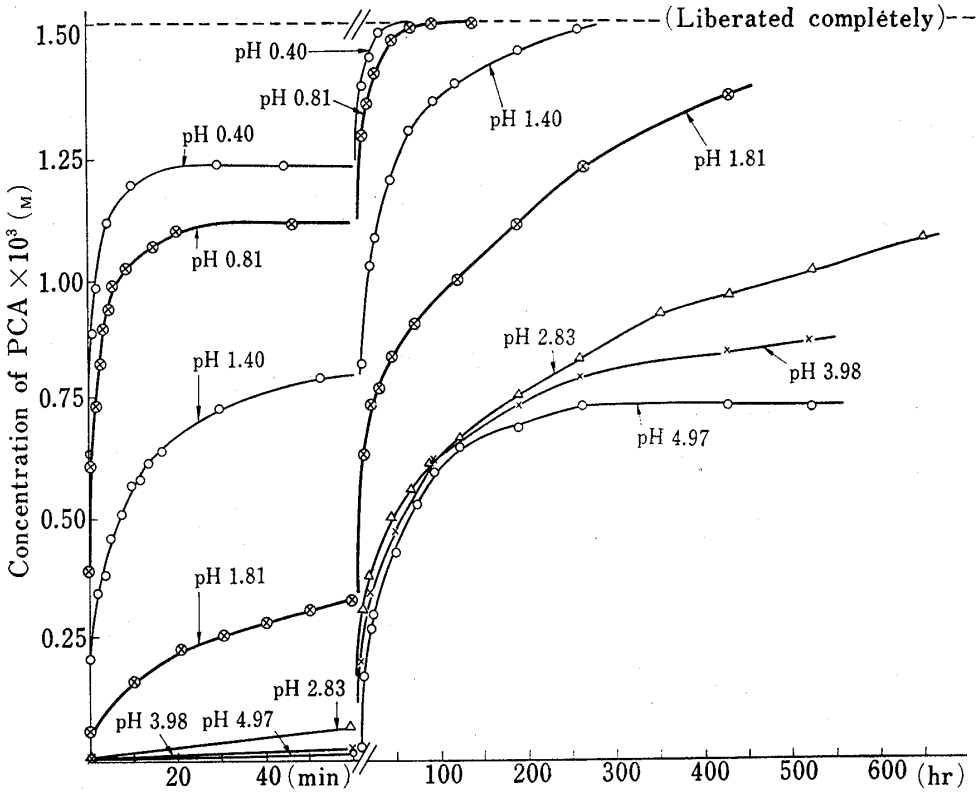

Fig. 1. Time Courses of PCA Liberation from PCA MSD in Acidic Conditions at 25°

The UV spectral changes in acid region also show two steps which occur simultaneously with the stepwise liberation of free amine. For an instance, Fig. 2 shows the first step change at pH 1.40 where two isobestic points at 223 and 210 nm are held within 30 minutes. The spectrum at 80 minutes shows some departure from the isobestic point at 210 nm which may be ascribed to the initiation of the secondary change. Figure 3 is the succeeding UV change observed at longer time intervals, where characteristic minimum is observed at 205 nm. The effect of buffer species and their concentration was not significant in both of UV change and the extraction results.

Figures 4, 5, and 6 are thin-layer chromatograms of the reaction solutions at various conditions. Figure 4 shows the change at pH 0.81 and 25°, where at 3 minutes and 3 hours three spots assigned to PCA, MSD of PCA, and unknown product were observed using *n*-butanol–acetic acid (4: 1) as developing solvent. At the final stage only PCA spot was detectable. Figure 5 shows the TLC at various pH after 90 hours. Only below pH 3.41 the spot of unknown product was observed.

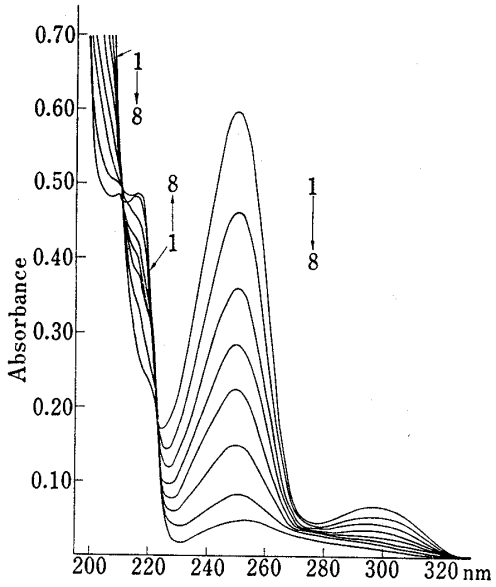

Fig. 2. UV Spectral Changes of 5.6×10^{-5}M PCA MSD Solution with Time at pH 1.40 and 25°

Time in minutes after reaction was initiated: 1: 0, 2: 3, 3: 6, 4: 9, 5: 12, 6: 21, 7: 30, 8: 80.

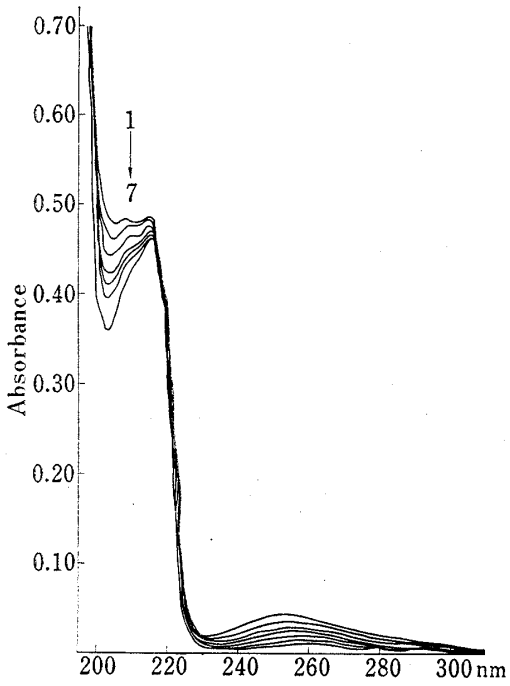

Fig. 3. UV Spectral Changes of 5.6×10^{-5}M PCA MSD Solution with Time at pH 1.40 and 25°

Time in hours after reaction was initiated: 1: 1.33, 2: 3.75, 3: 18.50, 4: 42.50, 5: 68.00, 6: 92.58, 7: PCA solution equivalent to PCA MSD concentration.

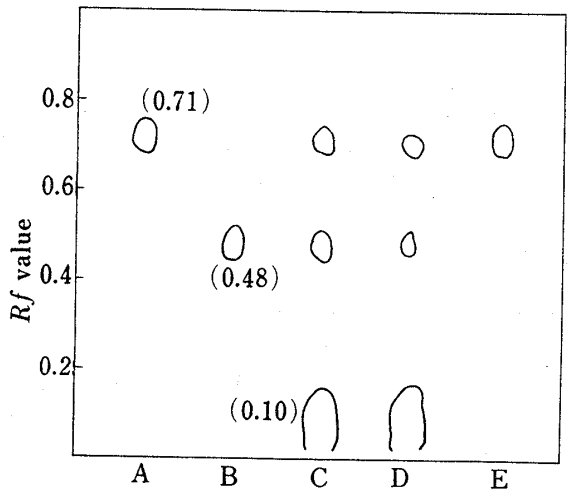

Fig. 4. Thin-Layer Chromatogram of PCA MSD Solution at Appropriate Intervals, pH 0.81 and 25°; Solvent, *n*-BuOH–Acetic Acid (4: 1)

A: PCA, B: PCA MSD, C: 3 minutes, D: 3 hours, E: 100 hours after reaction was initiated.

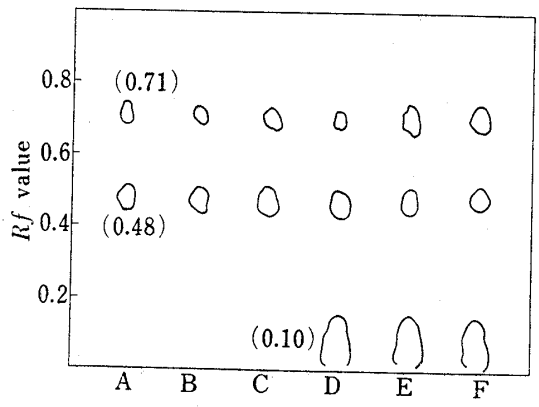

Fig. 5. Thin-Layer Chromatogram of PCA MSD Solution of Various pH at about 90 Hours after Initiation of Reaction; Solvent, *n*-BuOH–Acetic Acid (4: 1)

A: pH 7.43, B: pH 4.97, C: pH 3.98, D: pH 3.41, E: pH 2.83, F: pH 1.81

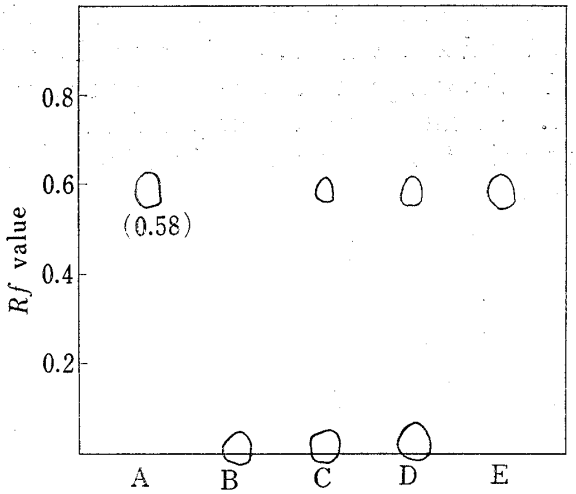

Fig. 6. Thin-Layer Chromatogram of PCA MSD Solution at Appropriate Intervals, pH 0.81 and 25°; Solvent, DCE

A: PCA, B: PCA MSD, C: 3 minutes, D: 3 hours, E: 100 hours after reaction was initiated.

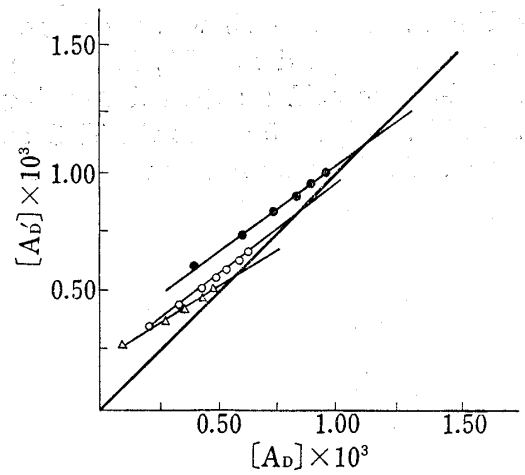

Fig. 7. Determination of $[A_D]_\infty$ by Modified Guggenheim Analysis

$$[A'_D]=e^{-k_1 t}[A_D]+\frac{k_1}{k}[A_0](1-e^{-k_1 t})$$

●: pH 0.81, ○: pH 1.40, △: pH 1.81

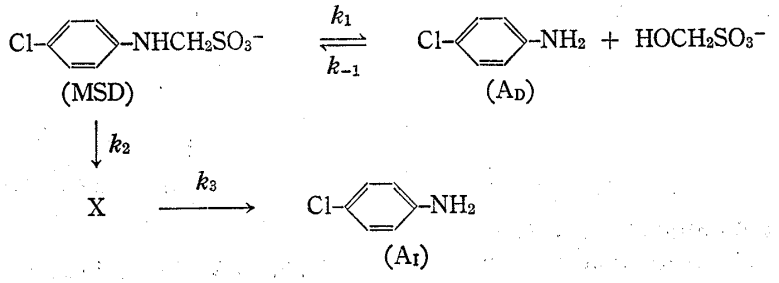

Chart 1

Figure 6 is the TLC using DCE as the developping solvent, where only the spots for PCA and MSD of PCA were detectable. This may prove that by the extraction with DCE only free PCA is extracted. Rate constants obtained by extraction procedure were consistent with those from UV change.

Possible Reaction Pathway in Acid Range

From the above observations Chart 1 may be rationally proposed as the reaction pathway. A_D and A_I denote the free PCA liberated by the direct hydrolysis and that by the concecutive process *via* unknown intermediate X, respectively. At acid conditions the reverse reaction of the direct hydrolysis may be disregarded as mentioned above. Although the chemical structure of unknown product X can not be determined at present, the possible structure may be the carbinolamine, *p*-Cl-C_6H_4-$NHCH_2OH$, or Schiff's base, *p*-Cl-C_6H_4-$N{=}CH_2$. These compounds may be produced by bisulfite ion spritting from MSD, which was suggested by S. Ono[7] and K. Kawamura[8] in the studies of iodometry of sulpyrin.

Estimation of Rate Constant

From the data such as in Fig. 1, k_1 and k_2 can not be determined directly and the rate constant for the first step change may be composed of k_1 and k_2. For the initial change such as at pH 0.40 in Fig. 1 the first order reaction rate, which is $k=k_1+k_2$ from Chart 1, can be obtained directly, because apparent end point of the first step can be estimated. For the reaction where the secondary reaction occurs continuously without apparent recess period

7) S. Ono, R. Onishi, and K. Kawamura, *Yakugaku Zasshi*, **86**, 11 (1966).
8) K. Kawamura and Y. Negoro, *Yakugaku Zasshi*, **88**, 554 (1968).

Guggenheim method,[9] which is pertinent to the first order process where end point is not known, was applied. Figure 7 shows the modified Guggenheim analysis on the data at pH 0.81, 1.40, and 1.81 shown in Fig. 1. The total amount of PCA liberated through direct hydrolysis, $[A_D]_\infty$, can be estimated from the cross point of the line of the plots and that of $[A_D']=[A_D]$, in which $[A_D]$ and $[A_D']$ are the concentrations of PCA at time t and $t+\Delta t$ respectively and Δt is a constant time increment. For the parallel first order reactions supposed as Chart 1 the rat ioof $[A_D]_\infty$ to the initial concentration of MSD, $[A_0]$, can be expressed as

$$\frac{[A_D]_\infty}{[A_0]} = \frac{k_1}{k} = \frac{k_1}{k_1+k_2} \qquad (1)$$

As the value $[A_D]_\infty$ and k can be obtained by Guggenheim analysis and $[A_0]$ is known, k can be divided into k_1 and k_2. The first order value of k_3 can be directly estimated below pH 2.0 from the final stage curve in Fig. 1.

From pH 2.0 to 4.0 the stepwise process becomes obscure, *i.e.* the initial PCA liberation is small and irresponsible for the accurate kinetic analysis and the very slow secondary change succeeds with obscure inflection as seen in Fig. 1. Furthermore reverse reaction, k_{-1}, would not be negligible in these conditions. However even in this pH region reaction rate constant, $k=k_1+k_2$, can be determined from the spectral change which intensively occurs with the decrease of MSD as seen in Fig. 2.

Above pH 5.0 the UV spectral change is simply first order because the initial concentration of MSD is as low as about 5×10^{-5}M and the reverse reaction is negligible. When the initial concentration of MSD was about 2×10^{-3}M and the reaction was followed by the extraction procedure, the reverse reaction of the hydrolysis became significant. As will be shown later, the reaction rate determined by the UV method agreed with that obtained from the extraction procedure assuming the reversible process. Above pH 5.0 the reaction rate k was pH-independent as has been reported previously.[3]

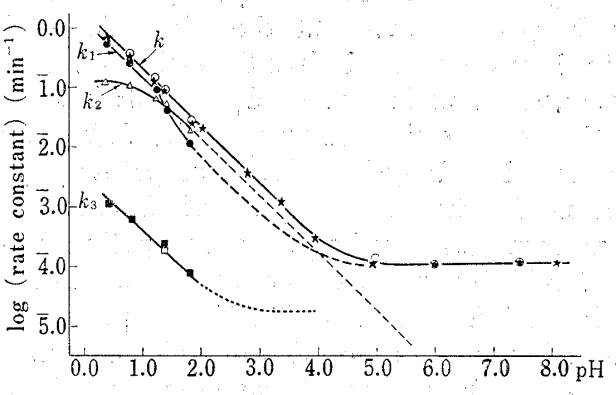

Fig. 8. The pH-Profile of Various Rates Determined and Estimated at 25°

★: k determined by UV spectral change
○: k determined from data obtained by extraction procedure
●: k_1 estimated by equation (1) from data obtained by extraction procedure
△: k_2 estimated by equation (1) from data obtained by extraction procedure
■: k_3 determined from data obtained by extraction procedure
□: k_3 determined by UV spectral change

pH-Rate Profile of the Reaction

Figure 8 shows pH-profiles of the logarithms of rate constants determined by various methods described above. In the acid conditions below pH 3.5 the pH-profile of k determined by two methods had a slope of -1. Above pH 5.0 the profile was pH-independent. Below pH 1.0 k_2 became pH-independent, while k_1 had a slope steeper than -1 around pH 1.0—2.0. The estimated k_3 was found to be about one thousand times smaller than k and the slope of the pH-profile was about -1.

To explain these pH-profiles and to connect the data below pH 2.0 to those above pH 5.0, the protonation of MSD (with the dissociation constant of K_s) and the reaction pathyways shown in Chart 2 are conceivable.

If the pH-profiles of log k_1 and log k_2 below pH 2.0 are attributed to the protonation of MSD (formation of zwitterionic MSDH), the pH-profile of log k_2 may be extrapolated linearly

9) E.A. Guggenheim, *Phil. Mag.*, 2, 538 (1926) [A.A. Frost and R.G. Pearson, "Kinetics and Mechanism," 2nd ed., Willy International Edition, New York, N. Y., 1961, p. 49].

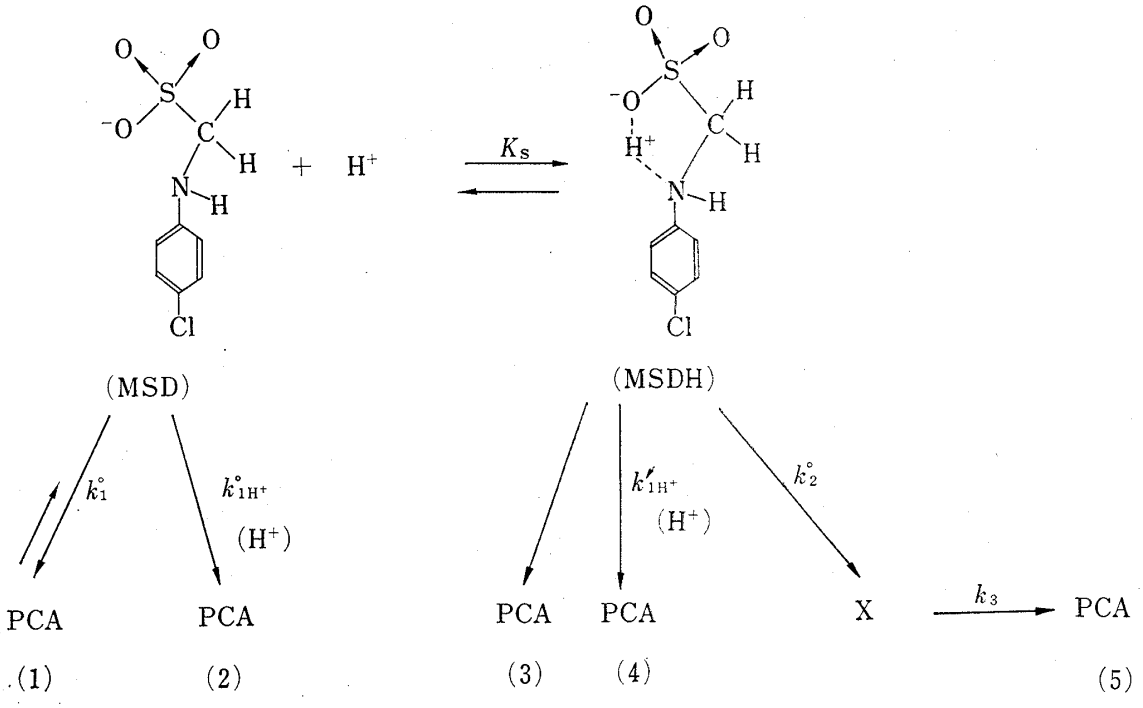

Chart 2

with slope of -1, which was represented by dashed line in Fig. 8. Moreover, the fact that the slope of log k_1 *v.s.* pH was steeper than -1 may be attributed to the hydrolysis of MSDH which is catalyzed by H^+ (pathway (4)). In the pH range 2.0—4.0 the subtraction of k_2 estimated by the extrapolation from k determined by UV method gives the estimated log k_1 profile with slope of -1 (thick dashed line in Fig. 8), which can be connected to the pH-independent profile above pH 5.0. For the interpretation of the estimated profile of log k_1 between pH 2.0—4.0 the reaction pathway (2), H^+-catalyzed hydrolysis of MSD, or pathway (3), water reaction of MSDH, are conceivable. From the structure of hydrolysis intermediate posturated in the previous study,[10] reaction pathway (2) may be prefered to pathway (3) where the intramolecular H-bonded structure of MSDH is not apt to make the proposed structure of the intermediate. The intramolecular H-bonded structure of MSDH was posturated from the observation that MSD was eluted directly without adsorption on anion-exchanger column.[4] The pathway (1) is the pH-independent hydrolysis which has been studied previously[3,4] and the hydrolysis rate is denoted by $k°_1$. Then if the reaction pathways (1), (2), (4), and (5) are responsible for the decay of total MSD, $(MSD)_T$, the following equation is obtained.

$$-\frac{d(MSD)_T}{dt} = k(MSD)_T = \left\{\frac{K_S}{K_S+[H^+]}(k°_1+k°_{1H^+}[H^+]) + \frac{[H^+]}{K_S+[H^+]}(k'_{1H^+}[H^+]+k°_2)\right\}(MSD)_T = (k_1+k_2)(MSD)_T \qquad (2)$$

The estimated values of $k°_1$, $k°_{1H^+}$, k'_{1H^+}, $k°_2$ and K_S were 1.24×10^{-4} (min^{-1}), 6.99×10^{-1} (M^{-1} min^{-1}), 2.29 (M^{-1} min^{-1}), 1.85×10^{-1} (min^{-1}), and 1.02×10^{-1} (M), respectively.

For the confirmation of the above discussion following calculations were carried out. From the kinetic differential equations according to Chart 1, $[A_D]$, $[A_I]$, and the total of them, $[A_T]$, can be expressed as;

10) Y. Kurono, K. Ikeda, and K. Uekama, *Chem. Pharm. Bull.* (Tokyo), **23**, 340 (1975).

$$[A_D] = [A_0]\frac{k_1}{k}(1-e^{-kt}) \quad (3)$$

$$[A_I] = [A_0]k_2\left\{\frac{1}{k}-\frac{1}{k-k_3}\left(e^{-k_3t}-\frac{k_3}{k}e^{-kt}\right)\right\} \quad (4)$$

$$[A_T] = [A_D] + [A_I] = [A_0]\left\{1-\frac{1}{k-k_3}\left(k_2\,e^{-k_3t}+(k_1-k_3)e^{-kt}\right)\right\} \quad (5)$$

When k, k_1, k_2, and k_3 values estimated from the linear extrapolation between pH 2.0—4.0 were applied to equation (5), the calculated $[A_T]$ *v.s.* time curve deviated from the experimental results at the advanced stage of the reaction, indicating that pH-log k_3 profile can not be extrapolated linearly above pH 2.0. Trial and error calculations revealed that when the value k_3 shown on the curved dotted line was used good agreement to experimental results was obtained. Thus it may be reasonable to assume that the rate constant, k_3, would be pH-independent above pH 2.0. This may indicate that liberation of PCA *via* intermediate X is also composed of H^+-catalyzed reaction and pH-independent reaction.

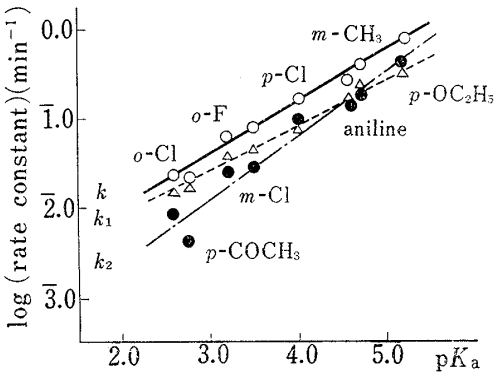

Fig. 9. Relationships between log k, k_1, and k_2 at pH 1.25 and 25° and pK_a of Corresponding Anilinium Ion

○: log k, ●: log k_1, △: log k_2

Effect of Substituents on Reaction Rates

Figure 9 shows the relationships between the logarithms of k, k_1, and k_2 at pH 1.25 and pK_a of the corresponding anilinium ion. All the rates were facilitated by the electron-donating groups on benzene ring and the electron-withdrawing groups retarded the rates. The slope of the apparent linear correlation between log k and pK_a was 0.583 and the effect of the substituents on the decay of the substituted aniline MSD catalyzed by proton was about half to that on the hydrolysis of MSD at neutral pH.[10] The slopes of the plots of log k_1 *v.s.* pK_a and log k_2 *v.s.* pK_a were 0.709 and 0.507, respectively.