

Stabilization of Drugs. I. The Quantitative Prediction of the pH-Dependency of Amide and Anilide Hydrolyses by Neighboring Hydroxyl Groups

TSUKINAKA YAMANA, AKIRA TSUJI, and YŪZŌ MIZUKAMI

Faculty of Pharmaceutical Sciences, Kanazawa University¹⁾

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Intramolecular hydrolyses of amides and anilides were examined over wide pH ranges at 90°. Shape of pH-rate profiles indicates that the relative reactivity of ionized (k'_{RO^-}) and un-ionized (k'_{ROH}) alcohol functions toward amide linkage is governed by difference in pK_a between attacking group alcohol and leaving group amine. As predicted from the theoretical consideration, their rate constants, k'_{RO^-} and k'_{ROH} , were found to have good linear free-energy correlation.

The problem of stabilization of unstable drugs in aqueous solution has long plagued pharmacists in every area of the profession. Some cases have been attempted in the control of pH, solubility, the choice of solvent system, the use of adjuvants, or in the careful control of buffer capacity and constituents.²⁾ Another attempt, which has the possibility of the production of reasonably stable drugs, has been made in the modification of drugs by appropriate substituents, if therapeutic efficacy is not decreased. However, due to the difficulty of the quantitative prediction of the structure-reactivity relationship, much attention for drug stabilization has been paid to the pharmaceutical formulation such as the former.

Numerous compounds which are important from a pharmaceutical standpoint contain an amide function. In general, hydrolysis of amide linkage at room temperature in pharmaceutically useful pH ranges proceeds extremely slow.³⁻⁵⁾ Even in strong acidic and basic solutions, amide⁶⁻¹³⁾ and anilide⁵⁾ are relatively stable as compared, for example, with the structurally similar esters. These kinetic studies indicate that amide linkage in pharmaceutical system is sufficiently stable in aqueous solution to be marketed. The excellently stable amide linkage, however, is rapidly hydrolyzed by the intramolecular catalysis of neighboring hydroxyl groups¹⁴⁻¹⁶⁾ or carboxyl groups.¹⁷⁾ It has been reported in the previous papers that both

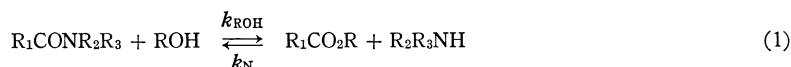
- 1) Location: Takara-machi, Kanazawa.
- 2) E.R. Garrett, "Advances in Pharmaceutical Sciences," Vol. 2, ed. by H.S. Bean, A.H. Beckett and J.E. Carless, Acad. Press, New York, 1967, p. 1.
- 3) P. Finhalt and T. Higuchi, *J. Pharm. Sci.*, **51**, 655 (1962).
- 4) T. Higuchi, A.D. Marcus, and C.T. Bias, *J. Am. Pharm. Ass., Sci., Ed.* **43**, 129 (1954); T. Higuchi and A.D. Marcus, *ibid.*, **43**, 530 (1954).
- 5) K.T. Koshy and J.L. Lach, *J. Pharm. Sci.*, **50**, 113 (1961).
- 6) a) T. Yamana, Y. Mizukami, A. Tsuji, Y. Yasuda, and K. Masuda, *Chem. Pharm. Bull. (Tokyo)*, **20**, 881 (1972); b) P.D. Bolton, J. Ellis, P.D. Frier, and P.C. Mancarrow, *Aus. J. Chem.*, **25**, 303 (1972).
- 7) a) P.D. Bolton, *Aust. J. Chem.*, **19**, 1013 (1966); b) P.D. Bolton and G.L. Jackson, *ibid.*, **22**, 527 (1969); c) *Idem, ibid.*, **24**, 471 (1971); d) *Idem, ibid.*, **24**, 969 (1971).
- 8) B.S. Rabinovitch and C.A. Winkler, *Can. J. Chem.*, **20**, 73 (1942).
- 9) I. Meloche and K.J. Laidler, *J. Am. Chem. Soc.*, **73**, 1712 (1951).
- 10) a) E.E. Reid, *Am. Chem. J.*, **21**, 284 (1899); b) *Idem, ibid.*, **24**, 397 (1900).
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- 13) A. Bruylants and F. Kezdy, *Record Chem. Progr.*, **21**, 213 (1960).
- 14) T.C. Bruice and S. Benkovic, "Bio-organic Mechanisms," Vol. 1, W.A. Benjamin, Inc., New York, 1966, p. 146.
- 15) T. Yamana, A. Tsuji, and Y. Mizukami, *Chem. Pharm. Bull. (Tokyo)*, **20**, 1217 (1972).
- 16) A. Tsuji, T. Yamana, and Y. Mizukami, *Chem. Pharm. Bull. (Tokyo)*, **20**, 2528 (1972).
- 17) For a summary of pertinent references see refs. 14, 15, and 16.

hydroxyl group and its anion participate through formation of corresponding five, six or seven membered lactone.^{15,16)} Their mechanisms in neutral and basic media are the rate-determining breakdown of the tetrahedral intermediates, and their reactivities correlate with the difference in pK_a of leaving group amine and attacking group alcohol.¹⁶⁾

The present study was attempted to generalize the structure-reactivity relationship of amides, anilides and probably lactams. This knowledge may enable to us to design the reasonably stable compounds toward intramolecular catalytic reactions.

Theoretical Consideration

The alcoholysis of amides can be formally written as



From Eqs. (1) and (2) the following relationship can be led

$$\frac{k_{RO^-}}{k_{ROH}} = \frac{K_w}{K_{a_{ROH}}} \cdot \frac{k_B}{k_N} \quad (3)$$

where $K_{a_{ROH}}$ and K_w are the dissociation constants of alcohol (ROH) and water, respectively. For aminolysis of esters, the rate constants k_N and k_B must obey the following Brønsted equations¹⁴⁾

$$\log k_N = \beta_N pK_{a_{R_2R_3NH}} + C_N \quad (4)$$

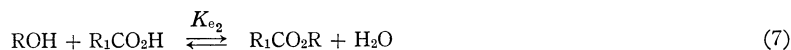
and

$$\log k_B = \beta_B pK_{a_{R_2R_3NH}} + C_B \quad (5)$$

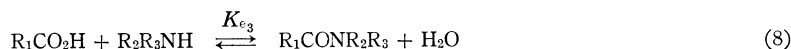
where β_N , β_B , C_N and C_B are constants which can be obtained from Brønsted plots. The logarithmic transformation of Eq. (3) and combination of Eqs. (4) and (5) yield

$$\log k_{RO^-} - \log k_{ROH} = pK_{a_{ROH}} - (\beta_N - \beta_B)pK_{a_{R_2R_3NH}} - (pK_w + C_N - C_B) \quad (6)$$

The equilibrium constant, K_{e_1} for reaction (1) can be calculated simply from the following two equilibria;



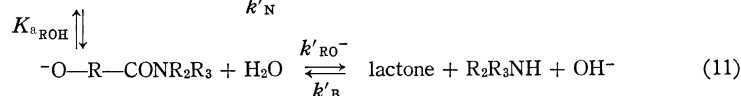
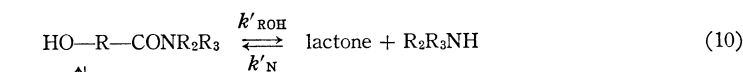
and



Thus

$$K_{e_1} = K_{e_2}/K_{e_3} \quad (9)$$

Similar formal schema may be written for the intramolecular alcoholysis of amides¹⁶⁾ and anilides which produces corresponding intraesters (*i.e.*, lactones) (see Eqs. (10) and (11)).



In the case of the equilibria with methyl formate and formamide, the data are precise. Unfortunately, there was no information on the hydrolysis of other amides because of the

excessively slow rates of uncatalyzed reactions. By rough approximation for other amide hydrolyses, Fersht¹⁸⁾ obtained the empirical equations. That is, Fersht¹⁸⁾ reported from the analyses of the data of Jencks¹⁹⁻²¹⁾ that k_B and k_N for the aminolysis at 25° of phenylacetate and methylformate by amines in the pK_a range 8.3–11.4 fit the empirical equation (12)

$$\log k_B = \log k_N - 1.0 pK_{a_{R_2R_3NH}} + 15.34 \quad (12)$$

From Eqs. (4) and (5), Eq. (12) gives $\beta_N - \beta_B = 1.0$ and $C_N - C_B = -15.34$. The introduction of this relationship and $pK_w = 15.74$ to Eq. (6) leads to

$$\log k_{RO^-} - \log k_{ROH} = pK_{a_{ROH}} - pK_{a_{R_2R_3NH}} - 0.4 \quad (13)$$

Furthermore, the combination of Brønsted relationships for the equilibria of ester formation (Eq. (7)) and amide formation (Eq. (8)) leads to Eq. (14) by using Eq. (9) for alcoholysis rate constant k_{ROH}

$$\log k_{ROH} = \beta_{\text{attack}} pK_{a_{ROH}} + \beta_{\text{leav}} pK_{a_{R_2R_3NH}} + \text{constant} \quad (14)$$

Fersht¹⁸⁾ showed, for alcoholysis of amide, a rough correlation of β_{attack} values for attacking group with β_{leav} values for leaving group according to mechanism. This correlation provides that β_{attack} and β_{leav} are opposite sign and that their absolute values are approximately equal, although this is not always the case. Such a correlation was also found in other acyl transfer reactions.²²⁾ These reactions show a definite trend for β values between attacking group and leaving group to change in a parallel manner (roughly 1:1).²²⁾ Fersht and Jencks concluded that most of acyl transfer reactions are coupled in such a way that a given degree of bond formation is accompanied by a similar amount of bond cleavage.²²⁾

On the basis of these linear free-energy relationships (Eqs. (13) and (14)) and of the argument^{18, 22)} for β values, it is assumed that intramolecular alcoholyses of amides and anilides may obey the similar equations.

$$\log k'_{RO^-} - \log k'_{ROH} = \beta_R (pK_{a_{ROH}} - pK_{a_{R_2R_3NH}}) + C_R \quad (15)$$

$$\log k'_{ROH} = \beta_{ROH} (pK_{a_{ROH}} - pK_{a_{R_2R_3NH}}) + C_{ROH} \quad (16)$$

From the above relationships, Eq. (17) can be easily led

$$\log k'_{RO^-} = \beta_{RO^-} (pK_{a_{ROH}} - pK_{a_{R_2R_3NH}}) + C_{RO^-} \quad (17)$$

where $\beta_{RO^-} - \beta_{ROH} = \beta_R$ and $C_{RO^-} - C_{ROH} = C_R$.

Experimental

Materials—*o*-Hydroxyphenylacetic acid- γ -lactone (**3a**) used was the material prepared in an earlier study.¹⁶⁾ Aniline and *m*-chloroaniline were purified by distillation. 5-Nitro-2-hydroxyphenylacetic acid- γ -lactone (**5a**) was prepared by nitration of **3a**, mp 189° (lit.²³⁾ 189°). 5-Nitro-2-hydroxyphenylacetamide (**5**) was prepared as follows; Five grams of **5a** was dissolved in 50 ml of liq. NH_3 . The mixture was allowed to stand at room temperature for 8 hr and residual NH_3 was evaporated at 40°. The solid was recrystallized (see Table I). The reflux at 210° for 2 hr with **5a** and excess aniline gave 5-nitro-2-hydroxyphenylacetanilide (**6**). The other anilides (**7** and **8**) were prepared from **3a** and the corresponding anilines by the method of Stoermer.²⁴⁾ Properties of the amide and anilides are listed in Table I.

All other chemicals were of reagent grade quality and were used without further purification.

Kinetics—The medium used for the kinetic studies was 5% ethanol-water (v/v). Buffers employed were of the same systems in an earlier paper.¹⁶⁾ The rate of hydrolysis of amide ($1.00 \times 10^{-3} M$) was deter-

18) A.R. Fersht, *J. Am. Chem. Soc.*, **93**, 3504 (1971).

19) W.P. Jencks and J. Carriulo, *J. Am. Chem. Soc.*, **82**, 765 (1960).

20) A.R. Fersht and W.P. Jencks, *J. Am. Chem. Soc.*, **92**, 5432 (1970).

21) W.P. Jencks and M. Gilchrist, *J. Am. Chem. Soc.*, **88**, 104 (1966).

22) A.R. Fersht and W.P. Jencks, *J. Am. Chem. Soc.*, **92**, 5442 (1970).

23) P. Tobias, J.H. Heidema, K.W. Lo, E.T. Kaiser, and F.J. Kezdy, *J. Am. Chem. Soc.*, **91**, 202 (1969).

24) R. Stoermer, *Ann.*, **313**, 79 (1900).

TABLE I. Hydroxyamide and Hydroxyanilides

No.	R ₁	R ₂	Method	mp (Recryst. solvt)	Formula	Analysis (%)		
						Calcd.	Found	
						C	H	N
5	NO ₂	H	a)	225° (acetone)	C ₈ H ₈ O ₄ N ₂	49.47 49.24	4.16 4.22	14.42 14.61
6	NO ₂	C ₆ H ₅	a)	192° (acetone)	C ₁₄ H ₁₂ O ₄ N ₂	61.75 61.76	4.45 4.59	10.29 10.26
7	H	C ₆ H ₅	b)	151° ^c (EtOH)	C ₁₄ H ₁₃ O ₂ N	73.97 73.83	5.77 5.69	6.16 6.34
8	H	<i>m</i> -ClC ₆ H ₄	b)	139° (peteronium ether)	C ₁₄ H ₁₂ O ₂ NCl	64.24 64.37	4.63 4.73	5.35 5.64

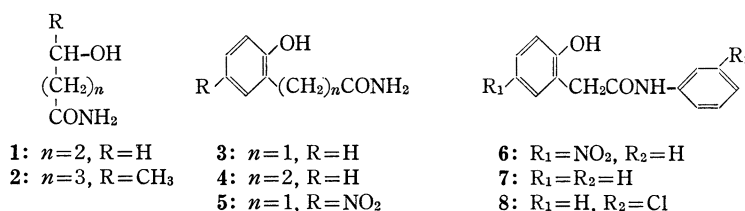
a) present method b) ref. 23 c) lit²⁰ mp 151–152°

mined by following the liberated ammonia in the same manner reported previously.^{15,16} The hydrolysis rates of anilides (5.00×10^{-4} M) were determined by following the appearance of the corresponding anilines by the diazotization assay.²⁵

pK_a Determination—The pK_a of phenol groups were determined kinetically from the pH-rate profiles or spectrophotometrically, in the same manner as reported previously.¹⁶ Other pK_a values were determined spectrophotometrically or potentiometrically under the conditions of kinetic experiments.²⁶

Result and Discussion

Pseudo first-order rate constants (k_{obs}) of the hydrolyses at 90° ($\pm 0.05^\circ$) of the compounds **1**–**8** were determined at various pH and in the presence of a large excess of buffer over amide and anilide. A constant ionic strength of 0.6 was maintained except in solutions of greater acid or base concentration. With the exception of the hydrolysis of **5**, there were large buffer-concentration effects on the rate constants, and plots of k_{obs} vs. buffer-concentration were



found to be linear. The experimental data for the hydrolyses of **6** and **7** are shown in Figs. 1 and 2. Extrapolation to zero buffer concentration supplies the pH dependent first-order rate constant, k_{pH} .

The Linear Free-Energy Relationship of k'_{ROH} and k'_{RO^-} for Amides

In Fig. 3 is plotted $\log k_{\text{pH}}$ vs. pH for **1**, **3**, **4** and **5**. The plots for **1**, **3** and **4** are from previous works.^{15,16} The curves drawn through the experimental points were generated from the rate expression as previously interpreted¹⁶;

25) T. Yamana and H. Koike, *Yakugaku Zasshi*, **81**, 783 (1961).

26) A. Albert and E.P. Sergant, "Ionization Constants of Acid and Bases," John Wiley and Sons, Inc., London, 1962, p. 16 and p. 69.

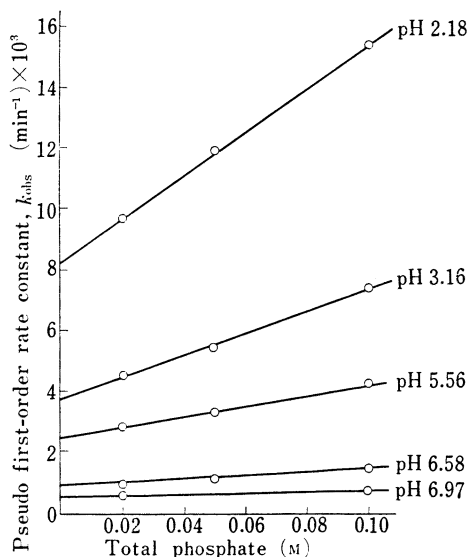


Fig. 1. Plots of Pseudo First-Order Rate Constants against Phosphate Buffer Concentration in the Hydrolysis of 6 at Constant Ionic Strength ($\mu=0.6$) and at 90°

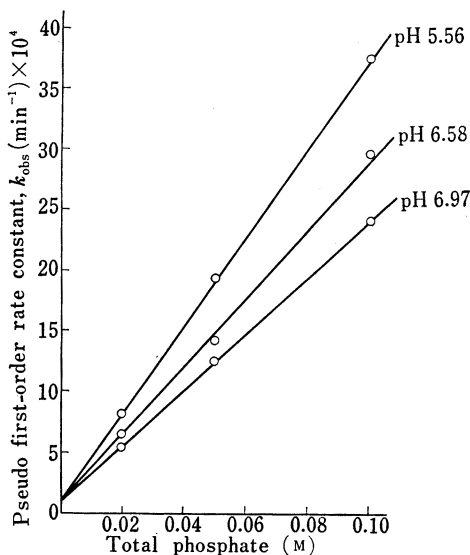


Fig. 2. Plots of Pseudo First-Order Rate Constants against Phosphate Buffer Concentration in the Hydrolysis of 7 at Constant Ionic Strength ($\mu=0.6$) and at 90° .

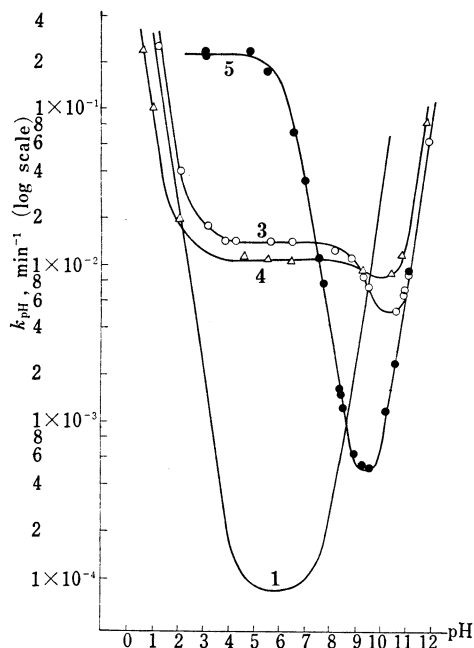


Fig. 3. Log k_{pH} - pH Profiles at 90° ($\mu=0.6$) for the Hydrolyses of Hydroxyamides, 1, 3, 4 and 5

The solid line for 1 is that calculated from the activation energy at each region (ref. 16).

TABLE II. Rate Constants and Dissociation Constants for Hydroxyamides^{a)}

Amide No.	$pK_{s_{ROH}}$	$pK_{s_{R_2R_3NH}}$ ^{b)}	k'_{ROH} (min ⁻¹)	k'_{RO^-} (min ⁻¹)
1 ^{c)}	14.5 ^{d)}	7.88	8.10×10^{-5}	$1.00 \times 10^{3e)}$
2 ^{c)}	14.5 ^{d)}		1.62×10^{-4}	$3.16 \times 10^{2f)}$
3 ^{c)}	9.53 ^{g)} ; 9.50 ^{h)}		1.40×10^{-2}	1.60×10^{-3}
4 ^{c)}	9.79 ^{g)} ; 9.80 ^{h)}		1.10×10^{-2}	2.00×10^{-3}
5	6.25 ^{h)}		2.22×10^{-1}	1.05×10^{-4}

a) Temp. = 90° , $\mu=0.6$ (adjusted with KCl)

b) potentiometrically determined under the conditions of kinetic experiments

c) data from ref. 16

d) estimated^{d)} from the following data; $\Delta H_s = -3.5 + \Delta H_w$ (kcal/mole) where ΔH_s and ΔH_w are the heat of ionization of the alcohol and of water, respectively. $\Delta H_w = 16.5$ at 90° ($\mu=0.6$) was obtained from the literature (H.S. Harned and B.B. Owen, "The Physical Chemistry of Electrolytic Solution," 3rd. Ed., Reinhold Publishing Co., New York, 1963, p. 754). $pK_{s_{ROH}}$ at 25° of 1 and 2 are assumed to be equal to those of butanol and hexanol at 25° ($pK_{s_{ROH}} = 16.10^4$).

e) $k_{pH} = 3.16 \text{ min}^{-1}$ at pH 12. k'_{RO^-} was calculated from equation; $k_{pH} = k'_{RO^-} \cdot K_{s_{ROH}} / (K_{s_{ROH}} + [H^+])$.

f) $k_{pH} = 1.05 \text{ min}^{-1}$ at pH 12. k'_{RO^-} was calculated from equation; $k_{pH} = k'_{RO^-} \cdot K_{s_{ROH}} / (K_{s_{ROH}} + [H^+])$.

g) photometrically determined

h) kinetically determined

i) J. Murto, *Acta Chem. Scand.*, **18**, 1048 (1963).

$$k_{pH} = k_H[H^+] + k'_{ROH} \frac{[H^+]}{K_{a_{ROH}} + [H^+]} + k'_{RO^-} \frac{K_{a_{ROH}}}{K_{a_{ROH}} + [H^+]} + k_{OH}[OH^-] \quad (18)$$

where k_H and k_{OH} represent, respectively, acid- and hydroxide-catalyzed hydrolyses of amides. The parameters concerning with the present discussion are listed in Table II.

As obviously seen in Fig. 3 and in Table II, the reactivity of the ionized and un-ionized alcohols toward amides is governed by pK_a of the hydroxyl group. When the pK_a of alcohol is smaller than that of leaving group ammonia, the un-ionized alcohol is more nucleophilic

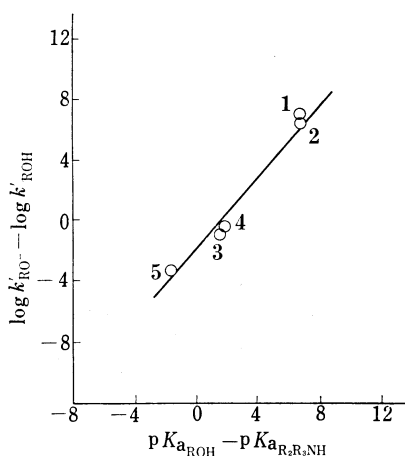


Fig. 4. Plot of $\log k'_{RO^-}/k'_{ROH}$ against $pK_{a_{ROH}} - pK_{a_{R_2R_3NH}}$ for the Intramolecular Alcoholysis of Hydroxyamides

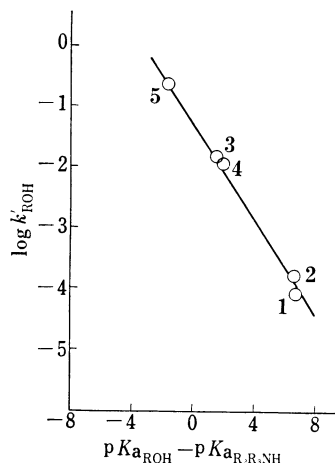


Fig. 5. Plot of $\log k'_{ROH}$ against $pK_{a_{ROH}} - pK_{a_{R_2R_3NH}}$ for the Intramolecular Alcoholysis of Hydroxyamides

(*e.g.*, **5**), and for the opposite pK_a difference the ionized alcohol becomes more effective nucleophile (*e.g.*, **1**). If the nucleophilicities of ROH and RO⁻ are nearly equal, a plot of k_{pH} vs. pH would be almost flat as seen in the cases of **3** and **4**. As noted previously,^{15,16,27} the hydrolyses by the participation of both forms of hydroxyl groups proceed through formation of corresponding lactones, and follow the reaction of Eqs. (10) and (11). These situations suggest that k'_{ROH} and k'_{RO^-} may fit the relationship of Eqs. (15), (16) and (17). Plots of these types provide the reasonable straight lines, as illustrated in Figs. 4, 5 and 6, with slope of $\beta_R = 1.20$, $\beta_{ROH} = -0.40$ and $\beta_{RO^-} = 0.80$ and with intercepts of $C_R = -1.9$, $C_{ROH} = -1.3$ and $C_{RO^-} = -3.2$. For intermolecular alcoholysis of amides whose mechanism is the rate-determining breakdown of the tetrahedral intermediate, Fersht¹⁸) found β value of $-(0.2-0.4)$ for alcohol variation and of $+(0.2-0.5)$ for amine variation. In the previous paper,¹⁶) it was concluded that the hydrolysis mechanism by both participations of un-ionized and ionized hydroxyl groups to neutral amides is the rate-determining breakdown of the tetrahedral intermediate. As seen in the present case, β values of -0.40 for hydroxyl variation and of $+0.40$ for amine variation probably reflect this mechanism. It must be emphasized, therefore, that β value in linear free-energy relationship for the alcoholysis of amides is almost unaffected even if reaction systems are of intramolecular catalyzed systems. These results indicate that the linear free-energy relationships of Eqs. (15), (16) and (17) must be widely applicable to alcoholyses of all other amides including anilides and lactams.

27) T.C. Bruice and F-Hans Marquardt, *J. Am. Chem. Soc.*, **84**, 365 (1962).

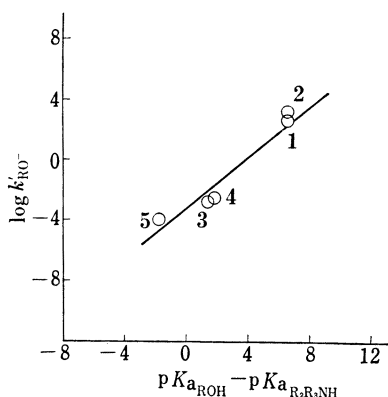


Fig. 6. Plot of $\log k'_{RO-}$ against $pK_{a_{ROH}} - pK_{a_{R_2R_3NH}}$ for the Intramolecular Alcoholysis of Hydroxyamides

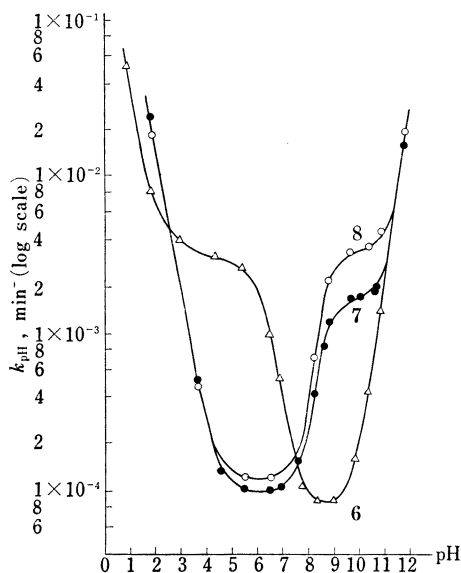


Fig. 7. $\log k_{pH}$ - pH Profiles at 90° ($\mu=0.6$) for the Hydrolyses of Hydroxyanilides, 6, 7 and 8

The Prediction of pH-Rate Profile of Hydroxyanilide Hydrolysis

The linear free-energy relationship as observed in the intramolecular alcoholysis of amides suggests that the interconversion of the shape of pH-rate profile may occur also for hydroxyanilides having appropriate pK_a difference, ΔpK_a ($\Delta pK_a = pK_{a_{ROH}} - pK_{a_{R_2R_3NH}}$). In Fig. 7 are plotted the $\log k_{pH}$ vs. pH for various anilides. The lines for these anilides were generated from Eq. (18). The values of rate constants and dissociation constants for various anilides are given in Table III. The shape of the $\log k_{pH}$ -pH profiles indicates that the hydrolysis of **6** is dependent on the mole fraction of hydroxyanilide in the un-ionized form, and on the contrary, that the hydrolyses of **7** and **8** are dependent on the mole fraction of hydroxyanilides in the ionized forms. Therefore, the neighboring un-ionized phenol is more nucleophile than phenol anion in the hydrolysis of **6**, while phenolate anion is more effective than un-ionized phenol in the hydrolyses of **7** and **8**.

TABLE III. Rate Constants and Dissociation Constants for Hydroxyanilides^{a)}

Anilide No.	$pK_{a_{ROH}}^b$	$pK_{a_{R_2R_3NH}}^c$	k'_{ROH} (min^{-1})	k'_{RO-} (min^{-1})
6	6.22; 6.25 ^{c)}	4.03	3.13×10^{-3}	6.20×10^{-5}
7	8.86	4.03	1.00×10^{-4}	1.75×10^{-3}
8	8.69	2.97	1.10×10^{-4}	3.39×10^{-3}

a) Temp. = 90° , $\mu=0.6$ (adjusted with KCl)

b) kinetically determined

c) photometrically determined under the condition of kinetics experiments

These relative reactivities are also interpreted in the plots of $\log k'_{RO-}$ or $\log k'_{ROH}$ vs. $pK_{a_{ROH}} - pK_{a_{R_2R_3NH}}$ as illustrated in Fig. 8 which provides $\beta_{ROH} = -0.40$ and $\beta_{RO-} = 0.60$. It

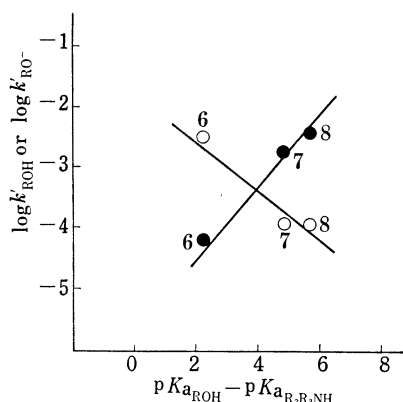


Fig. 8. Plot of $\log k'_{ROH}$ (○) or $\log k'_{RO-}$ (●) against $pK_{a_{ROH}} - pK_{a_{R_2R_3NH}}$ for the Intramolecular Alcoholysis of Hydroxyanilides

is evident from Fig. 8 that the conversion of the shape of $\log k_{pH}$ -pH profile may occur in the hydrolysis of hydroxyanilide having $\Delta pK_a = ca. 4$. In the hydrolyses of hydroxyanilides having larger ΔpK_a than this value, un-ionized hydroxyl may be less nucleophile than ionized hydroxyl, whereas smaller ΔpK_a , the opposite reactivities may be observed.

At room temperature acetamide and acetanilide are hydrolyzed with a half-life of several hundred years at pH 6. The compounds having the neighboring hydroxyl groups such as **5**, however, hydrolyze 10^6 times faster and exhibit half-lives of several hours under the same conditions. Even if the amide linkage would be put under a favorable circumstance to the intramolecular catalysis by neighboring hydroxyl groups, reasonably stable compound at given pH can be designed by an appropriate choice of ΔpK_a value according to the equation;

$$k_{pH} = k_H[H^+] + 10^{(\beta_{ROH}\Delta pK_a + C_{ROH})} \frac{[H^+]}{K_{a_{ROH}} + [H^+]} + 10^{(\beta_{RO-}\Delta pK_a + C_{RO-})} \frac{K_{a_{ROH}}}{K_{a_{ROH}} + [H^+]} + k_{OH}[OH^-] \quad (19)$$

It has recently been suggested¹⁴⁾ that the powerful catalytic reactivity of enzymes is due to the intramolecular catalysis of enzyme-substrate complex by one or more functional groups. The description and discussion of the linear free-energy relationship thus obtained will be useful for controlling the rate of enzymic hydrolysis of amide linkage.

Acknowledgement The authors are grateful to Mr. Y. Itatani for the elemental analysis.