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Kinetics of Hydrolysis of Oxazolam in Aqueous Solution[1a)]

MASARU IKEDA*,[a] and TSUNEJI NAGAI[b]

*Product Development Laboratories, Sankyo Co., Ltd.,[a] 1–2–58, Hiro-machi,
Shinagawa-ku, Tokyo 140, Japan and Faculty of Pharmaceutical Sciences,
Hoshi University,[b] 2–4–41, Ebara, Shinagawa-ku, Tokyo 142, Japan*

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The hydrolysis reaction of oxazolam, a representative of 1,4-benzodiazepinooxazoles (BDOZ), was investigated kinetically. The reaction product was identified by thin layer chromatography (TLC) and infrared (IR) spectroscopy, and it was concluded that irreversible hydrolytic cleavage took place at the diazepinone nucleus. The rate constant was determined by ultraviolet (UV) spectroscopy. The reaction was a first-order process consisting of two parallel reactions with different reacting species of oxazolam, depending on the pH of the medium.

The pH-rate profile obtained for the reaction suggested that the reaction was independent of hydrogen ion concentration in acidic media, while it was catalyzed by hydroxide ion in alkaline media. The nonlinear least-squares fit method was employed to determine the catalytic rate constants involved in the equation describing the pH–rate constant relationship. Activation energies as well as other thermodynamic parameters were obtained in media of pH 2.0 and 8.0, and the values confirmed that different chemical species of oxazolam were involved. The observed effect of buffer concentration indicated that general base catalysis was involved in the reaction of the ionized species of oxazolam.

A mechanistic consideration indicated that the rate-determining step may be the nucleophilic attack of a water molecule or hydroxide ion at the 11b-position in the diazepinone nucleus.

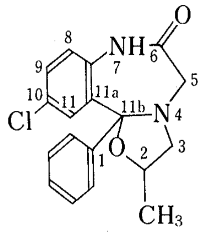
Keywords——oxazolam; benzodiazepine; first-order reaction rate; pH-rate profile; general base catalysis

The degradation kinetics of 1,4-benzodiazepines in aqueous solution have been studied by many workers. Carstensen[2)] reported details of diazepam degradation in parenteral formulations. Other papers[3)] on diazepam and its homologs revealed that the hydrolytic degradation took place at two sites on the diazepinone ring to give an aminobenzophenone compound as the final product.

Recently, Yoneda *et al.*,[4)] Hong *et al.*,[5)] and Nakano *et al.*[6)] independently found that the cleavage reaction of the diazepinone nucleus was reversible depending on the pH of the medium, and obtained the rate constants of the forward and reverse reactions.

In the present study, kinetic investigations were carried out on the cleavage reaction of the diazepinone ring in oxazolam, which is the typical drug among 1,4-benzodiazepino-oxazoles (BDOZ) which are commonly used as minor tranquilizers for oral use. It is necessary to investigate the kinetics in solution for development of a parenteral dosage form, as well as to obtain an understanding of the drug behavior after administration.

Although oxazolam was exclusively studied in this paper, other BDOZs might have broadly similar kinetic properties.



oxazolam (I)

Experimental

Materials——Oxazolam (I) (10-chloro-2,3,5,6,7,11b-hexahydro-2-methyl-11b-phenylbenzo[6,7][1,4]diazepino-[5,4-*b*]oxazol-6-one) was from the same manufacturing batch as that used in the previous paper.[1b)] Authentic samples of 2-(β-hydroxyisopropylamino)acetanilide-5-chlorobenzophenone (III), and 2-amino-5-chlorobenzophenone (IV) were the intermediates in the manufacturing synthesis of oxazolam; they were used without further purification.

Buffer Solution——Buffer solutions used were glycine (pH 2—4), acetate (pH 3.5—6), phosphate (pH 6—8), borate (pH 8—10), and carbonate (pH 10—11). Concentrations of the buffer solutions were 0.0005—0.10 M, and the ionic strength was adjusted to $\mu=0.1$ by addition of sodium chloride. The observed shift of pH in buffer solution was no more than 0.05 pH unit during the kinetic experiments. All the reagents were of reagent grade, and solutions were prepared using distilled water.

Apparatus——Ultraviolet (UV) absorption spectroscopy was carried out with a Hitachi 323 spectrophotometer. The pH measurements were made on a Hitachi-Horiba F-7 pH meter. A Taiyo M-1 incubator was used as a constant temperature bath, controlling the temperature to within 0.2 °C of the setting. Infrared (IR) spectra were measured on a Hitachi 260-50 IR spectrophotometer.

Procedure for Kinetic Study——An ethanolic stock solution of oxazolam was prepared at 200 μg/ml concentration, and kept in a refrigerator. The stock solution, preheated to the experimental temperature, was diluted to 10 μg/ml (3.04×10^{-5} M) with a buffer solution, which had also been preheated. The diluted experimental solution, which consequently contained 5% (v/v) ethanol, was kept in the constant temperature bath, and aliquots were withdrawn at appropriate intervals to measure the absorbance (A) at the wavelength preliminary determined. The procedure was continued until the absorbance value reached a nearly constant level, and the value at infinite time (A_∞) was read after allowing the solution to stand overnight in the bath. An apparent first-order rate constant (k_{obs}) was calculated using Eq. (1) from the slope of the straight line obtained by plotting $\log(A_\infty \sim A)$ against time t.

$$\log(A_\infty \sim A) = -\frac{k_{\text{obs}}}{2.303}\, t + \text{constant} \qquad (1)$$

The wavelengths for the absorbance measurement were 245 nm for solutions with pH below 4.0, and 238 nm for solutions with pH higher than 7.0. Since the absorbance change was tiny in solutions of pH 4.0—7.0, near the pK_a value of oxazolam (5.74),[1b)] the procedure was slightly modified. A 3 ml aliquot of the sample was acidified with 2 drops of 1.5 N hydrochloric acid in the optical cell so that I in the solution was completely converted to the immonium species (II), then absorbance measurement was carried out at 245 nm. All the kinetic experiments were done in duplicate, and the average values of k_{obs} were used.

Product Determination——The experimental solutions used in the kinetic procedures at pH 2.0 and 9.5 were kept standing at room temperature for another 96 h. The pH of the former solution was alkalinized to pH 8—9 by addition of 0.1 N sodium hydroxide. Then both solutions were evaporated to dryness under reduced pressure at 35 °C or below, and the residues were extracted with 1 ml of chloroform. Each extract was spotted on a thin layer plate of Kiesel Gel F_{254}, and developed with benzene–ethyl acetate (1 : 1). Reference standards of I, III, IV, and desmethyldiazepam (V) were developed simultaneously, and the spots were detected by UV irradiation (254 nm). IR spectrograms were obtained from compressed disks of KBr powder on which the chloroform extract had been adsorbed, and were compared with these of the reference standards.

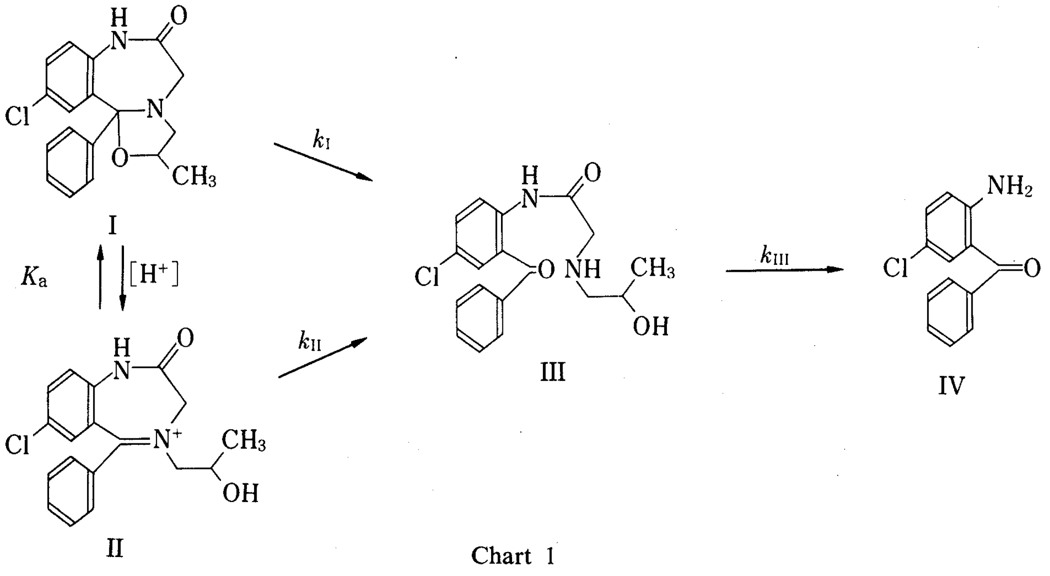


Chart 1

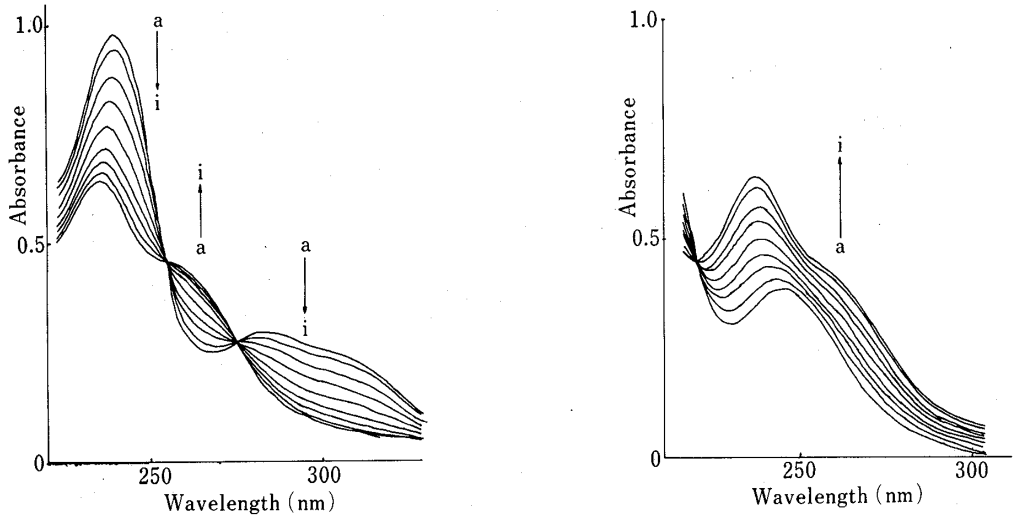
Results and Discussion

Hydrolysis Reaction of Oxazolam

The UV spectra in Fig. 1 show typical time courses for an aqueous solution of oxazolam. According to the acid–base equilibrium reported in the previous paper,[1b] oxazolam exhibited different initial spectra in acidic and alkaline media corresponding to the chemical species II and I, respectively. However, those spectra finally shifted to almost identical curves at the end of reaction, suggesting that the reaction product was the same independently of medium pH.

The spectral change is due to the hydrolytic cleavage reaction taking place at the carbon–nitrogen bond on the diazepine nucleus as shown in Chart 1. It was also verified by thin layer chromatography (TLC) and IR studies as described in the experimental section that the reaction product was only III either in acidic or alkaline medium, and no further decomposition took place under the conditions of the present study. TLC gave only one spot corresponding to III, and the IR spectrum was identical to that of III for the reaction products obtained through the kinetic procedure at pH 2.0 or 9.5. The result indicates that much more severe conditions or enzymatic hydrolysis are required to obtain the further product reported previously.[7] Consequently, the rate constant k_{III} was considered to be relatively small, and negligible compared to the rate constant k_{I} or k_{II}.

Although the hydrolytic ring-opening was reported to be a reversible reaction in other 1,4-benzodiazepines,[4-6] it appeared to be irreversible in oxazolam (a 1,4-benzodiazepino-oxazole) in the present study. The difference might be due to the alkyl substituent on the position-4 nitrogen, since Hong *et al.*[5] observed in a study on pyrazolodiazepinone that the equilibrium of the reversible ring-opening reaction was quite unbalanced, and no significant amount of ring-closed compound was detected in the case of alkyl-substituted derivatives on the nitrogen. Therefore, the ring-opening reaction would appear to be irreversible due to the extreme unbalance in favor of ring opening in oxazolam, which is an N-alkyl substituted benzodiazepine.



(a) (b)

Fig. 1. Change of the UV Spectrum of Oxazolam in Aqueous Solution at 37 °C, $\mu=0.1$

(a): in 0.07 M glycine buffer of pH 3.0.
a, time 1 min; b, 5 min; c, 13 min; d, 22 min; e, 37 min; f, 57 min; g, 76 min; h, 110 min; i, infinity (26 h).

(b): in 0.05 M phosphate buffer of pH 7.5.
a, time 1 min; b, 8 min; c, 21 min; d, 33 min; e, 47 min; f, 69 min; g, 90 min; h, 161 min; i, infinity (27 h).

Rate Constants of the Hydrolysis Reaction

The UV absorption change observed at constant wavelength was plotted against the reaction time t, according to the first-order kinetic equation (Eq. (1)). Typical plots are shown in Fig. 2, and the obtained straight lines indicate that the reaction followed first-order kinetics at any pH. Apparent rate constants (k_{obs}) were obtained from the slope, and are listed in Table I. The k_0 values, which were obtained by extrapolating k_{obs} to zero buffer concentration at the given pH, are also given in the table. Although buffer concentration dependencies were not always linear, the extrapolation was done in the lower concentration range of buffer solution.

The apparent rate constant k_{obs} is given by Eq. (2) at constant pH and very low buffer concentration;

$$k_{obs} = k_0 + k_B \cdot [\text{buffer}] \tag{2}$$

where [buffer] is the total buffer concentration, and k_B is the 2nd-order rate constant for the buffer catalyzed reaction. Since two species, I and II, are reacting, k_{obs} can also be expressed as follows;

$$k_{obs} = k^{I}_{obs} \cdot f^{I} + k^{II}_{obs} \cdot f^{II} \tag{3a}$$

$$= (k^{I}_{0} + k^{I}_{B} \cdot [\text{buffer}]) \cdot f^{I} + (k^{II}_{0} + k^{II}_{B} \cdot [\text{buffer}]) \cdot f^{II} \tag{3b}$$

$$= k^{I}_{0} \cdot f^{I} + k^{II}_{0} \cdot f^{II} + (k^{I}_{B} \cdot f^{I} + k^{II}_{B} \cdot f^{II}) \cdot [\text{buffer}] \tag{3c}$$

Therefore, k_0 should be expressed by Eq. (4);

$$k_0 = k^{I}_{0} \cdot f^{I} + k^{II}_{0} \cdot f^{II} \tag{4}$$

where f^{I} and f^{II} are the molar fractions of oxazolam species I and II, respectively, and suffixes I and II on k indicate the rate constants for species I and II, respectively. The fractions, f^{I} and f^{II}, are given by the following equations;

$$f^{I} = \frac{[\text{I}]}{[\text{I}] + [\text{II}]} = \frac{K_a}{K_a + a_H} \tag{5}$$

$$f^{II} = \frac{[\text{II}]}{[\text{I}] + [\text{II}]} = \frac{a_H}{K_a + a_H} \tag{6}$$

where K_a is the acid dissociation constant of the species II, and a_H is the activity of hydrogen ion.

Equation (3a) means that k_{obs} value reflects both rate constants, k^{I}_{obs} and k^{II}_{obs}, depending on the mole fractions of I and II in the solution. When the solution pH is very different from the pK_a value, the fraction of one species becomes very large, so that k_{obs} becomes essentially equal to k^{I}_{obs} or k^{II}_{obs}. Consequently, k_0 is equal to k^{I}_{0} or k^{II}_{0}. Both species should be taken into

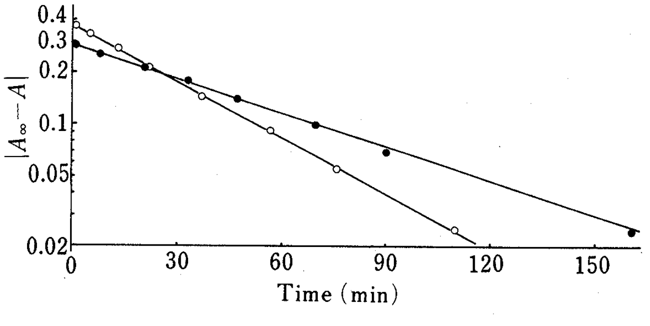


Fig. 2. Typical First-Order Plots for Hydrolysis Reaction of Oxazolam at 37 °C, $\mu = 0.1$

○, 0.07 M glycine buffer of pH 3.0; ●, 0.05 M phosphate buffer of pH 7.5.

TABLE I. First-order Rate Constants for the Hydrolysis Reaction of Oxazolam at 37 °C, $\mu=0.1$

Buffer system pH	$k_{\mathrm{obs}} \times 10^2$ (min^{-1})								$k_0 \times 10^2$ (min^{-1})
HCl									
1.1	1.09								1.09
Glycine HCl	(0.001 M)	(0.003 M)	(0.005 M)	(0.007 M)	(0.01 M)	(0.02 M)	(0.03 M)	(0.05 M)	
1.5					1.13	1.12	1.07		1.10
2.0	1.04	1.12	1.09	1.01	1.04	1.16	1.23		1.04
2.0 (30 °C)	0.45	0.51	0.49						0.45
2.0 (45 °C)	1.95	2.04	2.05						1.94
3.0					1.22	1.46		2.11	1.01
Acetate	(0.0005 M)	(0.001 M)	(0.002 M)	(0.003 M)	(0.01 M)	(0.02 M)	(0.03 M)	(0.05 M)	
3.5	1.08	1.06	1.07		2.20$^{a)}$	3.30$^{a)}$	4.30$^{a)}$		0.970
4.0	1.00	1.18	1.42		2.86$^{a)}$		4.88$^{a)}$	5.96$^{a)}$	0.880
4.5	1.12	1.40	1.82		4.13$^{a)}$		6.60$^{a)}$	8.19$^{a)}$	0.910
5.0	0.94	1.37	1.65	2.45	4.22$^{a)}$	5.39$^{a)}$	5.94$^{a)}$	8.10$^{a)}$	0.800
5.5	0.73	0.92		1.60	3.25$^{a)}$		4.94$^{a)}$	6.54$^{a)}$	0.565
5.75	0.59	0.76		1.15					0.509
6.0	0.45	0.56	0.82		2.35$^{a)}$		3.36$^{a)}$	4.25$^{a)}$	0.320
Phosphate	(0.0005 M)	(0.001 M)	(0.002 M)	(0.003 M)	(0.005 M)	(0.01 M)	(0.03 M)	(0.05 M)	
6.5	0.57	0.79	1.20			1.82$^{a)}$	2.37$^{a)}$	3.01$^{a)}$	0.365
7.0	0.38	0.53	0.70	0.85	1.11$^{a)}$	1.30$^{a)}$		1.91$^{a)}$	0.290
7.5	0.33	0.38	0.53			1.13$^{a)}$		1.56$^{a)}$	0.255
8.0			0.47	0.56	0.66	0.92$^{a)}$		1.33$^{a)}$	0.360
8.0 (30 °C)		0.27	0.31	0.37	0.39				0.230
8.0 (45 °C)		0.78	0.93	1.06	1.32				0.645
Borate						(0.01 M)	(0.03 M)	(0.05 M)	
8.35						0.34	0.41	0.48	0.305
8.55						0.39	0.43	0.52	0.349
9.1						0.55	0.66	0.85	0.462
9.35						0.65	0.71	0.82	0.600
9.6						0.80	0.95	1.06	0.742
9.9						0.97	1.02	1.16	0.908
Carbonate						(0.01 M)	(0.03 M)	(0.05 M)	
10.4						1.83	2.02	2.10	1.71
10.65						2.09	2.22	2.48	1.88$^{b)}$
11.1						2.99	3.45	3.83	2.58$^{b)}$
NaOH									
11.8	6.18								6.18$^{b)}$

a) Values neglected in the extrapolation for k_0.
b) Values neglected in the nonlinear least-squares estimation for $k'^{\mathrm{I}}_{\mathrm{H_2O}}$ and $k^{\mathrm{I}}_{\mathrm{OH}}$ in Eq. (8b).

account in the solutions of pH between approximately 3.5 and 8.

pH-Profile of the Rate Constant

The logarithm of the rate constant k_0 at 37 °C is plottd against the pH of the experimental solutions in Fig. 3. After a plateau in the low pH range, there is a decrease from pH 5 to the minimum at approximately pH 7.5, then an increase in the alkaline region. The plateau portion suggests that k_0 is independent of hydrogen ion concentration, and the steep rising portion in the alkaline region implies that hydroxide ion catalysis is important.

The rate constant k_0 described by Eq. (4) should be given more exactly by Eq. (7), taking

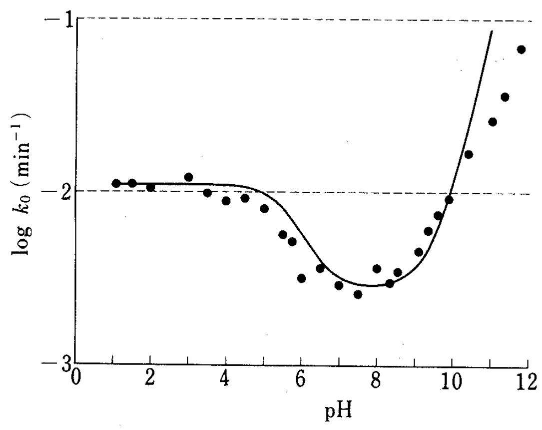


Fig. 3. logk_0–pH Profile for the Hydrolysis Reaction of Oxazolam at 37 °C, $\mu=0.1$

into consideration the hydrogen ion concentration;

$$k_0=(k^{\mathrm{I}}_{\mathrm{H}}\cdot a_{\mathrm{H}}+k^{\mathrm{I}}_{\mathrm{H_2O}}+k^{\mathrm{I}}_{\mathrm{OH}}\cdot a_{\mathrm{OH}})\cdot f^{\mathrm{I}}+(k^{\mathrm{II}}_{\mathrm{H}}\cdot a_{\mathrm{H}}+k^{\mathrm{II}}_{\mathrm{H_2O}}+k^{\mathrm{II}}_{\mathrm{OH}}\cdot a_{\mathrm{OH}})\cdot f^{\mathrm{II}} \tag{7}$$

where $k^{\mathrm{I}}_{\mathrm{H}}$, $k^{\mathrm{II}}_{\mathrm{H}}$ are 2nd-order rate constants of the reactions catalyzed by hydrogen ion for species I and II, respectively; $k^{\mathrm{I}}_{\mathrm{H_2O}}$ and $k^{\mathrm{II}}_{\mathrm{H_2O}}$ are first-order rate constants of the reactions catalyzed by H_2O molecule; $k^{\mathrm{I}}_{\mathrm{OH}}$ and $k^{\mathrm{II}}_{\mathrm{OH}}$ are 2nd-order rate constants of the reactions catalyzed by hydroxide ion; and a_{OH} is the activity of hydroxide ion. In this equation, the term $k^{\mathrm{I}}_{\mathrm{H}}\cdot a_{\mathrm{H}}\cdot f^{\mathrm{I}}$, which describes the reaction of I catalyzed by H^+, is kinetically equivalent to the term $k^{\mathrm{II}}_{\mathrm{H_2O}}\cdot f^{\mathrm{II}}$, which describes the reaction of II catalyzed by H_2O.[3d)] Therefore, those two terms can be combined into one, $k'^{\mathrm{II}}_{\mathrm{H_2O}}\cdot f^{\mathrm{II}}$. In the same manner, $k^{\mathrm{I}}_{\mathrm{H_2O}}\cdot f^{\mathrm{I}}$ and $k^{\mathrm{II}}_{\mathrm{OH}}\cdot a_{\mathrm{OH}}\cdot f^{\mathrm{II}}$ can be combined into $k'^{\mathrm{I}}_{\mathrm{H_2O}}\cdot f^{\mathrm{I}}$. Then Eq. (7) is simplified as follows;

$$k_0=(k'^{\mathrm{I}}_{\mathrm{H_2O}}+k^{\mathrm{I}}_{\mathrm{OH}}\cdot a_{\mathrm{OH}})\cdot f^{\mathrm{I}}+(k^{\mathrm{II}}_{\mathrm{H}}\cdot a_{\mathrm{H}}+k'^{\mathrm{II}}_{\mathrm{H_2O}})\cdot f^{\mathrm{II}} \tag{8a}$$

$$=\left(k'^{\mathrm{I}}_{\mathrm{H_2O}}+k^{\mathrm{I}}_{\mathrm{OH}}\cdot\frac{K'_{\mathrm{w}}}{a_{\mathrm{H}}}\right)\frac{K_{\mathrm{a}}}{K_{\mathrm{a}}+a_{\mathrm{H}}}+(k^{\mathrm{II}}_{\mathrm{H}}\cdot a_{\mathrm{H}}+k'^{\mathrm{II}}_{\mathrm{H_2O}})\frac{a_{\mathrm{H}}}{K_{\mathrm{a}}+a_{\mathrm{H}}} \tag{8b}$$

where K'_{w} is the product of a_{H} and a_{OH}.

The value of $k^{\mathrm{II}}_{\mathrm{H}}$, which is the rate constant for the reaction of II catalyzed by H^+, can be estimated to zero, because no increase of k_0 was observed with decrease of the medium pH. The value of $k'^{\mathrm{II}}_{\mathrm{H_2O}}=1.1\times10^{-2}\,\mathrm{min}^{-1}\cdot\mathrm{M}^{-1}$ was estimated from the k_0 value at the plateau portion in the acidic range, where $f^{\mathrm{I}}=0$ and $f^{\mathrm{II}}=1$ could reasonably be assumed. Other unknown constants, $k'^{\mathrm{I}}_{\mathrm{H_2O}}$ and $k^{\mathrm{I}}_{\mathrm{OH}}$, were estimated by the nonlinear least-squares fit method,[8a)] and the values $k'^{\mathrm{I}}_{\mathrm{H_2O}}=2.8(\pm0.4\ \mathrm{S.D.})\times10^{-3}\,\mathrm{min}^{-1}\cdot\mathrm{M}^{-1}$ and $k^{\mathrm{I}}_{\mathrm{OH}}=4.8(\pm0.9\ \mathrm{S.D.})\times10\,\mathrm{min}^{-1}\cdot\mathrm{M}^{-1}$ were obtained. Here, $K_{\mathrm{a}}=1.82\times10^{-6}\,\mathrm{M}$ ($\mathrm{p}K_{\mathrm{a}}=5.74$)[1b)] and $K'_{\mathrm{w}}=1.8\times10^{-14}\,\mathrm{M}^2$ were used,[9a)] and the last three k_0 data in the strongly alkaline region were omitted in the calculation since they deviated significantly from the preceding curve.

The solid line depicted in Fig. 3 based on Eq. (8b) with the values obtained above. The observed data points fit fairly well to the line, and the validity of Eq. (8b) was thus confirmed. The inflection point observed near pH 5 should be due to the change of the main reacting species from II to I, since the pK_{a} was 5.74 for oxazolam.[1b)] In the range above pH 10, where I is the main species and the OH^- catalyzing rate constant ($k^{\mathrm{I}}_{\mathrm{OH}}\cdot a_{\mathrm{OH}}$) is predominant in k_0, remarkable deviation was observed. Although the reason remained unclear in the present study, the second ionization of I at the nitrogen of position 7 may occur, causing this inflection of the curve.

Temperature Dependency of the Rate Constant

The temperature dependency of the rate constant was studied at two different pHs, pH 2.0 and 8.0, where k_{II} and k_{I} are the main reactions, respectively. Three temperature levels, 30,

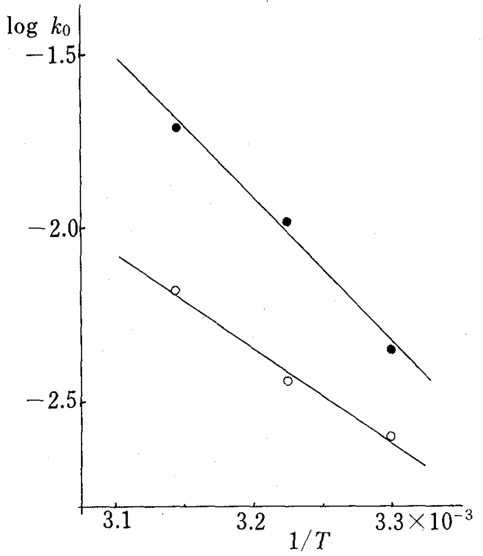


Fig. 4. Arrhenius Plots for the First-Order Rate Constant k_0 at $\mu=0.1$

●, in pH 2.0 glycine buffer; ○, in pH 8.0 phosphate buffer.

TABLE II. The Activation Energy and Other Thermodynamic Parameters for Hydrolysis of Oxazolam at 37 °C, $\mu=0.1$

pH	Activation energy, E_a (kcal/mol)	Enthalpy of activation, $\Delta H^{\neq}$ (kcal/mol)	Free energy of activation, $\Delta G^{\neq}$ (kcal/mol)	Entropy of activation, $\Delta S^{\neq}$ (cal/mol·deg)
2.0	18.8	18.2	23.4	−16.8
8.0	12.4	11.5	24.2	−42.3

37 and 45 °C, were employed for the kinetic study.

The Arrhenius plot of k_0 is shown in Fig. 4. The calculated activation energies are given in Table II together with other thermodynamic parameters obtained from the plot of $\log(k_0/T)$ against $1/T$, where T is the reaction temperature in the absolute unit. The negative and fairly large value of the entropy of activation implies that the transition state of the rate-determining step may be a moderately restrained one. The mechanism of the reaction will be discussed later.

Effect of Ionic Strength

The effect of ionic strength on the rate constant was studied in experimental solutions of pH 2.0, 5.5, and 8.0 with various ionic strength value (μ) between 0.002 and 0.2. A stronger ionic solution was not prepared because of crystallization of sodium chloride added for adjustment of the strength. No significant effect was observed on the rate constant in the range of ionic strength studied.

Effects of the Buffer System and Concentration

The hydrolytic rate constant k_{obs} was strongly affected by the concentration of buffering components. The relationship between k_{obs} and the concentration is shown in Fig. 5; the pattern of the relationship depended on the pH of the solution as well as the buffer system.

The linearity of the relationship was sufficient to obtain k_0 by extrapolation, although two-phase patterns were observed in acetate and phosphate systems. The values of k_{obs} in the low concentration range were used for k_0 in these systems. In borate and carbonate systems, the low concentration range was omitted because of the difficulty of the pH control.

The relationship was linear over the whole range of concentration studied in the glycine

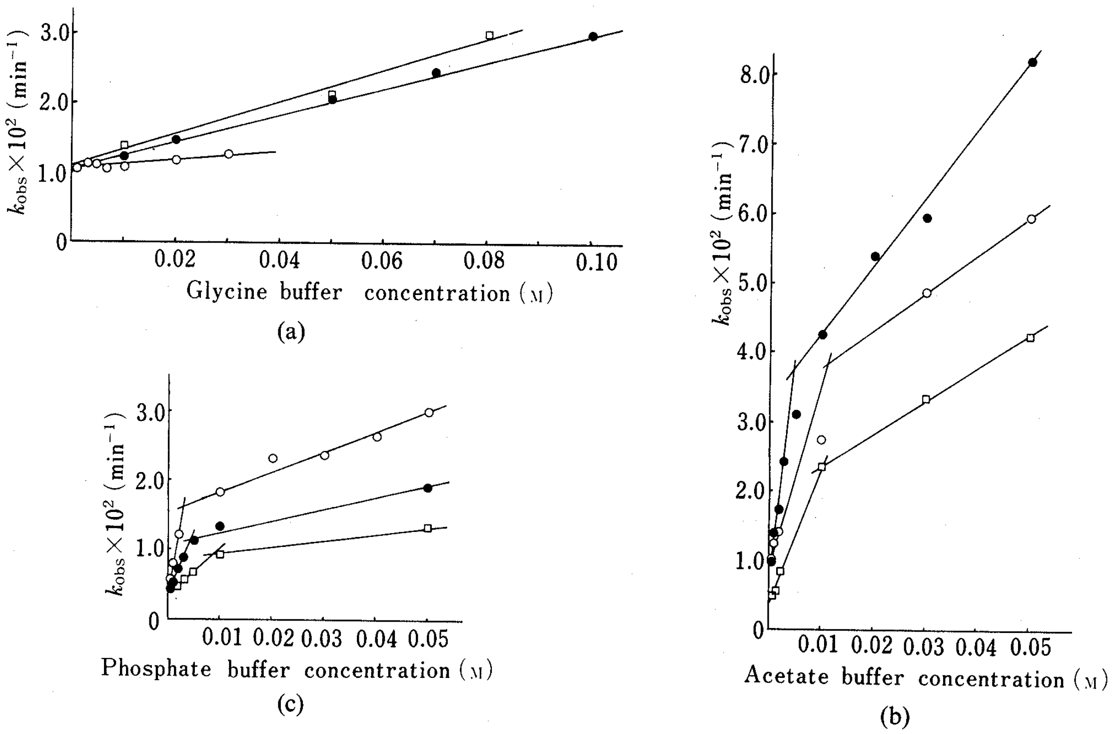


Fig. 5. Relationships between the Apparent Rate Constant k_{obs} and the Buffer Concentration at 37 °C, $\mu=0.1$

(a): in glycine buffer.
○, pH 2.0; ●, pH 3.0; □, pH 3.5.
(b): in acetate buffer.
○, pH 4.0; ●, pH 5.0; □, pH 6.0.
(c): in phosphate buffer.
○, pH 6.5; ●, pH 7.0; □, pH 8.0.

system, where the reacting species was only II. Therefore, k_{obs} in Eq. (3) can be written as follows;

$$k_{obs}=k_{obs}^{II}=k_0^{II}+k_B^{II}\cdot[\text{buffer}] \qquad (9a)$$

$$=k_0^{II}+k_{HA}^{II}\cdot[\text{HA}]+k_A^{II}\cdot[\text{A}^-] \qquad (9b)$$

$$=k_0^{II}+\left(k_{HA}^{II}\cdot\frac{a_H}{K_{HA}}+k_A^{II}\right)\cdot[\text{A}^-] \qquad (9c)$$

where [HA] and [A$^-$] are the concentration of the conjugate acid and base in the buffer system, K_{HA} is the dissociation constant of HA (4.46×10^{-3} M for glycine[10]), and k_B^{II}, k_{HA}^{II}, k_A^{II} are 2nd-order rate constants of the reaction catalyzed by the total buffer, HA, A$^-$, respectively. Although the slope of the lines in Fig. 5a depended on the solution pH, the same intercept was obtained and thus k_0 was independent of the solution pH.

When a plot was made according to Eq. (9c), as shown in Fig. 6, all the points fell on the same line independently of pH, and the result indicated that the value of $k_{HA}^{II}\cdot a_H/K_{HA}$ was negligibly small compared to that of k_A^{II}. Since a_H/K_{HA} should be of the order of 10^{-1}—10 in this range of pH, the comparative relationship $k_A^{II}\gg k_{HA}^{II}$ was obtained. In other words, k_{obs} was dependent only on the concentration of neutral glycine, [$H_3N^+CH_2COO^-$], but not that of glycinium ion, [$H_3N^+CH_2COOH$]. The slope of the line gave $k_A^{II}=k_{H_3N^+CH_2COO^-}^{II}=2.5\times 10^{-1}\ \text{min}^{-1}\cdot\text{M}^{-1}$.

In acetate buffers, however, the result (Fig. 7) was quite different from that in the glycine system in that the k_{obs} values were still pH-dependent. This may be because I and II co-exist in this pH range and the ratio of these species changes with the pH of solutions. The expression

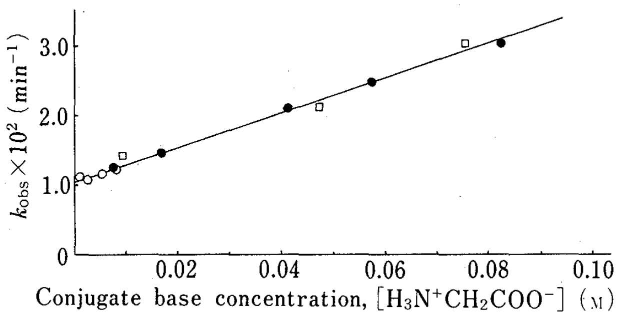


Fig. 6. Relationship between k_{obs} and the Conjugate Base Concentration in Glycine Buffer at 37 °C, $\mu=0.1$

○, pH 2.0; ●, pH 3.0; □, pH 3.5.

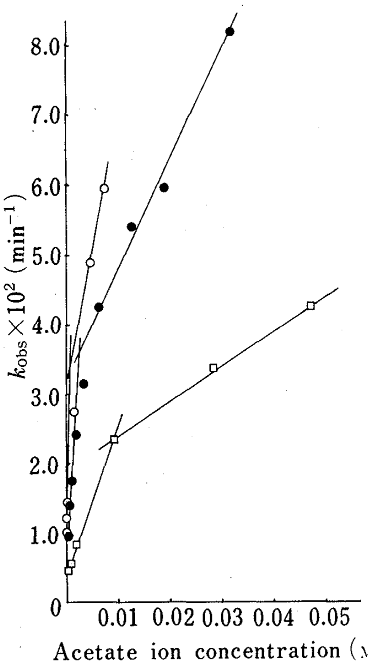


Fig. 7. Relationship between k_{obs} and the Conjugate Base Concentration in Acetate Buffer at 37 °C, $\mu=0.1$

○, pH 4.0; ●, pH 5.0; □, pH 6.0.

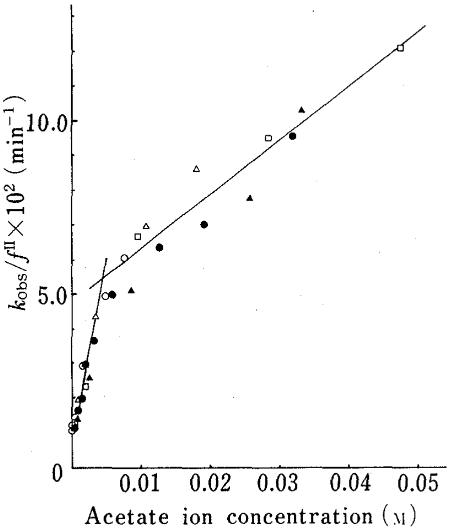


Fig. 8. Relationship between k_{obs}/f^{II} and the Conjugate Base Concentration in Acetate Buffer at 37 °C, $\mu=0.1$

○, pH 4.0; △, pH 4.5; ●, pH 5.0; ▲, pH 5.5; □, pH 6.0.

for k_{obs} in Eq. (3a) can be approximated to Eq. (10), provided that the first term is sufficiently small compared to the second term. The assumption may be acceptable for the pH range where the relation $f^{II}>f^{I}$ is established, since $k'^{II}_{H_2O}$ is almost four times greater than $k'^{I}_{H_2O}$.

$$k_{obs}=k^{II}_{obs}\cdot f^{II} \tag{10}$$

The following equation is obtained;

$$k^{II}_{obs}=k_{obs}/f^{II} \tag{11a}$$

$$=k^{II}_{0}+\left(k^{II}_{HA}\cdot\frac{a_H}{K_{HA}}+k^{II}_{A}\right)\cdot[A^-] \tag{11b}$$

where the conjugate acid and base, HA and A^-, are acetic acid and acetate ion, respectively.

Figure 8 shows the plot according to Eq. (11b). All the data points are on the same line independently of the solution pH. The result confirmed that the rate constant k^{II}_{obs} changed depending on the concentration of the general base, acetate ion. Although the line exhibited a two-phase pattern having an inflection point at 0.005 M of acetate ion concentration, the value of $k^{II}_{A}=k^{II}_{CH_3COO^-}=1.0\times10\,\text{min}^{-1}\cdot\text{M}^{-1}$ was obtained from the first slope. This suggested that acetate ion was a 40 times stronger catalyst than glycine.

Therefore, the reaction constant $k^{\mathrm{II}}_{\mathrm{obs}}$ was proved to be of general base catalysis, so that the step k_{II} in Chart 1 has the rate-determining step of a general base catalyzed reaction. On the other hand, k_{I} could be considered as a reaction of specific base catalysis, as well as water molecule catalyzed one, due to the prominent increase of the rate constant k_0 in the alkaline region, where the species I was the main reactant.

The buffer effect on k_{obs} also showed the two-phase pattern in the case of phosphate. However, no detailed discussion was possible since both k_{I} and k_{II} were involved and could not be discriminated. The buffer effect became smaller and closer to a one-phase pattern with increase of the pH. In borate and carbonate systems, though no investigation was done in the low concentration range, the error in estimating k_0 should be small, since the two-phase feature seemed to almost disappear in the pH range higher than pH 8.0. Some examples were given in other papers[3c,11] of such the two-phase dependency of k_{obs} on buffer concentration. The phenomena were attributed to the change of the rate-determining-step of the reaction in higher buffer concentration. This might also be the case here.

Mechanism of the Hydrolytic Reaction

The reaction pathway between I and II was previously reported,[1b] and it was shown that the hydrolytic cleavage of the diazepinone nucleus took place through two different routes depending on the pH of the solution in the present study. A possible mechanism for the reaction is presented in Chart 2.

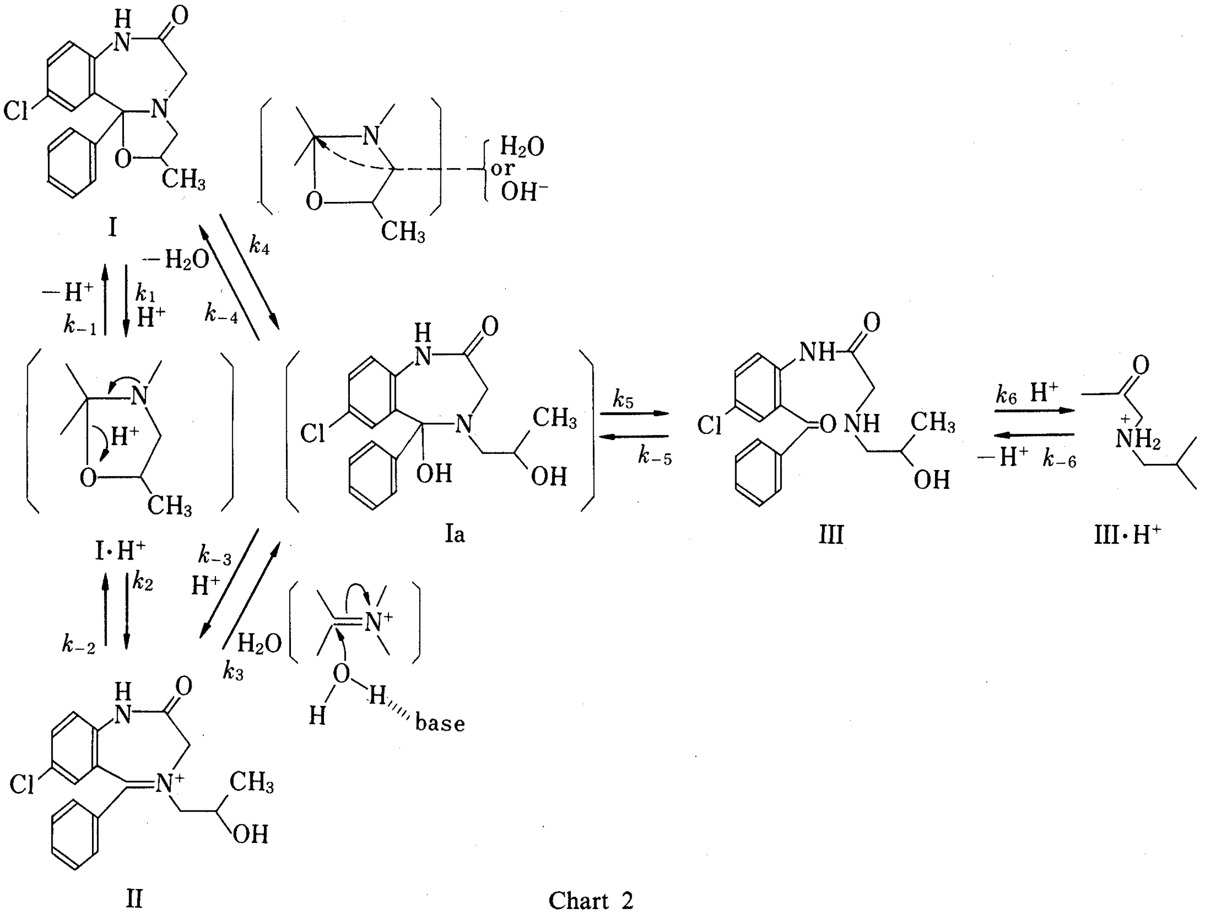


Chart 2

The carbinolamine intermediate (Ia) was presumed in the already established mechanistic principle for imine hydrolysis,[12] and it seems reasonable to apply this to the reaction II→III in acidic media. Such a reaction route was also proposed by Hong *et al.*[5] for 1,4-

benzodiazepines. Although the principle suggests that k_5 is the rate-determining step in acidic media and k_3 is rate-determining in neutral or alkaline solution, it is also mentioned that electron-withdrawing substituents reduce the boundary pH. Therefore, it may be feasible to consider that the rate-determining-step (RDS) is k_3, which is the nucleophilic attack of a water molecule on the 11b carbon in aid of the general base. The carbon has two phenyl substituents as electron-withdrawing groups, and the kinetic results in the previous section suggested that the RDS was a general base-catalyzed reaction. The inflection point observed in Fig. 8 might be due to the change of the RDS from k_3 to k_5.

In neutral or alkaline media, in which the reaction route is I→III, the formation of Ia through k_4 may be the RDS and k_5 may be fast. This hypothesis is supported by the kinetic results shown in Fig. 3, in which increase of the reaction rate constant was observed with increase of hydroxide ion concentration. The RDS, k_4, is the step of nucleophilic attack of a water molecule or a hydroxide ion on the carbon atom at position 11b.

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