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Studies on the Stability of Drugs. XVII.[1] Stability of Benzothiadiazines. (8).[2] Kinetics of Acid-Base Catalysed Hydrolysis of Chlorothiazide

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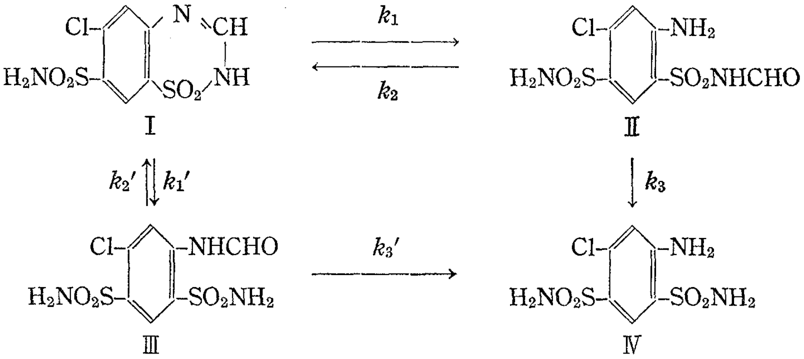
The present paper has established the kinetics and mechanisms for the general aicd-base catalysed hydrolysis of chlorothiazide (I) over the pH range 0.08—9.96 at 87.5°, which decomposes to 4-amino-6-chloro-*m*-benzenedisulfonamide (IV) *via* parallel consecutive first-order reaction in aqueous solution: one leading to the formation of N-(2-amino-4-chloro-5-sulfamoylphenylsulfonyl)formamide (II) in aqueous alkali, and the other leading to the formation of 5-chloro-2,4-disulfamoylformanilide (III) in acidic media.

$$\begin{tikzcd}
\mathrm{I} \arrow[r, shift left, "k_1"] \arrow[d, shift right, "k_1'"'] & \mathrm{II} \arrow[l, shift left, "k_2"] \arrow[d, "k_3"] \\
\mathrm{III} \arrow[u, shift right, "k_2'"'] \arrow[r, "k_3'"'] & \mathrm{IV}
\end{tikzcd}$$

In our previous papers[4,5] of this series, it has been reported that chlorothiazide (I) in aqueous solutions decomposes *via* parallel consecutive first-order reactions indicated in Scheme 1, one leading to the formation of 5-chloro-2,4-disulfamoylformanilide (III) in acidic media, and the other leading to the formation of N-(2-amino-4-chloro-5-sulfamoylphenylsulfonyl)formamide (II) in alkaline media, and this decomposition results in the end product of 4-amino-6-chloro-*m*-benzenedisulfonamide (IV).



Scheme 1

In view of these investigations, it was deemed to be of value to determine the degradation mechanisms over a broad pH range.

The present paper has established the kinetics and mechanisms for the acid-base catalyzed hydrolysis of this drug over the pH range 0.08—9.96.

Experimental

Materials——Chlorothiazide (I), supplied by Nippon Merck-Banyu Co., Ltd, was used. All reagents used were of analytical grade. Distilled water was used throughout this study.

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1) Part XVI: T. Yamana, Y. Mizukami, M. Nishida, M. Izaki, N. Kitade, and K. Shirai, *Yakuzaigaku*, **27**, 203 (1967).
2) Part XV. (7): T. Yamana and Y. Mizukami, *Yakugaku Zasshi*, **87**, 1304 (1967).
3) Location: *Takara-machi*, *Kanazawa*.
4) T. Yamana, H. Koike, Y. Mizukami, T. Motoya, and Y. Insemi, *Yakugaku Zasshi*, **84**, 971 (1964); T. Yamana, Y. Mizukami, F. Ichimura, and H. Koike, *ibid.*, **84**, 974 (1964).
5) T. Yamana and Y. Mizukami, *Yakugaku Zasshi*, **87**, 1304 (1967).

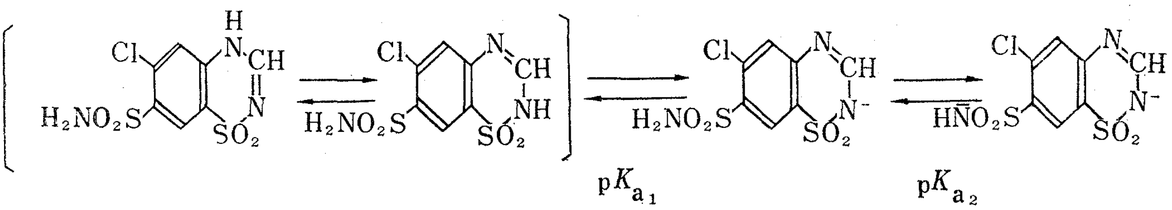
Buffer Solution——Buffer solutions were utilized in order to keep the hydrogen ion activities constant during the kinetic runs. To obviate any error due to the effect of ionic strength on the reaction rates, all buffers were made to have ionic strength of 1.5 by adjustment with potassium chloride. Buffer solutions employed were phosphate buffer and were made to 0.10, 0.05, and 0.02 M with respect to total buffer concentrations.

The pH of the solutions was determined with the Beckman Model G pH meter at the experimental temperature. In all kinetic studies, prior and subsequent to the kinetic runs, no change in pH was observed.

Degradation Studies——In most cases the following procedure was used: 59.2 mg (1.00×10^{-3} M) of I was dissolved in 200 ml of the appropriate buffer solution. The solution was heated to 87.50±0.05° in the thermostatically controlled bath. Samples were removed from the reaction–flask at appropriate intervals, cooled on ice, and the solution was analyzed.

Assay Procedure——According to the previously reported[4)] diazo-coupling method by Tsuda's reagent, the concentrations of II, III and IV were followed, by determining colorimetrically the change in absorbance against a blank at 530 mμ in Hitachi Model 101 Spectrophotometer. The amount of II, III and IV in the reaction mixture was read from the standard curve at the same buffer system.

Determination of Dissociation Constant——Novello, *et al.*,[6)] from the comparison of the ultraviolet spectra of the parent compound, 1,2,4–benzothiadiazine–1,1–dioxide and several of its derivatives, including I, indicated that the structure of I was undoubtedly a tautomeric system (2,3–$chH_2 \rightleftarrows$ 3,4–chH_2), and that the double bond might occupy either the 2,3 or 3,4 positions. From their view and our ultraviolet spectra data over a broad pH region, it appears that under acid conditions I exist predominantly in the form 2,3–chH_2, however, in aqueous alkali, the sturcture of I shifts toward the form 3,4–chH_2. I may exist neutral form (chH_2) (where chH_2=2,3–chH_2+3,4–chH_2), anion form (chH^-) and dianion form (ch^-) in the experimental pH region, in consideration of the dissociation of the hydrogen on the nitrogen atom at the position 2 and of the dissociation of sulfonamide, as indicated in Scheme 2.



2,3–cH_2 3,4–chH_2 anion form dianion form

neutral form

chH_2 chH^- ch^-

Scheme 2

Charnicki, *et al.*[7)] determined pK_{a_1} to be 6.7 and pK_{a_2} to be 9.5 at room temperature. The pK_{a_1} and pK_{a_2} determination are required to calculate the concentrations of chH_2, chH^- and ch^- in the experimental pH region at 87.5°. For this purpose, these pK_a values were determined spectrophotometrically at 87.5°.

The pK_{a_1} was calculated according to the following equation.

$$pK_{a_1} = pH - \log \frac{E_{chH^-} - E_{obs}}{E_{obs} - E_{chH_2}} \qquad (1)$$

where, E_{chH_2}, E_{chH^-} represent each absorbance of chH_2 and chH^-, and E_{obs} is the observed absorbance. These absorbances were determined in Shimadzu QV–50 Spectrophotometer, keeping the cell box at 87.5°. Prior to the addition of the sample, 100 ml of appropriate buffer solutions was heated at 87.5°, then, 1 ml of 5.00×10^{-3} M chlorothiazide solution was added to the heated buffer solutions, and the mixture was immediately determined the absorbance at 256 mμ at one minute intervals. From the absorbance at zero–time, pK_{a_1} was calculated using Eq. (1). Thus, $pK_{a_1}=6.63$ ($K_{a_1}=2.34\times10^{-7}$) at 87.5° was estimated.

pK_{a_2} was calculated to be 9.84 ($K_{a_2}=1.45\times10^{-10}$) at 87.5° according to the similarly method.

The Rate Determination——In the pH region 0.08—3.36, because of a low amount of III, it was difficult to estimate the concentration of III found in the degradation of I by the usual manner. Therefore, from the data obtained by the degradation of III, the rate constants k_1', k_2' and k_3' were calculated by the method previously reported.[4)]

In the pH region 3.36—4.44, k_2 and k_3 were measured by the degradation of II, since it was impossible to determine the concentration of II in the degradation of I for the similarly reason. These rates were determined according to the following technique: when k_1 was so small compared to k_2 and k_3, in the degrada-

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6) F.C. Novello, S.C. Bell, E.L.A. Abrams, C. Ziegler, and J.M. Sprague, *J. Org. Chem.*, **25**, 977 (1960).

7) W.F. Charnicki, F.A. Bacher, S.A. Freeman, and D.H. Decesare, *J. Am. Pharm. Assoc.*, **48**, 656 (1959).

tion of II, the parallel reaction $\mathrm{I} \xleftarrow{k_2} \mathrm{II} \xrightarrow{k_3} \mathrm{IV}$ can be considered in the initial stage of the reaction. Then, the rate of the consumption of II may be expressed as the following equation.

$$-\frac{d[\mathrm{II}]}{dt}=(k_2+k_3)[\mathrm{II}] \tag{2}$$

Integration of Eq. (2) is, therefore, given

$$\frac{1}{t}\log\frac{[\mathrm{II}]}{[\mathrm{II}]_0}=-(k_2+k_3)/2.303 \tag{3}$$

or

$$[\mathrm{II}]=[\mathrm{II}]_0\, e^{-(k_2+k_3)t} \tag{4}$$

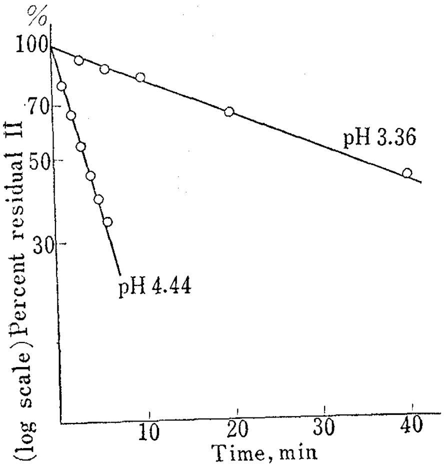

Fig. 1. Relation between Logarithm II % and Time by the Degradation of N-(2-amino-4-chloro-5-sulfamoylphenyl sulfonyl)formamide (II) at pH 3.36 and pH 4.44 at 87.5°

In the degradation II, if the residual II concentration are plotted logarithmically *versus* time, should yield a straight line. The rate k_2+k_3 was obtained by multiplying the slope by 2.303. The tyipcal plots are shown in Fig. 1.

On the other hand, the following equation is obtained.

$$\frac{d[\mathrm{I}]}{dt}=k_2[\mathrm{II}] \tag{5}$$

Substituting of Eq. (4) into (5), yield

$$\frac{d[\mathrm{I}]}{dt}=k_2[\mathrm{II}]_0 e^{-(k_2+k_3)t} \tag{6}$$

If $[\mathrm{I}]=0$ at $t=0$, the integral equation for Eq. (6) is given.

$$[\mathrm{I}]=\frac{k_2[\mathrm{II}]_0}{k_2+k_3}(1-e^{-(k_2+k_3)t})$$

$$=\frac{k_2}{k_2+k_3}([\mathrm{II}]_0-[\mathrm{II}]) \tag{7}$$

Eq. (7) suggests that a plot of $[\mathrm{II}]_0-[\mathrm{II}]$ *versus* [I] should yield a straight line through the origin with slope $k_2/(k_2+k_3)$. Figs. 2 and 3 show that the experimental points fit quite well to a straight line through the origin at pH 3.36 and 4.44.

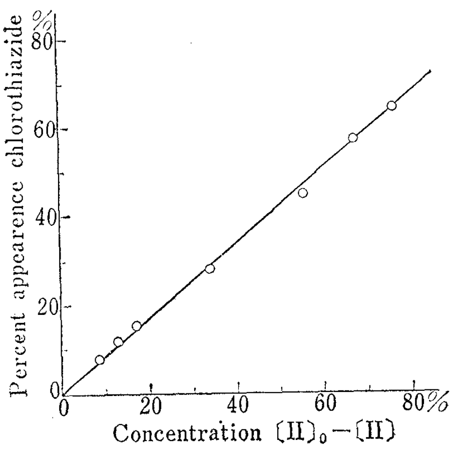

Fig. 2. Relation between Concentration of Chlorothiazide (I) and $[\mathrm{II}]_0$-[II] by the Degradation of N-(2-amino-4-chloro-5-sulfamoylphenylsulfonyl)formamide (II) at pH 3.36 and 87.5°

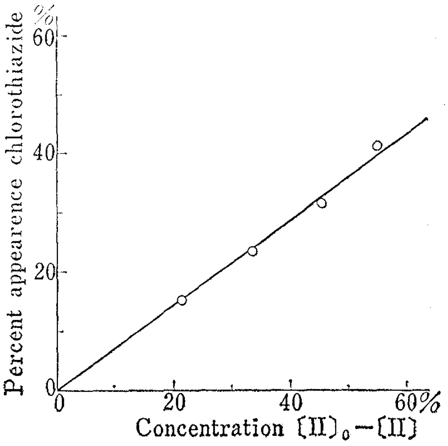

Fig. 3. Relation between Concentration of Chlorothiazide (I) and $[\mathrm{II}]_0$-[II] by the Degradation of N-(2-amino-4-chloro-5-sulfamoylphenylsulfonyl)formamide (II) at pH 4.44 and 87.5°

Each k_2 and k_3, therefore, can be calculated from these data.

In the pH region 5.36—9.96, the rate constants k_1, k_2 and k_3 were calculated by the method previously reported.[4]

Results and Discussion

Catalytic Effect of General Acids and Bases on the Hydrolysis of Chlorothiazide

The catalytic effect of different concentrations of buffers was determined by making runs with each buffer varing the total buffer concentration by keeping the pH and the ionic strength constant ($\mu=1.5$). Straight lines were obtained by plotting the observed k values against buffer concentration as indicated in Figs. 4—8, and in Figs. 4′—6′.

Log k-pH Profile of Degradation of Chlorothiazide

1) Determination of pH Dependency of Rate Constants k_1, k_2 and k_3 in the Alkaline Media

The rate constants k_1, k_2 and k_3 at zero buffer concentration can easily be picked from Figs. 4—8. These rate constants are shown in Fig. 9 as function of pH.

The log k_1–pH profile (Fig. 9) suggests that the overall reaction rate represents a summation of the following several separate reactions.

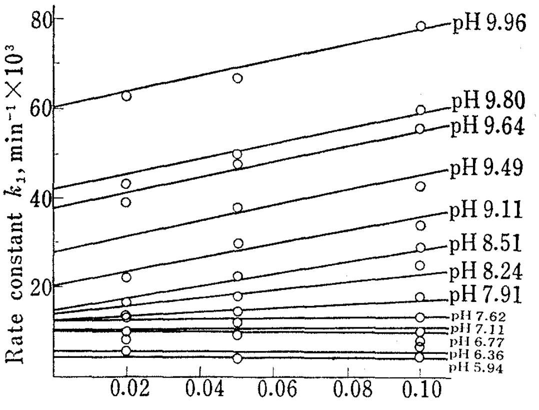

Fig. 4. Effect of Phosphate Concentration on the Pseudo First-order Rate Constant k_1 of the Degradation of Chlorothiazide at Fixed pH Values

All the runs made at 87.5° and an Ionic Strength of 1.5.

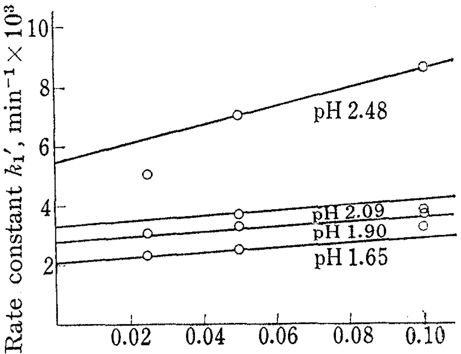

Fig. 4′. Effect of Phosphate Concentration on the Pseudo First-order Rate Constant k_1' of the Degradation of Chlorothiazide at Fixed pH Values

All the runs made at 87.5° and an ionic strength of 1.5.

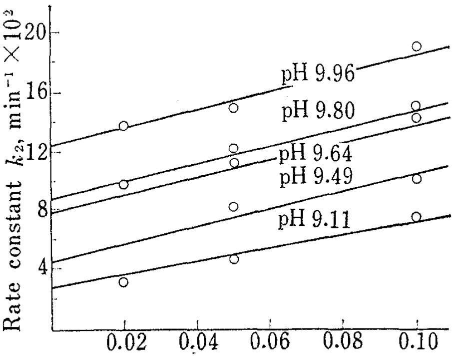

Fig. 5. Effect of Phosphate Concentration on the Pseudo First-order Rate Constant k_2 of the Degradation of Chlorothiazide at Fixed pH Values

All the runs made at 87.5° and an ionic strength of 1.5.

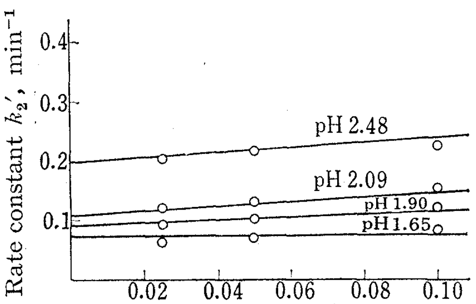

Fig. 5′. Effect of Phosphate Concentration on the Pseudo First-order Rate Constant k_2' of the Degradation of Chlorothiazide at Fixed pH Values

All the runs made at 87.5° and an ionic strength of 1.5.

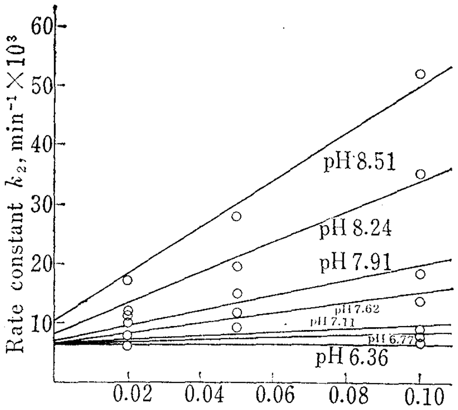

Fig. 6. Effect of Phosphate Concentration on the Pseudo First-order Rate Constant k_2 of the Degradation of Chlorothiazide at Fixed pH Values

All the runs made at 87.5° and an ionic strength of 1.5.

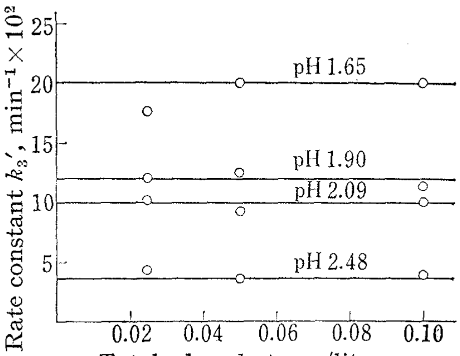

Fig. 6′. Effect of Phosphate Concentration on the Pseudo First-order Rate Constant k_3' of the Degradation of Chlorothiazide at Fixed pH Values

All the runs made at 87.5° and an ionic strength of 1.5.

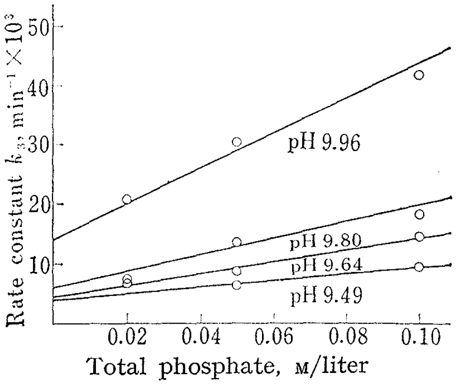

Fig. 7. Effect of Phosphate Concentration on the Pseudo First-order Rate Constant k_3 of the Degradation of Chlorothiazide at Fixed pH Values

All the runs made at 87.5° and an ionic strength of 1.5.

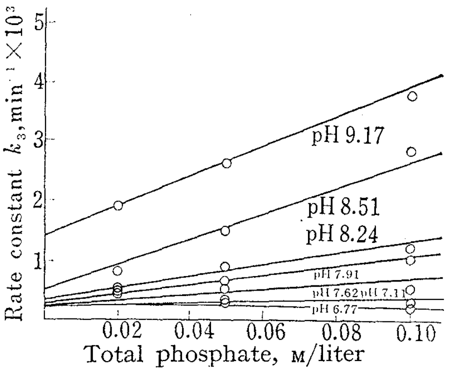

Fig. 8. Effect of Phosphate Concentration on the Pseudo First-order Rate Constnt k_3 of the Degradation of Chlorothiazide at Fixed pH Values

All the runs made at 87.5° and an ionic strength of 1.5.

$$\begin{array}{llll} \mathrm{chH_2+OH^-} & \xrightarrow{k_{\mathrm{OH^-}}} & \mathrm{II} & \text{Reaction 1} \\ \mathrm{chH_2+H_2O} & \xrightarrow{k_{\mathrm{H_2O}}} & \mathrm{II} & \text{Reaction 2} \\ \mathrm{cH+OH^-} & \xrightarrow{k_{\mathrm{OH^-}}'} & \mathrm{II} & \text{Reaction 3} \\ \mathrm{cH+H_2O} & \xrightarrow{k_{\mathrm{H_2O}}'} & \mathrm{II} & \text{Reaction 4} \end{array}$$

The rate constant k_1, therefore, is equal to the sum of the rates of these reactions

$$k_1[\mathrm{ch}]_\mathrm{T}=k_{\mathrm{OH^-}}[\mathrm{chH_2}][\mathrm{OH^-}]+k_{\mathrm{H_2O}}[\mathrm{chH_2}][\mathrm{H_2O}] \\ +k_{\mathrm{OH^-}}'[\mathrm{cH}][\mathrm{OH^-}]+k_{\mathrm{H_2O}}'[\mathrm{cH}][\mathrm{H_2O}] \quad (8)$$

where $k_{\mathrm{OH^-}}$, $k_{\mathrm{H_2O}}$, $k_{\mathrm{OH^-}}'$ and $k_{\mathrm{H_2O}}'$ are the specific catalytic constants, $[\mathrm{cH}]=[\mathrm{chH^-}]+[\mathrm{ch^-}]$ and $[\mathrm{ch_T}]$ represents the total concentration of I.

while, $$K_{\mathrm{a}_1}=\frac{[\mathrm{H^+}][\mathrm{chH^-}]}{[\mathrm{chH_2}]} \quad (9)$$

$$K_{a_2}=\frac{[H^+][ch^-]}{[chH^-]} \tag{10}$$

and
$$[ch]_T=[chH_2]+[chH^-]+[ch^-] \tag{11}$$

both $[chH_2]$ and $[cH]$ can be expressed in the following equations by combining Eqs. (9), (10) and (11).

$$[chH_2]=\frac{[H^+]^2}{[H^+]^2+K_{a_1}[H^+]+K_{a_1}\cdot K_{a_2}}\cdot[ch]_T \tag{12}$$

and
$$[cH]=\frac{K_{a_1}[H^+]+K_{a_1}\cdot K_{a_2}}{[H^+]^2+K_{a_1}[H^+]+K_{a_1}\cdot K_{a_2}}\cdot[ch]_T \tag{13}$$

where $[H^+]=10^{-pH}$, $[OH^-]=10^{pH-pK_w}$ and K_w is the ionization constant of water. $K_w=3.78\times10^{-13}$ at 87.5° was obtained from the equation in the literature.[8,9] Substituting Eq. (12) and Eq. (13) into Eq. (8), we obtain

$$k_1=(k_{OH^-}[OH^-]+k_{H_2O}[H_2O])\frac{[H^+]^2}{[H^+]^2+K_{a_1}[H^+]+K_{a_1}\cdot K_{a_2}}$$
$$+(k_{OH^-}'[OH^-]+k_{H_2O}'[H_2O])\frac{K_{a_1}[H^+]+K_{a_2}\cdot K_{a_1}}{[H^+]^2+K_{a_1}[H^+]+K_{a_1}\cdot K_{a_2}} \tag{14}$$

To evaluate the each specific rate constnat k_{OH^-}, k_{H_2O}, k_{HO^-}' and k_{H_2O}', we treated Eq. (14) by the following simplification.

In the experimental pH region, each concentration of chH_2 and cH can be approximately expressed as the following equations.

$$[chH_2]=\frac{[H^+]}{K_{a_1}+[H^+]}[ch]_T \tag{15}$$

and
$$[cH]=\frac{K_{a_1}}{K_{a_1}+[H^+]}[ch]_T \tag{16}$$

Therefore, k_1 may be changed as follow

$$k_1=\frac{(k_{OH^-}[OH^-]+k_{H_2O}[H_2O])[H^+]}{K_{a_1}+[H^+]}+\frac{(k_{OH^-}'[OH^-]+k_{H_2O}'[H_2O])K_{a_1}}{K_{a_1}+[H^+]} \tag{17}$$

At a relatively high pH region (≧9.0), the first term in Eq. (17) may be negligible compared to the other, and $K_{a_1}/(K_{a_1}+[H^+])$ will be approximately equal to 1.00, thus we may write

$$k_1=k_{OH^-}'[OH^-]+k_{H_2O}'[H_2O] \tag{18}$$

Eq. (18) suggests that a plot of k_1 as a function of $[OH^-]$ should give a straight line with slope k_{OH^-}' and intercept $k_{H_2O}'[H_2O]$. Such a plot is shown in Fig. 10. The constants calculated from the best fit line were: $k_{H_2O}'[H_2O]=13.0\times10^{-3}$ min^{-1}, $k_{OH^-}'=1.22\times10$ min^{-1} mole^{-1} liter.

Eq. (19) may be obtained substituting the above k values into Eq. (17)

$$k_1=\frac{3.78\times10^{-13}k_{OH^-}+k_{H_2O}[H_2O][H^+]+2.85\times10^{-6}[H^+]+3.04\times10^{-9}}{2.34\times10^{-7}+[H^+]} \tag{19}$$

We may rearrange Eq. (19) to

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8) H.S. Harned and B.B. Owen, "The Physical Chemistry of Electrolytic Solution," Reinhold Publishing Corporation, New York, 3rd Ed., 1963, p. 645.

9) However, no correction in K_w was applied for the ionic strength of the solution.

$$k_1\cdot(2.34\times10^{-7}+[H^+])-3.04\times10^{-9}$$
$$=3.78\times10^{-13}k_{OH^-}+k_{H_2O}[H_2O][H^+]+2.85\times10^{-6}[OH^-] \quad (20)$$

At a relatively low pH region ($\leqq 6.77$), since $2.85\times10^{-6}[OH^-]$ will be negligible compared to $k_1\cdot(2.34\times10^{-7}+[H^+])-3.04\times10^{-9}$, a plot of $k_1\cdot(2.34\times10^{-7}+[H^+])-3.04\times10^{-9}$ as a function of $[H^+]$ should give a straight line with slope $k_{H_2O}[H_2O]$ and intercept $3.78\times10^{-13}k_{OH^-}$. Such a plot is shown in Fig. 11. Hence, $k_{OH^-}=2.65\times10^2$ min^{-1} mole^{-1} liter, $k_{H_2O}[H_2O]=1.86\times10^{-3}$ min^{-1} were obtained. There were small contribution of the term k_{OH^-} to the total rate constant k_1. However, from the kinetical point of view, we neglected the effect of the degradation of chH_2 by OH^-. Eq. (21), therefore, is obtained by substituting the above k values into Eq. (14).

$$k_1=\frac{1.86\times10^{-3}[H^+]^2+3.04\times10^{-9}[H^+]+4.14\times10^{-16}[OH^-]+1.52\times10^{-18}}{[H^+]^2+2.34\times10^{-7}[H^+]+3.39\times10^{-17}} \quad (21)$$

The theoretical profile, shown in Fig. 9, was in good agreement with the observed points.

From spectrophotometic measurements, pK_a of II was estimated to be 2.73 at 25°. But it was impossible to determine the value at 87.5°, since the instability of II precluded the determination. It seems rather likely that there is no significant variation on the pK_a value under these

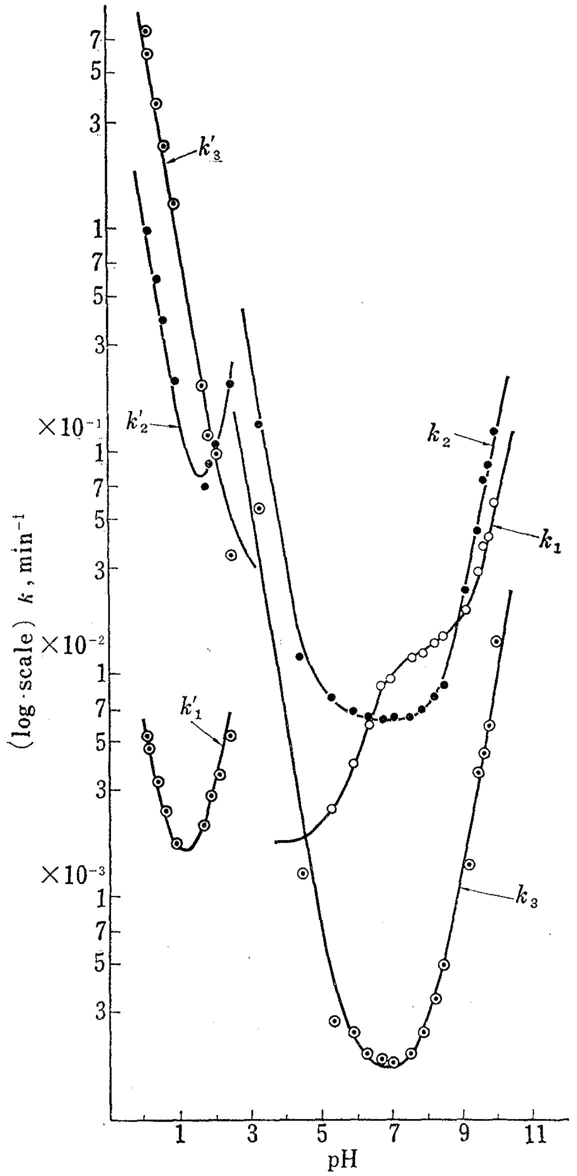

Fig. 9. Log k-pH Profile of the Degradation of Chlorothiazide at 87.5°

The circles represent the experimental results. The lines correspond to that expected theoretically from the proposed reactions.

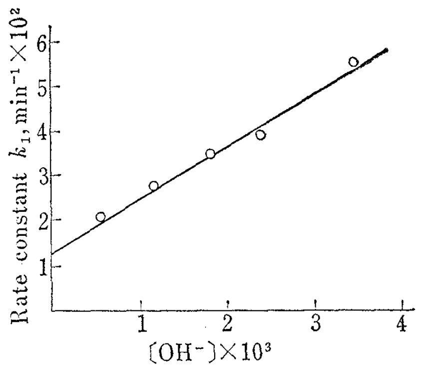

Fig. 10. Plot of k_1 vs. $[OH^-]$ in Accord with the Equation: $k_1=k_{OH^-}'[OH^-]+k_{H_2O}'[H_2O]$

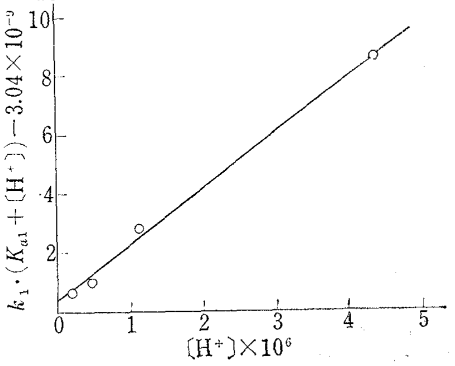

Fig. 11. Plot of $k_1\cdot(K_{a_1}+[H^+])-3.04\times10^{-9}$ vs. $[H^+]$ in Accord with the Equation (20)

temperature region (25°—90°), therefore, II may exist as protonated form (II^-) in the experimental pH region.

In log k_2 and log k_3–pH profiles, the straight lines of both lower and higher pH regions are extended and cross points are obtained, respectively. The observed minimum rate constants were larger than the double values of the cross points. This results may show the presence of water catalysis. Each specific catalytic constants of water for k_2 and k_3 is obtained by the differences between the observed minimum rate constants and the double values of the cross points. The following reactions would give the pH rate k_2 and k_3 profiles that appear to fit the experimental points well.

$$\mathrm{II^-+H^+} \xrightarrow{k_{2\cdot \mathrm{H}^+}} \mathrm{I} \qquad \text{Reaction 5}$$

$$\mathrm{II^-+H_2O} \xrightarrow{k_{2\cdot \mathrm{H_2O}}} \mathrm{I} \qquad \text{Reaction 6}$$

$$\mathrm{II^-+OH^-} \xrightarrow{k_{2\cdot \mathrm{OH}^-}} \mathrm{I} \qquad \text{Reaction 7}$$

Similarly in relation to k_3

$$\mathrm{II^-+H^+} \xrightarrow{k_{3\cdot \mathrm{H}^+}} \mathrm{IV} \qquad \text{Reaction 8}$$

$$\mathrm{II^-+H_2O} \xrightarrow{k_{3\cdot \mathrm{H_2O}}} \mathrm{IV} \qquad \text{Reaction 9}$$

$$\mathrm{II^-+OH^-} \xrightarrow{k_{3\cdot \mathrm{OH}^-}} \mathrm{IV} \qquad \text{Reaction 10}$$

where $k_{2\cdot \mathrm{H}^+}$, $k_{2\cdot \mathrm{H_2O}}$, $k_{2\cdot \mathrm{OH}^-}$, $k_{3\cdot \mathrm{H}^+}$, $k_{3\cdot \mathrm{H_2O}}$ and $k_{3\cdot \mathrm{OH}^-}$ are the specific catalytic constants. The rate constants k_2 and k_3 are equal to the sum of the rates of all these reactions.

$$k_2=k_{2\cdot \mathrm{H}^+}[\mathrm{H}^+]+k_{2\cdot \mathrm{H_2O}}[\mathrm{H_2O}]+k_{2\cdot \mathrm{OH}^-}[\mathrm{OH}^-] \qquad (22)$$

and

$$k_3=k_{3\cdot \mathrm{H}^+}[\mathrm{H}^+]+k_{3\cdot \mathrm{H_2O}}[\mathrm{H_2O}]+k_{3\cdot \mathrm{OH}^-}[\mathrm{OH}^-] \qquad (23)$$

From the experimental results, the following k values have been calculated.

$k_{2\cdot \mathrm{H}^+}=3.00\times 10^2$ min^{-1} mole^{-1} liter. $k_{3\cdot \mathrm{H}^+}=7.00\times 10$ min^{-1} mole^{-1} liter.

$k_{2\cdot \mathrm{OH}^-}=3.40\times 10$ min^{-1} mole^{-1} liter. $k_{3\cdot \mathrm{OH}^-}=2.98$ min^{-1} mole^{-1} liter.

$k_{2\cdot \mathrm{H_2O}}[\mathrm{H_2O}]=6.23\times 10^{-3}$ min^{-1}. $k_{3\cdot \mathrm{H_2O}}[\mathrm{H_2O}]=1.60\times 10^{-4}$ min^{-1}.

The theoretical lines in Fig. 8 have been calculated by substituting the above k value into Eq. (22) and (23).

2) Determination of pH Dependency of Rate Constants k_1', k_2' and k_3' in Acidic Media

The rate constants k_1', k_2' and k_3' at zero buffer concentration can be picked from Figs. 4'—6'. These rate constants are shown in Fig. 9 as function of pH.

In the pH region 0.08—3.40, I may exist as $\mathrm{chH_2}$. In relation to k_1', the shape of the pH rate profile may show the presence of the water catalysis as in the case of k_2 and k_3, therefore, the profile may be explained by assuming that the following reactions take place at zero buffer concentration.

$$\mathrm{chH_2+H^+} \xrightarrow{k_{1\cdot \mathrm{H}^+}'} \mathrm{III} \qquad \text{Reaction 11}$$

$$\mathrm{chH_2+H_2O} \xrightarrow{k_{1\cdot \mathrm{H_2O}}'} \mathrm{III} \qquad \text{Reaction 12}$$

$$\mathrm{chH_2+OH^-} \xrightarrow{k_{1\cdot \mathrm{OH}^-}'} \mathrm{III} \qquad \text{Reaction 13}$$

where $k_{1\cdot H^+}'$, $k_{1\cdot H_2O}'$ and $k_{1\cdot OH^-}'$ are the specific catalytic constants.

The rate constant k_1' is equal to the sum of the rates of these reactions.

$$k_1' = k_{1\cdot H^+}'[H^+] + k_{1\cdot H_2O}'[H_2O] + k_{1\cdot OH^-}'[OH^-] \qquad (24)$$

It was impossible to take into account the existence of different species of III in solution, since III was unstable to determine pK_a. However, in consideration of the degradation of III by water as in the case of k_1', the following reactions would give the pH rates k_2' and k_3' profiles that appear to fit the experimental points well.

In relation to k_2',

$$\mathrm{III} + H^+ \xrightarrow{k_{2\cdot H^+}'} \mathrm{I} \qquad \text{Reaction 14}$$

$$\mathrm{III} + H_2O \xrightarrow{k_{2\cdot H_2O}'} \mathrm{I} \qquad \text{Reaction 15}$$

$$\mathrm{III} + OH^- \xrightarrow{k_{2\cdot OH^-}'} \mathrm{I} \qquad \text{Reaction 16}$$

and in relation to k_3',

$$\mathrm{III} + H^+ \xrightarrow{k_{3\cdot H^+}'} \mathrm{IV} \qquad \text{Reaction 17}$$

$$\mathrm{III} + H_2O \xrightarrow{k_{3\cdot H_2O}'} \mathrm{IV} \qquad \text{Reaction 18}$$

$$\mathrm{III} + OH^- \xrightarrow{k_{3\cdot OH^-}'} \mathrm{IV} \qquad \text{Reaction 19}$$

where $k_{2\cdot H^+}'$, $k_{2\cdot H_2O}'$, $k_{2\cdot OH^-}'$, $k_{3\cdot H^+}'$, $k_{3\cdot H_2O}'$ and $k_{3\cdot OH^-}'$ are the specific catalytic constants.

The rate constants k_2' and k_3' are equal to the sum of the rates of all these reactions.

$$k_2' = k_{2\cdot H^+}'[H^+] + k_{2\cdot H_2O}'[H_2O] + k_{2\cdot OH^-}'[OH^-] \qquad (25)$$

and

$$k_3' = k_{3\cdot H^+}'[H^+] + k_{3\cdot H_2O}'[H_2O] + k_{3\cdot OH^-}'[OH^-] \qquad (26)$$

From the experimental results, the following k values have been calculated.

$k_{1\cdot H^+}' = 5.29 \times 10^{-3}$ min^{-1} mole^{-1} liter, $k_{1\cdot OH^-}' = 5.30 \times 10^{7}$ min^{-1} mole^{-1} liter,
$k_{1\cdot H_2O}'[H_2O] = 1.05 \times 10^{-3}$ min^{-1}, $k_{2\cdot H^+}' = 1.46$ min^{-1} mole^{-1} liter,
$k_{2\cdot OH^-}' = 1.64 \times 10^{9}$ min^{-1} mole^{-1} liter, $k_{2\cdot H_2O}'[H_2O] = 2.00 \times 10^{-2}$ min^{-1},
$k_{3\cdot H^+}' = 9.25$ min^{-1} mole^{-1} liter, $k_{3\cdot OH^-}' = 0$ min^{-1} mole^{-1} liter,
$k_{3\cdot H_2O}'[H_2O] = 2.00 \times 10^{-2}$ min^{-1}.

The theoretical lines in Fig. 9 have been calculated by substituting the above k values and $K_w = 3.78 \times 10^{-13}$ into Eqs. (24), (25) and (26).

The relatively good agreement of the experimental data and the theoretical profile does not, of course, prove that proposed reactions 11—13, 14—16 and 17—19 are the correct ones. It is, however, difficult to explain these rate profiles by alternative reactions.

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