[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, YALE SCHOOL OF MEDICINE]

Imidazole Catalysis. I. The Catalysis of the Hydrolysis of Phenyl Acetates by Imidazole

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In view of the suggested importance of the imidazolyl group of histidine to the activity of hydrolytic enzymes, the ability of imidazole to catalyze the hydrolysis of phenyl acetates was investigated. It was found that the hydrolysis is first order in phenyl acetate and imidazole to at least 60% completion of reaction (pH 8.0). The rate of the catalyzed hydrolysis was found to be pH dependent, and it was shown that the effective catalyst is the non-protonated imidazole species. In addition, it was demonstrated that catalysis increases with the basicity of the imidazolyl group. Thus, for the hydrolysis of p-nitrophenyl acetate by a series of 4(5)-substituted imidazoles, the calculated second-order rate constant obeyed the expression $k_2 = 2.24 \times 10^{-4} K_b^{0.67}$ and the 4(5)-substituted imidazole having maximum activity at any given pH was calculated to have a $pK'_a = 0.3 + p$H. That the catalysis may be an inherent property of the imidazole structure was demonstrated in the inability of pyridines and 8-hydroxyquinoline to catalyze the hydrolysis of p-nitrophenyl acetate at concentrations to 5×10^{-4} M in amine, conditions under which imidazoles of similar pK'_a were effective. Additional data which corroborate the suggested participation of imidazole as a particularly effective basic catalyst are obtained by comparison of ρ-values for the hydrolysis of phenyl acetates by imidazole, hydroxide ion and hydronium ion and by comparison of the activation energy terms. The mechanism of the catalysis is discussed in the light of the above data and the catalytic efficiency of N-methylimidazole as compared to imidazole.

Introduction

In recent years experimental evidence of several types has indicated that an imidazolyl group of histidine may form a portion of the catalytic site of several hydrolytic enzymes. Thus, from studies on the pH dependence of their catalytic action, it has been inferred that acetylcholine esterase,[2] chymotrypsin,[3] trypsin[4] and kidney dialkylfluorophosphatase[5] owe their esteratic properties at least in part to the imidazolyl group of a histidine residue. Loss of enzymatic activity upon the photooxidation of histidine residues has been interpreted to indicate that the activity of chymotrypsin,[6] lysozyme,[7] ribonuclease[8] and intestinal carbohydrases[9] depends on the presence of intact imidazolyl groups. Aside from oxidative and pH dependence studies, it has been reported that an imidazolyl group of chymotrypsin, normally labile to attack by 2,4-dinitrofluorobenzene, is protected by a competitive inhibitor[10] or, on reaction of the enzyme with dialkylfluorophosphate,[11] to form the inactive dialkylphosphochymotrypsin.

In view of these findings, it is of obvious interest to determine whether imidazole possesses "esteratic properties" toward carboxylic esters, and in this paper we describe our studies of the catalysis of hydrolysis of phenyl acetates by imidazole and some substituted imidazoles.[12,13] The phenyl acetates were chosen for this study because they have served as non-specific substrates for esterases[14-16] and also because their acid and base catalyzed hydrolyses have been well studied.

Experimental

Phenyl acetates were prepared by the method of Spasov.[17] m- and p-nitrophenyl acetates were recrystallized from ligroin and ethyl ether, respectively, to constant melting points, m.p. 55–56° (lit.[18] 55–56°) and m.p. 79–80° (lit.[19] 79.5–80°). The liquid phenyl acetates were distilled through a 3-foot vacuum jacketed column packed with stainless steel turnings and a middle cut of the correctly distilling fraction taken. The $n^{27}D$ values found were for phenyl acetate, 1.5012 (lit.[20] 1.5030), p-chlorophenyl acetate, 1.5180, and p-cresyl acetate, 1.5011 (lit.[21] 1.4991).

Imidazoles.—Eastman Kodak Co. White Label imidazole and benzimidazole were employed without further purification, m.p. 89–90° (lit.[22a] 90°) and m.p. 171–173° (lit.[22b] 170°), respectively. 4-Bromoimidazole, m.p. 132–134° (lit.[23] 130–131°), 4-hydroxymethylimidazole hydrochloride, m.p. 105–108° (lit.[24] 107–109°), 4-methylimidazole hydrochloride, m.p. 114° (lit.[25] 118°), N-methylimidazole, b.p. 191° (lit.[26] 195°), and 2-methylimidazole, m.p. 144° (lit.[27] 139°), were prepared by recorded procedures.

Apparatus.—Water-bath temperatures were maintained to ±0.02° by means of bar heaters connected in series with phase-shift thyratron relay[28] and a Braun model 4.5 contact thermometer. Spectrophotometric readings were made with a Beckman model DU spectrophotometer and the pH of solutions determined by means of a Beckman model G pH meter.

Kinetic Method.—In the studies reported herein the solvent was 28.5% ethanol-water (v./v.), phosphate was em-

(1) Lafayette B. Mendel Predoctoral Fellow in Biochemistry, 1955–1956.

(2) I. B. Wilson and F. Bergmann, *J. Biol. Chem.*, **186**, 683 (1950).

(3) R. B. Hammond and H. Gutfreund, *Biochem. J.*, **61**, 187 (1955).

(4) H. Gutfreund, *Trans. Faraday Soc.*, **51**, 441 (1955).

(5) L. A. Mounter, *J. Biol. Chem.*, **219**, 677 (1956).

(6) L. Weil and A. R. Buchert, *Fed. Proc.*, **11**, 307 (1952).

(7) L. Weil, A. R. Buchert and J. Maher, *Arch. Biochem. Biophys.*, **40**, 245 (1952).

(8) L. Weil and T. S. Seibles, *ibid.*, **54**, 368 (1955).

(9) J. Larner and R. E. Gillespie, *ibid.*, **58**, 252 (1955).

(10) B. S. Hartley, *Ann. Repts. Progr. Chem.* (Chem. Soc., London), **51**, 309 (1954).

(11) H. Fraenkel-Conrat, *Ann. Rev. Biochem.*, **25**, 312 (1956), see also V. Massey and B. S. Hartley, *Biochim. Biophys. Acta*, **21**, 361 (1956).

(12) For previous observations of the catalysis of hydrolytic reactions by imidazole compounds, see (a) T. Wagner-Jauregg and B. E. Hackley, THIS JOURNAL, **75**, 2125 (1953); (b) p. 311 of ref. 10; (c) Th. Müller, T. Rathlev and Th. Rosenberg, *Biochim. Biophys. Acta*, **19**, 563 (1956).

(13) A portion of this study has been previously presented as a preliminary communication (*cf. Arch. Biochem. Biophys.*, **63**, 484 (1956)).

(14) B. S. Hartley and B. A. Kilby, *Biochem. J.*, **56**, 288 (1954).

(15) O. Gawron, C. J. Grelecki and M. Duggan, *Arch. Biochem Biophys.*, **44**, 455 (1953).

(16) C. Huggins and J. Lapides, *J. Biol. Chem.*, **170**, 467 (1947).

(17) A. Spasov, *Ann. Univ. Sofia II, Faculte Phys. Math.*, Livre II, **35**, 289 (1938–1939); *C. A.*, **34**, 2343 (1940).

(18) F. Arnall, *J. Chem. Soc.*, **125**, 816 (1924).

(19) A. Kaufmann, *Ber.*, **42**, 3482 (1909).

(20) E. H. Huntress and S. P. Mulliken, "Identification of Pure Organic Compounds. Order I," John Wiley and Sons, Inc., New York, N. Y., 1941, p. 319.

(21) *Ibid.*, p. 323.

(22) (a) K. Hofmann, "Imidazole and its Derivatives." Part I, Interscience Publishers, Inc., New York, N. Y., 1953, p. 6; (b) p. 379.

(23) I. E. Balaban and F. L. Pyman, *J. Chem. Soc.*, **121**, 947 (1922).

(24) J. R. Totter and W. J. Darby, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 460.

(25) A. Kirby and A. Neuberger, *Biochem. J.*, **32**, 1146 (1938).

(26) O. Wallach, *Ber.*, **15**, 644 (1882).

(27) G. Dedichen, *ibid.*, **39**, 1838 (1906); R. G. Fargher and F. L. Pyman, *J. Chem. Soc.*, **115**, 217 (1919).

(28) N. A. Frigerio, *Rev. Sci. Instr.*, **27**, 1022 (1956).

ployed as buffer and *p*-nitrophenyl acetate was most generally employed as substrate. The final compositions of the various reaction mixtures are indicated in footnotes to the appropriate tables. In general, the components of the reaction mixture, with the exception of the substrate, were mixed and allowed to equilibrate in a constant temperature bath maintained at the desired temperature of the study. The substrate made up freshly in aqueous ethanol prior to each run was then added and the contents of the reaction vessel thoroughly mixed. The reaction was then followed by withdrawing samples at regular intervals and by determining spectrophotometrically the concentration of liberated phenol. The wave lengths employed were as follows: *p*-nitrophenol, 400 mμ; *m*-nitrophenol, 420 mμ; *p*-chlorophenol, 285 mμ; and phenol, 275 mμ; *p*-cresol, 280 mμ. In the case of *p*-chlorophenol and *p*-cresol, the spectrum of the acetate overlapped that of the corresponding phenol and the concentration of liberated phenol was determined by comparing the absorbance of samples of the reaction solution at various times to that of a plot of absorbance *vs.* mole fraction phenol where (phenol) + (phenyl acetate) = (phenyl acetate)$_0$. Light absorption by the mixtures followed Beer's law over the concentration ranges used in these studies.

When *p*-nitrophenyl acetate was employed as substrate, the rate constants k_w and k'_2 were calculated from the rate expressions using the optical density at infinite time (determined after suitable dilution). In the case of the other substrates, the optical density at infinite time was calculated by extrapolation from the standard curves.

Results

The hydrolysis of phenyl acetates in the presence of imidazole was found to be first order in imidazole and phenyl acetate (Fig. 1). The first-order rate constants obtained for the hydrolysis of phenyl acetates, corrected for hydrolysis in the absence of catalyst (*i.e.*, $k_1 - k_w$), where k_1 is the observed first-order rate constant and k_w is the rate constant for "aqueous" hydrolysis under the same conditions, is proportional to the imidazole concentration. For *p*-nitrophenyl acetate at *p*H 7.9–8.0, the apparent second-order rate constant, $k_2' = (k_1 - k_w)/I_0$, where I_0 is the total molar concentration of added imidazole, was found to be 12.7 ± 0.2 l. mole^{-1} min.$^{-1}$ (25°) with phosphate buffer at 0.0054 *M* and 19.7 ± 0.9 l. mole^{-1} min.$^{-1}$ (30°) with phosphate buffer at 0.20 *M* (Table I).

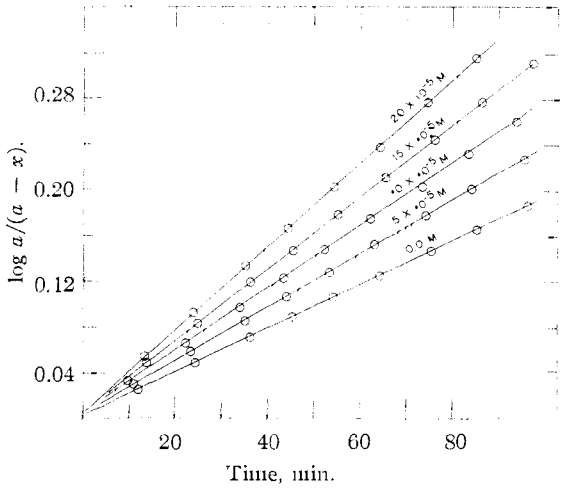


Fig. 1.—The first-order formation of *p*-nitrophenol in the hydrolysis of *p*-nitrophenyl acetate in the presence and absence of imidazole.

The determination of the dependence of the rate of *p*-nitrophenol liberation on *p*H (Table II) clearly shows that catalysis is mediated by the non-protonated imidazole species. This suggests that, for a series of imidazole derivatives, k_2' should depend not only on *p*H but also on the base strength of the catalyst. Since pK' is an approximate index of base strength, the catalysis of *p*-nitrophenyl acetate hydrolysis by various 4(5)-substituted imidazoles of known pK' was studied. At the *p*H employed (8.0) these compounds were either completely or at least 90% in the unprotonated form, and it was found that k_2' (Table III) increased with increasing pK_a'. From a plot of pK_a' *vs.* log k_2, where $k_2 = k_2'(1 + (H^+)/K_a)$, the best linear relationship (least squares) affords the equation $k_2 = 2.24 \times 10^{-4} K_b^{0.67}$ (Fig. 2). From these relationships and the equivalence of $K_b = 1/K_a$ it follows that

$$k_2' = \frac{2.24 \times 10^{-4} K_b^{0.67}}{(1 + K_b(H^+))}$$

The maximum of k_2' as a function of K_b, is obtained by setting the expression $dk_2'/dK_b = 0$ with the result that $p\bar{K}_a = 0.3 + p$H, where $p\bar{K}_a$ is now the value of pK_a' for the imidazole compound of this series which gives a maximum value of k_2' at any given *p*H. From this expression it follows

Table I

Rate Constants for the Hydrolysis of *p*-Nitrophenyl Acetate (*p*H 7.9–8.0)

Buffer, *M*	Temp., °C.	Imidazole, *M*	*p*-Nitrophenyl acetate, *M* × 10^5	k_2', l. mole^{-1} min.$^{-1}$	$k_w \times 10^4$, min.$^{-1}$
0.0054	25	5 × 10^{-5}	5	12.3	5.2
.0054	25	5 × 10^{-5}	5	13.1	5.6
.0054	25	5 × 10^{-5}	5	12.7	5.4
.0054	25	5 × 10^{-5}	5	11.7	5.5
.0054	25	5 × 10^{-5}	5	12.9	6.2
.0054	25	5 × 10^{-5}	5	12.8	5.6
.2	30	5 × 10^{-5}	2	20.0	44.0
.2	30	1 × 10^{-4}	2	18.7	44.0
.2	30	1.5 × 10^{-4}	2	19.2	44.0
.2	30	2 × 10^{-4}	2	19.9	44.0
.2	30	1 × 10^{-4}	2	17.2	38.0
.2	30	1 × 10^{-4}	2	22.2	43.4
.2	30	1 × 10^{-4}	2	20.2	46.3
.2	30	1 × 10^{-4}	2	20.1	46.0

Table II

The *p*H Dependence of the Rate of Imidazole-catalyzed Hydrolysis of *p*-Nitrophenyl Acetate at 25°[c]

*p*H	$k_1 \times 10^4$, min.$^{-1}$	$k_w \times 10^4$, min.$^{-1}$	k_2', l. mole^{-1} min.$^{-1}$ Found	Calcd.[a]
7.9	11.9	5.6	12.7	(12.7)
7.4	7.45	2.3	10.3	10.6
6.9	4.72[b]	0.70	8.0	7.0
6.3	2.00[b]	0.53	2.9	2.8

[a] The calculated values are based on the k_2' found at *p*H 7.9 and the assumption that only unprotonated imidazole is the catalytic agent. The concentration of free base present is calculated on the basis of pK_a' of 6.9 for imidazole. See ref. 29. [b] At the lower *p*H values, first-order kinetics were followed only to 10% completion of reaction. [c] In all experiments the phosphate buffer was employed at a calculated ionic strength of 1.5×10^{-2} *M*. In a previous communication this value was incorrectly reported as 3×10^{-2} *M*.

(29) R. W. Cowgill and W. M. Clark, *J. Biol. Chem.*, **198**, 33 (1952).

TABLE III

THE CATALYSIS BY 4(5)-SUBSTITUTED IMIDAZOLES OF *p*-NITROPHENYL ACETATE HYDROLYSIS AT *p*H 8.0 AS A FUNCTION OF pK_a' (*T* 25°)[a]

Catalyst	Concn. of catalyst, *M*	pKa'	k_2', l. mole^{-1} min.$^{-1}$	k_2, l. mole^{-1} min.$^{-1}$
4(5)-Bromoimidazole	5×10^{-4}	3.60[b]	0.1	0.1
Benzimidazole	5×10^{-4}	4.98[c]	0.3	0.3
4(5)-Hydroxymethyl-imidazole	5×10^{-5}	6.3[b]	2.35	2.4
Imidazole	5×10^{-5}	6.9[b]	12.7	14.0
4(5)-Methylimidazole	5×10^{-5}	7.4[b]	16.1	20.2

[a] In all cases, the concentration of *p*-nitrophenyl acetate was 5×10^{-4} *M* and phosphate buffer 0.0054 *M*. [b] Determined in 23.3% ethanol–water (v./v.) solvent. Ref. 29 of paper. [c] Determined in 50% ethanol–water (v./v.) solvent, by M. T. Davies, *et al.*, *J. Pharm. Pharmacol.*, **3**, 420 (1951).

that imidazole would be the most effective catalyst for the hydrolysis of *p*-nitrophenyl acetate at *p*H 6.6.

That the catalysis observed for imidazole is not merely a function of *pK* is borne out by the finding that pyridine, 2,6-dimethylpyridine, 2,4,6-trimethylpyridine (pK_a' 5.23, 6.62 and 7.45, respectively)[30] and 8-hydroxyquinoline do not catalyze the hydrolysis of *p*-nitrophenyl acetate at concentrations up to 5×10^{-4} *M* in amine. The lack of catalysis by the 2,6-disubstituted pyridines may be inconclusive because of the strain that may be expected in any complex or transition state, but it should be noted that the pK_a' of pyridine exceeds those of 4-bromoimidazole and benzimidazole which proved to be effective catalysts.

In Table IV there is given the variation of k_w and k_2', for the hydrolysis of *p*-nitrophenyl acetate, as a function of temperature, and in Table V the

TABLE IV

THE DEPENDENCE OF RATE OF *p*-NITROPHENYL ACETATE HYDROLYSIS ON TEMPERATURE AT *p*H 8.0[a]

T, °K.	$k_w \times 10^4$, min.$^{-1}$	Imidazole k_2', l. mole^{-1} min.$^{-1}$
298	5.6	12.7
303	10.2	17.6
308	18.3	21.7
312	27.8	24.0

[a] In all experiments the phosphate concentration was 0.0054 *M* and *p*-nitrophenyl acetate was from 2 to 5×10^{-4} *M* while the imidazole concentration was 5×10^{-5} *M*.

TABLE V

ENERGY AND ENTROPY OF ACTIVATION FOR *p*-NITROPHENYL ACETATE HYDROLYSIS

Energy term[a]	"Aqueous"	Basic[b]	Imidazole
E_a, kcal. mole^{-1}	20.7	11.1	8.4
$\Delta H^{\ddagger}$, kcal. mole^{-1}	20.1	10.5	7.8
$\Delta F^{\ddagger}$, kcal. mole^{-1}	21.9	16.2	15.9
$\Delta S^{\ddagger}$, e.u.	−6.0	−19.2	−27.2

[a] E_a was calculated from the slope of the linear relationship of ln *k* *vs.* $1/T$ by the method of least squares. From the value of E_a, $\Delta H^{\ddagger}$ was obtained from the relationship $\Delta H^{\ddagger} = E_a - RT$. The value of $\Delta F^{\ddagger}$ was obtained by means of the equation $\ln k_{rate}\, h/KT = -\Delta F^{\ddagger}/RT$ (see A. Frost and R. Pearson, "Kinetics and Mechanism," John Wiley and Sons, Inc., New York, N. Y., 1953, p. 95). [b] Calculated from the data of Tommila and Hinshelwood. See ref. 31 of paper.

(30) A. Gero and J. J. Markham, *J. Org. Chem.*, **16**, 1835 (1951).

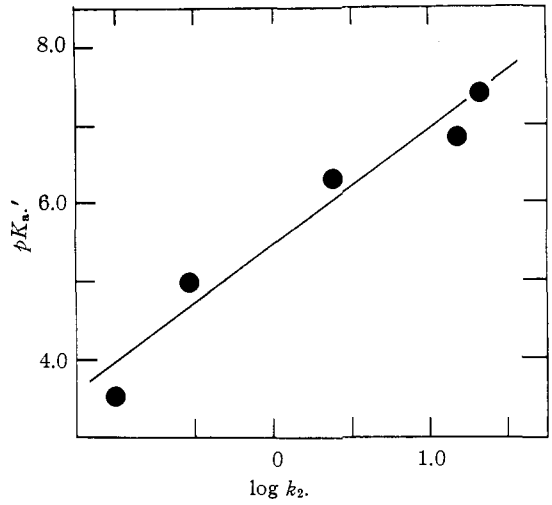


Fig. 2.—Brønsted plot for the catalysis of hydrolysis *p*-nitrophenyl acetate by 4(5)-substituted imidazoles ($pK_a' = 1.49 \log k_2 + 5.44$).

calculated activation terms are compared to those for hydroxide ion catalysis.

The recorded activation energy for the acid-catalyzed hydrolysis of phenyl acetate is 17.2 kcal.[31] Though the necessary data for the calculation of the activation terms for the acid hydrolysis of *p*-nitrophenyl acetate are not available, from the data which follow (Table VI and subsequently calculated ρ-values) it can be estimated that the activation energy is about 18 kcal./mole. The closer correspondence of $\Delta H^{\ddagger}$ and $\Delta F^{\ddagger}$ for imidazole and hydroxide ion catalysis, as compared

TABLE VI

RATES OF HYDROLYSIS OF *m*- AND *p*-SUBSTITUTED PHENYL ACETATES[a]

Substituent	σ[b]	$k_w \times 10^4$, min.$^{-1}$ "aqueous"	k_2, l. mole^{-1} min.$^{-1} \times 10^2$ Imidazole	OH^-[c]	H_3O^+[c]
p-NO_2	1.27	43.7	2130	48,300	
m-NO_2	0.71	18.8	454	32,900	0.117
p-COOH	.35				0.148
m-COOH	.265				0.142
p-COO^-	.32			5,600	
m-COO^-	.13			4,700	
p-Cl	.37	3.84	45.4		
H	.00	1.35	10.8	3,200	0.166
p-CH_3	−.17	1.73	5.3	1,900	0.176
p-NH_2	−.66			1,700	

[a] The concentrations of phenyl acetates employed were: for *p*-nitrophenyl acetate, $2.0–2.27 \times 10^{-4}$ *M*; *m*-nitrophenyl acetate, 1.05×10^{-2} *M*; *p*-chlorophenyl acetate, 4.19 to 4.4×10^{-3} *M*; phenyl acetate, 5.28 to 5.64×10^{-3} *M*; and *p*-cresyl acetate, 3.76 to 4.04×10^{-3} *M*. The concentration of imidazole was: for *p*-nitrophenyl acetate, 1×10^{-4} *M*; *m*-nitrophenyl acetate, 5×10^{-4} *M*; *p*-chlorophenyl acetate, 1×10^{-3} *M*; phenyl and *p*-cresyl acetates, 2×10^{-3} *M*. In all experiments the phosphate buffer was at 0.2 *M*, *p*H 8.0 and *T* 30°. The values of the rate constants for imidazole catalysis and "aqueous" hydrolysis are the average values obtained from no less than three separate experiments. [b] H. H. Jaffé, *Chem. Revs.*, **53**, 191 (1953). [c] Data of reference 31. The rate constants were calculated from the experimental data to 25° using the Arrhenius equation. The solvent in these experiments was 60% acetone in water.

(31) E. Tommila and C. N. Hinshelwood, *J. Chem. Soc.*, 1801 (1938).

to acid and "aqueous" hydrolysis, may then be noted. The more negative $\Delta S^{\ddagger}$ for imidazole catalysis suggests electrostriction of solvent in the transition state, an expected consequence of the interaction of uncharged species to form ionic products.

If the imidazole catalysis of the hydrolysis of *p*-nitrophenyl acetate is initiated by the attack of nucleophilic imidazole upon the carboxyl carbonyl group of the phenyl acetate, then the rate of this bimolecular reaction should decrease as the positive nature of the phenyl group diminishes. In Table VI there is presented the rates of hydrolysis of a series of *m*- and *p*-substituted phenyl acetates.

The relative sensitivities to changes in the electronic nature of the substituents are obtainable by means of the conventional Hammett[32] $\rho\sigma$ plot (Fig. 3). The ρ-values, as calculated from the data of Table VI by least squares, are

Catalyst	Imidazole	OH^-	"Aqueous"	H_3O^+
ρ	1.90	1.00	1.15	−0.22

It can be seen that whereas the sensitivity of the rate of hydrolysis to the electronic nature of the *m* and *p*-substituents for OH^- catalysis and "aqueous" hydrolysis are about that for the ionization

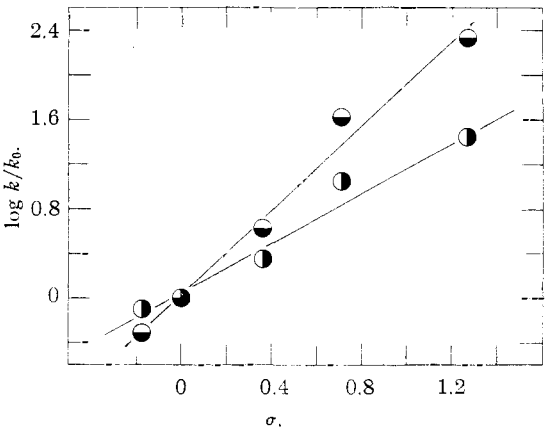


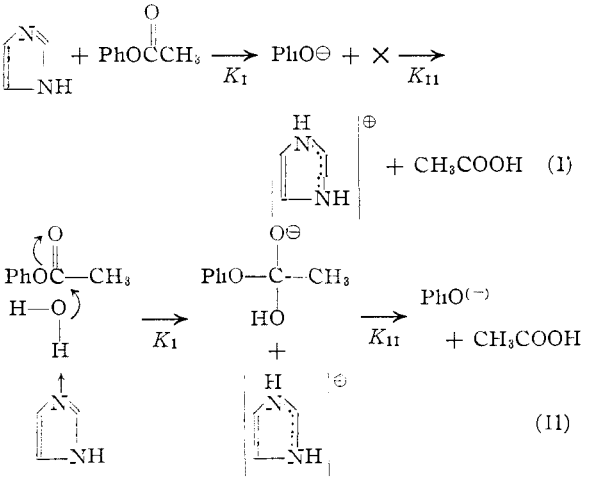
Fig. 3.—$\rho\sigma$ plot for the hydrolysis of phenyl acetates at *p*H 8.0: ◒, treatment applied to apparent second-order rate constants (k'_2) for the reaction of substituted phenyl acetates with imidazole; ◑, treatment applied to the "aqueous" hydrolysis (k_w) of substituted phenyl acetates.

of benzoic acids, the sensitivity to imidazole catalysis is almost twice this value and that for acid catalysis is but two-tenths this value and of opposite sign. The greater sensitivity of the catalysis by imidazole may possibly be explained as follows. From the *o*-complex CH_3–$\overset{O(-)}{\overset{|}{C}}$$^-$ (imidazole)–OC_6H_4X either the phenolate ion or imidazole may dissociate. That group which will most often succeed in leaving is that which can most easily accommodate a pair of electrons. On this basis it would be expected that the dissociation of the imidazole group, to yield either uncharged imidazole or a highly resonance stabilized imidazolium ion, could compete with the dissociation of the phenolate ion. As the electron-attracting power of the *m*- or *p*-substituent on the phenolic residue decreases, the dissociation of imidazole would be increasingly favored and catalysis of hydrolysis would, therefore, rapidly wane. In the case of hydroxyl ion catalysis, the dissociation of the complex CH_3–$\overset{O(-)}{\overset{|}{C}}$(OH)–$OC_6H_4X$ would yield either a hydroxide ion or a resonance stabilized phenolate ion, and catalysis by hydroxide ion should be less sensitive to substitution since in all cases the hydroxide ion would be much the poorer leaving group. These results suggest that as the electron-donating power of X increases, k_1 should decrease to a point where $k_1 - k_w = 0$ and catalysis by imidazole would no longer be experimentally perceptible.

(32) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, Chapter VII.

Discussion

From the above data it can be concluded that imidazole is an effective basic catalyst for the hydrolysis of phenyl acetates. The data presented are consistent with either of two over-all mechanisms.



where $K_1 = k_2'$ as determined and $K_1 \ll K_{11}$

In (I) imidazole reacts directly with phenyl acetate to form X and phenol, X then reacting with water to form acetic acid and catalyst. In (II) imidazole-bound water[33] reacts with phenyl acetate to form an unstable intermediate as is formed in the hydroxide ion catalyzed hydrolysis of certain esters.[34]

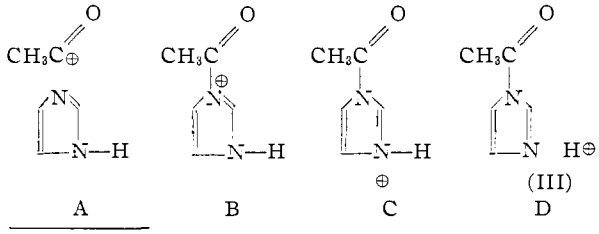
Though the data presented do not differentiate between (I) and (II), the authors favor (I). This choice is based, in part, on the values of ρ- for the imidazole, hydroxide ion and "aqueous" hydrolysis of phenyl acetates. The much greater ρ for imidazole catalysis would seem to indicate a direct attack of a highly polarizable species upon the carboxyl carbonyl group. The direct attack by imidazole would be in accord with this expectation, whereas participation of imidazole-bound water would be expected to be characterized by a ρ-value

(33) Mechanism II finds its counterpart in the activation of amines by glycols, etc., for the aminolysis of esters; A. R. Day and co-workers, THIS JOURNAL, **70**, 1946 (1948); **71**, 1245 (1949); **72**, 5635 (1950); **73**, 5393 (1951); **78**, 4372 (1956).

(34) M. L. Bender, *et al.*, THIS JOURNAL, **76**, 3350 (1954); **77**, 398 (1955).

more nearly consistent with those of hydroxide ion and "aqueous" hydrolysis.

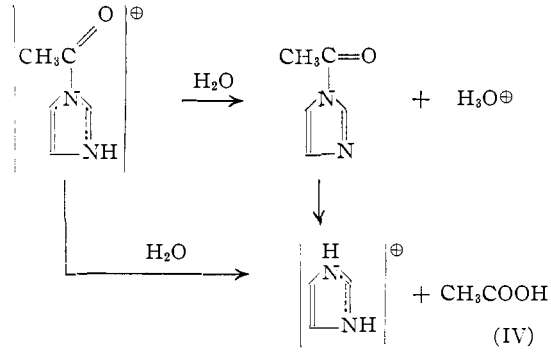
The nature of the proposed intermediate X in mechanism I is at present unclear. It has been suggested[2,35] that an imidazolyl group becomes acylated in the course of the reaction of esterases with substrate to produce an N-acylimidazolyl grouping. It is known[36-39] that the hydrolysis of acyl imidazoles is characterized by very low heats of activation. The identity of acetylimidazole as X is thus consistent with equation I. On this basis it would be expected that N-methylimidazole (pK_a' 7.2),[29] as in the case of the pyridines, would not exhibit catalysis at the low concentrations employed herein for other imidazoles. Actually this is not the case, since when N-methylimidazole was employed as catalyst the value of k_2' was found to approximate 7.6 l. mole^{-1} min.$^{-1}$, and this value compares favorably to that of 12.7 l. mole^{-1} min.$^{-1}$ obtained for imidazole under identical experimental conditions. It is therefore evident that the formation of an N-acetylimidazole intermediate is not a necessary prerequisite for catalysis. The catalyst may act by the formation of an acylium ion–imidazole complex (III) in which



A B C D (III)

the acylium ion is stabilized by an additional ionic resonance effect through the participation of forms A, B, C and D.

With N-methylimidazole, form D would be very improbable, whereas in the case of imidazole, D could be of major importance and the resultant resonance hybrid might be expected to yield either its proton or acylium ion readily to water with the formation of N-acetylimidazole or imidazole and acetate[40] (IV).



(IV)

Addenda: During the preparation of this manuscript, we learned from Dr. M. L. Bender of his studies on the imidazole catalysis of ester hydrolysis (THIS JOURNAL, **79**, 1656 (1957)). The general concordance of the results obtained in these two independent studies is most gratifying.

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(35) F. Bergmann, *Disc. Faraday Soc.*, **20**, 133 (1955).

(36) E. R. Stadtman, "The Mechanism of Enzyme Action," W. D. McElroy and B. Glass, eds., Johns Hopkins Press, Baltimore, Md., 1954, p. 596.

(37) E. R. Stadtman and F. H. White, THIS JOURNAL, **75**, 2022 (1953).

(38) M. Bergmann and L. Zervas, *Z. physiol. Chem.*, **175**, 145 (1928).

(39) T. Wieland and G. Schneider, *Ann.*, **580**, 159 (1953).

(40) The mechanism proposed herein is analogous to that of Gold and co-workers (*J. Chem. Soc.*, 1406, 1409, 1416 (1953)) for the catalysis of hydrolysis of acetic anhydrides by pyridines. The more profitable association of the acylium ion with imidazole, as compared to pyridine, could well rest on the greater orbital overlap and subsequent delocalization of the formal positive charge of the acylium ion with the former base.

NEW HAVEN, CONNECTICUT

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF TEXAS]

Alkylpyridines. Extension of the Sodamide–Liquid Ammonia Alkylation Method

BY H. L. LOCHTE AND TOM H. CHEAVENS[1]

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Methyl iodide alkylation using the sodamide–liquid ammonia method has been applied to 2,3-, 2,4-, 2,5- and 2,6-lutidines to effect the synthesis of 2-ethyl-3-methyl-, 4-ethyl-2-methyl-, 2-methyl-4(2-propyl)-, 2-ethyl-5-methyl-, 2-ethyl-6-methyl- and 2,6-diethylpyridines. A marked preferential reactivity of the 4-alkyl group in 2,4-lutidine is exhibited. The previously reported activity of alkyl groups in the 3-position on the pyridine ring has been further confirmed by the synthesis of 3-propyl- and 3-(3-pentyl)-pyridines from 3-picoline and ethyl bromide.

In an attempt to identify a base isolated from Colorado shale-oil, a survey of the isomeric $C_8H_{11}N$ pyridines was made. Of the 22 possible isomers in this series, only 2-ethyl-3-methylpyridine and 3,4,-5-trimethyl-pyridine were unreported. However 3-propylpyridine was characterized only by means of its boiling point[2] which seemed too low for a substance of this constitution. In addition, some confusion existed as to the melting point of the picrate of 2-ethyl-5-methylpyridine.[3]

The first synthetic route leading to compounds of this type investigated by the authors was that developed by Brown and Murphy,[4] the side-chain

(1) From the Ph.D. Dissertation of Tom H. Cheavens, University of Texas, 1955; Union Carbide and Carbon Fellow, 1953–1954.

(2) A. Cahours and A. Etard, *Compt. rend.*, **92**, 1082 (1881).

(3) (a) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **129**, 79 (1939); **143**, 427 (1942); (b) W. A. Jacobs, L. C. Craig and G. J. Lavin, *ibid.*, **141**, 51 (1941); (c) V. Prelog and S. Szpilfogel, *Helv. Chim. Acta*, **25**, 1306 (1942).

(4) H. C. Brown and W. A. Murphy, THIS JOURNAL, **73**, 3308 (1951).