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Hydroxyl Group Catalysis. IV. The Mechanism of Intramolecular Participation of the Aliphatic Hydroxyl Group in Amide Hydrolysis

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The *p*H–rate profiles for the hydrolysis of acetamide and γ-hydroxybutyramide have been obtained at 100° in water at a calculated ionic strength of 1.0 *M*. The conclusion is reached that γ-hydroxybutryamide hydrolysis occurs at all *p*H values *via* formation of the intermediate γ-butyrolactone. Of particular interest is that the intramolecular attack of the hydroxyl group on the amide bond involves both the neutral hydroxyl group (at *p*H values near neutrality) and the alkoxide ion (at alkaline *p*H values). This finding may be compared to the fact that only the acid species of weak bases (carboxyl, imidazole) are kinetically implicated in intramolecular catalysis of amide hydrolysis. Evidence is presented that general acid catalysis of amide hydrolysis involved a concerted mechanism of proton addition and nucleophilic attack.

The acknowledged importance of the serine hydroxyl group in enzymic catalysis of hydrolytic reactions[2] has led us to a study of the aliphatic hydroxyl group as a catalyst of amide and ester hydrolysis.[3,5] The present kinetic investigation of the hydrolysis of γ-hydroxybutyramide and acetamide was designed to ascertain the mechanism of intramolecular hydroxyl group participation in amide hydrolysis.

Experimental

Materials.—γ-Butyrolactone was Eastman Kodak Co. white label, Acetamide was J. T. Baker analyzed. All buffered salts were Mallinckrodt analytical reagents.

γ-Hydroxybutyramide was prepared quantitatively from γ-butyrolactone *via* a modification of the procedure previously used by Levene and Haller[6] for the preparation of γ-hydroxyvaleramide. A mixture of 1.10 g. (0.0128 mole) of γ-butyrolactone and 3.4 g. (0.200 mole) of anhydrous ammonia was allowed to react for 4 days at room temperature in a sealed ampule. On evaporation of the excess ammonia, a solid product was obtained. The product was washed with anhydrous ether and recrystallized from hot ethyl acetate. γ-Hydroxybutyramide was obtained in this manner as white needles, m.p. 53–54° (uncor.) (lit. m.p. 46°).[7] The product was extremely hygroscopic. The product exhibited bands in the infrared at 6.16 and 7.0μ which are absent in the spectrum of γ-butyrolactam[8] and which are interpreted as the NH deformation and C–N absorption, characteristic of primary amides.[9] Nitrogen analysis on the product was carried out by the micro-Kjeldahl procedure.

Anal. Calcd. for $C_4H_9N_1O_2$: N, 13.60. Found: N, 13.66.

Potassium γ-Hydroxybutyrate.—γ-Butyrolactone (17.2 g., 0.20 mole) and 11.2 g (0.20 mole) of potassium hydroxide were dissolved in 30 ml. of water and refluxed for 3 hr. Flash evaporation yielded the salt, which was recrystallized from absolute ethanol (91% yield). The equivalent weight obtained by titration to thymol blue endpoint was found to be 143.3 (theoretical 142.2 as calculated for $C_4H_7O_3K$).

***p*H Determinations.**—The *p*H's of the buffer solutions employed in this study were determined at 99 ± 0.1° by means of a Metrohm S type H high temperature glass electrode and a Radiometer 22 *p*H meter. The constant temperature cell consisted of a steam-jacketed U-tube, the open ends of which were fitted with S joints to receive the S glass electrode and S salt bridge. To prevent leakage of KCl into the buffer solution, the end of the salt bridge was closed by a fine asbestos wick sealed in a collapsed glass capillary tube. The salt bridge led from the constant temperature cell to a calomel electrode kept at 25°. The bottom of the U-tube shaped cell was fitted with a stop-cock for draining and toward the top of one leg was a side arm consisting of a shallow water trap leading to a funnel (as in a thistle tube) for introduction of the buffers into the apparatus. To prevent evaporation of the buffer solutions, the water trap was filled with the buffer solution to seal the apparatus.

Prior to the measurements, the glass electrode was calibrated against standard buffers.

Kinetics.—The hydrolysis of acetamide and γ-hydroxybutyramide were carried out at 100 ± 0.1° in aqueous solutions buffered by potassium dihydrogen phosphate–dipotassium hydrogen phosphate buffers and boric acid–potassium dihydrogen borate buffers. At each *p*H at which the reactions were run, several different buffer concentrations were employed, so that the rate could be extrapolated to zero buffer concentration. The buffer concentrations employed were 0.4. 0.266, 0.2 and 0.133 *M* in total borate for the borate buffers and 0.33, 0.22, 0.20, 0.132 and 0.11 *M* in total phosphate concentration for the phosphate buffers. To minimize the salt effects, all the buffer solutions were brought to a calculated "ionic strength" of 1.0 *M* with KCl.

Operationally, 1-ml. aliquots of the solutions of amide in aqueous buffer were placed into 16 × 150 mm. Kimax screw cap vials with rubber liners and heated at 100° in a constant temperature oil-bath. At periodic intervals, tubes were withdrawn and the reaction quenched in ice. Best results were obtained when all the tubes for one run were developed at one time. For this reason the tubes were stored at −4° until such time that the rate-run was completed and then assayed for remaining amide by the hydroxamic acid method. For reactions that were carried out over prolonged time intervals, sealed Pyrex ampules were employed in place of the screw cap vials from which 1.0-ml. aliquots were withdrawn for the assay.

The hydroxamic acid method employed for the assay of remaining amide was adapted from literature procedure.[10,11] The following reagent solutions were used: (A) equal volumes of a 28% aqueous hydroxyl amine solution and a 14% aqueous sodium hydroxide solution were mixed and to this was added an equal volume of a buffer which was 0.02 *M* in acetic acid and 0.08 *M* in sodium acetate; (B) aqueous 3 *N* hydrochloric acid; (C) an aqueous solution of 5% ferric chloride in 0.1 *N* hydrochloric acid. To convert the remaining amide to the corresponding hydroxamic acid ferric ion complex, the reaction tubes were opened and 2 ml. of reagent A added to them. The vials were then capped by a marble and heated in a thermostated bath at 100° for 1 hour. Next, the vials were removed from the bath,

(1) Postdoctoral Fellow, Department of Chemistry, Cornell Univ.

(2) M. L. Bender, *Chem. Revs.*, **60**, 53 (1960).

(3) T. C. Bruice and J. L. York, *J. Am. Chem. Soc.*, **83**, 1382 (1961).

(4) T. C. Bruice, T. H. Fife, J. J. Bruno and N. F. Brandon, *Biochemistry*, **1**, 1079 (1961).

(5) T. C. Bruice and T. H. Fife, *Tetrahedron Letters*, No. **8**, 263 (1961); *J. Am. Chem. Soc.*, **83**, 1124 (1961).

(6) P. A. Levene and H. L. Haller, *J. Biol. Chem.* **69**, 165 (1928).

(7) L. Zürn, *Ann.*, **631**, 56 (1960).

(8) H. M. Randall, *et al.*, "Infrared Determination of Organic Structure," D. van Nostrand Co., Inc., New York, N. Y., 1949, p. 162.

(9) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2d Ed., John Wiley and Sons, Inc., New York, N. Y., 1959, p. 205.

(10) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).

(11) S. Hestrin, *ibid.*, **180**, 249 (1949).

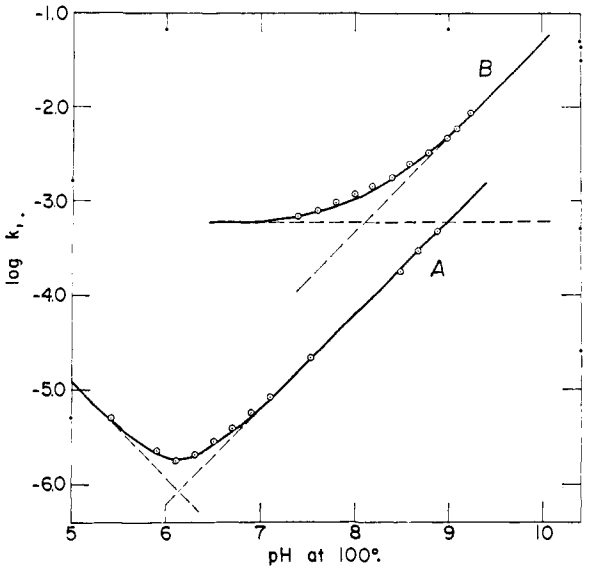


Fig. 1.—pH-rate profile for amide hydrolysis in 1 M potassium chloride at 100°: A. acetamide; B, γ-hydroxybutyramide.

quenched in ice and 1 ml. of reagent B was added followed by 1 ml. of reagent C. The absorbance of the hydroxamic acid ferric ion complex was then measured at 540 mμ on a Zeiss PM QII spectrophotometer. The color of the hydroxamic acid ferric ion complex was found to follow Beer's law to an absorbance of about 2.5. The measurements of absorbance were made against blanks prepared from the buffer employed wihout added amide.

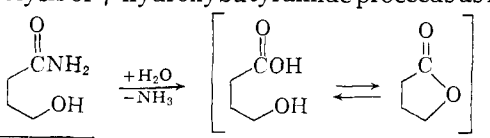
Acetamide.—The hydrolysis of acetamide, in all the buffer solutions employed, followed first-order kinetics. Extrapolation of the experimentally determined first-order rate constants (k_{obs}), obtained at constant pH but with varying buffer composition, to zero buffer concentration afforded pseudo-first-order rate constants (k_1) which are only a function of the pH. A plot of log k_1 *vs.* pH is provided in Fig. 1-A. From Fig. 1-A it is apparent that acetamide hydrolysis in the pH range studied follows the kinetic expression (A = amide).

$$\frac{-dA}{dt} = k_H(H^+)(A) + k_{OH}(OH^-)(A) = k_1A \quad (2)$$

$$k_1 = k_Ha_H + k_{OH}(K_w/a_H) \quad (3)$$

The second-order rate constants, k_H and k_{OH}, were obtained from (3) at high and low pH values where the magnitudes of a_H and K_w/a_H are respectively negligible (a_H is the hydrogen ion activity as measured by the glass electrode and K_w is the autoprotolysis constant of water which at 100° is 48 × 10^{-14}).[12] Interestingly $k_H = k_{OH} = 1.20$ l. mole^{-1}. min.$^{-1}$ with the minimum in the pH-rate profile at the pH of neutrality at 100° (pH 6.16). The theoretical curve of Fig. 1-A was calculated from the appropriate expression log k_1 = log 1.20 ($a_H + K_w/a_H$).

γ-Hydroxybutyramide.—In aqueous solution the hydrolysis of γ-hydroxybutyramide proceeds as in 4



(4)

(12) A. A. Noyes and Y. Kato, Carnegie Institution Publication, **63**, 188 (1907).

The lactone, which is a fraction of the hydrolysis product, is indistinguishable from the amide in the hydroxamic acid assay[13] and will thus influence the apparent kinetics of the hydrolysis reaction as followed by this assay procedure. The acidity dependence of the lactonization of γ-hydroxybutyric acid in aqueous solution has received only sketchy treatment in the literature.[14]

In these studies it is stated that in the range of 1.0 to 0.2 N $H^{\oplus}$ the equilibrium constant (K = lactone/acid) is about 2.7 at 25°, that the equilibrium constant is hydrogen ion dependent and that for pure γ-hydroxybutyric acid in water the equilibrium constant is about 1.5. Because the solution of buffered hydroxylamine, after addition of the aliquot of reaction mixture, is essentially neutral (pH ~ 6.5) the distinct possibility of some lactone formation during the assay procedure arose. If this were to occur, this lactone, as well as that formed on solvolysis of the hydroxyamide, would also be trapped as hydroxamic acid and compound the error in the assay procedure. These possibilities had, therefore, to be investigated.

When freshly prepared, buffered solutions of potassium γ-hydroxybutyrate of various pH values were subjected to the standard hydroxamic acid assay procedure, it was found that the values of "e" = O.D./(salt) at t = 0 ("e_0") were a function of the pH of the assayed solution (2 ml. of buffered hydroxylamine solution plus 1 ml. of buffered hydroxybutyrate solution) but independent of the concentration of buffer salts. When the same experiment was carried out with potassium acetate in place of potassium γ-hydroxybutyrate, very little color was produced by the hydroxamate method. From these results one can assume that part of the hydrolysis product has to lactonize during the assay procedure and that the lactone so produced was trapped by reaction with hydroxylamine to yield hydroxamic acid. The amount of lactone formed in this way is very small but not completely negligible.

Heating the buffered solutions of γ-hydroxybutyrate to 100° prior to the analytical hydroxamate determination resulted in an increase of "e" with time to a constant value "e_∞"; "e_∞" was found to be a function of the pH of the buffered solutions and the buffer system employed (*i.e.*, phosphate or borate). The time required to reach "e_∞" was shorter by a factor 7 than the time required for completion of the hydrolysis of the amide at any pH. Also, from a previous study[13] and the $\Delta H^{\ddagger}$ for alakline hydrolysis of butyrolactone,[15] we know that the rate of lactone hydrolysis is much faster than amide solvolysis. Therefore, it was assumed that the ratio of lactone to free acid during the entire course of hydrolysis was given by this apparent equilibrium value ("e_∞"). The value of the apparent equilibrium or "e" is a sum of the lactone at equilibrium at each

(13) T. C. Bruice and J. J. Bruno, *J. Am. Chem. Soc.*, **83**, 3494 (1961).

(14) (a) R. Fittig and H. B. Chanlaroff, *Ann.*, **226**, 322 (1884); (b) P. Henry, *Z. physik. Chem.*, **10**, 96 (1892); (c) H. S. Taylor and H. W. Close, *J. Am. Chem. Soc.*, **39**, 422 (1917); (d) H. Johansson and H. Sebelius, *Ber.*, **51**, 480 (1918); (e) A. Kailan, *Z. physik. Chem.*, **94**, 111 (1920); (f) A. Kailan and E. F. Neumann, *ibid.*, **101**, 63 (1922); (g) E. J. Boorman and R. P. Linstead, *J. Chem. Soc.*, 577 (1933).

(15) D. S. Hegan and J. M. Wolfenden, *ibid.*, 508 (1939).

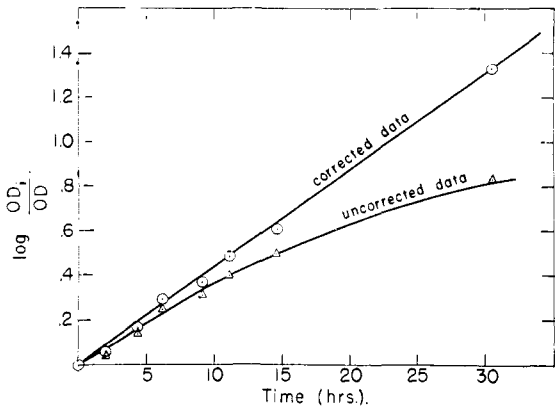


Fig. 2.—First-order plots of the hydrolysis of γ-hydroxybutyramide in 0.266 *M* borate buffer of $p\mathrm{H}_{100°}$ = 7.33 and ionic strength of 1.0 *M* with and without corrections for lactone formation.

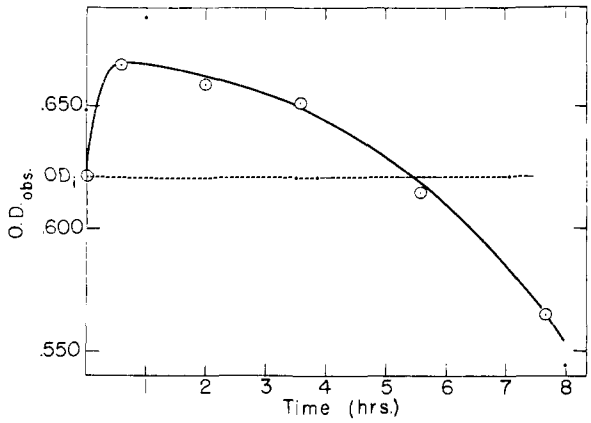


Fig. 3.—Time course of the solvolysis of γ-hydroxybutyramide at 100° in a 0.2 *M* phosphate buffer ($p\mathrm{H}_{100°}$ = 6.71), as followed by the hydroxamate procedure.

*p*H plus that amount of acid converted to hydroxamate during the assay (*vide supra*). Finally, it was established that the observed absorbance of the ferric ion–hydroxamate complex was a linear function of the concentration of the potassium γ-hydroxybutyrate solution employed in the hydroxamic acid assay.

The absorbance values (O.D.) obtained *via* the hydroxamic acid–ferric ion complex assay are, therefore, the sum of an hydroxamate absorbance due to unreacted amide ($\mathrm{O.D._a}$) and one due to lactone of the hydrolysis product ($\mathrm{O.D._m}$), formed in the course of the run and during the assay procedure.

$$\mathrm{O.D.} = \mathrm{O.D._a} + \mathrm{O.D._m} \qquad (5)$$

If *S* is the fraction of initial amide remaining at time *t*, $\mathrm{O.D._i}$ the absorbance at $t = 0$ and $\mathrm{O.D._\infty}$ the absorbance at $t = \infty$ then

$$S = \mathrm{O.D._a/O.D._i} \qquad (6)$$
$$1 - S = \mathrm{O.D._m/O.D._\infty}$$

and

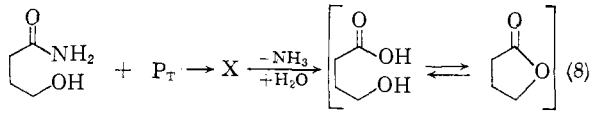
$$\mathrm{O.D.} = \mathrm{O.D._i}S + \mathrm{O.D._\infty}(1 - S)$$

the value of $\mathrm{O.D._\infty}$ can be calculated from "e_∞" and the concentration of amide employed. From 5 and 6 the value of $\mathrm{O.D._a}$ is given by

$$\mathrm{O.D._a} = \frac{\mathrm{O.D.} - \mathrm{O.D._\infty}}{1 - \mathrm{O.D./O.D._i}} \qquad (7)$$

In Fig. 2 a comparison is made of the results of a typical kinetic run, where the first-order plots have been made employing the experimental absorbance values (O.D.) and the corrected values ($\mathrm{O.D._a}$). The negative deviation of the points for the first-order rate calculated from O.D. is due to formation of lactone from the product.

The corrected first-order rate plots for the hydrolysis of γ-hydroxybutyramide in the borate buffer solutions were all linear passing through the origin. Reactions carried out in phosphate buffered solutions, on the other hand, exhibited additional complications. Proceeding from $t = 0$ and withdrawing samples in the usual way, the absorbance values obtained by the hydroxamic acid procedure first increase (Fig. 3) and then slowly decrease. This abnormal behavior was found not to be due to any alteration of the buffer during the run since buffers preheated to 100° for 11 hr. provided results essentially identical to freshly prepared buffers. The shape of the absorbance *vs.* time curves (Fig. 3) suggest a sequence of reactions, the simplest case being 8. In 8, P_T represents the phosphate buffer



species involved in a fast reaction with amide to produce an intermediate, X, which then solvolyzes to products. The initial increase in the absorbancy would then be accounted for through the greater reactivity of X with hydroxylamine (*i.e.*, greater fractional conversion of X to hydroxamate as compared to amide). In one experiment the rate of solvolysis of the hydroxyamide was followed in phosphate buffer by determining the rate of formation of ammonia *via* Nesslerization. It was found that the rate of ammonia formation was comparable to the rate of decrease of absorbance and not that of its initial increase. Therefore, the hypothetical species X cannot be lactone (*e.g.*, a rapid formation of lactone catalyzed by phosphate buffer). The decreasing portion of the hydroxamate absorbance *vs.* time plot in phosphate buffers did not afford good first-order rate plots, possibly a consequence of the rate of formation and solvolysis of X not being sufficiently different.

Extrapolation of the K_{obs} values calculated from the $\mathrm{O.D._a}$ values (7) for borate buffers to zero buffer concentration at constant *p*H afforded the buffer-independent pseudo-first-order rate constants k_1. The profile of log k_1 *vs.* *p*H for γ-hydroxybutyramide is presented in Fig. 1-B. The points of Fig. 1-B are experimental and the curve is theoretical, being calculated from eq. 10

$$\frac{-dA}{dt} = k_{OH}(K_w/a_H)A + k_0A = k_1A \qquad (9)$$

$$\log k_1 = \log\left[\frac{k_{OH}K_w}{a_H} + k_0\right] \qquad (10)$$

the appropriate values of k_{OH} and k_0 being 9.5 l. mole^{-1} min.$^{-1}$ and 5.75 × 10^{-4} min.$^{-1}$, respectively.

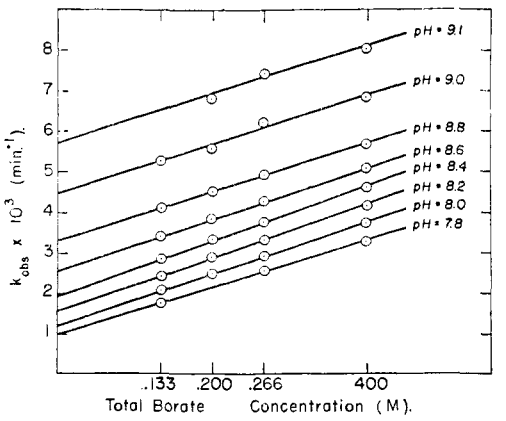


Fig. 4.—Plots of k_{obs} *vs.* borate buffer concentration in the hydrolysis of γ-hydroxybutyramide.

Catalysis of Amide Hydrolysis by Phosphate and Borate Salts.—The catalysis of the hydrolysis of acetamide by acetate buffer in aqueous solution has been investigated by Wyness.[16] The observed pseudo-first-order rate constants for amide hydrolysis were found to be dependent on the concentration of acetic acid but not on acetate anion. In this study we find that the slopes of the plots of buffer concentration *vs.* k_{obs}—for phosphate and borate buffers in the case of acetamide and for borate buffers in the case of γ-hydroxybutyramide—are parallel at all *p*H's studied (see for example Fig. 4). Thus, the two phosphate and the two borate species present must have the same rate constants. On the basis of experiments performed at 8 *p*H values between *p*H 5.4 and 7.1, the second-order rate constants for catalysis by phosphate ions are $k_{H_2PO_4^-} = k_{HPO_4^{=}} = 2.60 \pm 0.06 \times 10^{-5}$ l. mole^{-1} min.$^{-1}$. Similarly, for the borate catalysis $k_{H_3BO_3} = k_{H_2BO_3^-} = 3.20 \pm 0.06 \times 10^{-4}$ l. mole^{-1} min.$^{-1}$ as calculated from experiments at four *p*H values between *p*H 8.4 and *p*H 8.9. For γ-hydroxybutyramide $k_{H_3BO_3} = k_{H_2BO_3^-} = 5.94 \pm 0.50 \times 10^{-1}$ l. mole^{-1} min.$^{-1}$ calculated from experiments carried out at eleven *p*H values between *p*H 7.4 and 9.25.

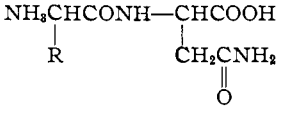
Discussion

Nucleophilic displacement reactions at the ester and amide carbonyl groups by weak bases have been noted to be kinetically distinct processes (11 and 12).

$$-\text{d ester}/dt = k_r\,(\text{ester})(\text{B:}) \qquad (11)$$

$$-\text{d amide}/dt = k_r\,(\text{amide})(\text{BH}) \qquad (12)$$

where B: and BH are the base and its conjugate acid. This possible generality has been most firmly established in intramolecular displacement reactions. Perhaps the first pertinent observations were made by Leach and Lindley[17] who found that the hydrolysis of glycyl-L-asparagine and L-leucyl-L-asparagine (I) at intermediate *p*H value obeyed



(I)

(16) K. G. Wyness, *J. Chem. Soc.*, 2934 (1958).

(17) S. J. Leach and H. Lindley, *Trans. Faraday Soc.*, **49**, 915 (1953).

the rate expression 13—the intramolecular counterpart of 12. Where K_a' is the

$$\frac{-\text{d amide}}{dt} = \frac{k_1(\text{amide})a_H}{K_a' + a_H} \qquad (13)$$

dissociation constant of the carboxyl group of I. The expression 13 strongly supported the authors suggestion that the neighboring carboxyl group was functioning as an acid catalyst. Bender and co-workers,[18,19] found the *p*H–rate profiles for the hydrolysis of monomethyl phthalate and phthalamide to support mechanisms in which the carboxyl anion participated in ester hydrolysis and the protonated carboxyl group in amide hydrolysis. O^{18}-Isotope experiments gave support to the anhydride being the intermediate in the carboxyl-catalyzed ester hydrolysis. More recently the anhydride has been identified kinetically in a similar system.[20] Results similar to those of Bender were reported by Bruice and Sturtevant[21] for the intramolecular catalysis of amide hydrolysis by the imidazole group of γ-(4-imidazolyl)-butyramide. The support for a pre-equilibrium protonation, rather than a concerted displacement accompanied by a proton transfer, arises from the studies of Wyness[16] on the acetic acid catalysis of acetamide hydrolysis in H_2O and D_2O.

The driving force which determines the difference in the course of nucleophilic displacement at the ester and amide carbonyl group resides in the order of leaving tendencies: $NH_3 > \overset{\oplus}{O}CH_3 > \overset{\oplus}{N}H_2>$. This order is supported by the fact that the specific rate associated with carboxyl participation and imidazolium ion participation in amide hydrolysis are much greater than the specific rates associated with carboxlate and imidazolyl participation in ester hydrolysis.[18,19,21]

Facilitation of amide hydrolysis by a neighboring aliphatic hydroxyl group was first proposed by Wolfrom, Bennett and Crum[22] to explain the observed hydrolysis of aldonamides. A consideration of the admitted[24] difficulty in the reporduction of rate runs and the sometimes observed long lag periods preceding the reaction suggests that aldonamide hydrolysis is more complicated than suggested. Zahn and Zürn[23] postulated an intramolecular nucleophilic attack of an aliphatic hydroxyl group to account for the rapid hydrolysis of allohydroxylysylglycinamide in strong acid. Justification for hydroxyl group participation comes from the more recent observations of Zürn[7] who found the hydrolysis of δ-hydroxyvaleramide and γ-hydroxybutyramide (1 *N* HCl) to be much faster than the rates of hydrolysis of the corresponding *n*-alkylcarboamides.

From the present study and that of Zürn[7] at higher acidity (1.0 *N* HCl) it is apparent that the *p*H–rate profile for γ-hydroxybutyramide solvolysis

(18) M. L. Bender, Y. Chow and F. Chloupek, *J. Am. Chem. Soc.*, **80**, 5380 (1958).

(19) M. L. Bender, F. Chloupek and M. C. Neveu, *ibid.*, **80**, 5384 (1958).

(20) T. C. Bruice and U. K. Pandit, *ibid.*, **82**, 5858 (1960).

(21) T. C. Bruice and J. M. Sturtevant, *ibid.*, **81**, 2860 (1959).

(22) M. L. Wolfrom, R. B. Bennett and J. D. Crum, *J. Am. Chem. Soc.*, **80**, 944 (1958).

(23) H. Zahn and L. Zürn, *Ann.*, **613**, 76 (1958).

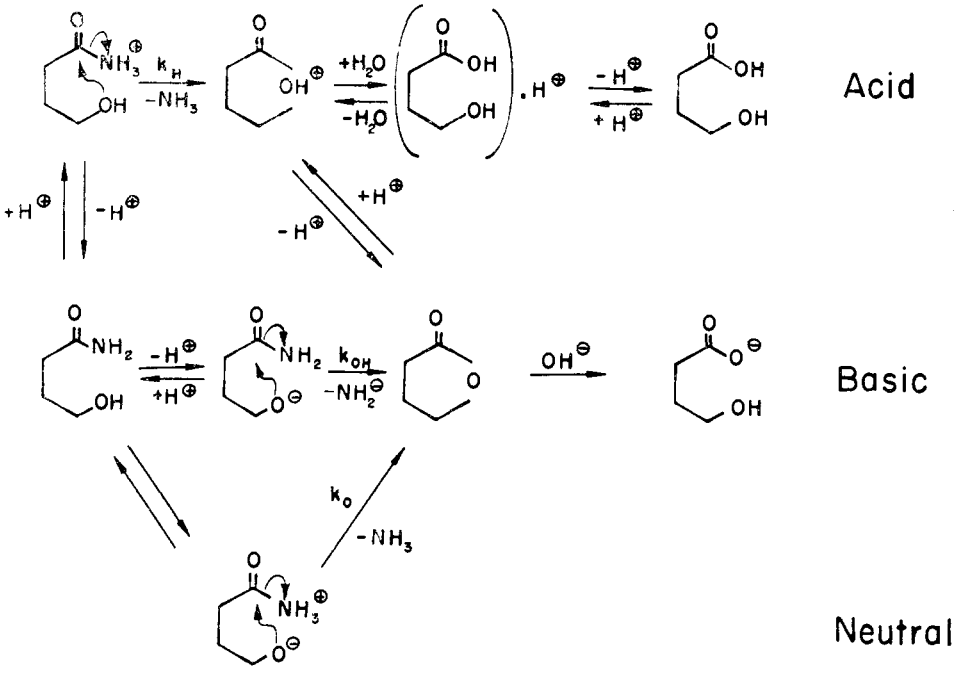


Chart I

has three distinct regions (not including the expected results at very high acidity).[24] In moderately strong acid and base the pseudo-first-order rates of solvolysis are (H^+) and (OH^-) dependent. This feature is shared by the *p*H–rate profile for acetamide hydrolysis (Fig. 1-A).[25] The values of the related second-order rate constant are

	k_H (30°). l. mole^{-1} × 10^4	k_{OH} (100°). min.$^{-1}$
CH_3CONH_2	5.6	1.2
$CH_3(CH_2)_2CONH_2$	3.7	(0.44)[26]
$HOCH_2(CH_2)_2CONH_2$	65.0	9.5

Thus, the ratio of the rate constants for the acid-catalyzed solvolysis of γ-hydroxybutyramide to that for acetamide is 9:1 and for butyramide 15:1 while the comparative ratios for base catalysis are 8:1 and 22:1. We see, therefore, that the introduction of a hydroxyl group in the γ-position of butyramide increases the ratio of alkaline and acid hydrolysis by a factor of 15 to 20. Acid-catalyzed hydrolysis of amide is almost completely insensitive to the electronic effects of substituents on the acyl portion of the amide[27,28] and in any event the substitution is too far removed from the seat of the reaction to account for an electronic effect. It is also difficult to envision a neighboring group participation that could speed the attack of H_2O in an (H^+) catalyzed reaction. The most logical conclusion is, therefore, that the rates of γ-hydroxybutyramide solvolysis in acidic and basic media represents an intramolecular participation of the γ-hydroxyl group.

The unique feature of the *p*H–rate profile for the solvolysis of γ-hydroxybutyramide at 100° is the plateau occurring near neutrality. This indicates that in the neutral *p*H range γ-hydroxybutyramide undergoes spontaneous solvolysis. Furthermore, this monomolecular solvolysis is quite efficient so that at neutrality (*p*H 6.16 at 100°) the hydroxamide solvolyses 300 times as fast as acetamide (or −800 times as fast as butyramide). This spontaneous solvolysis must also be due to the participation of the γ-hydroxyl group.

Reasonable mechanisms for the observed participation of the hydroxyl group of γ-hydroxybutyramide in its hydrolysis are presented in Chart I. In comparison to the weak bases (as $-COO^\ominus$ and imidazole) the hydroxyl group can participate in intramolecular amide hydrolysis as either the acid or base forms (*i.e.*, –OH or $-O^\ominus$ as in 11 and 12).

It is of considerable interest to know if the reaction of BH with amide occurs in a step wise pre-equilibrium protonation mechanism 14 or a concerted process as in 15.

The contributions of Wyness[16] to this problem have been mentioned. Our finding that $k_{H_2PO_4^\ominus}$ =

(24) J. T. Edwards, *J. Chem. Soc.*, 2000, 2007 (1957).

(25) The kinetics of acetamide hydrolysis have been studied many times in the past but no complete *p*H–rate profiles have been reported and, of course, studies under our experimental conditions were desired. Previous references to acetamide hydrolysis are: (a) J. C. Crocker, *J. Chem. Soc.*, **91**, 593 (1907); (b) J. C. Crocker and F. H. Lowe, *ibid.*, 952 (1907); (c) N. von Peskoff and J. Meyer, *Z. physik. Chem.*, **82**, 129 (1913); (d) H. von Euler and E. Rudberg, *Z. anorg. allgem. Chem.*, **127**, 244 (1923); (e) E. Calvert, *J. Chem. Phys.*, **30**, 140 (1933); (f) M. Willems and A. Bruylants, *Bull. Soc. Chim. Belg.*, **60**, 191 (1951); (g) S. Widequist, *Arkiv Kemi*, **4**, 429 (1952); (h) J. Packer, A. L. Thomson and J. Vaugham, *J. Chem. Soc.*, 26001 (1955); (i) A. Bruylants and F. Kezdy, *Rec. Chem. Progr.*, **21**, 213 (1960).

(26) The value of k_{OH} for butyramide hydrolysis has been calculated for our conditions (100°, μ 1.0 *M*) from the ratio of rate constants for the alkaline hydrolysis of acetamide and butyramide between 0–95.5° (ref. 25b,e and c) with the knowledge that the E_a* values and salt effects are similar for *n*-alkyl amides (ref. 25f,i).

(27) J. A. Leisten, *J. Chem. Soc.*, 765 (1959).

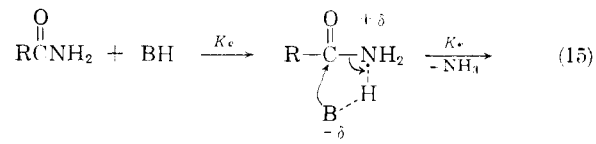
(28) J. T. Edwards, H. S. Chang, K. Yates and S. Stewart, *Am. J. Chem.*, **38**, 2271 (1960).

$$BH \overset{K_a}{\rightleftharpoons} B^{\ominus} + H^{\oplus}$$

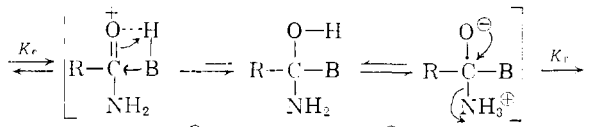
$$H^{\oplus} + RCONH_2 \overset{K_a'}{\rightleftharpoons} RCONH_3^{\oplus} \qquad (14)$$

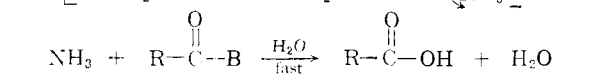
$$B^{\ominus} + RCONH_3^{\oplus} \xrightarrow{k_r} RCOB + BH$$

$$RCOB \xrightarrow[H_2O]{\text{fast}} RCOOH + BH$$



$$RCOB \xrightarrow[H_2O]{\text{fast}} RCOOH + BH, \text{ or } RCNH_2 + B{-}H$$





$k_{HPO_4^{\ominus}}$ and $k_{H_2BO_3} = k_{H_2BO_3^{\ominus}}$ for amide hydrolysis (when BH = $H_2PO_4^{\ominus}$ or $HPO_4^{\ominus}$, etc.) can only be explained through mechanism 15. In the preprotonation mechanism of Wyness[16] (14) the concentration of protonated amide is dependent only on the pH and K_a' while the value of k_r will depend on K_a (from the Brönsted relationship). One would then predict a large difference in the rates for catalysis by H_2PO_4 (pK_a 6.5) and HPO_4^- (pK_a 11-12). If, on the other hand, the process of proton transfer and nucleophilic attack are concerted (15) then one might expect the observed rates to be insensitive to the pK_a of BH. Thus, if BH were a strong acid the protonation of the amide would be favored but nucleophilic attack of B at the carbonyl group would not. If BH were a weak acid protonation would not be favored but nucleophilic attack by B would. It is reasonable to believe that these two factors would have an equal influence on the over-all rate so that it would be insensitive to the pK_a' of BH but sensitive only to the nature of B (borate or phosphate). This leads to the experimentally verifiable prediction that for a series of acids, as carboxylic acids, the slope of the Brönsted plot, α, (*i.e.*, $\log k_{rate} = \alpha pK_a' + C$) would be almost zero for the catalysis of amide hydrolysis. The finding that the observed over-all rate of amide hydrolysis by mechanism 18 is insensitive to pK_a' of BH relates to the observations of Leisten[27] and Edwards[28] on the electronic effects of substituents on acid-catalyzed benzamide hydrolysis (16). In 16 the ρ-for the preprotonation step is

$$ARCNH_2 + H_3O^{\oplus} \overset{K}{\rightleftharpoons} ARCNH_3^{\oplus} + H_2O \qquad (16)$$

$$ARCONH_3 + H_2O \xrightarrow{\text{slow}} ARCO_2H + NH_3$$

+1.40 while that for the rate-determining step is −1.30.[24] The over-all ρ-value is then but 0.1 and the over-all reaction is insensitive to electronic effects. The similarity in 15 and 16 then lies in the inverse importance in basicity and electronic effects in the protonation and nucleophilic attack.

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Conformational Analysis. XXI. The Ethyl Group[1,2]

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The thermodynamic constants of an axial ethyl group, relative to an equatorial, were calculated as follows: $\Delta H°_{298}$ = +1.71 kcal./mole, $\Delta S°_{298}$ = −0.51 e.u., $\Delta F°_{298}$ = +1.86 kcal./mole. Equilibration of the *cis* and *trans* isomers of 1,3-diethylcyclohexane, and of 1,4-diethylcyclohexane, by heating the compounds with a palladium catalyst at elevated temperatures gave $\Delta H°_{554}$ and $\Delta S°_{554}$ for these isomerizations. These quantities were also calculated for the ethyl group from the data and statistical considerations. Theory and experiment agree that the free energy of an axial ethyl group is only slightly greater than the corresponding value for an axial methyl group.

Perhaps the most important numerical quantity in the conformational analysis of cyclohexane systems is the value for the energy (actually enthalpy) of a methyl group axial (relative to equatorial) on a cyclohexane ring, 1.6–1.8 kcal./mole. This quantity can be evaluated in many ways; perhaps the earliest and most fundamental evaluation was that made by Beckett, Pitzer and Spitzer,[3] in their fundamental study of the dimethylcyclohexanes.

The corresponding value for the ethyl group has been much less thoroughly studied. The only numerical value for the conformational free energy of this group (2.1 kcal./mole) appears to be that reported by Winstein and Holness.[4] The quantity was determined in a round-about way and was regarded by those authors as only approximate.

In the present work the determination of the enthalpy of the change of an ethyl group from the axial to the equatorial position was determined independently in two separate compounds. The general experimental method is the same as was used earlier to determine $\Delta H°$ and $\Delta S°$ for the isomeriza-

(1) Paper XX, N. L. Allinger and M. A. DaRooge, *J. Am. Chem. Soc.*, **83**, 4256 (1961).

(2) In the discussion part of this paper all temperatures are given in °K. In the experimental part the temperatures are in °C. except where otherwise specified.

(3) C. W. Beckett, K. S. Pitzer and R. Spitzer, *J. Am. Chem. Soc.*, **69**, 977 2488 (1947).

(4) S. Winstein and N. J. Holness, *ibid.*, **77**, 5562 (1955).