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Studies on the Stability of Amides. IV.[1] Intramolecular Hydroxyl Group Participation in the Acidic Hydrolysis of Aliphatic Amides[2]

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In order to study the structure-reactivity relationship in the intramolecular catalyzed hydrolysis of amide, a series of aliphatic hydroxyamides was subjected to acid-catalyzed hydrolysis. Paper chromatogram verified that the hydroxyamides, which had high rates compared with those of the corresponding alkyl analogues, produced the corresponding lactones in their hydrolytic processes. Their reaction pathways were classified theoretically into three schema. The Arrhenius parameters of entropy and enthalpy or activation were determined and the mechanisms of lactonization and hydroxy acid formation were discussed from entropy-enthalpy relationship. The proposed mechanisms were supported by the appropriate kinetic data.

The chemical and physical properties of amide linkage have long been the subject of the investigation, since amide group is the repeating unit in the biologically important polypeptide macromolecules. The first paper of this series[4] clarified the mechanism of the hydrolysis of N-substituted acetamide in aqueous solution, and showed that alkyl substituted acetamides were generally more resistant to hydrolysis than acetamide. In addition to the stabilization by the steric hindrance of these alkyl substituents, more stability of amides than corresponding esters is expected owing to the resonance structure of amide group.[5] On the other hand, it has been reported that a suitably substituted hydroxyl group,[6–11] carboxyl group[12–18] or imidazoyl group[19] facilitates amide hydrolysis by intramolecular

1) Part III: T. Yamana, Y. Mizukami, A. Tsuji and M. Ikuta, *Chem. Pharm. Bull.* (Tokyo), accepted.
2) This work was presented at the 90th and 91st Annual Meetings of the Pharmaceutical Society of Japan, Sapporo, July 1970 and Fukuoka, April 1971.
3) Location: *Takara-machi, Kanazawa.*
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catalyzed process.[20] Because, among other substituent effects[21] on the hydrolysis rates of amides, these intramolecular participation effects by such functional groups may cause the instability of amides, they have wide significance in studies on the stability of drugs for pharmacists[20a] and on models of enzyme reaction for biochemists.[20b,c]

Facilitation of amide hydrolysis by a neighboring aliphatic hydroxyl group has been recognized in the hydrolyses of aldonamides,[6] γ-hydroxybutyramide,[7b,8a,9] γ-hydroxybutyranilide,[10] and 2-hydroxymethylbenzamide.[11] Bruice, *et al.*[8a] explained the rapid hydrolysis of γ-hydroxybutyramide on the basis of a pathway involving intramolecular nucleophilic displacement by the hydroxyl group on the amide reaction center through intermediate lactone formation. Recently, neighboring hydroxyl groups, which presumably do not form lactone as the intermediate, have been found to facilitate the hydrolysis rates of carboxylic acid derivatives.[22] As reported in the salicylamide hydrolysis,[8b] for instance, when direct nucleophilic catalysis through intermediate lactone formation is prohibited by the molecular geometry, a neighboring hydroxyl group may assist amide hydrolysis by the intramolecular general base or kinetically equivalent general acid catalysis.[8b] In the previous study on the hydrolysis of glucuronamide,[23] it was shown that the amide was hydrolyzed to produce the corresponding five membered lactone (glucurono-γ-lactone) and hydroxy acid (glucuronic acid) in the simultaneous process. It is interesting to ascertain the mechanism of such different reaction routes in some hydroxyamide hydrolyses in connection with the above explanations.

The present investigation was intended to clarify the mechanism of the intramolecular hydroxyl group participation in the simultaneous formation of lactone and hydroxy acid in various aliphatic hydroxyamide hydrolyses.

Experimental

Materials——β-Propiolactone, γ-butyrolactone and γ-valerolactone were purchased from Wako Pure Chemical, Ltd. and Tokyo Kasei Co., Ltd. and purified by distillation. D-Arabono-γ-lactone was kindly supplied by Fuji Chemical Industries, Ltd. and recrystallized from an appropriate solvent (Table I). Other lactones were prepared according to the known method[24–32] (Table I). Table I summarizes the characters of these lactones.

γ,δ-Dihydroxyvaleramide was prepared from δ-hydroxy-γ-valerolactone: A mixture of 5 g of the lactone and 50 ml of liq. ammonia was allowed to stand for 5 hr at room temperature. The solution was heated at 40° *in vacuo* to remove the last of ammonia. The syrup residue cooled on CO_2-acetone mixture overnight gave crude crystals. This solid was recrystallized (Table II). *cis*-3-Hydroxycyclohexanamide was prepared from the corresponding γ-lactone: The lactone (2 g) was dissolved in 50 ml of MeOH and dry NH_3 gas was bubbled into the mixture until saturation. The solution was placed in a sealed tube for a month at room

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Table I. Aliphatic Lactone

Lactone	Method	mp (Recryst. solvt) bp (mmHg)	Formula	Analysis (%) Calcd. Found C	H
δ-Hydroxy-γ-valero lactone	lit. 25	bp 162° (10) [a]	$C_5H_8O_3$	51.72 51.17	6.90 7.38
δ-Valerolactone	lit. 26	bp 120° (20) [b]	$C_5H_8O_2$	—	—
δ-Methyl-δ-valero lactone	lit. 29	bp 114—115° (22) [c]	$C_6H_{10}O_2$	63.13 62.98	8.83 9.00
α,β-Dihydroxy-γ-valerolactone	d)	mp 100—101° [e] (ethyl acetate)	$C_5H_8O_4$	45.45 45.42	6.12 6.08
D-Arabono-γ-lactone	—	mp 98° [f] (acetone)	$C_5H_8O_5$	60.54 60.63	5.45 5.61
3-Hydroxycyclohexanoic acid γ-lactone	g)	bp 120° (20) [h] mp 118—119° [i] (petroleum ether)	$C_7H_{10}O_2$	—	—

a) lit [25] bp 165—166° (11 mmHg)
b) lit [26] bp 120—124° (20 mmHg); lit [27] 114° (17 mmHg)
c) lit [29] bp 95° (9 mmHg); lit [28] 113° (20 mmHg)
d) lit 30; prepared by oxidating β-angelicalactone[31] with $KMnO_4$
e) lit [30] mp 100°
f) lit [24] mp 98—99°
g) lit 32; prepared by the distillation of 3-hydroxycyclohexanoic acid [34] pre-heated for 1.5 hr at 170—180°
h) lit [34] bp 120—123° (19 mmHg)
i) lit [32] mp 118°; lit [34] 119°

Table II. Aliphatic Hydroxyamide

Amide	Method	mp (Recryst. solvt.)	Formula	Analysis (%) Calcd. Found C	H	N
β-Hydroxypropionamide	lit. 35	65 [a] (EtOH)	$C_3H_7O_2N$	40.43 40.69	7.93 7.93	15.73 15.92
γ-Hydroxybutyramide	lit. 36	47 [b] (EtOH–ether)	$C_4H_9O_2N$	46.58 46.24	8.81 8.72	13.58 13.42
γ-Hydroxyvaleramide	lit. 37	56 [c] (ether)	$C_5H_{11}O_2N$	51.25 51.56	9.48 9.62	11.96 11.61
γ,δ-Dihydroxyvaleramide	d)	64 (EtOH–water)	$C_5H_{11}O_3N$	45.09 44.91	8.34 8.28	10.52 10.34
δ-Hydroxyvaleramide	lit. 39	108 [e] (ethyl acetate)	$C_5H_{11}O_2N$	51.25 51.54	9.48 9.52	11.96 11.86
δ-Hydroxycapronamide	lit. 40	72 [f] (ethyl acetate)	$C_6H_{13}O_2N$	54.92 55.19	9.99 10.14	10.67 10.73
D-Arabonamide	lit. 24	138 [g] (MeOH)	$C_5H_{11}O_5N$	36.34 35.99	6.73 6.73	8.48 8.41
α,β,γ-Trihydroxyvaleramide (Rivo form)	lit. 41	107 [h] (EtOH)	$C_5H_{11}O_4N$	40.26 40.45	7.43 7.55	9.39 9.53
cis-3-Hydroxycyclo hexanamide	d)	172 (EtOH)	$C_7H_{13}O_2N$	58.70 58.67	9.17 9.16	9.78 9.88

a) lit [35] mp 65—66°. This amide is very hygroscopic
b) lit [7b] mp 46°; lit [8a] 53—54°; lit [36] 46—48°. This amide is extremely hygroscopic
c) lit [37,38] mp 56°
d) present method
e) lit [39] mp 107.0—107.5°
f) lit.[40a] mp 70°; lit.[40b] 74°
g) lit.[24] mp 138—139°
h) lit.[41] mp 107—108°

temperature. Evaporation of MeOH gave the crude amide, which was recrystallized (Table II). Cyclohexanamide was prepared from cyclohexane carbonyl chloride and cooled conc. NH_4OH (28%).[33] After the precipitate was washed with H_2O, the amide was recrystallized from EtOH, mp 184—185° (lit.[33] mp 184—186°). The other hydroxyamides were prepared by previously reported procedure[34-41] (Table II). The melting points and the result of elemental analyses of the synthesized aliphatic hydroxyamides are listed in Table II.

Other reagents used were of reagent grade without further purification. The deuterium oxide (99.75% pure) and deuterochloric acid used were purchased from E. Merck AG. (Germany).

Buffer Solutions——Buffer solutions were made up to a total concentration of 0.2 M buffer (H_3PO_4–NaH_2PO_4 system) and were adjusted to an ionic strength of 0.6 with potassium chloride. The pH value of the buffer solutions was determined at the experimental temperature, 80.0°. A Hitachi-Horiba pH meter, Model F5, was used with a Horiba type 1027-05T glass electrode designed for 5—110° over the pH range 0—14 and a high temperature calomel electrode, type 2631-05T.

pK_a Determination——The ionization constants of β-hydroxypropionamide, γ-hydroxybutyramide and γ-hydroxyvaleramide were determined by the photometric method described in the previous paper.[1] All spectral measurements were made with a Shimadzu QV-50 spectrophotometer in aqueous perchloric aicd solutions at 25° and at 200 mμ. The appropriate solvent was used as a blank for each determination. Because of the facile hydrolysis of these amides in concentrated acid solution, the optical density for these solutions has been obtained by extrapolation to time zero of the photometric measurements as a function of time.

The concentration ratio of the protonated amide C_{SH^+} and the neutral amide C_S was obtained from the equation:

$$\frac{C_{SH^+}}{C_S} = \frac{E_S - E}{E - E_{SH^+}} \tag{1}$$

where E, E_S and E_{SH^+} have their usual significance. The experimental pK_a values (H_0 value[42] at $C_{SH^+}/C_S = 1.00$) for the three compounds were found to be in close agreement and are -1.65 for β-hydroxypropionamide, -1.10 for γ-hydroxybutyramide and -1.30 for γ-hydroxyvaleramide.

Hydrolysis and Analytical Procedure——Hydrolyses were carried out under pseudo first-order conditions: A known weight of amide and lactone corresponding to the concentration of approximately 5×10^{-3} M (1×10^{-3} M for the experiments of deuterium solvent isotope effect) was dissolved in 100 ml of the appropriate reaction solution (50 ml of the deuterium oxide-deuterochloric acid solution) heated previously to the desired temperature within ± 0.05°. Aliquot portions were withdrawn at suitable intervals for analysis.

During hydrolysis of hydroxyamide, residual amide concentration was followed by determination of liberated ammonia using indophenol method and the produced lactone concentration was determined by hydroxamic acid method[43] in the same manner described previously.[23] Optical densities were determined with a Hitachi Model 101 spectrophotometer.

Paper Chromatography——Hydroxyamide or lactone (*ca.* 0.5 g) was dissolved in 10 ml of aqueous 0.05N HCl solution. The solution was stored at 40° and spotted on a filter paper (Toyo Roshi, Co., No. 51) at appropriate intervals. The paper was developed with acetonitril–acetone–water–acetic acid (80:5:15:1) mixture.[44] Hydroxamic acid method was employed for detection. The developed paper was separated lengthwise into two portions. One portion was used for the detection of lactone, and in case of difficulty

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41) S. Sasaki, *Nippon Kagaku Zasshi*, **78**, 1464 (1957).
42) J.E. Lefler and E. Grunwald, "Rate and Equilibria of Organic Reactions," John Wiley and Sons, Inc., New York, 1963, p. 272.
43) When residual amide was trapped as indophenol blue and/or hydroxyamic acid, the concentration of amide and lactone was corrected. All hydroxy acids used in the present investigation were not colored in hydroxamic acid method, using the corresponding potassium salts.
44) T. Takahashi and M. Mitsumoto, *Nature*, **199**, 765 (1963).

with the color reaction of amide, the other portion was used for detection of amide after conversion to lactone by spraying 5N HCl solution. The spots were identified by spotting knowns on paper and developing under the same condition.

Result

Identification of Hydrolysis Products

The nature of the products of the hydrolyses of hydroxyamides and lactones was determined by paper chromatography. This was necessary not only for the assay of the hydrolyzates and remaining amide, but also for the establishment of the hydrolysis pathway.

Chromatograms of the solution of D-arabonamide hydrolysis in aqueous 0.05N HCl solution held at 40° gave two spots corresponding to the amide and the γ-lactone, as seen in Fig. 1. Under the same condition, paper chromatograms of the solutions of arabono-γ-lactone gave only one spot due to the unchanged γ-lactone.

TABLE III. *Rf* Values of Paper Chromatogams Obtained on Solutions of Hydrolyzed Hydroxyamides

Amide	Degraded sample		Standard	
	Amide	Lactone	Amide	Lactone
β-Hydroxypropionamide	0.23	—	0.25	0.98
γ-Hydroxybutyramide	0.48	0.95	0.46	0.95
γ-Hydroxyvaleramide	0.30	0.65	0.32	0.65
δ-Hydroxyvaleramide	0.00	0.51	0.00	0.51
δ-Hydroxycarproanmide	0.00	0.56	0.00	0.57
γ,δ-Dihydroxyvaleramide	0.28	0.84	0.30	0.85
α,β,γ-Trihydroxyvaleramide	0.34	0.82	0.34	0.81
D-Arabonamide	0.07	0.56	0.09	0.54
cis-3-Hydroxycyclohexanamide	0.10	—	0.08	0.62

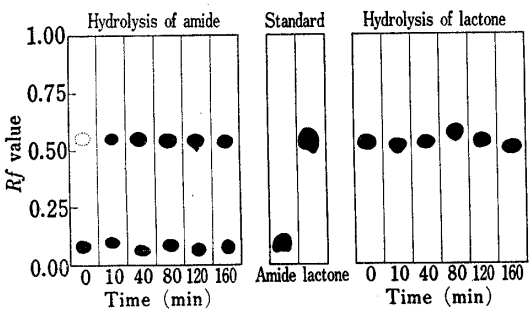

Fig. 1. Paper Chromatograms of Hydrolyzed D-Arabonamide and D-Arabono-γ-lactone in Aqueous 0.05 N HCl Solution at 40.0°

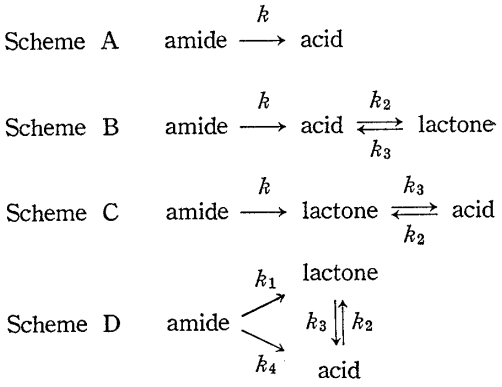

Chart 1. Possible Reaction Schema of Hydroxyamide Hydrolysis

It was found that β-hydroxypropionamide and *cis*-3-hydroxycyclohexanamide did not produce lactone and the other hydroxyamides produced only the corresponding γ- or δ-lactone in their hydrolytic processes. These results are listed in Table III.

Reaction Pathways

Hydrolysis of each hydroxyamide in aqueous hydrochloric acid solution was found to follow the first-order kinetics by the determination of liberated ammonia concentration (typical plot is shown in Fig. 2). Moreover, in view of the time course of lactone formation from hydroxyamide, the Schema A—D are considered as possible reaction pathways (Chart

TABLE IV. Observed and Calculated Concentration of Lactone Formed from the Some Hydroxyamide Hydrolyses

Time (min)	Found Lactone (%)	Calcd. lactone (%)		
		Scheme B	Scheme C	Scheme D
γ,δ-Dihydroxyvaleramide [a]				
10	8.7	3.7	11.9	8.6[b]
20	17.5	11.2	21.9	17.6
30	26.2	19.8	30.7	26.3
40	33.9	28.0	38.2	34.1
50	41.2	35.6	44.7	41.1
60	47.4	42.3	50.5	47.2
70	52.6	48.2	55.5	52.5
80	57.3	53.5	59.9	57.3
90	61.2	58.2	63.8	61.5
100	65.3	62.3	67.2	65.2
γ-Hydroxybutyramide [c]				
15	10.8	2.9	9.9	—
30	17.8	8.7	17.5	—
45	24.7	15.0	24.0	—
60	28.9	20.4	28.8	—
75	34.4	26.8	34.3	—
90	38.7	31.0	38.0	—
105	42.4	36.4	42.5	—
γ-Hydroxyvaleramide [d]				
20	6.3	1.0	5.7	—
40	12.2	3.5	11.0	—
60	15.5	6.6	15.9	—
80	19.9	9.5	20.5	—
100	24.5	13.6	24.8	—
120	28.5	17.6	28.9	—
α,β,γ-Trihydroxyvaleramide [e]				
20	16.8	1.8	16.8	—
40	30.8	6.3	30.3	—
60	41.9	12.3	41.0	—
80	50.5	19.1	49.6	—
100	57.3	26.0	56.5	—
120	62.9	32.8	62.6	—

a) Hydrolyzed in 0.1004 N HCl at 50.0°. Kinetic parameters based on the calculations are as follows; $k_1+k_4=1.21\times10^{-2}$ min^{-1}, $k_2=7.79\times10^{-2}$ min^{-1}and $k_3=0.72\times10^{-2}$ min^{-1}

b) Calculated average value, $k_1=0.80\times10^{-2}$min^{-1}, was used.

c) Hydrolyzed in 0.0597 N HCl at 60.0°. Kinetic parameters based on the calculations are as follows; $k=0.77\times10^{-2}$min^{-1}, $k_2=4.67\times10^{-2}$ min and $k_3=1.66\times10^{-2}$ min^{-1}

d) Hydrolyzed in 0.0489N HCl at 40.0°. Kinetic parameters based on the calculations are as follows; $k=0.30\times10^{-2}$ min^{-1}, $k_2=1.97\times10^{-2}$ min^{-1} and$k_3=0.12\times10^{-2}$ min^{-1}

e) Hydrolyzed in 0.0544N HCl at 40.0°. Kinetic parameters based on the calculations are as follows; $k=0.94\times10^{-2}$ min^{-1}, $k_2=1.13\times10^{-2}$ min^{-1} and $k_3=0.19\times10^{-2}$ min^{-1}

1). In the same manner as described previously in D-glucuronamide hydrolysis,[23] the reaction scheme selected as reasonable for each hydroxyamide was one in which the observed concentrations of lactone at various times during the hydrolysis were in good agreement with those calculated from the kinetic equation.[45]

45) These equation are as follow: For Scheme B,

$$[\text{lactone}]=\frac{k_2C_o}{k_2+k_3}\{1-\exp[-(k_2+k_3)t]\}-\frac{k_2C_o}{k_2+k_3-k}\{\exp(-kt)-\exp[-(k_2+k_3)t]\}$$

for Scheme C,

$$[\text{lactone}]=\frac{k_2C_o}{k_2+k_3}\{1-\exp[-(k_2+k_3)t]\}+\frac{(k-k_2)C_o}{k_2+k_3-k}\{\exp(-kt)-\exp[-(k_2+k_3)t]\}$$

and for Scheme D,

$$[\text{lactone}]=\frac{k_2C_o}{k_2+k_3}\{1-\exp[-(k_2+k_3)t]\}+\frac{(k_1-k_4)C_o}{(k_2+k_3)-(k_1+k_4)}\{\exp[-(k_1+k_4)t]-\exp[-(k_2+k_3)t]\}$$

Figure 2 and Table IV show the observed and calculated lactone concentrations as a function of time for the amide hydrolysis, and the classification of the amides into the reaction Schema A—D is shown in Table V.

Buffer Concentration Effect on the Hydrolysis Rates

In acidic hydrolysis of γ-hydroxybutyramide, the catalytic effect by buffer components was determined in each buffer solution, varying the total buffer concentration but keeping the pH and ionic strength constant at 0.6. Plotting the pseudo first-order rate constants against the total concentration of phosphate gave straight lines. The slopes of these lines are essentially equal as shown in Fig. 3. On the bases of experiments performed in various pH buffer solutions between pH 1.59 and 2.70, the second-order catalytic rate constants were calculated; $k_{H_3PO_4}=k_{H_2PO_4^-}=8.10\times10^{-3}M^{-1}\,min^{-1}$ at 80°. The catalytic effect in the γ-hydroxybutyramide hydrolysis shows the same behavior as seen in the acidic hydrolysis of acetamide, of which the catalytic rate constants are $k_{H_2PO_4^-}=k_{HPO_4^{2-}}=2.60\times10^{-5}M^{-1}\,min^{-1}$ at 100°.[8a] These results indicate that the reasonable mechanism of amide hydrolysis by general acid (BH) is the one insensitive to pK_a of BH as proposed by Bruice, *et al.*[8a] Therefore, it is expected that the process of proton transfer and nucleophilic attack are concerted in the hydrolysis of amide.[8a]

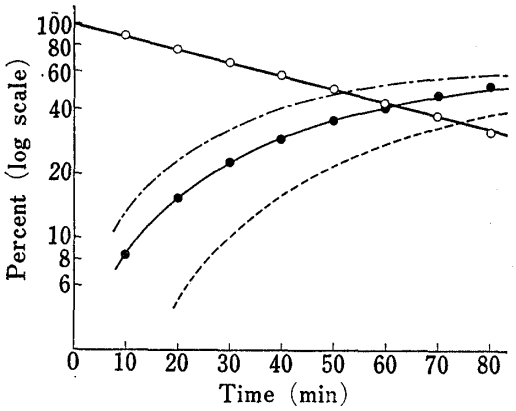

Fig. 2. Disappearance of D-Arabonamide and Formation of D-Arabono-γ-lactone in 0.1021 N HCl at 50.0°

Found: D-arabonamide: ○
D-arabono-γ-lactone: ●
Calculated D-arabono-γ-lactone:
----------: Scheme B
————: Scheme C
————: Scheme D

Kinetic parameters on the basis for the calculation are: $k_1=0.78\times10^{-2}$ min^{-1}, $k_2=2.44\times10^{-2}$min^{-1}, $k_3=0.63\times10^{-2}$ min^{-1} and $k_4=0.59\times10^{-2}$ min^{-1}

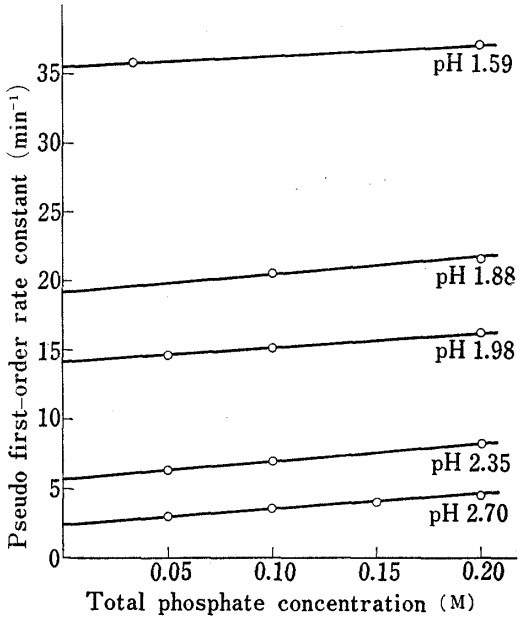

Fig. 3. Plots of Pseudo First-Order Rate Constansts aganist Phosphate Buffer Concentration in the Hydrolysis of γ-Hydroxybutyramide at Constant Ionic Strength ($\mu=0.6$) and at 80.0°

The relation between pH and logarithm of k extrapolated to zero buffer concentration shows that γ-hydroxybutyramide hydrolysis follows the first-order kinetics with respect to the hydronium ion (Fig. 4).

Rate Constants and Activation Parameters

Table V summarizes the second-order rate constants (k, k_1 and k_4) measured at various temperatures, the activation enthalpies and entropies (at 60°) for the acidic hydrolysis of the 12 amides studied in the present work. Suitable data [21a,21b] on alkyl amides are also included for comparison in this table.

Table V. Activation Enthalpy and Entropy of the Acidic Hydrolysis of Aliphatic Amides

No.	Amide	Reaction temp.(°C)	Rate constants $10^2 k$, k_1 and k_4 (M^{-1} min^{-1})	Activation parameter ΔH^* (kcal/mole)	ΔS^*(at 60°) (*e.u.*)
Scheme A					
1	β-hydroxypropionamide	50	0.21	19.4	—20.6
		70	1.42		
		80	3.38		
		90	8.07		
2	*cis*-3-hydroxycyclohexanamide	50	0.29	19.6	—19.8
		60	0.68		
		80	3.76		
3	cyclohexanamide [a]	60	0.30	20.3	—19.4
		80	1.75		
4	acetamide [b]	60	1.70	19.7	—18.4
5	propionamide [b]	60	2.30	18.1	—20.2
6	butyramide [b]	60	1.04	18.9	—19.5
7	valeramide [b]	60	1.06	18.7	—20.0
8	isovaleramide [b]	60	0.22	19.4	—20.9
Scheme C					
9	γ-hydroxybutyramide	30	0.78	19.9	—12.9
		40	2.10		
		50	5.60		
		60	12.7		
		70	32.2		
10	γ-hydroxyvaleramide	30	2.59	18.4	—13.3
		40	6.30		
		50	15.4		
		60	42.7		
11	δ-hydroxycapronamide	40	6.20	19.6	—11.9
		50	15.9		
		60	36.0		
		70	77.0		
12	α,β,γ-trihydroxyvaleramide	20	2.50	19.4	—12.2
		30	7.03		
		40	18.0		
		50	49.3		
		60	127		
13	δ-hydroxyvaleramide	30	1.70	20.0	—11.5
		40	4.10		
		50	10.1		
		60	23.8		
Scheme D			k_1 k_4	k_1 k_4	k_1 k_4
14	D-arabonamide	40	2.74 2.55	22.2 16.0	—5.2 —24.9
		50	7.64 5.78		
		60	21.7 13.5		
15	D-glucouronamide	50	0.40 0.30	23.3 16.5	—7.4 —29.2
		60	1.40 0.67		
		70	4.00 1.30		
		90	26.0 5.70		
16	γ,δ-dihydroxyvaleramide	30	1.20 1.00	21.7 15.3	—6.5 —27.8
		40	2.99 1.99		
		50	7.97 4.08		
		60	23.9 7.97		

a) The amide was hydrolyzed in acidic 50% EtOH–water solution.
b) Data taken from ref. 21.

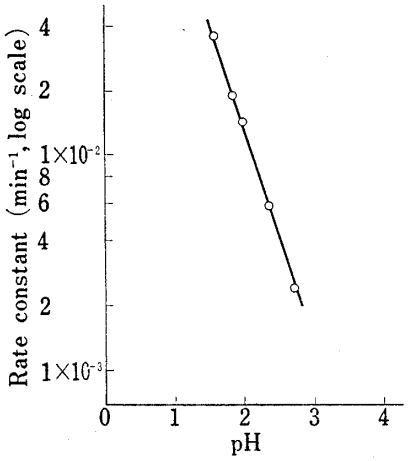

Fig. 4. pH-Rate Profile for γ-Hydroxybutyramide at Constant Ionic Strength (μ=0.6) and at 80.0°

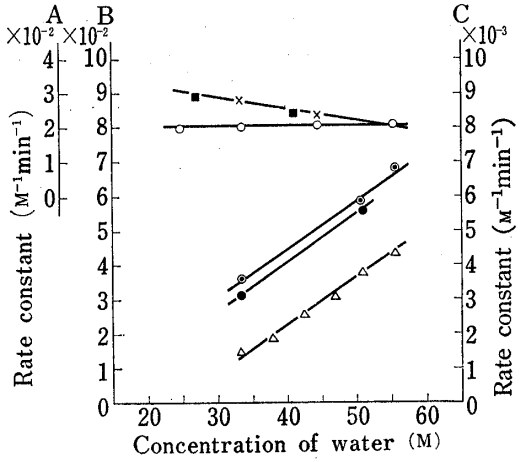

Fig. 5. Plots of the Second-Order Rate Constants against Concentration of Water for the Acidic Hydrolysis of Amides in Mixed Solvents

γ-hydroxybutyramide in ethanol-water (at 40.0°) —○—, in acetone-water (at 40.0°) —×—, in dioxane-water (at 40.0°) —■—, *cis*-3-hydroxycyclohexanamide in ethanol-water (at 60.0°) —⊙—, cyclohexanamide in ethanol-water (at 60.0°) —●—, acetamide in ethanol-water (at 80.0°) —△—
scale: A, γ-hydroxybutyramide; B, acetamide; C, *cis*-3-hydroxyclohexanamide and cyclohexanamide

Solvent Effect on Reaction Rate Constants

The effects of solvent composition on the rate constants of the acidic hydrolysis of some amides were studied. The plots of the rate constant against water concentration are shown in Fig. 5. The rate constant of amide hydrolysis following Scheme A decreased with a decrease in the concentration of water. On the other hand, the addition of an organic solvent had no effect on (EtOH–water) or facilitated (acetone–water, dioxane–water) the hydrolysis following Scheme C. In the acidic hydrolysis of D-arabonamide which proceeds according to Scheme D, such interesting results were obtained that the addition of an organic solvent increased the rate constant (k_1) of the lactonization, and on the contrary, decreased the rate constant (k_4) of the hydroxy acid formation (Table VI).

TABLE VI. Acidic Hydrolysis of D-Arabonamide in Ethanol- and Dioxane–Water Mixtures at 50.0°

Solvent composition	$10^2\,k_1$ ($M^{-1}\,min^{-1}$)	$10^2\,k_4$ ($M^{-1}\,min^{-1}$)
H_2O	8.2	6.0
25% EtOH–H_2O	8.7	4.7
50% EtOH–H_2O	10.3	4.4
50% dioxane–H_2O	17.0	4.1

TABLE VII. Deuterium Solvent Isotope Effects for Acidic Hydrolysis of Amides

Amide	Acid concentration (Reaction temp.)	k_{D_2O}/k_{H_2O}
γ-Hydroxybutyramide	0.1N HCl (40.0°)	1.19
Acetamide[a]	0.1N HCl (25.0°)	1.45

a) Data taken from ref. 46.

Solvent isotope effect for the acidic hydrolysis of γ-hydroxybutyramide is smaller than that found for the acidic hydrolysis of acetamide.[46] These results are listed in Table VII.

Relation between Rate Constant and Water Activity

The following relation (Eq. 2) between the rate constant and the water activity of the reaction medium has been reported previously[1] for amide hydrolysis in concentrated acid solution.

$$\log \frac{km}{k_0 m_0} = (r - r_0) \log a_{H_2O} + \text{constant} \quad (2)$$

where

$$m = 1 + C_S/C_{SH^+} \quad (3)$$

In Eq. 2, k is the pseudo first-order rate constant, m is the reciprocal of the protonation ratio of amide obtained photometrically from Eq. 1, and r is a hydration parameter which is the same measure of the number of water molecules required to convert a protonated amide molecule to transition state as described previously.[1] k_0, m_0, and r_0 represent the same values for standard amide for which acidic hydrolysis is generally accepted to follow the bimolecular reaction mechanism.

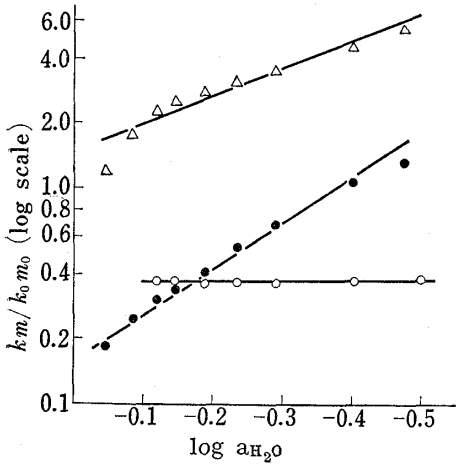

Fig. 6. Plots[a] of log km/k_0m_0 against log a_{H_2O} According to Eq. 2 for the Hydrolysis of Hydroxyamides in Aqueous Perchloric Acid Solution

—○—: β-hydroxypropionamide (at 60.0°)
—●—: γ-hydroxybutyramide[b] (at 25.0°)
—△—: γ-hydroxyvaleramide (at 30.0°)

a) Data for acetamide as the standard was obtained at 80.0°.
b) Data taken from ref. 9.

The plot of log km/k_0m_0 against log a_{H_2O}[42] should give a straight line with a slope of the difference between the hydration parameter of amide and that of standard amide. The r—r_0 values obtained from the data of several hydroxyamides and acetamide as standard (at 80.0°) are approximately: 0.0 for β-hydroxypropionamide (at 60.0°), −2.2 for γ-hydroxybutyramide (at 25°) and −1.3 for γ-hydroxyvaleramide (at 30.0°) (Fig. 6). From these results, it seems likely that the hydrolysis of β-hydroxypropionamide follows the same mechanism with acetamide.

Discussion

Evidence of Hydroxyl Group Participation

The introduction of a hydroxyl group in the γ- or δ-position of butyramide and valeramide increases the rate constant of these acidic hydrolyses. Since the acidic hydrolysis of amides is almost completely insensitive to polar effects by the substituents as discussed previously,[4,47] it is unlikely that such facilitation of the reaction could be caused by inductive electron withdrawal by the hydroxyl group. Accordingly, two possible explanations are considered for the amide hydrolysis facilitated by a neighboring hydroxyl group. One is based on the hydrogen bonding mechanism as suggested by Henbest and Lovell[48] and numerous other investigators.[20] In this case, it seems likely that the hydrogen bond will become much stronger in the transition state, and that the increase in hydrogen bond energy

46) K.B. Wiberg, *Chem. Rev.*, **55**, 713 (1955).
47) J.A. Leisten, *J. Chem. Soc.*, **1959**, 765; J.T. Edward, H.S. Chang, K. Yates and S. Stewart, *Am. J. Chem.*, **38**, 2271 (1960).
48) H.B. Henbest and B.J. Lovell, *J. Chem. Soc.*, **1957**, 1965.

in passing from the initial state to the transition for the reaction thereby increase the rate of hydrolysis by nucleophilic reagent. An alternative explanation is that a direct nucleophilic attack by the neighboring hydroxyl group can facilitate amide hydrolysis. Bruice, *et al.*[8a] gave the most reasonable explanation that, for the acidic hydrolysis mechanism, the intramolecular attack of the oxygen of the hydroxyl group on the carbonyl carbon of the protonated amide produces intermediate lactone. The present investigation verified, indeed, that γ-hydroxybutyramide produced the corresponding lactone in the hydrolytic process by determining the lactone concentration (Table III), as reported by these authors.

But, interestingly, it was also found that some of aliphatic hydroxyamides (*i.e.* γ,δ-dihydroxyvaleramide and D-arabonamide) were hydrolyzed simultaneously according to two different pathways, one leading to the formation of γ-lactones and the other leading to the direct formation of hydroxy acids. Moreover, these hydrolyses to the corresponding hydroxy acids were also accelerated as compared with that of corresponding alkyl analogues (Table V). This acceleration effect cannot be accounted for by the mechanism proposed by Bruice, *et al.*[8a]

Mechanisms

In the general comparison of the two equivalent intramolecular and intermolecular reaction cases, the entropy of activation of the intramolecular reaction is significantly more positive than that of the corresponding intermolecular one.[49] The activation entropy (−5 to −13 *e.u.*) of the reaction of lactone formation for acidic hydrolysis of hydroxyamides was more positive than that (−20 to −29 *e.u.*) of some hydroxyamides and ordinary alkylamides which produce directly corresponding carboxylic acids (Table V). These results certainly indicate that the contiguous positions of catalyst and substrate are important in controlling the reaction of lactonization.

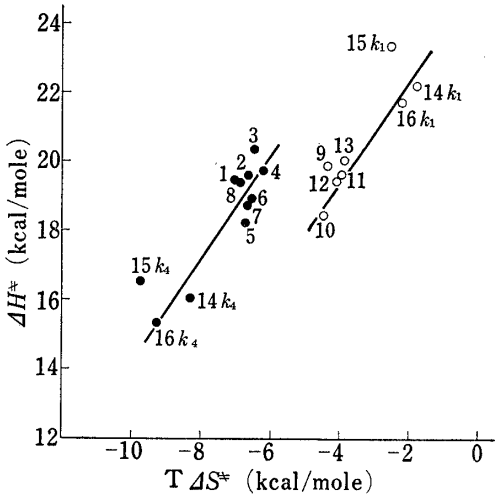

Fig. 7. Enthalpy and Entropy Relationship for Acidic Hydrolysis of Aliphatic Hydroxyamides and Alkyl Analogues[a]

a) The numbers in the figure are corresponded to the same number in Table V.

Plotting $\Delta H^{\neq}$ against $T\Delta S^{\neq}$ for the reaction of lactonization and carboxylic acid formation satisfied the isokinetic relationship,[50] falling into two separated parallel lines as shown in Fig. 7.[51] One of these lines involves the observed points for the lactone formation from hydroxyamide hydrolysis, and the other involves that for the carboxylic acid formation from some hydroxyamides and ordinary amide hydrolyses.

a) Reaction of Carboxylic Acid Formation——Figure 7 suggests that the mechanism of the hydroxy acid production from hydroxyamide (*i.e.*, amides of Scheme A and Scheme D as shown in Table V) is the same as one of the ordinary amides such as acetamide. That is, the mechanism acceptable for the reaction to give the rate constant k of Scheme A and k_4 of Scheme D is in accord with a bimolecular reaction mechanism in which the rate-determining step is the nucleophilic attack of water molecules on the conjugated acid of amide. This

49) M.L. Bender and M.C. Neveu, *J. Am. Chem. Soc.*, **80**, 5388 (1958).
50) Ref. 42, Chapter 9; J.E. Leffler, *J. Org. Chem.*, **20**, 1202 (1955).
51) Arrhenius plots of $\log k_{H^+}$ against 1/T pass through a common point in both sets which is a necessary consequence of true compensation.[50] Observed isokinetic temperature was 500°K in the both intramolecular and intermolecular catalyzed hydrolyses.

bimolecular reaction mechanism is also supported by the results, (1) that bimolecular catalyzed reaction is generally retarded[52] by the addition of organic solvent as observed in the hydrolysis of *cis*-hydroxycyclohexanamide and D-arabonamide (k_4), (2) that the entropy of activation is largely negative,[52] as presented in Table V, and (3) that the difference of the hydration parameter between β-hydroxypropionamide hydrolysis and acetamide hydrolysis in aqueous solution is nearly zero.

b) Reaction of Lactone Formation——The Leffler-compensation relationship (Fig. 7) indicates that the mechanism of the lactone formation from the hydroxyamide (k of Scheme C and k_1 of Scheme D) is quite different from the bimolecular reaction mechanism.[53] Witkop[54] and Bruice, *et al.*[8a] suggested that there is a nucleophilic attack of hydroxyl oxygen on the carbonyl carbon of the protonated amide and also a direct transfer of hydroxyl proton to nitrogen. This mechanism does not involve water molecules in the rate-determining step. The values r—r_0 for γ-hydroxybutyramide and γ-hydroxyvaleramide, however, are too high (−1.3 to −2.2) to assume no involvement of water molecules in the slow step. In order to accomodate a role for water molecules it can be proposed, on the basis of Martin, *et al.*'s hypothesis,[9] that proton transfer by one or two water molecules and nucleophilic attack by internal hydroxyl oxygen are concerted in the rate-determining step as presented in Chart 2. The deuterium isotope effect, $k_{D_2O}/k_{H_2O}=1.19$, found in the acidic hydrolysis of γ-hydroxybutyramide is in accord with the proposed mechanism (Chart 2) in which proton transfer from hydroxyl hydrogen to amide nitrogen through water molecules is assumed to be concerted in the rate-determining step.[55] This mechanism is also supported by the results of buffer concentration effect on the acidic hydrolysis of γ-hydroxybutyramide.

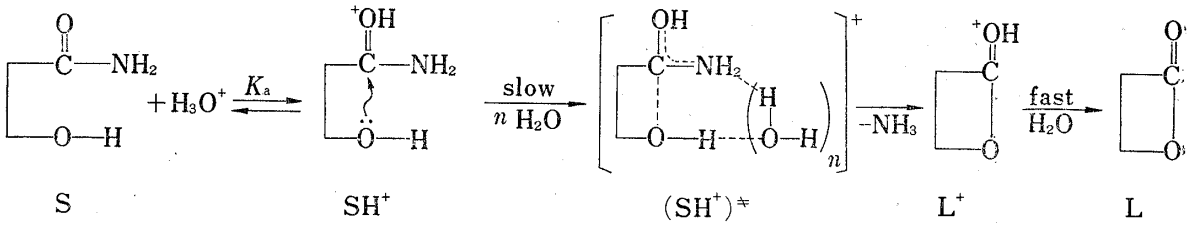

Chart 2

The transition state $(SH^+)^*$ is a positively charged species in which the positive charge is distributed among the amide moiety and hydroxyl group, whereas in an ionic substrate (SH^+) and product (L^+) the charge is more localized. This distribution of the charge in the transition state makes it more stable relative to the positively charged reactant, hence the lactonization rate increases in the solvent of low dielectric constants[56] (Fig. 5 and Table VI).

The reaction of lactonization of aliphatic hydroxyamide is controlled by the entropy of activation. In other words, the proximity effects of neighboring hydroxyl groups as the intramolecular nucleophilic catalysis cause more positive entropy of activation than that for the reaction involving water molecules as a nucleophile, while the enthaply of activation remains constant (*ca.* 19 kcal/mole) (Table V).

52) L.L. Schaleger and F.A. Long, "Advances in Physical Organic Chemistry," Vol. 1, ed. by V. Gold, Acad. Press, New York, 1963, p. 1.
53) In the glucuronamide hydrolysis, deviation from these plots was observed. Since it is expected that this hydrolysis may involve more complicate reaction, *e.g.*, the conversion of ether linkage, D-glucuronamide was omitted from the present discussion.
54) B. Witkop, *Advan. Protein. Chem.*, **16**, 221 (1961).
55) C.A. Bunton and S.J. Farber, *J. Org. Chem.*, **34**, 3396 (1969); R.H. De Wolfe, K.M. Ivanetich and N.F. Perry, *ibid.*, **34**, 848 (1969); C.A. Bunton and R.H. De Wolfe, *ibid.*, **30**, 1371 (1965).
56) E.D. Hughes and C.K. Ingold, *J. Chem. Soc.*, **1935**, 255; E.S. Amis. "Solvent Effects on Reaction Rates and Mechanisms," Acad. Press, New York, 1966, p. 209.

c) Synchronous Formation of Hydroxy Acid and Lactone——In the acidic hydrolysis of γ,δ-dihydroxyvaleramide and D-arabonamide according to Scheme D, it is concluded that the lactone and the carboxylic acids formed synchronously do not arise from the same transition state, *i.e.*, nucleophilic attacks by neighboring hydroxyl groups and by water molecules are operative respectively. In addition, this reaction scheme was found to be characteristic of amides involving hydroxyl groups both at γ- and δ-positions in the present hydroxyamide series. If these different transition states could be produced from the two different species of initial substrates such as Model 1 and Model 2 (Fig. 8), the large rate constant, k_4, in comparison with that of valeramide cannot be accounted for, since the distribution of positive charge at carbonyl carbon in Model 2 is expected to be the same as that in Model 1 and valeramide[57] (Fig. 8).

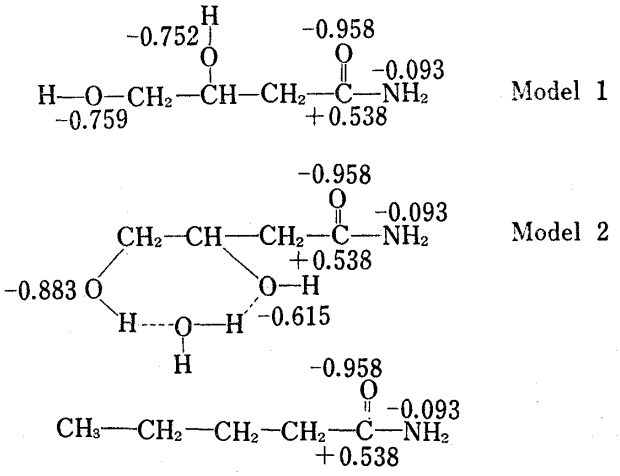

Fig. 8. Calculated Net Charge for the Some Models of γ,δ-Dihydroxyvaleramide and for Valeramide

In the hydrolysis of hydroxyamides following Scheme D, the activation enthalpies of lactonization are higher by *ca.* 2—3 kcal/mole and the activation entropies of this reaction series (k_1) are significantly lower negative (—5——6 *e.u.*) relative to those for the series of Scheme C. On the other hand, the enthalpies of activation for k_4 are lower by *ca.* 3—4kcal/mole compared to other intermolecular catalyzed hydrolysis of ordinary amides ($\Delta H^{\ddagger}$= *ca.* 19—20 kcal/mole). This lowered enthalpy of activation is probably due to a much stronger internal hydrogen bond in the transition state than in the initial state.[58] As a result of the low enthalpy of activation, the reaction of the hydroxy acid formation, in the hydrolyses of γ,δ-dihydroxyvaleramide and D-arabonamide, must be facilitated in comparison with the hydrolysis of *n*-butyramide and *n*-valeramide under the same condition, in spite of the more negative entropy of activation (Table V).

Conclusion

In the acidic hydrolysis of hydroxyamides, two different kinetically distinguishable reactions controlled by activation entropy and by activation enthalpy occur simultaneously, one proceeding intramolecularly with the nucleophilic attack by γ- or δ-hydroxyl group and with electron transfer by the assist of one or two water molecules concertedly through lactone formatin, and the other proceeding intermolecularly with the nucleophilic attack by water molecules through a tetrahedral intermediate. Consequently, in the case of the same contribution of these activation parameters (*i.e.* free energy of activation) the hydrolytic Scheme D is considered to be the hydrolysis pathway of hydroxyamides.

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57) Molecular orbital calculation was made in the same manner as described previously.[37a]

58) The intramolecular hydrogen bonding between δ-hydroxyl group and amide oxygen, such as verified in the γ-hydroxyvaleramide,[37a] may provide the necessary rigidity for the molecule to undergo catalysis by the internal γ-hydroxyl group in the manner suggested for asparagine N-acylated derivatives[16] and phthalamic acid.[12b]