3049(m), 2933(m), 2770(m), 1667(s), 1603(s), 1497(m), 1456(w), 1403(w), 1175(w), 1073(w), 1028(w), 977(w), 912(s), 850(w), 827(w), 748(m), 698(s); thioamide, 3311(s), 3279(s), 3021(m), 2907(m), 2817(m), 1692(w), 1681(w), 1664(w), 1647(m), 1634(m), 1603(m), 1582(m), 1536(w), 1497(m), 1464(s), 1458(s), 1362(w), 1311(w), 1272(w), 1205(w), 1068(w), 1032(w), 1015(w), 987(w), 934(m), 885(m), 754(s), 735(m), 698(s), 676(w), 672(w); amide, 3311(s), 3058(s), 2950(m), 2817(w), 1675(s), 1613(s), 1595(s), 1499(m), 1451(m), 1416(s), 1361(w), 1335(m), 1302(w), 1284(w), 1214(w), 1134(m), 1117(m), 1079(m), 1029(w), 1000(s), 953(s), 928(w), 903(m), 873(w), 849(w), 779(s), 766(w), 734(s), 700(s); acid, 3448(w), 3040(m), 2967(m), 2710(m), 2525(m), 2151(m), 1626(s), 1587(s), 1513(s), 1504(s), 1447(m), 1414(s), 1340(m), 1309(s), 1294(m), 1208(w), 1155(w), 1129(w), 1071(w), 1032(w), 984(w), 912(w), 852(m), 775(w), 745(m), 697(s), 677(w), with all values in cm.$^{-1}$ and with the intensity indicated as strong (s), medium (m) and weak (w).

PASADENA, CALIF.

[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND CHEMISTRY,[1] YALE UNIVERSITY]

Imidazole Catalysis. V. The Intramolecular Participation of the Imidazolyl Group in the Hydrolysis of Some Esters and the Amide of γ-(4-Imidazolyl)-butyric Acid and 4-(2′-Acetoxyethyl)-imidazole[2]

BY THOMAS C. BRUICE[3] AND JULIAN M. STURTEVANT

RECEIVED DECEMBER 13, 1958

The synthesis of a number of imidazoles including γ-(4-imidazolyl)-butyric acid (V) and four of its phenyl esters as well as the methyl ester and amide are recorded. Also, a new method for the easy preparation of N-acyl imidazoles is noted. The phenyl esters of V solvolyze rapidly in water due to the very effective anchimeric assistance of the neutral imidazolyl group. The fact that the *p*-nitrophenyl ester of V exhibits a rate of solvolysis almost identical to that of the α-chymotrypsin-*p*-nitrophenyl acetate complex is discussed in the light of the involvement of an imidazolyl group in both processes. The change in mechanism in going from inter- to intramolecular catalysis of substituted phenyl acetates is discussed in terms of the nucleophilic attack becoming concerted with the dissociation of the imidazolium species so that the rate-determining step becomes the collapse of the tetrahedral intermediate in the intramolecular reactions whereas in the bimolecular reactions the tetrahedral intermediate is at a very low and steady state concentration. Unlike the methyl ester of V, 4-(2′-acetoxyethyl)-imidazole undergoes hydrolysis with imidazole participation. This is the first reported instance of the nucleophilic catalysis of the hydrolysis of an aliphatic ester by an imidazole. In the hydrolysis of the amide of V the protonated imidazolyl group participates. The similarity between the effectiveness of the imidazole and carboxyl anion and imidazolium and carboxyl groups as anchimeric participants in ester and amide hydrolysis is pointed out.

Considerable evidence has been accumulated in recent years to indicate that esters and amides are catalytically hydrolyzed by esteratic enzymes through a double displacement reaction involving an acylated enzyme intermediate. The formation

$$\text{(a) EnzH + RCOX} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \left[\text{EnzH}\cdots\text{RCOX} \longrightarrow \text{Enz–}\overset{\overset{\text{O}}{\|}}{\text{C}}\text{R}\cdots\text{XH}\right] \xrightarrow{k_2} \text{EnzCOR + XH} \quad (1)$$

$$\text{(b) Enz–}\overset{\overset{\text{O}}{\|}}{\text{C}}\text{R + H}_2\text{O} \xrightarrow{k_3} \text{EnzH + RCOOH}$$

of acyl-enzyme in 1a takes place after formation of an enzyme–substrate complex, and undoubtedly involves the participation of specific amino-acid side chains (intracomplex participation) in the displacement of X; in at least one case[4] the formation of acyl-enzyme is kinetically first order in the enzyme–substrate complex. It follows, therefore, that appropriate models for esteratic enzymes should be sought among hydrolytic reactions which proceed *via* first-order processes with assistance of an intracomplex or intramolecular nature. Intracomplex participation, in the catalysis of the hydrolysis of amides and esters, has been realized through incorporation of the nucleophilic or electrophilic participants into polymers to which the substrate becomes bound.[5,6] Due to the ready availability of suitably substituted esters and amides, the carboxyl and carboxylate groups have received particular attention as intramolecular participants in ester and amide hydrolysis.[7-17]

In the case of numerous esteratic enzymes, there is much evidence to indicate that a non-protonated imidazolyl group of a histidine residue[18] and an aliphatic hydroxyl group of a serine residue[19]

(1) Contribution No. 1523.

(2) For previous papers in this series see: (a) T. C. Bruice and G. L. Schmir, THIS JOURNAL, **79**, 1663 (1957); (b) **80**, 148 (1958); (c) G. L. Schmir and T. C. Bruice, *ibid.*, **80**, 1173 (1958); (d) T. C. Bruice and R. Lapinski, *ibid.*, **80**, 2265 (1958).

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(4) H. Gutfreund and J. M. Sturtevant, *Biochem. J.*, **63**, 656 (1956).

(5) J. R. Whitaker and F. E. Deatherage, THIS JOURNAL, **77**, 3360, 5298 (1955); J. C. Paulson, F. E. Deatherage and E. F. Almy, *ibid.*, **75**, 2039 (1953).

(6) H. Samelson and L. P. Hammett, *ibid.*, **78**, 524 (1956).

(7) L. J. Edwards, *Trans. Faraday Soc.*, **46**, 723 (1950); *ibid.*, **48**, 696 (1952).

(8) E. R. Garrett, THIS JOURNAL, **79**, 3401 (1957).

(9) E. R. Garrett, *ibid.*, **79**, 5206 (1957).

(10) P. E. Zimmering, E. W. Westhead and H. Morawetz, *Biochem. Biophys. Acta*, **25**, 376 (1957).

(11) E. W. Westhead and H. Morawetz, THIS JOURNAL, **80**, 237 (1958).

(12) H. Morawetz and I. Oreskes, *ibid.*, **80**, 2591 (1958).

(13) M. L. Bender, Y. Chow and F. Chloupek, *ibid.*, **80**, 5380 (1958).

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

(15) M. L. Bender and M. C. Neveu, *ibid.*, **80**, 5388 (1958).

(16) E. R. Garrett, *ibid.*, **80**, 4049 (1958).

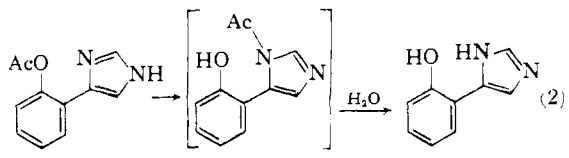
(17) S. J. Leach and H. Lindley, *Trans. Faraday Soc.*, **49**, 921 (1953).

(18) For pertinent references see ref. 2a and for more recent studies see: D. R. Davies and A. L. Green, *Biochem. J.*, **63**, 529 (1956); V. Massey and B. S. Hartley, *Biochim. Biophys. Acta*, **21**, 361 (1956); B. S. Hartley, *Biochem. J.*, **64**, 27 P (1956); L. A. Mounter, *et al.*, *J. Biol. Chem.*, **226**, 873 (1957); L. W. Cunningham and C. S. Brown, *ibid.*, **221**, 287 (1956); G. H. Dixon and H. Neurath, *ibid.*, **225**, 1049 (1957).

(19) H. Gutfreund and J. M. Sturtevant, ref. 4 and *Proc. Nat. Acad. Sci. (U. S.)*, **42**, 719 (1956); N. K. Schaffer, C. S. May and W. H. Summerson, *J. Biol. Chem.*, **202**, 67 (1953); R. A. Oosterbaan, P.

are the intracomplex participants. The various postulations for the mechanism of hydroxyl and imidazole intracomplex assistance in these cases are: (a) The adsorbed substrate undergoes a nucleophilic attack at the ester or amide bond by an imidazolyl group to yield an N-acylimidazolyl grouping; (b) the hydroxyl group of serine influenced by the juxtaposition of an imidazolyl group initiates the nucleophilic attack to form an O-acyl serine residue. The O-acyl group then undergoes an O to N migration to form an N-acyl imidazolyl group. The acyl-imidazolyl configuration formed by either a or b is then postulated to undergo rapid hydrolysis in alkaline media (as in the case of N-acetylimidazole[20,21]) or, in acid media, an N to O acyl transfer to serine to yield stable O-acylated enzyme[22]; (c) the serine hydroxyl serves as the nucleophile and the acyl group never resides on the imidazolyl group, but the latter acts as a proton acceptor[23]; or (d) the nucleophile center of the enzyme is the nitrogen of a Δ^2-oxazoline (formed by addition of a serine hydroxyl group across an amide carbonyl group with loss of water) which transfers the acylium group to an aspartic acid carboxyl group to form an anhydride which is then hydrolyzed with the possible participation of an imidazolyl group.[24] Since imidazole is postulated to be involved in a, b and perhaps d (but not c) it was felt that it would be worthwhile to explore the experimentally determinable points of the working hypothesis a, b and d. Since neither imidazole[25] nor serine catalyze the hydrolysis of aliphatic esters or amides in bimolecular processes, we may first ask ourselves if it is possible that an imidazole group as an intracomplex or intramolecular participant can bring about the nucleophilic displacement of alcohol and amine from aliphatic ester and amide bonds. This point is an important one since in a it is postulated to occur initially in reaction of enzyme with substrate and in b to occur by way of the proposed O to N acyl shift from serine to imidazole.

In earlier papers we have reported studies on the catalysis of the hydrolysis of phenyl acetates by numerous imidazoles in the bimolecular[2a,b] process and in one instance with intramolecular participation[2c] and have compared the nucleophilicity of imidazoles to other general bases in the displacement of *p*-nitrophenol from *p*-nitrophenyl acetate.[2d] In our earlier investigation of the intramolecular assistance of an imidazolyl group in the hydrolysis of 4-(2′-acetoxyphenyl)-imidazole we found the rate associated with the loss of acetic acid (2) was approximately 10^3 times that without assistance. It was our desire in the present study not only to determine the effectiveness of the imidazolyl



(2)

group in the intramolecular catalysis of aliphatic ester and amide hydrolysis but to determine if the effect of substitution on the leaving tendency of the phenolate group was the same in intra- as previously found for intermolecular nucleophilic displacement by imidazole on substituted phenyl acetates. For these purposes we have chosen to study the hydrolysis of esters and amides of γ-(4-imidazolyl)-aliphatic acids and -alcohols. In this paper the participation of the imidazole group in the hydrolysis of substituted phenyl esters (VII-X) and the methyl ester (VI) and amide (XI) of γ-(4-imidazolyl)-butyric acid (V) as well as O-acetyl-4-(2′-hydroxyethyl)-imidazole (III) is described.

The synthesis sequence employed in the preparation of the various compounds is given in Fig. 1. 1,4-Dihydroxybutyne was converted to 1,4-dihydroxybutanone-2 *via* a modification of the original procedures of Reppe.[26] In our hands we have found that the extended time period for the hydration of the butyne could be shortened. The conversion of 1,4-dihydroxy-butanone-2 to 4-(2′-hydroxyethyl)-imidazole (I) was effected by the general Weidenhagen method. This compound has been prepared previously by various methods[27–29] and has only been obtained once as a crystalline hydrochloride by Pyman after desiccation for many months. Since the purity of I was found to determine the success of the following reactions, a suitable crystalline derivative was desired. This was realized in the preparation of N-acetyl-4-(2′-acetoxyethyl)-imidazole hydrochloride (II). Partial hydrolysis of pure II afforded III and total hydrolysis yielded I as a colorelss product which was then converted to the hydrochloride of γ-(4-imidazolyl)-butyric acid (V) by way of the chloro compound (IV). Attempts to esterify V by the general Fisher technique with acidic methanol proved of no avail. The methyl ester was obtained by the direct reaction of the acid with a 10-fold excess of methanol in the presence of thionyl chloride. The hydrochloride so obtained could not be crystallized but when converted to the tosylate salt *via* displacement of HCl yielded crystalline VI.

Kunst and J. A. Cohen, *Biochim. Biophys. Acta*, **16**, 299 (1955); G. H. Dixon, S. Go and H. Neurath, *ibid.*, **19**, 193 (1956); G. H. Dixon, W. J. Dreyer and H. Neurath, THIS JOURNAL, **78**, 4810 (1956); N. K. Schaffer, L. Simet, R. R. Engle and R. W. Drisko, *J. Biol. Chem.*, **225**, 197 (1957); R. A. Oosterbaan, H. S. Jansz and J. A. Cohen, *Biochim. Biophys. Acta*, **20**, 402 (1956); J. A. Gladner and K. Laki, THIS JOURNAL, **80**, 1263 (1958).

(20) E. R. Stadtman, in "The Mechanism of Enzyme Action," W. D. McElroy and B. Glass, eds., Johns Hopkins Press, Baltimore, Md., 1954, p. 581.

(21) H. A. Staab, *Ber.*, **89**, 1927, 2088 (1956).

(22) For the case of chymotrypsin see: (a) L. W. Cunningham, *Science*, **125**, 1145 (1957); (b) G. H. Dixon and H. Neurath, THIS JOURNAL, **79**, 4558 (1957). (The experimental basis of the hypothesis of Dixon and Neurath has recently been questioned; see ref. 23); and (c) F. H. Westheimer, *Proc. Natl. Acad. Sci. (U. S.)*, **43**, 969 (1957).

(23) T. Spencer and J. M. Sturtevant, THIS JOURNAL, **81**, 1874 (1959).

(24) H. N. Rydon, *Nature*, **182**, 927 (1958).

(25) M. L. Bender and B. W. Turnquest, THIS JOURNAL, **79**, 1656 (1957).

(26) In Copenhaver and Bigelow, "Acetylene and Carbon Monoxide Chemistry," New York, N. Y., 1949, p. 138.

(27) R. A. Turner, THIS JOURNAL, **71**, 3476 (1949).

(28) B. Garforth and F. L. Pyman, *J. Chem. Soc.*, 489 (1935).

(29) F. Wrede and P. Holts, *Arch. ges. Physiol. (Pflügers)*, **234**, 432 (1934); *C. A.*, **28**, 5477 (1934).

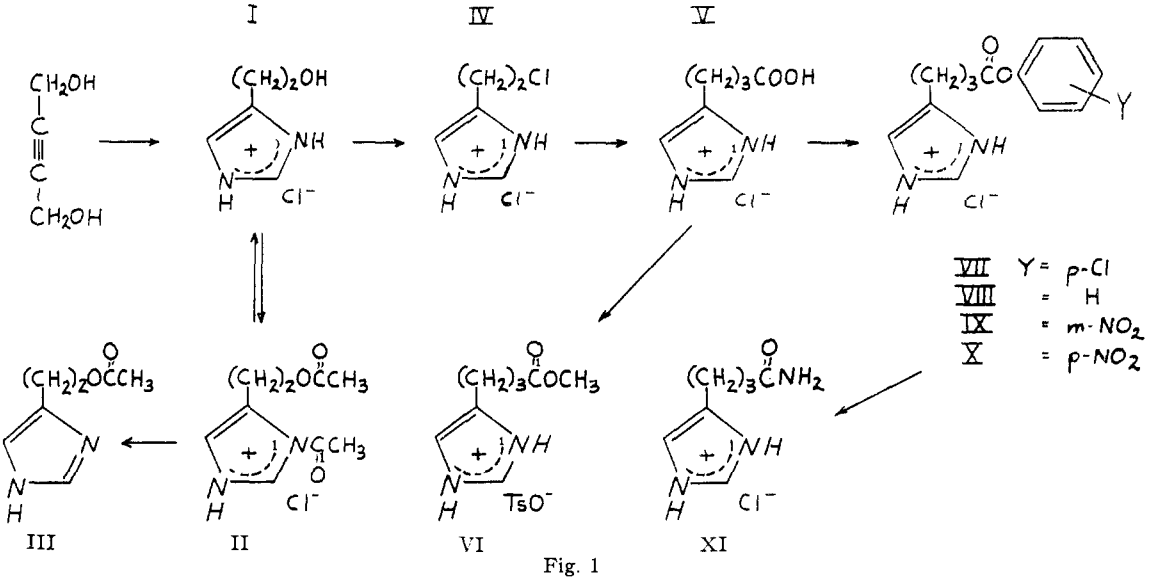


Fig. 1

The phenyl esters of V (VII, VIII, IX and X) were obtained by direct reaction of V and *p*-nitrophenol in the presence of a slight excess of thionyl chloride or by the reaction of the acid chloride of V with phenol, *p*-chlorophenol or *p*-cresol. All the esters of V exhibit negative heats of solution in organic solvents. The amide XI was obtained by aminolysis of X.

In the preparation of II an N-acylation of an imidazolium salt is involved. This is a new reaction.[30] We have subsequently found the acylation of imidazole hydrochlorides to be the method of choice for the preparation of the heretofore difficultly obtainable N-acyl imidazoles.[31]

The characterization of II as an N-acetylimidazolium hydrochloride rests on its correct analysis, typical acetyl-imidazole absorption in the ultraviolet (λ_{max} 254 mμ, ϵ 2,600), rapid reaction with neutral aqueous hydroxylamine and sensitivity to both acid- and base-catalyzed hydrolysis (Fig. 2).

Experimental

N-Acetyl-4-(2'-acetoxyethyl)-imidazole Hydrochloride (II).—At a temperature of 15° and with constant stirring a suspension of 9.65 g. (0.325 mole) of mercuric sulfate in 25 ml. of concentrated sulfuric acid was added slowly to a solution of 550 ml. of technical 35% aqueous butyne-1,4-diol[32] in 2.0 l. of water. After stirring for 8 hr. at 20–30° the reaction mixture was allowed to settle for 1 hr., filtered, stirred for an additional 6 hr. at 40° and adjusted to *p*H 4.5 by addition of calcium carbonate. The suspension was next filtered and the clear filtrate concentrated to a volume of 550 ml. by flash evaporation at 60–70 mm. and 40–50°.

The solution of 1,4-dihydroxybutanone-2 obtained in the above manner was added to a mixture of 870 g. of cupric acetate (4.35 moles), 310 ml. of 35% aqueous formalin (3.58 moles) and 6.9 l. of 35% aqueous ammonia (71 moles). The reaction mixture was heated by steam for 2 hr. and refrigerated overnight. The cuprous salt was collected, washed with water until neutral, suspended in 5.5 l. of water, made acidic (congo red) with hydrochloric acid and treated with hydrogen sulfide for 5 hr. The copper sulfide was removed by suction filtration through a Filter-cel pad and the precipitate washed with 1.5 l. of hot water. The washings and filtrate were concentrated to 400 ml. and brought to *p*H 8.0 by addition of sodium carbonate. The alkaline solution was heated to a boil, charcoal added and filtered. The filtrate was then evaporated to dryness under reduced pressure and the residue triturated with dry acetone. The extract was clarified by filtration, evaporated to dryness and the yellow oil taken up in an excess of 6 *N* hydrochloric acid and again evaporated to dryness. The yellow oil of the hydrochloride was then covered with 3 volumes of ethyl acetate and brought to reflux while acetic anhydride was added in small aliquots (caution). When solution was completed the mixture was refluxed for an additional 5 min., charcoal added and filtered through a steam-jacketed Büchner funnel. After 24 hr. of refrigeration the white crystalline product was collected and washed with a mixture of acetic anhydride and ethyl acetate and then with ether. After drying over sodium hydroxide and P_2O_5 *in vacuo* II was obtained as colorless needles, m.p. 118–120° (starting at 95°). A second batch of product of approximately the same yield and purity as the first was obtained by concentrating the filtrate from the first batch to dryness, dissolving the residue in water, neutralizing to *p*H 8.0 with sodium bicarbonate and working the product up as before. In this manner there is obtained in all about 160 g. (21%) of II.

For analysis a sample was recrystallized from ethyl acetate–acetic anhydride and dried at room temperature over P_2O_5 and KOH *in vacuo*, m.p. unchanged. *Anal.* Calcd. for $C_9H_{13}O_3N_2Cl$: C, 46.4; H, 5.64; N, 12.05. Found: C, 46.44; H, 5.44; N, 12.05. The compound reacted instantaneously with neutral aqueous hydroxylamine solution to yield the hydroxamic acid. The employment of the quantitative hydroxamic acid method of Lipmann and Tuttle[33] led to an equiv. wt. of 229 (calcd. 233). The compound is very hygroscopic, decomposing rapidly if left in an open container, and is light sensitive.

4-(2'-Acetoxyethyl)-imidazole (III).—To 100 ml. of water containing 15 g. of potassium bicarbonate (0.075 mole) was added 11.6 g. (0.05 mole) of II. After 24 hr. at 0° the solution was evaporated to dryness under vacuum and the residue triturated with dry chloroform. The chloroform was concentrated to a small volume and anhydrous carbon tetrachloride added until two layers formed. After seeding and refrigerating for 12 hr. the white fluffy needles were collected (6.46 g., 85%), m.p. 74–75°. An analytical sample was prepared by recrystallization from chloroform–carbon tetrachloride and chloroform–ether mixtures, m.p. 75–76°.

Anal. Calcd. for $C_7H_{10}O_2N_2$: C, 54.4; H, 6.52; N, 18.15. Found: C, 54.34; H, 6.60; N, 18.07.

4-(2'-Chloroethyl)-imidazole.—To 250 ml. of water was added 45 g. (0.194 mole) of II. The resultant clear, colorless solution was refluxed for 24 hr. and evaporated to dryness *in vacuo*. The colorless oil of the hydroxyethyl imidazole hydrochloride was then converted to IV *via* the proce-

(30) A. Patchornik, A. Berger and E. Katchalski (THIS JOURNAL, **79**, 6417 (1957)) recently reported an analogous case in the synthesis of 1,N-dicarbobenzoxyl-L-histidine methyl ester hydrochloride.

(31) A detailed study will be published shortly.

(32) Matheson Chemical Co.

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

dure of Turner.[37] The product so obtained (27.6 g., 85%), m.p. 120°, appeared to be identical to that reported by Turner except that it was colorless, as expected, rather than yellow.

γ-(4-Imidazolyl)-butyric Acid Hydrochloride (V).—To a solution of 1.175 g. of Na (0.051 mole) dissolved in 40 ml. of absolute ethanol contained in a three-neck flask equipped with stirrer, reflux condenser and drying tube there was added 4.1 ml. (0.027 mole) of diethyl malonate. The reaction mixture was heated to 50° for 10 min. and allowed to cool, when 4.25 g. (0.0254 mole) of IV was added. After refluxing with rapid stirring for 1 hr. and allowing to stand for 1 hr. the suspended NaCl was removed by filtration, the filtrate evaporated to a small volume, and the residue refluxed with excess concentrated hydrochloric acid for 8 hr. Upon evaporation to dryness a yellow crystalline residue of V was obtained. The residue was taken up in a minimum quantity of hot glacial acetic acid and filtered to remove a small quantity of sodium chloride. Concentration and seeding yielded 2.47 g. (58%) of V, m.p. 166–167°. An additional 15% yield could be obtained by precipitating the crude product from the filtrate by addition of ether and recrystallizing this material from ethyl acetate–acetic acid with charcoaling, m.p. 154–160°.

An analytical sample of the hydrochloride was obtained by recrystallizing several times from acetic acid or acetic acid–ether mixtures, m.p. 166–167°.

Anal. Calcd. for $C_7H_{11}O_2N_2Cl$: C, 44.1; H, 5.81; N, 14.69. Found: C, 43.85; H, 5.92; N, 14.72.

***p*-Nitrophenyl γ-(4-Imidazolyl)-butyrate Hydrochloride (X).**—To a finely ground mixture of *p*-nitrophenol (1.39 g., 0.01 mole) and V (1.91 g., 0.01 mole) contained in a flask fitted with a drying tube, there was added dropwise 0.8 ml. (0.011 mole) of pure thionyl chloride. When evolution of gas was completed the flask was placed in an oil-bath at 40° for 24 hours. The reaction mixture was quickly converted to a clear melt which then soon became solid. When reaction was completed the odor of thionyl chloride was not detectable. The solid mass of crystals were broken up with a stirring rod and dissolved in cold, anhydrous chloroform by addition of just sufficient anhydrous methanol. After treatment with charcoal and filtering the product was crystallized out by addition of ether and the suspension refrigerated. After collecting, washing with chloroform and ether and drying there was obtained 2.41 g. (78%) of X, m.p. 138–140°. Best results were obtained when the crystallization procedures were carried out as rapidly as possible since X has a rapid rate of decomposition in methanol. Also, it should be noted that the product is quite deliquescent, decomposing when left in an open container, and is photosensitive. An analytical sample was prepared by recrystallization from the same solvents, m.p. 148–149°.

Anal. Calcd. for $C_{13}H_{14}O_4N_3Cl$: C, 50.1; H, 4.53; N, 13.49. Found: C, 49.75; H, 4.58; N, 13.45. The ester reacts instantaneously with neutral hydroxylamine at room temperature to yield the hydroxamic acid. The reaction equivalent wt.[33] was found to be 314 (calcd. 312).

Methyl γ-(4-Imidazolyl)-butyrate *p*-Toluenesulfonate (VI).—To 0.79 ml. (0.02 mole) of absolute methanol contained in a test-tube there was added, at −5°, 0.2 ml. (0.0029 mole) of thionyl chloride followed by 0.5 g. of V (0.0026 mole). The test-tube was stoppered with a calcium chloride drying tube and heated at 40° for 21 hr. and at 80° for 5 min. The oily residue was taken up in absolute chloroform and the excess methanol and thionyl chloride removed by a stream of dry air. This procedure was repeated several times. The residue was next dissolved in anhydrous chloroform containing excess of pure, dry *p*-toluenesulfonic acid and the solution boiled until the odor of HCl could no longer be detected. After cooling, ether was added with scratching until some oil had precipitated. The supernatant was then decanted and diluted with a large volume of ether to yield (scratching) VI as sticky, colorless plates. The reaction mixture was then redissolved in absolute chloroform and ether again added until an oil had precipitated. Again the supernatant was decanted and diluted with additional ether to give more of the impure VI as sticky crystals. This procedure was continued until at last nothing remained on addition of ether to the chloroform extract of the reaction mixture save a small amount of yellow crystals which were discarded. The impure product was collected, suspended in methyl acetate and dissolved by addition of just sufficient methanol. Upon addition of ether, VI was obtained as pearly, colorless plates. After several recrystallizations from methyl acetate–methanol–ether the product was dried over P_2O_5 at 1.0 mm. and 70°, m.p. 87–89° (0.26 g., 30%).

Anal. Calcd. for $C_{15}H_{20}O_5N_2S$: C, 52.9; H, 5.92; N, 8.23. Found: C, 52.35; H, 6.17; N, 8.12.

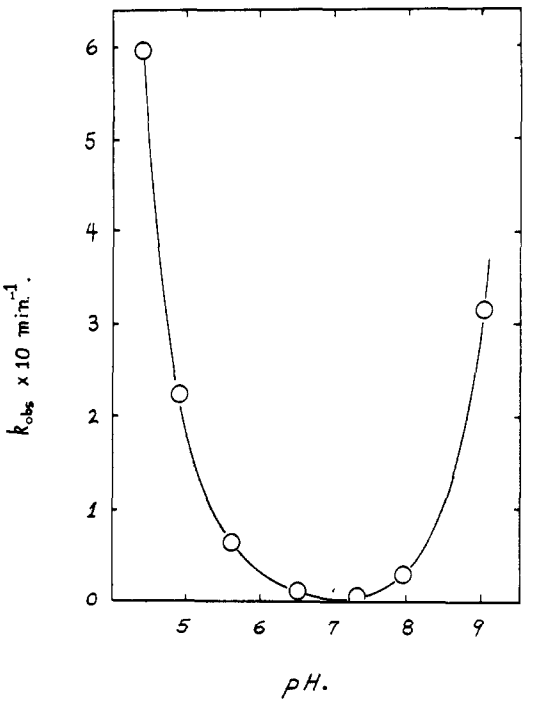


Fig. 2.—The *p*H dependence of the hydrolysis of the N-acetyl bond of N-acetyl-4-(2′-acetoxyethyl)-imidazole hydrochloride (II) at 30° (σ = 1.0 by KCl) in water. Buffers (0.2 *M*) were for *p*H 4.4, 4.9 and 5.6, citrate; 6.5, maleic; 7.3, 2,4,6-leutidine; 7.9, potassium bicarbonate or barbitol (same rate constant); and at 9.0, potassium carbonate and bicarbonate.

***p*-Chlorophenyl, Phenyl and *m*-Nitrophenyl γ-(4-Imidazolyl)-butyrate Hydrochlorides (VII, VIII and IX).**—To a small test-tube containing 0.19 g. (0.001 mole) of freshly dried and finely ground V there was added 0.08 ml. (0.0011 mole) of pure thionyl chloride. The tube was tightly stoppered with a calcium chloride drying tube and warmed at 55° for 45 min. with intermittent agitation. After cooling there was then added 0.002 mole of the phenol. The drying tube was set in place and the contents of the tube mixed by rotating in a water-bath at 55°. When mixing was completed the reaction mixture was heated at 100° for 5 min., allowed to cool and triturated many times with dry ether to remove unreacted phenol. After covering with an additional aliquot of ether and refrigerating overnight a small sample of the oil was ground under anhydrous ether in a soft glass test-tube until crystallization had occurred. The oil was then seeded with this material and allowed to stand under ether at room temperature for 48 hours when crystallization was completed. In this manner there was obtained between 75 and 95% of crude ester melting generally over an 8 to 10° range below that of the analytical sample.

The crude ester was dissolved in an appropriate solvent (anhydrous chloroform for IX and VII and anhydrous methyl acetate–methanol for VIII) and ether added until an oil began to precipitate. The cloudy supernatant was then decanted into a dry flask and allowed to crystallize (seeding and scratching). The residual oil was then taken up in the same solvent and the procedure repeated. After many such extractions and precipitations the remaining dark colored residue was discarded and the combined supernatant allowed to crystallize completely at room temperature. The colorless crystalline material was collected and for analysis

recrystallized several times from chloroform–ether or methyl acetate–methanol–ether mixtures.

Compd.	M.p., °C.	Formula	Nitrogen, % Calcd.	Found
VII	123–124	$C_{13}H_{14}O_2N_2Cl_2$	9.30	9.74
VIII	111–112	$C_{13}H_{15}O_2N_2Cl$	10.51	10.88
IX	121–123	$C_{13}H_{14}O_4N_3Cl$	13.49	13.89

These esters should not be placed in the Abderhalden drying pistol since they easily lose phenol or substituted phenol.

γ-(4-Imidazolyl)-butyramide Hydrochloride (XI).—Four hundred mg. (0.00128 mole) of X was added to 60 ml. of anhydrous chloroform previously saturated with dry ammonia at 0°. After shaking for 10 min. the precipitated ammonium chloride and ammonium *p*-nitrophenylate were removed by filtration and washed with anhydrous chloroform. The washings and filtrate were combined and evaporated at reduced pressure and the yellow oil pumped at 1.0 mm. until the odor of ammonia could not be detected. The oil was then treated with 40 ml. of anhydrous chloroform saturated with dry HCl. The solvent was again removed under reduced pressure and the sirup extracted 4 times with 30-ml. aliquots of ether to remove remaining *p*-nitrophenol. After pumping for a short time at 1.0 mm., 10 ml. of anhydrous methyl acetate and 4 ml. of anhydrous methanol were added. After addition of methyl acetate to a permanent turbidity the product began to crystallize. When crystallization was completed (4 hr.) the γ-(4-imidazolyl)-butyramide hydrochloride (120 mg., 50%) was collected, m.p. 154–158°. An analytical sample was prepared by further crystallization from methanol–methyl acetate, m.p. 157–158°.

Anal. Calcd. for $C_7H_{12}ON_3Cl$: C, 44.50; H, 6.41; N, 22.15. Found: C, 44.43; H, 6.47; N, 22.08.

Kinetic Measurements. (A) Hydrolysis of II.—The components of the buffer solution (see legend of Fig. 2) were mixed and allowed to equilibrate for 1 hr. at 30 ± 0.1°. The substrate (*ca.* 20 mg.) was weighed into a dry flask which was submerged in the constant temperature water-bath and 25 ml. of the equilibrated buffer added from a quick flow pipet. After thorough mixing, 1-ml. aliquots were rapidly withdrawn at appropriate time intervals and assayed for remaining substrate by the quantitative hydroxamate method of Lipmann and Tuttle.[33] The concentration of substrate at 0 time was determined from the first aliquot which was, in general, withdrawn within 20 sec. of the mixing of the reaction solution. First-order kinetics were followed within 1–5% up to 90% reaction.

(B) Lactamization of VII, VIII, IX and X.—These reactions were so rapid that it was necessary to employ the stopped-flow technique[34] in determining their rate constants. In each experiment equal volumes of the ester dissolved in absolute ethanol at a concentration of approximately 3×10^{-4} *M* and an aqueous THAM[35]–acetic acid buffer of desired *p*H and 0.04 *M* ionic strength were mixed in the stopped-flow apparatus. The rate change of absorption at the wave lengths listed in Table I was observed. The temperature of the reaction solutions was 25.0 ± 0.2°. The reactions were all found to follow first-order kinetics with good accuracy. The data for two typical experiments with the *p*-nitro ester X are given in Fig. 3 in the form of semi-logarithmic first-order plots.

TABLE I

WAVE LENGTHS EMPLOYED IN DETERMINING THE RATES OF LACTAMIZATION OF SUBSTITUTED PHENYL γ-IMIDAZOLYL-BUTYRATES

Substituent	H	*p*-Cl	*m*-NO_2	*p*-NO_2
Wave length, mμ	275	285	350	330

(C) Hydrolyses of III and VI were followed at constant temperature and *p*H by the automatic titration of liberated acid with an autotitration assembly consisting of a Radiometer T111a autotitrator with magnetic value which activated a Fitzpatrick[36] recorder equipped with an automatic drive for an Agla micrometer syringe which led to a three neck § Metrohm Microtitration cell by way of a glass capillary which extended to just above a small bar-magnet stirrer. The Metrohm Microtitration assembly was fitted with a § Metrohm type X glass electrode and a § salt bridge leading to an external calomel electrode. The problem of rapid flow of KCl from the salt bridge at the temperatures of the experiment (resulting in drastic changes in ionic strength during each run) was circumvented by fusion of a Beckman calomel electrode tip to its end. The microtitration vessel was maintained at 78° by circulation of water through the water jacket from a precision thermoregulated circulating water-bath heated by means of extra bar heaters. The glass electrode was maintained at 78° during the entire period of the investigation.

The use of an inert atmosphere after initial flushing with nitrogen was found to be unessential since the micro-titration cell, as assembled, was air-tight. The latter factor also prevented evaporation. All runs were made in 0.1 *N* KCl and followed to about 20–60% completion. In all instances first-order kinetics were followed with less than 1% deviation. In practice between 35 and 10 μmoles of ester in 2 ml. of solution were employed and approximately 0.035 *N* NaOH was used to maintain constant *p*H. The base was frequently standardized and only those runs which exhibited less than 0.02 *p*H unit drift were calculated.

(D) Hydrolysis of XI.—Approximately 3×10^{-3} *M* solutions of XI were prepared in 0.1 *M* citrate or phosphate buffers. The *p*H of the solutions were determined at 78° against a Metrohm X glass electrode. Aliquots of the solutions were then dispensed into Pyrex ampules and placed in a glycerine-bath heated to 78° by means of refluxing ethanol. The ampules were withdrawn at various time intervals and the ammonia formed determined by means of the micro-diffusion method of Conway.[37] Blank runs employing V showed no ammonia formation by decomposition of the imidazolyl ring at these temperatures. The reactions were followed to 60% completion and were first order in amide.

pK_a' determinations were performed at the various temperatures by use of the autotitration device previously described. All determinations were carried out in 0.1 *N* KCl solution. The values of pK_a' were obtained by fitting the experimental data to theoretical titration curves.

Results[38]

Because of the very rapid lactamization of the phenyl esters of V in water it was necessary to prepare the solutions of these substances in a less reactive solvent prior to mixing with the aqueous buffer in the stopped flow apparatus. Considerations of the construction of the apparatus (plastic), the formation of refractive index gradients and air bubbles on mixing, etc., led to the use of absolute ethanol for this purpose. Even in absolute ethanol the *p*-nitro and *m*-nitro esters underwent rapid solvolysis; however, the rate of phenol liberation from undecomposed ester could be followed readily. Therefore, the lactamizations of VII, VIII, IX and X were followed in 50% (v./v.) aqueous ethanol while the hydrolysis of III, VI and XI were studied in water. THAM-acetate buffers were employed to maintain constant *p*H for the phenyl esters, while citrate buffers were employed for XI and for the esters III and VI constant *p*H was maintained by the use of an autotitrator.

(34) The stopped-flow apparatus has been described by T. Spencer and J. M. Sturtevant, see ref. 23.

(35) Tri-(hydroxymethyl)-methylamine.

(36) J. B. Fitzpatrick, Machine Shop, Yale University School of Medicine, New Haven, Conn.

(37) R. B. Johnston, M. J. Mycek and J. S. Fruton, *J. Biol. Chem.*, **185**, 629 (1950).

(38) The various hydrolytic species and equilibrium constants associated with the imidazolyl group are abbreviated as: $IMH \underset{}{\overset{K_1}{\rightleftharpoons}} IM \underset{}{\overset{K_2}{\rightleftharpoons}} IM^-$; total ester and amide (E_T and A_T); protonated ester and amide (EH and AH); neutral ester and amide (E_N and A_N); ester anion (E_A). Other abbreviations employed are *p*-NPA for *p*-nitrophenylacetate, ATEE for acetyl-L-tyrosine ethyl ester, and THAM (see footnote 35).

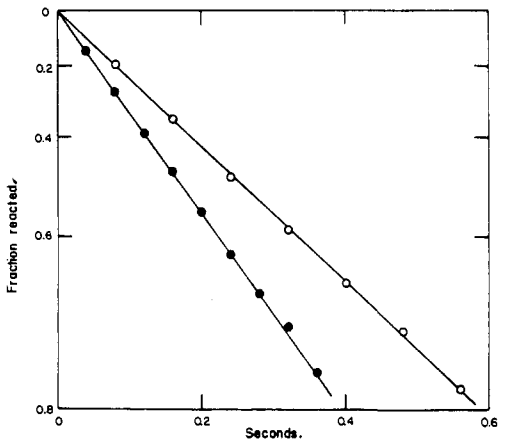


Fig. 3.—First-order plots for two typical experiments on the lactamization of *p*-nitrophenyl γ-(4-imidazolyl)-butyrate (X) at 25°, 0.04 *M* ionic strength, in THAM-acetate buffer in 50% (v./v.) ethanol–water. The reactions were followed by observation of the changes in optical density at 330 mμ; open circles, *p*H 6.89; filled circles, *p*H 7.81.

From studies on the bimolecular catalysis of phenyl acetate hydrolysis by imidazoles[2a,2b,25] it was felt that the imidazolyl group of compounds VII, VIII, IX and X would assist in solvolysis and do so in the neutral non-protonated form; it was also expected that, depending on solvent, buffer type and concentration, a solvolytic decomposition of the esters without imidazolyl participation might take place. The rate of appearance of substituted phenol would then be given by the expression

$$\text{d phenol}/dt = k_1E_T + k_2E_N \qquad (3)$$

The observed first-order rate constant at any *p*H would be (4)

$$k_{obs} = k_1 + k_2\frac{K_1}{K_1 + a_H} \qquad (4)$$

where a_H is the activity of hydrogen ion (here operationally defined as the quantity determined by the glass electrode). Rearrangement of equation 4 gives

$$k_{obs} - k_1 = k_2 - \frac{1}{K_1}(k_{obs} - k_1)a_H \qquad (5)$$

from which it is evident that a plot of $k_{obs} - k_1$ *vs.* $(k_{obs} - k_1)a_H$ should be a straight line of slope $-1/K_1$ and intercept k_2 at $a_H = 0$.

In the cases of the esters VII, VIII and IX it was found that non-vanishing values of k_1 had to be selected to give straight line plots. The values of k_1 arrived at in this way are not very accurate, but fortunately the values of k_2 and K_1 obtained from the plots are not sensitive to the value of k_1 selected.

In the case of the *p*-nitro ester X, no indication of solvolytic reaction without imidazolyl participation was observed at low *p*H ($k_1 = 0$), but a rapid rise in rate at high *p*H suggestive of hydroxide ion catalysis was encountered. A satisfactory plot was obtained by replacing k_1 in equation 5 by the quantity $(9.0/a_H) \times 10^{-7}$ min.$^{-1}$.[39] The data for this ester are summarized

(39) This is indicative of a very large second-order rate of reaction with hydroxide ion when compared to the value of $2.6/a_H \times 10^{-11}$ employed for *p*-NPA (see ref. 23, and also 2 and 25).

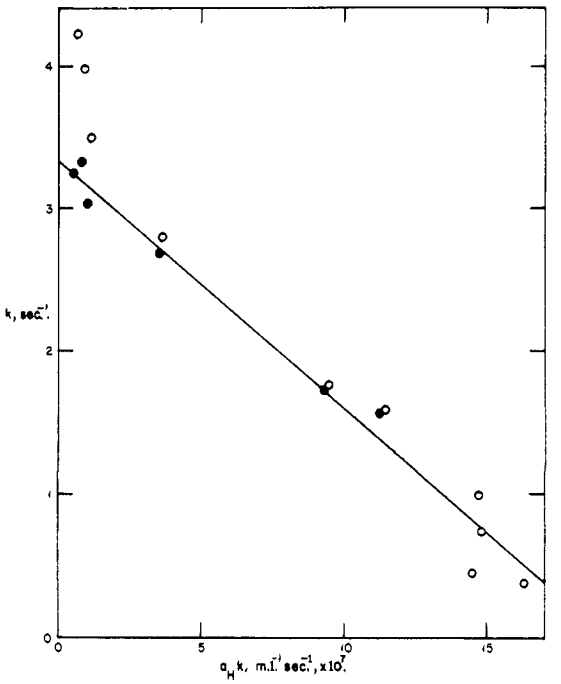


Fig. 4.—Plot of first-order constant against the product of rate constant times hydrogen ion activity for the lactamization of *p*-nitrophenyl γ-(4-imidazolyl)-butyrate (X) at 25°, 0.04 *M* ionic strength, in THAM-acetate buffers in 50% (v./v. ethanol–water; open circles, observed rate constants; filled circles, observed rate constants corrected for hydroxide-ion catalyzed hydrolysis as described in the text.

in Fig. 4, the open circles being the observed rates and the filled circles being the rates after correction for the assumed hydroxide ion catalysis. Table II lists the observed first-order rate constants (in each case the mean of three to five determinations usually agreeing to within better than 5%) for four phenyl esters, together with the derived values for k_1, k_2 and K_1.

Unlike the phenyl esters of V the aliphatic esters III and VI are quite stable in aqueous solution at 25° necessitating the employment of higher temperatures for the study of the *p*H dependence of their hydrolysis. A temperature of 78° was chosen as being the most convenient compromise between the instability of the glass electrode and the rate of proton release. The rates of hydrolysis at this temperature were such that the pK_a' of both reactants and products could be determined titrimetrically (Table III).

For the methyl ester VI a linear plot of k_{obs} *vs.* hydroxide ion activity (as calculated from the *p*H employing the value of K_w at 78°, *i.e.*, $10^{-12.5}$)[40] is permitted (Fig. 5). Since the intercept of Fig. 5 at zero hydroxide ion activity is zero, the hydrolysis of VI between *p*H 6.0 and 7.7 can be ascribed solely to specific base catalysis

$$\text{d acid}/dt = k_{OH}(OH^-)(E_T) \qquad (6)$$

The slope of Fig. 4 gives a specific rate constant for hydroxide ion catalysis of 2.53×10^2 l. mole^{-1} min.$^{-1}$.

(40) Extrapolated from data in "International Critical Tables," Vol. VI, p. 152.

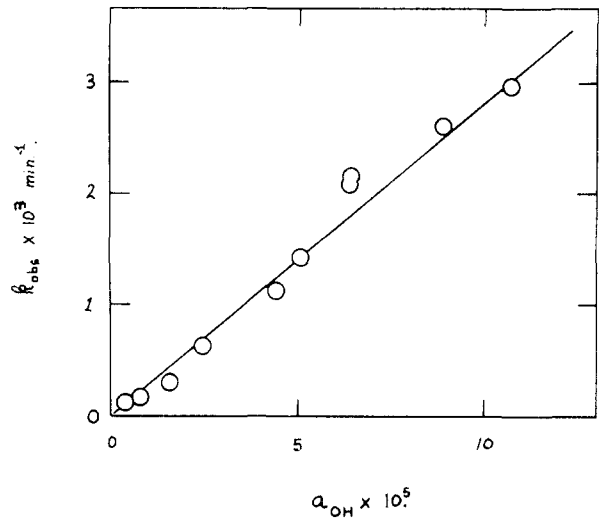


Fig. 5.—The observed pseudo-first-order rate constants (k_{obs}) for the hydrolysis of methyl γ-(4-imidazolyl)-butyrate (VI) plotted against hydroxide ion activity.

A linear plot of k_{obs} *vs.* hydroxide ion activity for the ester III is not possible, the rate of hydrolysis increasing most rapidly as the *p*H increases in the range of the pK_1' of the –IM group, suggesting participation of the latter. We then obtain

TABLE II

THE *p*H DEPENDENCE OF THE RATES OF HYDROLYSIS OF *m*- AND *p*-SUBSTITUTED PHENYL γ-(4-IMIDAZOLYL)-BUTYRATE AT 25° IN THAM-ACETATE BUFFER, 0.04 *M* IONIC STRENGTH, 50% (V./V.) ETHANOL-WATER

Substituent	*p*H	k_{obs}, min.$^{-1}$	k_1, min.$^{-1}$	k_3, min.$^{-1}$	pK_1
H	7.71	2.09			
	7.69	2.36			
	6.89	1.20			
	6.33	0.62			
	6.28	.618			
	5.79	.30			
	5.65	.30	0.14	2.58	6.91
p-Cl	7.71	9.60			
	6.93	6.66			
	6.33	3.42			
	5.82	1.56	0.36	10.4	6.69
m-NO_2	7.71	131			
	6.93	79.8			
	6.33	48.8			
	5.82	25.4	1.2	144	6.79
p-NO_2[a]	7.81	253			
	7.65	239			
	7.50	210			
	6.89	168			
	6.27	106			
	6.14	95.4	0.0	200	6.24
	5.83	59.8			
	5.71	44.5			
	5.49	26.8			
	5.39	22.4			

[a] Hydroxide ion catalysis given by $(9.0/a_H) \times 10^{-7}$ min.$^{-1}$ was deducted from the observed rate constants to get a straight line plot of rate constant *vs.* rate constant times hydrogen ion activity.

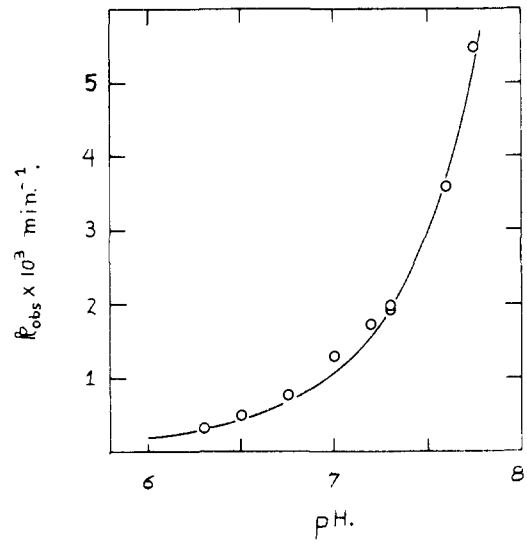


Fig. 6.—The observed pseudo-first-order rate constants (k_{obs}) for the hydrolysis of 4-(2′-acetoxyethyl)-imidazole (III) plotted against *p*H. The curve is calculated on the basis of a specific rate constant for OH^- (k_{OH}) of 2.82×10^2 l. mole^{-1} min.$^{-1}$ and a specific rate constant for imidazole participation of 2×10^{-4} min.$^{-1}$

equations 7 and 8

$$\text{d acid}/dt = k_{OH}(a_{OH})(E_T) + k_N(E_N) \quad (7)$$

$$k_{obs} = k_{OH}\frac{K_w}{a_H} + k_N\frac{K_1}{K_1 + a_H} \quad (8)$$

For the best fit to the experimental data k_N is assigned a value of 2×10^{-4} min.$^{-1}$ and k_{OH} a value of 2.82×10^2 l. mole^{-1} min.$^{-1}$, respectively. In Fig. 6 there is plotted the experimental rate data *vs.* the theoretical curve obtained from 8.

TABLE III

DETERMINABLE DISSOCIATION CONSTANTS OF REACTANTS AND PRODUCTS

Compound	°C.	pK_{1M}'	pK_{COOH}
4-(2′-Acetoxyethyl)-imidazole	26	6.97	
(III)	78	6.06	
Methyl γ-(4-imidazolyl)-butyrate	26	7.3	
(VI)	60	6.82	
	78	6.48	
γ-(4-Imidazolyl)-butyramide (XI)	78	6.52	
γ-(4-Imidazolyl)-butyric acid (V)	26	7.62	4.26
	60	7.0	4.43

A comparison of the second-order rate constants for specific base catalysis of III and VI (280 and 250 l. mole^{-1} min.$^{-1}$) to that for ethyl acetate and methyl butyrate (26.4 and 11.5 l. mole^{-1} min.$^{-1}$ at 78° in 70% ethanol–water)[41] reveals that the rates for the imidazole substituted esters are 10 to 20 times greater. The larger rates for the imidazole substituted esters could be due to the participation of the $-IM^-$ group because K_2 for a 4(5)-alkyl-substituted imidazole would be very close to K_w and participation of the $-IM^-$ species would result in an apparent greater value of k_{OH}.[2b]

In Fig. 7 the values of k_{obs} for the hydrolytic liberation of ammonia from XI are plotted *vs.*

(41) Extrapolated from studies of E. Tommila, *et al.*, *Ann. Acad. Sci. Fennicae*, **AII**, 47 (1952); *Acta Chem. Scand.*, **8**, 257 (1954).

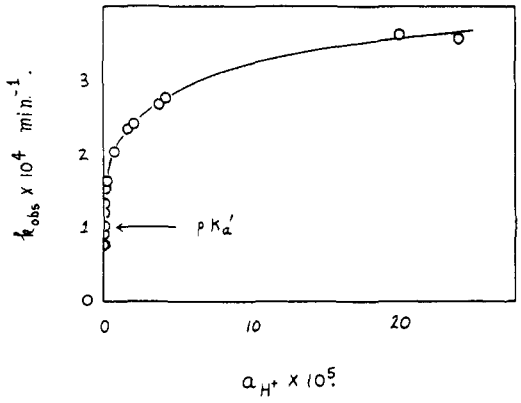


Fig. 7.—The pseudo-first-order rate constants (k_{obs}) for the hydrolysis of γ-(4-imidazolyl)-butyramide (XI) plotted against the hydrogen ion activity.

a_H. A cursory inspection of Fig. 7 reveals that the rate increases as the a_H increases but not in a linear fashion and that the increase of k_{obs} is particularly marked in the region of the pK_1' of the imidazolyl group—where butyramide is quite resistant to hydrolysis. The dependence of ammonia liberation on a_H has been found to follow equation 9

$$\text{d ammonia}/dt = k_2A_N + k_3AH + k_4(AH)(HB^-) + k_5(AH)(a_H) \quad (9)$$

various of the terms having several kinetic equivalents

$$k_2A_N + k_3AH = k_6A_T + k_5AH$$

$$k_2A_N = \frac{k_2k_1(AH)(a_{OH})}{K_w} \qquad k_3AH = \frac{k_3(A_N)(a_H)}{K_1} \text{ etc.} \quad (10)$$

From equation 9, k_{obs} at constant pH and constant total buffer (B_T) concentration becomes equation

$$k_{obs} = k_6 + \frac{a_H}{a_H + K_1}\left[k_1'\frac{a_H}{a_H + K_B} + k_5 + k_4a_H\right] \quad (11)$$

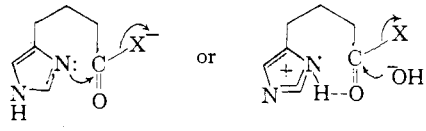
11 in which $k_1' = k_1B_T$. The values of K_1 (Table II) and K_B (1×10^{-5}) were determined titrimetrically at the temperature of the experiment (78°). The best fit of (11) to the experimental data is given in Fig. 8 and the various constants are presented below:

Constant	Value: min.$^{-1}$ × 10^4	Value: l. mole^{-1} min.$^{-1}$
k_1'	1.12	
k_1		1.12×10^{-3}
$k_6 = k_2$	0.45	
k_5	1.25	
k_4		3.74×10^{-1}

The term $k_1(AH)(HB^-)$, where HB^- represents buffer monoanion, may represent a case of general acid catalysis of amide hydrolysis.[42] In experiments where phosphate replaced citrate as buffer between pH 7 and 8, k_{obs} was increased by about fourfold.

Discussion

The assistance of the -IM group in the solvolysis of III and the phenyl esters of V can be rationalized by postulating either a direct attack of an amidine nitrogen on the ester bond or to the intramolecular solvation of the transition state for OH^- attack by the -IMH ion (12). These two possibilities cannot



(12)

be differentiated kinetically. Bender and Turnquest[25] have established that acetylimidazole is formed in high yield as an intermediate in the reaction of imidazole with p-NPA, and later Brouwer[43] reported that this intermediate accounts for 90% of the reaction path. More recently Schmir and Bruice[44] have established that in the reaction of benzimidazole with p-acetoxybenzoic acid the path through N-acetylbenzimidazole accounts for at least 90% of the conversion of p-NPA to hydrolytic products. The latter experiment is particularly noteworthy since the stability of acetylbenzimidazole (half-time of hydrolysis, 21 hours at room temperature)[45] allows for its accurate determination. Therefore, we feel quite confident that the mechanism involving direct attack of an amidine nitrogen on the carbonyl carbon is correct for the intramolecular catalysis of ester hydrolysis by -IM. However, it must be recalled that in the solvolysis of 4-(2′-acetoxyphenyl)-imidazole[2c] the N-acetyl compound could not be detected—a factor possibly attributable to its low steady state concentration—and Garrett,[16] in a study of the hydrolysis of α-pyrrolidylacetyl salicylate, established the participation of the pyrrolidinium group.

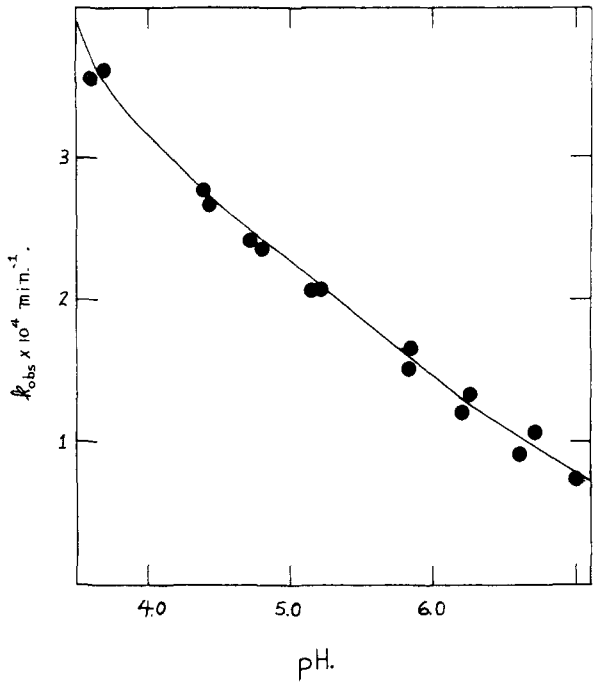


Fig. 8.—The relationship of the rate of hydrolysis of γ-(4′-imidazolyl)-butyramide (XI) to pH. The experimental points have been plotted along with the theoretical curve as calculated from equation 11.

(42) Wyness, *J. Chem. Soc.*, 2034 (1958).

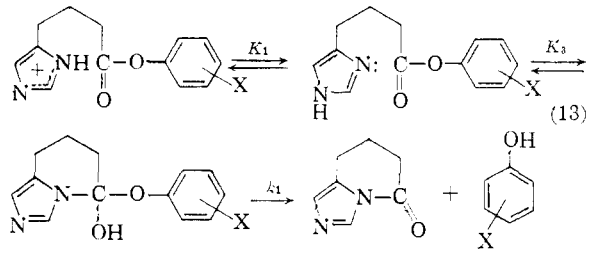
(43) D. M. Brouwer, Dissertation, Leiden, 1957.

(44) G. L. Schmir and T. C. Bruice, unpublished results.

(45) H. A. Staab, *Ber.*, **90**, 1320 (1957).

It is known that imidazole itself does not detectably catalyze the hydrolysis of aliphatic esters, and the establishment of an intramolecular catalysis by -IM in III is the first instance of the displacement of alkoxide from the carboalkoxy bond by an imidazole. In this regard the hydrolysis of III resembles the case of monomethyl phthalate in which the carboxyl anion assists,[14] whereas the bimolecular catalysis of the hydrolysis of an aliphatic ester by a carboxylate reagent has never been observed.

The participation of -IM in the hydrolysis of the phenyl acylate bonds of VII, VIII, IX and X possesses an unusual characteristic. Inspection of Table IV reveals that the apparent dissociation constant (pK_{app}) of the imidazolyl group—as determined kinetically—depends markedly on the *m*- or *p*-substituent of the carbophenoxy group; being much lower for the *p*-nitro ester. These results may be compared to those of Bruice and Schmir in the hydrolysis of 2-(4′-imidazolyl)-phenyl acetate[2c] where the value of pK_{app} (5.5) was found to be that of the imidazolyl group. The present results suggest that a substituent dependent equilibrium—in addition to the dissociation of the imidazolium ion—occurs prior to the rate limiting step. A most reasonable mechanism would be



The rate expression would then be[16]

$$\frac{d(\text{lactam})}{dt} = k_{app}\frac{K_{app}}{K_{app} + a_H}E_T \qquad (14)$$

$$\text{where } k_{app} = \frac{k_1K_3}{K_3 + 1};\ K_{app} = K_1(K_3 + 1)$$

If it is assumed that K_1 for the phenyl esters is identical to that for the methyl ester (pK_a' = 7.1 in 50% ethanol–H_2O (v./v.))—and this should be so—then the values of K_3 and k_1 may be calculated from the values of K_{app} and k_{app} by use of equation 14. These calculated values appear in Table IV.

TABLE IV

CALCULATED EQUILIBRIA AND RATE CONSTANTS FOR THE HYDROLYSIS OF THE PHENYL ESTERS OF V

Ester	pK_{app}	K_3	k_{app} (min.$^{-1}$)	k_1
Phenyl	6.91	0.55	2.58	7.27
p-Chlorophenyl	6.69	1.53	10.4	17.2
m-Nitrophenyl	6.79	1.06	144	180
p-Nitrophenyl	6.24	6.22	200	232

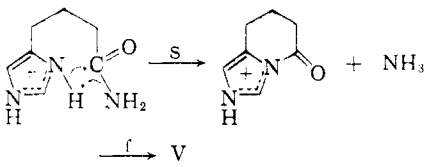
On the basis of the calculated constants K_3 and k_1 we may now compare the inter- to the intramolecular reaction of imidazole with substituted phenyl acetates. In the intermolecular reaction the pK_{app} is equal to the pK_a' of the imidazole regardless of the nature of the substituent on the phenyl ester.[2a] It would appear, therefore, that in converting the intermolecular to the intramolecular catalysis the ionization of the base tends to become concerted with the nucleophilic attack on the ester carbonyl and the rate of collapse of the tetrahedral intermediate to N-acylimidazole becomes of increasing importance in the over-all rate of phenol liberation. It is possible that the increase in rate of base catalysis of X over *p*-NPA (30,000) may be due to catalysis of the collapse of the tetrahedral intermediate through proton abstraction. For the intermolecular reaction the Hammett constant ρ- was found to be 1.9.[2a] Plots of K_3 and k_1 by the conventional Hammett equation are given in Fig. 9. In the equilibrium K_3 the substituents are not conjugated to the bond-forming center and electronic effects must pass to the carbonyl group by an inductive effect from the ester oxygen. Therefore, for plotting the equilibrium data the non-conjugative σ-value has been used for the *p*-nitro group. In the case of k_1 the stabilization of the negative charge of the leaving phenoxide ion is of great importance in the rate-determining bond breaking process and, therefore, in this case the conjugative σ-constant has been employed for the *p*-nitro group. Inspection of Fig. 9 reveals that the *m*-nitro ester deviates greatly from the line passing through the points for the *p*-substituted esters. Though the data are scarcely numerous enough to warrant Hammett plots, the divergence of the *m*- as compared to the *p*-substituents suggests that perhaps $\Delta S^{\ddagger}$ varies with the position of substitution. It can be surmised from the $\rho\sigma$ plots that the electronic effects of the substituents are of approximately equal importance in the scission of the C–O bond (k_1) and in the attack of the amidine nitrogen on the carbonyl carbon. There appears to be a decrease in sensitivity to electronic effects in going from inter- to intramolecular catalysis by imidazole.

Bender and Neveu[15] have pointed out that the few kinetic data at present available for intramolecular catalysis and closely similar intermolecular catalysis indicate that the major factor favoring intramolecular catalysis is a much less negative entropy of activation. A particular striking example of this is furnished by the cases of mono-*p*-nitrophenyl glutarate and *p*-NPA plus acetate ion.[10] Here the enthalpy of activation is actually about 4 kcal. per mole smaller for the intermolecular reaction, but this difference is completely overshadowed by the difference in entropies of activation of 25 e.u. in favor of the intramolecular reaction. This type of result is to be expected since in the intermolecular case there must be a considerable loss in translational entropy in forming the activated state. It should be remembered that entropies of activation are standard state quantities, and that in the intermolecular case, but not in the intramolecular case, the actual value of $\Delta S^{\ddagger}$ depends importantly on the choice of standard state, or what amounts to the same thing, on the choice of concentration units. The standard entropy difference observed in this case actually approaches in magnitude the calculated loss in

(46) T. C. Bruice and G. L. Schmir, THIS JOURNAL, **81**, in press (1959).

translational entropy of two perfect gas molecules coalescing to form a new molecule, all at a concentration of 1 mole per liter. Unfortunately activation parameters are of little use for comparative purposes in the present study because of the large heat of ionization of imidazoles which is reflected in the $\Delta S^{\ddagger}$ values.[2c]

On the same bases employed to establish the participation of the carboxylate group in the hydrolysis of acetyl salicylate,[7,8] mono-*p*-nitrophenyl glutarate,[10] monomethyl phthalate[14] and related esters (*i.e.*, a rapid increase in the pseudo-first-order rate constant on dissociation of the carboxyl group) the –IMH group is implicated as a participant in the solvolysis of XI. That the basic –IM group assists in ester hydrolysis and the IMH group in amide hydrolysis finds a parallel in the catalysis of ester hydrolysis by participation of the carboxylate group and catalysis of amide hydrolysis by participation of the undissociated carboxyl group.[13,14,17] The specific rate associated with carboxyl participation in the hydrolysis of phthalamide is much greater than that for carboxylate participation in the hydrolysis of monomethyl phthalate and the specific rate constant for –IMH participation in the hydrolysis of the amide XI is greater than that of –IM participation in the hydrolysis of the corresponding ester VI ($k_r = 0$). These results add credence to the four center nucleophilic–electrophilic mechanisms proposed by Bender for general acid intramolecular catalysis of amide hydrolysis[13] and indicate the greater leaving tendency of the NH_3 as compared to the NH_2^- species. The mechanism for –IMH participation in the hydrolysis of XI may then be tentatively written as



(15)

The rate constant associated with –IMH assistance is about 10^2 smaller than that reported by Leach and Lindley[17] (90°) for the hydrolysis of glycyl-L-asparagine and L-leucyl-L-asparagine with carboxyl assistance, whereas the constant for specific acid catalysis is 10 times that for L-asparagine. The assistance of the –IMH group is, therefore, much less effective in the case of XI than the –COOH group is for the asparagine peptides. This is accounted for by the greater acidity of the latter and its closer proximity to the amide bonds of the asparagine peptides.

The fact that the rate constants for the loss of *p*-nitrophenol from X and from the chymotrypsin *p*-NPA complex are almost identical (200 *vs.* 190 min.$^{-1}$),[4] as are the "apparent" basicities (determined in both cases by the dependence of k_{obs} on *p*H) of the participating imidazolyl groups is most interesting. This comparison demonstrates that if a molecule of *p*-NPA were bound to a protein so as to possess the same or equivalent steric restraint relative to a histidine residue as the ester bond of X does to the imidazolyl ring, then an

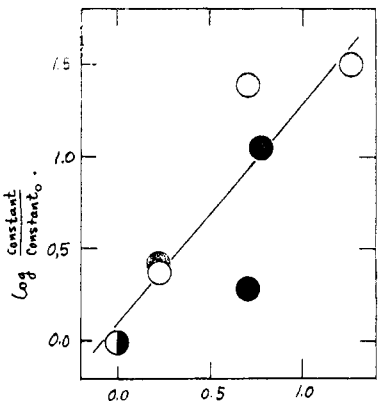


Fig. 9.—Hammett $\rho\sigma$ plot for the equilibrium between ester and tetrahedral intermediate (open circles) and conversion of tetrahedral intermediate into lactam (shaded circles) in the intramolecular nucleophilic catalysts of the hydrolysis of the phenyl esters of γ-(4-imidazolyl)-butyric acid (ρ 1.3).

"enzymic-like" rate of hydrolysis would be observed and, furthermore, for this substrate, no other group would need to participate. This conclusion is given added importance from the conclusion that the sites of reaction of *p*-NPA and of ATEE with chymotrypsin are probably identical.[23] The same steric requirements which allowed assistance to such a degree with the *p*-nitrophenyl ester of V failed to give any assistance with the methyl ester. Chemically speaking, this is not at all surprising. However, the rate of enzymic hydrolysis of the ethyl ester ATEE is much greater than that for *p*-NPA, whereas the latter is more subject to specific acid and base or to general base catalysis. If the enzymic mechanism involves a nucleophilic attack at the ester bond then regardless of the nature of the nucleophile (serine hydroxyl, Δ^2-oxazoline, imidazolyl, etc.) the rate for ATEE could only be greater than that for *p*-NPA if the former were compressed against the nucleophile to a much greater extent than the latter. This conclusion is not only chemically sound but receives added support from the consideration that the easily hydrolyzed *p*-NPA molecule is a general substrate for many esteratic enzymes irrespective of their steric requirements whereas ATEE possesses the basis structure associated with chymotryptic activity. Examples of the type of steric compression which is being suggested are not rare in organic chemistry, being seen for example in the so-called "*gem*-dimethyl" effect[47] and in an interesting example of the Smiles rearrangement,[48,49] where substitution of a methyl group for a hydrogen results in sufficient compression to give an increase of rate of 10.[6] Similar additional increases in rate should be noted in intramolecular catalysis of ester and amide hydrolysis if participation by –IM, or other nucleophile is so enhanced.

(47) C. K. Ingold, *J. Chem. Soc.*, 305 (1923); R. F. Brown and Norman M. van Gulick, *J. Org. Chem.*, **21**, 1046 (1956).

(48) C. S. McClement and S. Smiles, *J. Chem. Soc.*, 1016 (1937); J. F. Bunnett and R. E. Zahler, *Chem. Revs.*, **49**, 273 (1951).

(49) J. F. Bunnett and T. Okamoto, THIS JOURNAL, **78**, 5363 (1956).

Studies toward this end are now in progress in this Laboratory.

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Proton N.S.R. Spectroscopy. V. Studies of Amino Acids and Peptides in Trifluoroacetic Acid

BY F. A. BOVEY AND G. V. D. TIERS

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The proton spin resonance spectra of trifluoroacetic acid solutions of all the common and several less common amino acids and of many of their glycyl peptides and N-acetyl derivatives are reported. The use of trifluoroacetic acid as solvent has the advantage that an internal reference standard, tetramethylsilane, may be employed, enabling peak positions to be very accurately determined; in addition, it is an excellent solvent for these compounds. By measurement and comparison of peak positions, widths and multiplicities, conclusions of considerable interest may be drawn concerning charge distribution, inductive effects of polar groups and positive charges, rates of proton exchange with solvent, base strength and (in one case) molecular conformation. In addition, the tabulation of peak positions is very useful in the interpretation of the n.s.r. spectra of proteins.

Introduction

The proton nuclear spin resonance (n.s.r.) spectra of glycine, alanine, β-alanine, cysteine, proline, hydroxyproline, glycylglycine and alanylalanine have been reported by Takeda and Jardetzky,[1] who examined 2–3 *M* solutions in strongly acidic, neutral and strongly basic aqueous medium. These studies have recently been extended to include 22 amino acids.[2] The protons of the $-NH_3^+$ group can be distinguished in 6 *N* HCl, in concentrated H_2SO_4 and in $H_2SO_4 \cdot H_2O$; in less strongly acidic solutions the peak due to these protons coalesces with that of the water, in the fashion typical for rapid exchange between the species. In the spectra of the dipeptides the peak due to peptide hydrogen likewise appears only in strongly acid solutions. Takeda[3] has extended these studies to β-alanine, α-aminobutyric acid and γ-aminocaproic acid.

We have studied the proton n.s.r. spectra in trifluoroacetic acid solvent of all the common and of several less common amino acids and of many of their glycyl peptides and N-acetyl derivatives. Trifluoroacetic acid is an excellent solvent for these substances, 20% (weight/volume) solutions of nearly all of them being readily prepared. Glycine is soluble to the extent of about 15% and cysteine and cystine are still less soluble but sufficiently so (5–8%) to give excellent spectra. In trifluoroacetic acid, for which the Hammett acidity function H_0 is -4.4,[4] those groups acquire protons which would normally do so in strongly acidic aqueous medium. Peptide and amide protons show n.s.r. peaks, as does the sulfhydryl group of cysteine, but those of hydroxyl and carboxyl exchange too rapidly with the solvent to be observable; small concentrations of water likewise give no n.s.r. peak distinguishable from that of the solvent. The present study provides information useful in the interpretation of the n.s.r. spectra of proteins, as will be demonstrated in a later publication.

Referencing.—In D_2O or H_2O solutions the n.s.r. peak for water protons in the solution is variable in position and hence not satisfactory as a reference point for a scale of shielding values. The use of an "external reference," for example, H_2O or toluene in a capillary tube, would be satisfactory, *provided that the required*[5–7] *extrapolation of the solute peak position to infinite dilution were carried out.* Unfortunately this is virtually never done, unreliable values being reported instead.[8] Careful temperature control and measurement also are necessary because of the differing magnetic susceptibilities and coefficients of expansion of the solvent and reference. On the basis of work done in organic liquids[8] we suspect that an internal reference might be preferable even in aqueous media; however, the proper choice of such a reference for aqueous solutions has not been discussed in the scientific literature, and is not dealt with in this research.

One of the significant advantages provided by the use of trifluoroacetic acid as solvent is that tetramethylsilane may be employed as the internal reference.[8] It does not appear to be sufficiently soluble in purely aqueous solutions. The usual concentration of tetramethylsilane is 1% by volume. Its n.s.r. peak is very sharp and falls beyond the usual range of proton resonances. It is nonassociative, magnetically isotropic, and is usually

(1) M. Takeda and O. Jardetzky, *J. Chem. Phys.*, **26**, 1346 (1957).

(2) O. Jardetzky and C. D. Jardetzky, *J. Biol. Chem.*, **233**, 383 (1958).

(3) M. Takeda, paper presented to Div. of Biol. Chem., 131st Meeting of American Chemical Society, April 7–12, 1957.

(4) (a) G. V. D. Tiers, THIS JOURNAL, **78**, 4165 (1956), and (b) E. L. Mackor, P. J. Smit and J. H. Van der Waals, *Trans. Faraday Soc.*, **53**, 1309 (1957).

(5) A. A. Bothner-By and R. E. Glick, THIS JOURNAL, **78**, 1071 (1956).

(6) A. L. Allred and E. G. Rochow, *ibid.*, **79**, 5361 (1957).

(7) J. S. Waugh and R. W. Fessenden, *ibid.*, **79**, 846 (1957).

(8) G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).