action of 100 g. of furfural with 98.2 g. of cyclohexanone, when conducted exactly as described above, gave 31.3 g. (18%) of XXI, n = 1, m.p. 46–47° (lit.[16] 47°). The purple dinitrophenylhydrazone, prepared and purified as were those above, melted at 190–192°.

Anal. Calcd. for $C_{17}H_{16}O_5N_4$: C, 57.30; H, 4.53. Found: C, 57.21; H, 4.66.

In addition, there was obtained 81.5 g. (32%) of 2,6-bisfurfurylidenecyclohexanone (XXII, n = 1), m.p. 143–145° (lit.[16] 145°).

Acid hydrolysis of the mono derivative gave resinous material, from which nothing could be extracted.

6-Furfurylidenebenzosuber-5-one (XVIII).—A solution of 80.0 g. of benzosuberone[17] and 50.0 g. of furfural in 200 ml. of ethanol was treated with 3 ml. of 45% potassium hydroxide solution and cooled under running water as required to keep the temperature below 50°. After the exothermic reaction had subsided, the mixture was allowed to stand for 2 hr. at 25°, diluted with 250 ml. of water, cooled and filtered with suction. Recrystallization of the damp cake from ethyl acetate gave, in two crops, 116 g. (98%) of XVIII, m.p. 126–127°. The m.p. was not changed by further purification.

(16) N. Wolff, *Ann. chim.*, [9] **20**, 82 (1923).

(17) W. J. Horton and F. E. Walker, THIS JOURNAL, **74**, 758 (1952).

Anal. Calcd. for $C_{16}H_{14}O_2$: C, 80.65; H, 5.92. Found: C, 80.28; H, 5.95.

Acid Hydrolysis of XVIII.—A mixture of 112 g. of XVIII, 600 ml. of 95% ethanol and 275 ml. of concd. hydrochloric acid was heated under reflux on a steam-bath for 18 hr. Nearly all solvent was removed by slow distillation through a 24-in. Vigreux column (20 mm.). The black residue was refluxed with 700 ml. of acetic acid for 4 hr. and diluted with 2 l. of water. The mixture was extracted with ether (2 × 1 l.) and the ethereal layer was washed with water (2 × 500 ml.) and then with 500 ml. of 10% sodium hydroxide solution. Acidification of the basic wash afforded 13 g. of black semi-solid which, after three recrystallizations from benzene, afforded 3.3 g. (3%) of 5,6-dihydrobenzocyclohepta[3.4-b]furan-2-propionic acid (XX), m.p. 133–134°. A colorless sample, having the same m.p., was obtained by sublimation at 0.1 mm. This substance did not form carbonyl derivatives.

Anal. Calcd. for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 74.98; H, 6.28.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, YALE UNIVERSITY]

Imidazole Catalysis. II. The Reaction of Substituted Imidazoles with Phenyl Acetates in Aqueous Solution

BY THOMAS C. BRUICE AND GASTON L. SCHMIR[1]

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The reaction of imidazole with p-nitrophenyl acetate in aqueous solution, previously shown to be pseudo first order in the liberation of p-nitrophenol, has now been shown to be first order in acetate liberation by the same rate law. The study of the dependence of rate on basicity has been extended through the determination of the second-order rate constants for the reaction with p-nitrophenyl acetate of 21 imidazoles of various pK_a'. The second-order rate constants do not obey a simple Brönsted catalysis law since both the neutral and anionic species exhibit catalytic properties. By means of a linear relationship of pK_2' to pK_1' for the imidazoles (*i.e.*, $pK_2' = 0.94pK_1' + 7.43$) equation 15 was derived and found to adequately correlate the apparent second-order rate constant, k_2', to the value of pK_1' for 4(5)-substituted imidazoles. The application of this equation, as well as the factors influencing the fit of the experimental data to the equation are discussed. Some attempts to realize a Lowry type catalysis of p-nitrophenyl acetate and phenyl acetate hydrolysis are described and discussed in the light of catalyzed reactions which have been proposed to be of this type. The hydrolysis of p-nitrophenyl acetate was found to be catalyzed by pyrimidoimidazoles (purines) at 2×10^{-4} M but not by pyrimidines at this concentration. For the purines there is no apparent direct relationship of pK_a' to k_2'.

Introduction.—The implication of an imidazolyl group of histidine as essential to the activity of numerous hydrolytic enzymes[2] has led to studies of the ability of imidazoles to catalyze the hydrolysis of substrates of several hydrolytic enzymes. Thus, it has been found that imidazole compounds catalyze the hydrolysis of thiol esters,[3,4] phenyl acetates,[4,5] N-acetylamides,[6] fluorophosphates[7] and, at high catalyst concentrations, phosphoamides.[8] In this paper we report on the continuation of our studies of the reaction of imidazoles with phenyl acetates.

(1) Public Health Predoctoral Fellow of the National Institutes of Health, 1956–1957.

(2) For pertinent references on this topic see ref. 4 and 5.

(3) E. R. Stadtman, "The Mechanism of Enzyme Action," W. D. McElroy and B. Glass, eds., The Johns Hopkins Press, Baltimore, Md., 1954, pp. 581–598.

(4) M. L. Bender and B. W. Turnquest, THIS JOURNAL, **79**, 1652, 1656 (1957).

(5) T. C. Bruice and G. L. Schmir, *ibid.*, **79**, 1663 (1957).

(6) T. C. Bruice, unpublished.

(7) T. Wagner-Jauregg and B. E. Hackley, THIS JOURNAL, **75**, 2125 (1953).

(8) T. Rathlev and T. Rosenberg, *Arch. Biochem. and Biophys.*, **65**, 319 (1956).

Experimental

Imidazoles.—Benzimidazole, imidazole, 4-hydroxymethylimidazole hydrochloride, 4-methylimidazole hydrochloride and 4-bromoimidazole were samples used in a previous study.[5] 6-Nitrobenzimidazole was an Eastman Kodak Co. white label product while 2-(2'-hydroxyphenyl)-benzimidazole,[9] 4-nitroimidazole,[10] 2-methylbenzimidazole,[11] L-histidine methyl ester dihydrochloride,[12] 6-aminobenzimidazole·H_2O·$^1/_2HCl$[13] and 2-methylimidazole[14] were prepared by recorded procedures. Dr. C. E. Carter provided us with a sample of histamine acid phosphate while the various purines and pyrimidines were obtained from Dr. H. G. Mautner, and N-acetyl-DL-histidine hydrate was obtained from Dr. J. S. Fruton. Dr. Morris Engelman and Dr. H. B. Gillespie of the Department of Biochemistry, Columbia University, College of Physicians and Surgeons, provided us with the following compounds[15]: 6-amino-4-hydroxy-2-methylbenzimidazole sulfate·$^1/_2H_2O$, 6-amino-4-hydroxy-

(9) W. L. Walter and H. Freiser, *Anal. Chem.*, **25**, 127 (1953).

(10) R. Fargher and F. Pyman, *J. Chem. Soc.*, **115**, 217 (1919).

(11) R. Weidenhagen, *Ber.*, **69**, 2263 (1936).

(12) Prepared by the general method of R. L. M. Synge [*Biochem. J.*, **42**, 99 (1948)] and found to agree in physical properties with that originally prepared by E. Fischer and L. Cone [*Ann.*, **363**, 107 (1908)].

(13) M. Staüble, *Helv. Chim. Acta*, **32**, 135 (1949).

(14) G. Dedichen, *Ber.*, **39**, 1838 (1906).

(15) H. B. Gillespie, M. Engelman and S. Graff, THIS JOURNAL, **76**, 3531 (1954); **78**, 2445 (1956).

benzimidazole sulfate monohydrate, 4-hydroxy-6-nitrobenzimidazole and 4-hydroxy-2-methyl-6-nitrobenzimidazole. 4-Hydroxy- and 4-methoxybenzimidazole were obtained from Dr. E. S. Lane[16] of the Atomic Energy Research Establishment (England).

4-(2',4'-Dihydroxyphenyl)-imidazole.—2,4-Dimethoxyacetophenone was prepared from *m*-dimethoxybenzene[17] *via* the procedures of Noller and Adams.[18] This compound was converted to 2,4-dimethoxyphenylglyoxal by the general procedure of Perkins.[19] The phenyl glyoxal (5 g., 0.025 mole), dissolved in 80 ml. of hot water, was added to a solution of 13 g. of cupric acetate monohydrate (0.065 mole) dissolved in a mixture of 100 ml. of 38% ammonium hydroxide and 5 ml. of 37% aqueous formalin. The reaction mixture was heated on a steam-bath for 30 min., cooled, and the precipitated copper salt collected by filtration and washed with water. With stirring, hydrogen sulfide was passed for 30 min. through a suspension of the copper salt in 150 ml. of hot water acidified with 2 ml. of 6 *N* hydrochloric acid. The cupric sulfide was removed by filtration and washed with a little hot water. The washings and filtrate were then degassed, by heating, and saturated with sodium carbonate. After cooling, the solution was extracted four times with ether, the ether extract evaporated to dryness and the residue taken up in absolute ethanol, filtered and the ethanol solvent removed *in vacuo*. In this manner the 4-(2',4'-dimethoxyphenyl)-imidazole was obtained as an oil (3.7 g.).

Without purification the dimethoxy compound was refluxed for 8 hr. with a solution of 37 ml. of 48% aqueous hydrobromic acid and 2.0 ml. of 50% aqueous hypophosphorous acid. The reaction mixture was chilled in ice and the hydrobromide collected by filtration. The salt was dissolved in a little water made alkaline with 10% sodium carbonate solution and extracted four times with ether. After drying over anhydrous sodium sulfate, the ethereal solution was saturated with dry hydrogen chloride, chilled and the hydrochloride collected and recrystallized from absolute ethanol by addition of dry ether. There was thus obtained 2 g. of 4-(2',4'-dihydroxyphenyl)-imidazole hydrochloride monohydrate (44% over-all). After drying at 60° and 0.7 mm. over P_2O_5, the compound was found to melt at 248–250° when placed in a bath at 240°.

Anal. Calcd. for $C_9H_{11}O_3N_2Cl$: C, 46.9; H, 4.79; N, 12.12. Found: C, 46.59; H, 4.98; N, 11.92.

Carbobenzoxy-L-histidyl-L-tyrosine Ethyl Ester.[20]—A mixture of 2.2 g. (0.009 mole) of L-tyrosine ethyl ester hydrochloride and 20 ml. of ethyl acetate was cooled in an ice-bath and 12 ml. of a 50% (w./v.) aqueous potassium carbonate solution added. The mixture was allowed to equilibrate and the ethyl acetate layer separated and dried over anhydrous sodium sulfate at 0° during the preparation of the azide described below.

A solution of 1.8 g. (0.006 mole) of carbobenzoxy-L-histidine hydrazide[21] in 15 ml. of 1.2 *N* hydrochloric acid (0.018 mole) was mixed with 24 ml. of ethyl acetate and cooled in an ice-bath when a cooled solution of 420 mg. (0.006 mole) of sodium nitrite in 1.5 ml. of water was added. After 2 min., 7.2 ml. of cold 50% potassium carbonate solution was added, and the mixture was equilibrated. The ethyl acetate layer was separated and the aqueous solution extracted with an additional 3 ml. of ethyl acetate. The combined ethyl acetate solutions were dried a few minutes over anhydrous sodium sulfate at 0°.

The solutions of L-tyrosine ethyl ester and carbobenzoxy-L-histidine azide were filtered and combined. The reaction mixture was then allowed to stand at room temperature for 21 hr. after which it was washed twice with water, dried over anhydrous sodium sulfate and concentrated to a small volume. On addition of petroleum ether, an oil appeared which readily was induced to crystallize. After cooling for 8 hr., the product was collected (1.96 g., 69%). After recrystallization from ethyl acetate by addition of petroleum ether (1.54 g., 54%) the product was found to melt at 143–146°. For analysis the compound was recrystallized in a like manner and dried at 80° over P_2O_5 at 1.0 mm., melting point unchanged.

Anal. Calcd. for $C_{25}H_{28}O_6N_4$: C, 62.5; H, 5.9; N, 11.7. Found: C, 62.33; H, 5.76; N, 11.65.

pK_a' determinations were made by one of two methods. (a) A solution of the imidazolyl compound (5 to 10×10^{-3} *M*) in 28.5% ethanol–water (v./v.), 0.05 *M* in KCl, was half neutralized with approximately 0.04 *N* standard HCl (or in the case of an acid salt, 0.04 *N* standard NaOH) and serially diluted with a solution of 0.05 *M* KCl in 28.5% ethanol–water (v./v.). The *p*H values of the resultant solutions were then determined immediately (T = 25 to 27°) with a Beckman model G *p*H meter. The values obtained, as apparent pK's, were plotted against the concentration of the imidazolyl compound. The pK_a' was taken as that *p*H value which did not change on going to more concentrated solution since in this concentration range neither the hydrolysis of the conjugate acid nor small amounts of CO_2 absorption need be taken into account.[22]

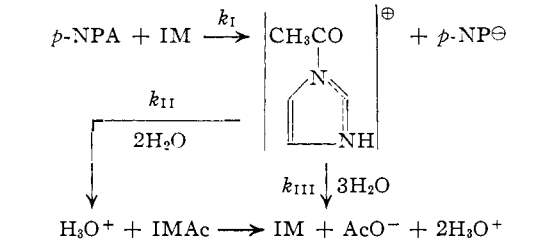
(b) The imidazolyl compound (2 to 5×10^{-3} *M*) in 28.5% ethanol–water (v./v.), 0.2 *M* in KCl, was titrated at 30 ± 1° under nitrogen with 0.04 *M* standard HCl (or in the case of an acid salt, 0.04 *M* standard NaOH) utilizing a Radiometer type TTT 1a *p*H meter equipped with micro-buret. To obtain pK_a' the titration data were corrected for hydrolysis of the conjugate acid by subtraction of a blank acid titration curve and then fitted to theoretical titration curves calculated from the Henderson–Hasselbalch equation. The pK_a' also was calculated from the half-neutralization point. The two calculated values were found to be in close agreement.

Kinetics.—Experiments, except where otherwise indicated, were performed at 30 ± 0.1° in 28.5% ethanol–water (v./v.). The spectrophotometric data for the calculation of the rates of hydrolysis of phenyl and *p*-nitrophenyl acetate were obtained as in a previous study.[5] The apparent second-order rate constants, k_2', were obtained from the expression $k_2' = (k_1 - k_w)/IM_0$, where k_1 and k_w are the experimentally determined first-order constants in the presence and absence of catalyst, respectively, and IM_0 is the molar concentration of added imidazole. The specific second-order rate constant, k_2, was calculated from the expression $k_2 = k_2'[1 + (H^+)/K_a']$.

The titrimetric rate data were obtained at a constant *p*H of 8.0 (without buffer) maintained by addition of standard NaOH using a Radiometer type TTT 1a *p*H meter equipped with automatic buret and recorder. The first-order rate constants were calculated directly from the automatically recorded time (in cm.) *vs.* ml. standard base (in cm.) plot by the Guggenheim method.[23]

Discussion and Results

Mechanism of the Imidazole Catalysis of *p*-NPA Hydrolysis.[24]—The reaction of IM with *p*-NPA, in aqueous solution, is believed to proceed as



Step k_I is believed to be rate limiting since the appearance of *p*-NP^- is first order with respect to *p*-NPA and imidazole to over 90% completion of reac-

(16) E. S. Lane and C. Williams, *J. Chem. Soc.*, 569 (1956).

(17) S. A. Flood and J. A. Nieuwland, THIS JOURNAL, **50**, 2570 (1928).

(18) C. R. Noller and R. Adams, *ibid.*, **46**, 1892 (1924).

(19) W. H. Perkins, *J. Chem. Soc.*, **93**, 1108 (1908).

(20) While this work was in progress N. C. Davis [*J. Biol. Chem.*, **223**, 935 (1956)] reported the preparation of this compound and recorded the m.p. as 94–95°.

(21) R. W. Holley and E. Sondheimer, THIS JOURNAL, **76**, 1326 (1954).

(22) R. Swidler and G. M. Steinberg, *ibid.*, **78**, 3594 (1956).

(23) E. A. Guggenheim, *Phil. Mag.*, **2**, 538 (1926).

(24) Abbreviations used are: imidazole (IM), imidazolium ion (IMH^+), imidazole anion (IM^-), *p*-nitrophenyl acetate (*p*-NPA), *p*-nitrophenol (*p*-NP), *p*-nitrophenolate (*p*-NP^-), acetate (AcO^-), ethyl acetate (EtOAc) and acetylimidazole (IMAc).

tion.[5] Evidence for IMAc as an intermediate has been obtained *via* ultraviolet spectroscopy when IM was employed at a high concentration.[4] The postulation of an intermediate imidazolium acetate complex finds support in the fact that pyridines[4] and especially N-methylimidazole[4,5] are effective catalysts of *p*-NPA hydrolysis.[25] It is not known what portion of the acetylimidazolium complex passes to imidazole and acetate *via* paths k_{II} and k_{III}. Also, it is known that IMH^+ is non-catalytic.[4,5]

If step k_I is rate limiting, then not only *p*-NP formation but H^+ formation should be first order with respect to both *p*-NPA and imidazole. Also, IMAc might be expected to be at a negligible concentration because of its very short half-life in neutral or alkaline media.[3,4,26] Thus, the effective concentration of IM can be assumed to be equal to IM_0, the concentration of added imidazole.

In the aqueous ethanol solvent employed in this and previous studies[5] and at *p*H 8.0, the decomposition of *p*-NPA in the presence of IM proceeds according to equations B to F, where IMAc and acetylimidazolium complex are not distinguished.

$$\underset{(P_0 - p)}{p\text{-NPA} + IM} \xrightarrow{k_2'} \underset{(x - m)}{IMAc} + \underset{x}{p\text{-NP}} \quad (B)$$

$$p\text{-NPA} + OH^- \xrightarrow{k_c} \underset{r}{AcO^-} + \underset{d}{p\text{-NP}} + \underset{r}{H^+} \quad (C)$$

$$p\text{-NPA} + EtO^- \xrightarrow{k_d} \underset{s}{EtOAc} + \underset{e}{p\text{-NP}} \quad (D)$$

$$\underset{(x - m)}{IMAc} + OH^- \xrightarrow{k_e} \underset{y}{AcO^-} + \underset{y}{IM} + H^+ \quad (E)$$

$$IMAc + EtO^- \xrightarrow{k_f} \underset{z}{EtOAc} + IM \quad (F)$$

where p = total *p*-NP liberated at time t (*i.e.*, $p = x + d + e = x + n$), m = acetate and ethyl acetate formed by decomposition of IMAc at time t (*i.e.*, $m = y + z$), and n = total phenol or acetate + ethyl acetate formed in the uncatalyzed reaction (*i.e.*, $n = r + s = d + e$). Integration of the expression

$$dp/dt = (P_0 - p)(k_2'IM + k_c + k_d) \quad (1)$$

gives

$$\ln \frac{[P_0]}{[P_0 - p]} = (k_2'IM + k_c + k_d)t \quad (2)$$

where $k_c + k_d = k_w$ in the general treatment (see Experimental). Combination of the integrated expressions for the rate of formation of y, z, r, s, x, n and $(x - m)$ with the assumption of a steady state in IMAc, $d(x - m)/dt = 0$, leads to

$$y = Ax \quad \text{where } A = k_e/(k_e + k_f) \quad (3)$$

$$r = Bn \quad B = k_c/(k_c + k_d) \quad (4)$$

$$x = Cn \quad C = (k_2'IM)/(k_c + k_d) \quad (5)$$

Since the total protons produced $= y + r$ and $p = n + x$, then from 3, 4 and 5

$$p = \frac{(y + r)(C + 1)}{AC + B} \quad (6)$$

combining equations 6 and 1 and integrating gives

$$\ln \frac{(y + r)_0}{(y + r)_0 - (y + r)} = (k_2'IM + k_c + k_d)t \quad (7)$$

It can be seen that if the assumptions are valid, then following either the rate of formation of *p*-NP (equation 2) or the rate of production of protons (equation 7) should yield the same pseudo first order rate constant.[27] That these assumptions are probably correct is attested to by comparison of k_2' as obtained from equations 2 and 7, respectively. Thus, the value of k_2' (*p*H 8.0) obtained from the slope of a plot of k_1 (where $k_1 = k_2'IM + k_c + k_d$) *vs.* IM concentration calculated from equation 7 is 17.0 l. mole^{-1} min.$^{-1}$ (Fig. 1). Following *p*-NP liberation and employing equation 2 yields a value of k_2' (*p*H 8.0) of 19.7 l. mole^{-1} min.$^{-1}$.[5] The contribution of reactions D and F (formation of ethyl acetate) to the over-all disappearance of *p*-NPA is shown by the fact that only about 75% of the quantity of standard base required to saponify the total of the *p*-NPA employed was needed to maintain constant *p*H in the titrimetric experiments.

In the titrimetric experiments $k_w = 12 \times 10^{-4}$ min.$^{-1}$, while in the spectrophotometric experiments $k_w = 44 \times 10^{-4}$ min.$^{-1}$. The greater value of k_w for the latter was found to be due to phosphate ion of the buffer. It was also found that changes in the organic portion of the solvent had little effect on k_2', though k_w was greatly altered (Table I).[28]

TABLE I[a]

Concn. of phosphate, *M*	Solvent, % (v./v.)	k_2', l. mole^{-1} min.$^{-1}$	$k_w \times 10^4$, min.$^{-1}$
0.0054	28.5 ethanol–water	17.6	10.2
.2	28.5 ethanol–water	19.7 ± 0.7	44
.2	15 acetone–water	24	39.1
.2	15 ethylene glycol–water	19.5	140
.2	30 ethylene glycol–water	24	204

[a] Imidazole at 10^{-4} *M*, *p*-NPA at 2×10^{-4} *M*.

Basicity and Catalysis.—Aside from its possible biochemical implications, the catalysis of hydrolysis of phenyl acetates by heterocyclic tertiary amines represents the first instance where reliable rate data have been obtained for the correlation of nucleophilicity of these bases toward the ester carbonyl group. Therefore, further clarification

(25) In the catalysis of the hydrolysis of acetic anhydride and acetyl phosphate by pyridine, pyridinium acetate has been postulated to be the steady state intermediate [V. Gold, *et al.*, *J. Chem. Soc.*, 1406, 1409 (1953); D. E. Koshland, THIS JOURNAL, **74**, 2286 (1952)].

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

(27) The contribution to total proton production due to ionization of *p*-NP at *p*H 8.0 has been neglected in these derivations. However, it can be shown that inclusion of this factor would simply multiply $(y + r)$ and $(y + r)_0$ by a constant term, which then can be canceled out from all terms in equation 7.

(28) A. R. Day [THIS JOURNAL, **70**, 1946 (1948); **71**, 1245 (1949); **72**, 5635 (1950); **73**, 5393 (1951); **78**, 4372 (1956)] has previously noted the catalysis of aminolysis and ammonolysis by ethylene glycol and has postulated the glycol to be involved in the activation of the acyl acceptor. Watanabe and De Fonso [*ibid.*, **78**, 4552 (1956)] suggest the glycol participates in a transesterification reaction resulting in the formation of acyl glycols which are then more reactive toward acceptor than the original esters. The latter mechanism is identical to that proposed by Langenbeck [for discussion and references see: R. Ammon and M. Jararma in "The Enzymes," Vol. I, Part I (eds. J. B. Sumner and K. Myrbäck), Academic Press, Inc., New York, N. Y., 1950, p. 396] who studied systems of this type as model esterases. In earlier experiments we found Tris to have a potentiating effect on the hydrolysis of *p*-NPA and suggest that the mechanism of this reaction resembles that for glycol.

TABLE II

The pK_a' OF IMIDAZOLES AND THEIR RATES OF HYDROLYSIS OF *p*-NPA[a]

No.	Catalyst[b]	pK_1'[c]	k_2', l. mole^{-1} min.$^{-1}$	n
1	2-Methylimidazole	7.75[d]	2.7 ± 0.3	2
2	4-Methylimidazole	7.45[d]	25.1 ± .23	2
3	N-Acetylhistidine	7.05	11.2 ± .2	2
4	Imidazole	6.95	20.2 ± .7	12
5	2-Methyl-4-hydroxy-6-aminobenzimidazole[e]	6.65	1.50 ± .4	2
6	4-(2',4'-Dihydroxyphenyl)-imidazole	6.45	9.4 ± .5	3
7	4-Hydroxymethylimidazole	6.45[d]	5.6 ± .02	2
8	Carbobenzoxy-L-histidyl-L-tyrosine ethyl ester	6.25	8.9 ± .4	2
9	2-Methylbenzimidazole	6.1[d]	0.0375 ± .0025	2
10	Histamine	6.0	7.0 ± .1	2
11	6-Aminobenzimidazole	6.0 (NH_2 3.0)	2.95 ± .05	2
12	4-Hydroxy-6-aminobenzimidazole[e]	5.9	6.15 ± .4	3
13	Benzimidazole	5.4	0.96 ± .03	2
14	4-Hydroxybenzimidazole[f]	5.3 (OH 9.5)	2.80 ± .25	2
15	Histidine methyl ester	5.2 (NH_2 7.1)	5.6 ± .3	2
16	4-Methoxybenzimidazole	5.1	0.31 ± .01	2
17	2-Methyl-4-hydroxy-6-nitrobenzimidazole	3.9	1.1 ± .2	3
18	4-Bromoimidazole	3.7[d]	0.28 ± .02	3
19	6-Nitrobenzimidazole	3.05 (pK_2' 10.6)	4.8 ± .2	3
20	4-Hydroxy-6-nitrobenzimidazole	3.05	3.75 ± .15	2
21	4-Nitroimidazole	1.5 (pK_2' 9.1)	35.5 ± .5	2
	Pyridine	4.9[d]	0.088 ± .002	2
	4-Picoline	5.7[d]	0.49 ± .07	2

[a] *p*-NPA at about 2×10^{-4} *M*, *p*H 8.0 provided by potassium phosphate buffer at 0.2 *M* (calculated $\Sigma/2 = 0.55$ *M*). [b] Concentrations of the catalysts varied from 10^{-4} *M* for the more active compounds (*e.g.*, imidazole) to 4×10^{-3} *M* for the least active compounds (*e.g.*, 4-methoxybenzimidazole). [c] Where: $IMH^+ \underset{}{\overset{K_1}{\rightleftharpoons}} IM \overset{K_2}{\rightleftharpoons} IM^-$. [d] Obtained by half neutralization. Other values obtained from titration values. [e] Solutions of these compounds in the reaction medium deposited a black precipitate upon prolonged standing, probably due to oxidation of these catalysts to polymeric substances. There was no evidence for appreciable decomposition during the short time employed for determination of rate constants (about 2 hours). 6-Aminobenzimidazole appeared stable even for prolonged periods of time in the reaction medium. [f] Formed colored products upon extended standing, but not during the reaction time employed.

of the relationship of pK_a' to rate for the imidazole catalysis of *p*-NPA hydrolysis was desired. Toward this end, we have determined the rates of reaction of a series of imidazoles of various pK_a' values with *p*-NPA (Table II).

From inspection of Table II it is evident that a conventional Brönsted plot cannot be employed to correlate basicity to catalysis. Thus, 4-nitroimidazole with $pK_1' = 1.5$ is a better catalyst than imidazole with $pK_1' = 6.9$, at *p*H 8.0, where both compounds are essentially in the free base form. Reactions run at various *p*H values establish that those imidazoles with a low value of pK_1' owe their catalytic ability primarily to the presence of their anionic species (Table III). That a substantial

TABLE III

k_2' l. mole^{-1} min.$^{-1}$

*p*H	4-Nitroimidazole Found[a,b]	4-Nitroimidazole Calcd.[c]	6-Nitrobenzimidazole Found[a,b]	6-Nitrobenzimidazole Calcd.[c]
7.0	3.6	3.2	0.54	0.43
7.5	11.0	13.2	1.75	1.35
8.0	35.5	30.0	4.8	4.3
8.45	74.5	74.7	12.6	12.0
8.65	107	(107)	19	(19)

[a] Determined in phosphate buffer at a calculated ionic strength of 0.55 *M*. Phosphate concentration varied from about 0.29 *M* at *p*H 7.0 to 0.19 *M* at *p*H 8.65. [b] *p*-NPA concentration was about 2×10^{-4} *M*, 4-nitroimidazole varied in concentration from 4.4×10^{-4} *M* at *p*H 7.0 to 4.1×10^{-5} at *p*H 8.65, whereas 6-nitrobenzimidazole concentration varied from 4×10^{-3} *M* at *p*H 7.0 to 2×10^{-4} *M* at *p*H 8.65. [c] Calculated on the basis of pK_2' of 9.1 for 4-nitroimidazole and 10.6 for 6-nitrobenzimidazole and the determined k_2' values at *p*H 8.65.

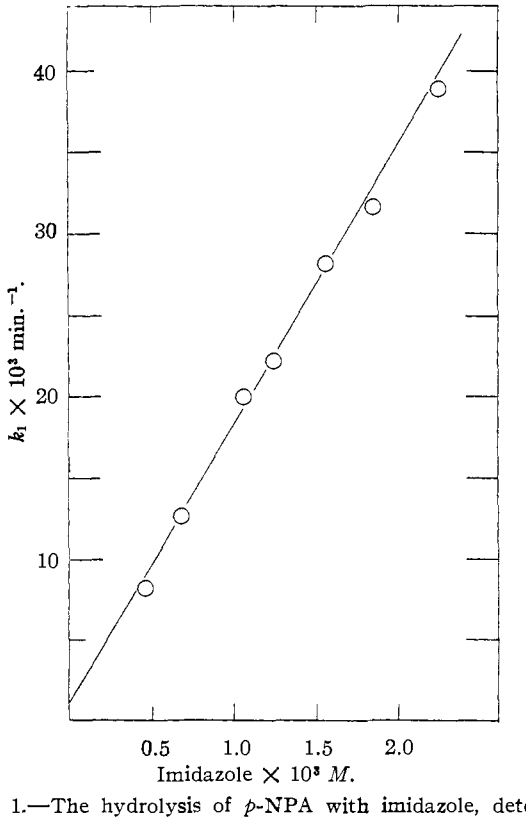


Fig. 1.—The hydrolysis of *p*-NPA with imidazole, determined by following the appearance of acid (*p*H 8.0).

portion of the catalytic ability of imidazoles with pK_1' as high as 4.0 is still due to the anionic species is shown in the variation of k_2' from 0.05 to 0.86 l. mole^{-1} min.$^{-1}$ for 4-bromoimidazole when the pH was varied from 7.0 to 8.7.

It might be anticipated that the observed rate constants for all imidazoles will be composed of terms for their neutral and anionic species.[29] The value of k_2' should be a composite rate constant related to the apparent second-order rate constants for anionic (k_A') and neutral (k_N') species by the simple expression

$$k_2' = k_A' + k_N' \quad (8)$$

It can be shown that k_A' and k_N' are related to the specific rate constants k_A and k_N *via* expressions 9 and 10.

$$k_N' = k_N \left[\frac{K_1H^+}{K_1K_2 + (H^+)^2 + K_1H^+}\right] \quad (9)$$

$$k_A' = k_A \left[\frac{K_1K_2}{K_1K_2 + (H^+)^2 + K_1H^+}\right] \quad (10)$$

where K_1 and K_2 are the first and second acid dissociation constants, respectively. Further, it appears that pK_1' and pK_2' for the imidazoles are linearly related (Table IV and Fig. 2).

TABLE IV

THE VALUES OF pK_1' AND pK_2' EMPLOYED IN DETERMINING A AND B

Compounds	pK_1'	pK_2'
4-Nitroimidazole	1.5[b]	9.1[b]
6-Nitrobenzimidazole	3.05[b]	10.6[b]
2-Phenylbenzimidazole	5.0[c]	11.7[a]
Benzimidazole	5.4[b]	12.57[a]
4-Phenylimidazole	6.0[a]	13.20[a]
2-Phenylimidazole	6.4[d]	13.10[a]
Imidazole	6.9[b]	14.52[a]

[a] H. Walba and R. W. Isensee, THIS JOURNAL, **77**, 5488 (1955). [b] From Table II. [c] From data of A. H. Kirby and A. Neuberger [*Biochem. J.*, **32**, 1146 (1938)] corrected from water to ethanol–water. [d] M. T. Davies, *et al.*, *J. Pharm. Pharmacol.*, **3**, 420 (1951).

$$pK_2' = ApK_1' + B \quad (11)$$

If it is assumed that the usual Brönsted relationship holds for k_N and k_A, then these values can be related to pK_1' and pK_2', respectively.

$$\log k_N = \alpha_N pK_1' + c_N \quad (12)$$

$$\log k_A = \alpha_A pK_2' + c_A \quad (13)$$

Combining 8, 9, 10, 12 and 13 and eliminating K_2 by use of 11, we arrive at a general expression for k_2' at any pH.

$$k_2' = \frac{10^{c_N}}{K_1^{\alpha_N}}\left[\frac{K_1H^+}{\frac{K_1^{A+1}}{10^B} + (H^+)^2 + K_1H^+}\right] + \frac{10^{c_A}(10^B)^{\alpha_A}}{K_1^{A\alpha_A}}\left[\frac{K_1^{A+1}}{\frac{K_1^{A+1}}{10^B} + (H^+)^2 + K_1H^+}\right] \quad (14)$$

The first term of the sum represents the contribution of k_N' to k_2', while the second term represents the contribution of k_A' to k_2'.

The least square values (Fig. 2) for A and B of equation 11 are found to be 0.94 and 7.43, respectively. For the limiting case where pK_2' is far removed from the pH of the experiment, $k_N' >> k_A'$ and a conventional Brönsted plot may be employed to evaluate α_N and c_N. From Fig. 3 these values have been determined (least squares) to be $\alpha_N = 0.8$ and $c_N = -4.30$. Insufficient examples of imidazoles whose k_2' values are composed entirely of k_A' were available for the determination of α_A and c_A as was done for α_N and c_N. The value of α_A must be smaller than α_N, since, if the k_2 for the anion of 4-nitroimidazole and 6-nitrobenzimidazole could be plotted with the neutral imidazoles as in Fig. 3, their expected k_2 values would be 950 and 15,000 l. mole^{-1} min.$^{-1}$ as compared to the experimental values of 450 and 1,800 l. mole^{-1} min.$^{-1}$, respectively. The values of $\alpha_A = 0.15$ and $c_A = 1.35$ have been chosen as reasonable constants, α_A being necessarily significantly smaller than α_N. Substituting the stated values of A, B, α_N, α_A, c_N and c_A into equation 14 and simplifying terms provides equation 15. The value of α_A employed in equation 15 is in the lower limits, whereas α_N is in the upper limits previously encountered in basic catalysis[30] (the latter comparing favorably to that previously reported at 25° for a limited series of imidazoles[5]).

$$k_2' = \frac{10^{2.46}K_1^{0.2}\,[10^{0.67}H^+ + K_1^{1.67}]}{K_1^{1.94} + 10^{7.43}\,[(H^+)^2 + K_1H^+]} \quad (15)$$

In Fig. 4 the calculated curve provided by equation 15 at pH 8.0 is presented along with a plot of the experimental data of Table II. Aside from the usual assumption of the applicability of the Brönsted relationship to reactions not involving proton transfer, the successful employment of equation 15 is dependent on: (1) the accuracy of the pK_1' and pK_2' values used in the determination of A and B; (2) the reliability of the pK_1' values subsequently used in plotting the data; (3) the values of α_N and c_N as determined from the limiting case where $k_N' >> k_A'$; (4) the approximated values of α_A and c_A; (5) the reliability of the experimental values of k_2'; and (6) the assumption that complicating factors as steric interference in the formation of the tetrahedral complex and the reaction of substituents other than the amidine nitrogens need not be taken into account. Of these it is felt certain that factors 3 and 5 need not cause concern. In factor 1 there is room for error since: either pK_1' or pK_2' is the better known value (*i.e.*, lies within the pK range for accurate titrimetric determination); the pK_2' values employed in determining A and B were literature values in most cases and not determined under the same conditions; and there may well be exceptions to equation 11. Factor 2 need not cause concern except in the instance of 4-nitroimidazole. The 50%

(29) Other imidazole reactions should also possess composite rate constants, the kinetics of such reactions being characterized by continually increasing second-order rate constants with increase in pH, even at pH values far exceeding pK_1' where the compound can be considered to be entirely in the free base form. In the reaction of 2,4-dinitrofluorobenzene with chymotrypsin, J. R. Whitaker and B. J. Jandorf [*J. Biol. Chem.*, **223**, 751 (1956)] found that the second-order rate constant continually increased from pH 6 to 10.7. There was also found to be a direct relationship between decrease in enzymatic activity and decrease in free histidine as found on hydrolysis of the DNP-protein. This suggests that in the reaction of chymotrypsin with 2,4-dinitrofluorobenzene the histidyl anion might well be contributing to the over-all reaction.

(30) R. P. Bell, "Acid-Base Catalysis," Oxford Univ. Press, London, 1941, Ch. 5 and 7.

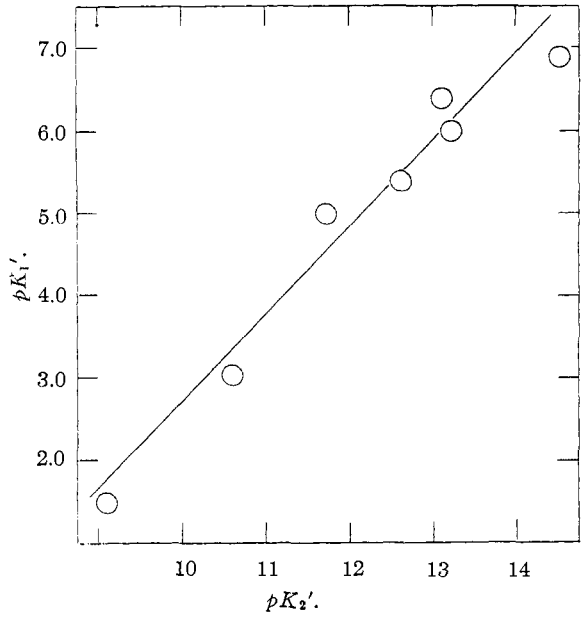


Fig. 2.—The linear relationship of pK_1' and pK_2' for the imidazoles and benzimidazoles [$pK_2' = 0.94pK_1' + 7.43$].

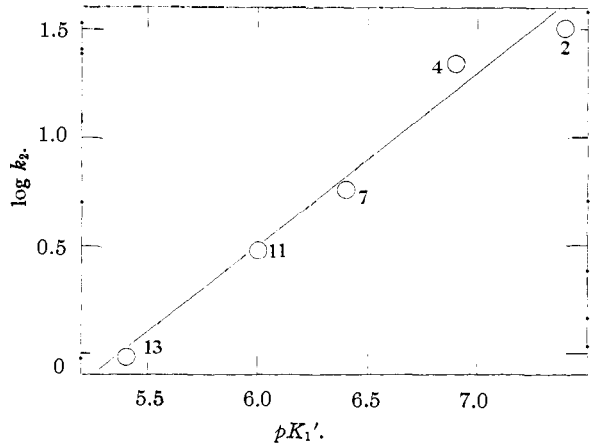


Fig. 3.—Brönsted plot for simple imidazoles when $k_N' \gg k_A'$ (log $k_2 = 0.8pK_1' - 4.30$). The numbers refer to the compounds of Table II.

negative deviation of this compound from the calculated curve of Fig. 4 can be corrected by employing a pK_1' of 1.7 in place of 1.5. The method employed to determine the pK_1' of this substance is probably no more accurate than ± 0.4 unit in this low range and, therefore, we could just as well assume a pK_1' of 1.7 for 4-nitromidazole. However, there is little gained in doing so since factor 4 could also account for this particular deviation since α_A and c_A can only be approximated. In general 6 should cause greatest concern. Sterically, the 4- and 5-positions of imidazole are *ortho* to the attacking amidine nitrogens. In the benzene series the steric requirements of *o*-substituents are reflected in erratic alteration of ΔS^* preventing the use of linear free energy relationships for the correlation of the electronic effects of *o*-substituents on rates.[31] In the imidazole series these steric requirements appear not to be as large as in the case of benzene, allowing the use of equation 15 with some degree of success for the 4(5)-monosubstituted imidazoles and 4-substituted benzimidazoles. However, in the case of substitution in the 2-position of imidazoles, steric effects become maximized. Thus, the 2-methylimidazoles and 2-methylbenzimidazoles (Table II) have such large negative deviations

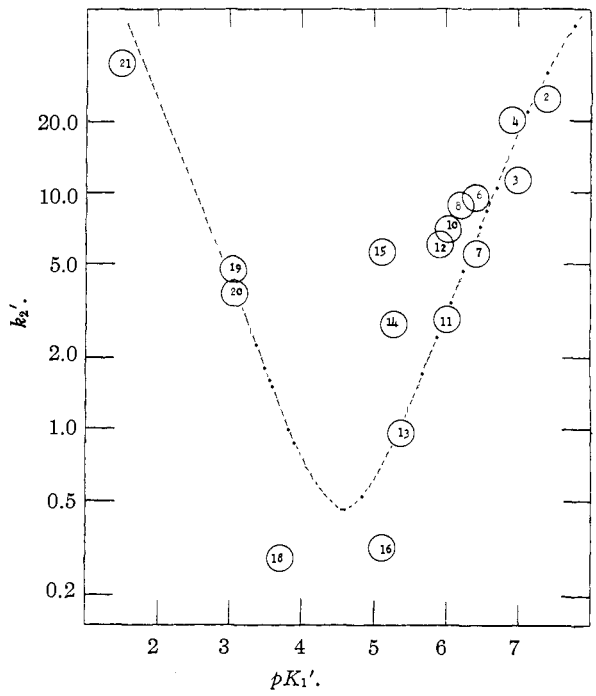


Fig. 4.—Plot of k_2' for the reaction of imidazoles with *p*-NPA (*p*H 8.0) *vs.* pK_1'. The dotted line represents the expected relationship of k_2' to pK_1' as determined from equation 15 and the numbers refer to the compounds of Table II.

from equation 15 that the rate data for these compounds could not be included in Fig. 4, although a plot of k_2' *vs.* pK_1' for these compounds has the same general shape as Fig. 4. It is known also that the phenolic hydroxyl,[32] the aliphatic amino[32] and the sulhydryl group[33] can act as acceptors of the acyl moiety of *p*-nitrophenylesters and, therefore, their presence should lead to higher values of k_2'. Thus, k_2' for histidine methyl ester has a positive deviation from the calculated value by a factor of 10. The pK_a' of the aliphatic amino group in this compound is 7.1 and, therefore, the amino group is present mostly in the unprotonated form at *p*H 8.0. Since the basicity of the α-amino group is comparable to that of imidazole, it is expected to be a good acyl acceptor.[34] The value of k_2' for the reaction of histidine methyl ester with *p*-NPA (Table II) was determined under conditions such that there was a twenty-fold excess of catalyst to *p*-NPA reacted and under these conditions the reaction of both the imidazolyl and amino groups are

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

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

(33) L. Perenyi, *Acta Physiol. Acad. Sci. Hung.*, **5**. 87, 97, 103 (1954); P. D. Boyer and B. M. Dirkes, *Cereal Chem.*, **28**. 483 (1951).

(34) S. A. Bernhard has found that the amino group of histidine methyl ester is acetylated by *p*-NPA (private communication).

pseudo first order. We can, therefore, assign a second-order rate constant to the α-amino group of about 5 l. mole^{-1} min.$^{-1}$ as compared to 0.5 l. mole^{-1} min.$^{-1}$ expected for the imidazolyl group in this compound (Fig. 4). Though the second-order rate constant for the amino group is appreciable, it is only one-sixth that of an imidazole of same pK_1'. It also should be noticed that when the α-amino group is masked, as in the case of N-acetylhistidine, there is no large positive deviation from the calculated value of k_2'. Similar arguments can be advanced for the positive deviation of k_2' for the 4-hydroxy- and 4-hydroxy-6-aminobenzimidazoles, histamine and carbobenzoxyhistidyltyrosine ethyl ester. Thus the pK_a' of the hydroxyl group of 4-hydroxybenzimidazole is 9.5. This value is identical to pK_1' of catechol[35] and in earlier experiments we found k_2' for the reaction of catechol with p-NPA (T = 25°, pH 8.0) to be 1.7 l. mole^{-1} min.$^{-1}$. The rate constant for the reaction of catechol with p-NPA compares favorably to the deviation of 1.9 l. mole^{-1} min.$^{-1}$ found for 4-hydroxybenzimidazole. Again the second-order rate constant for the phenolic group is only a fraction of that expected for an imidazolyl group of same pK_1'.

Though the values of k_2' for the entire series of imidazoles were not determined at a pH value other than 8.0, occasion arose in the course of this study to examine certain members at other pH values and in Table V these rate data are compared to the values calculated from equation 15. Also from equation 14 the expected curves relating pK_1' and k_2' may be constructed for any pH. In Fig. 5 there are presented the calculated curves at pH values of 10 and 6 and these are compared to that of 8.0. The maximum occurring at the left of the curve for pH 10 depicts the increase in the concentration of the anionic species followed by the decrease in the catalytic ability of the species.

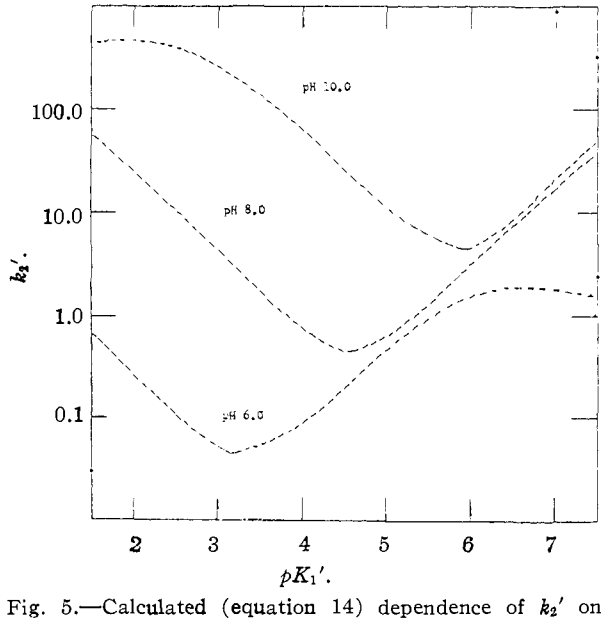


Fig. 5.—Calculated (equation 14) dependence of k_2' on pK_1' at pH 6, 8 and 10.

(35) J. Epstein, D. H. Rosenblatt and M. M. Demek, THIS JOURNAL, 78, 341 (1956).

TABLE V

COMPARISON OF k_2' (EXPTL.) TO k_2' (CALCD.) FROM EQUATION 15

pH	Compound[b]	k_2', l. mole^{-1} min.$^{-1}$ Calcd.	Exptl.[a]
7.0	4-Nitroimidazole	6.6	3.58 ± 0.05[c]
	6-Nitrobenzimidazole	0.40	0.54 ± .01[c]
	4-Bromoimidazole	0.17	0.056 ± .008[c]
	Benzimidazole	1.00	0.67 ± .01[c]
	4-Hydroxymethyl-imidazole	5.25	3.39 ± .01[c]
	Imidazole	9.3	9.25 ± .01[c]
7.5	4-Nitroimidazole	20.0	11.0 ± .9[c]
	6-Nitrobenzimidazole	1.24	1.75 ± .05[c]
	4-Hydroxy-6-nitro-benzimidazole	1.24	2.15 ± .05
	4-(2',4'-Dihydroxy-phenyl)-imidazole	6.12	7.6 ± .3[c]
	Imidazole	16.6	17.1 ± .1[c]
8.45	4-Nitroimidazole	135	74.5
	6-Nitrobenzimidazole	10.7	12.6
	Imidazole	16.1	23.5
8.65	4-Nitroimidazole	183	107
	6-Nitrobenzimidazole	17.0	19.0
	Imidazole	16.3	24.9

[a] Phosphate buffer at a constant calculated $\Gamma/2$ = 0.55 M, the phosphate concentration varying from about 0.29 M at pH 7.0 to 0.19 M at pH 8.65; p-NPA concentration 2 × 10^{-4} M. [b] Catalyst concentration varied from 4 × 10^{-5} M to 7 × 10^{-3} M. [c] On the basis of duplicate experiments.

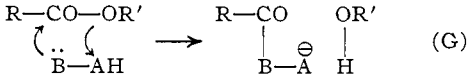
In like manner, the maximum occurring to the right of the pH 6.0 plot depicts a simultaneous increase in neutral species and catalytic efficiency followed by a decrease in the concentration of this species as the catalytic ability of the species continually increases. The pK_1' corresponding to the maximum in k_2' at high pK_1' values is obtained from the relationship pK_1' = 0.6 + pH; and the maximum at lower pK_1' values is given by the equation, pK_1' = 1.06 pH − 8.7. From equation 14 it follows that the pK_1' expected to be associated with a minimum of k_2' at any pH is given by the expression, pK_1' = (pH −0.67)/1.60. Also from Fig. 5, it can be seen that changes in acidity of 10^4 result in only a threefold change in k_2' (calcd.) for an imidazole of pK_1' 6.0 but a 2,500-fold change in k_2' (calcd.) for an imidazole of pK_1' 3.0.

Bifunctional Catalysis.—Compounds RCO–XR′ may be divided into two groups depending on their lability to imidazole-catalyzed hydrolysis. In the labile group of substrates, –XR′ = –SR, –OPh, –NHCOR′ and in the non-labile group, –XR′ = OAlk, –NHAlk and –NHAr. It has been suggested[4,5] that the determining factor for catalysis is the competition for elimination between –XR′ and imidazole in the tetrahedral complex. For the labile esters –XR′ is capable of competing with the imidazole group for the pair of electrons of the tetrahedral complex, while for the non-labile esters imidazole is much the better leaving group. This hypothesis gets support in the finding that the rate of hydrolysis of p-substituted phenyl acetates, as catalyzed by imidazole, fall precipitously as the electronegativity of the p-substituent decreases (*i.e.*, decrease in resonance stabilization of the negative charge of –XR′).[5] That this decrease in

rate is not due to a diminution of the electronegativity of the carboxyl carbonyl is supported by the observation that the hydrolysis of methyl *p*-nitrobenzoate (as contrasted to *p*-nitrophenyl acetate) is not catalyzed by imidazole.[4]

Imidazole catalysis of ester hydrolysis, then, differs in two significant respects from enzymatic hydrolysis. The rates of hydrolysis are always less and the sensitivity of the imidazole catalyzed reaction to the leaving tendency of $-XR'$ is much greater. Thus, the Hammett[31] ρ factor for the hydrolysis of phenyl acetates by wheat germ lipase[36] is exceedingly small (0.12)[37] as compared to that for imidazole catalysis (1.95).[5] If histidine does form a portion of the "active-site" of some esterases then there must be other contributing structural factors that overcome this limitation of imidazole catalysis.

It has been postulated for a large number of esterases that an imidazolyl group of histidine participates in a Lowry[38] type mechanism.[39] Wilson and Bergmann[40] first proposed such a mechanism for acetylcholine esterase. The basic imidazolyl and the acidic group are postulated to be so fixed as to stereoelectronically favor a concerted attack of both (G).



(G)

It would be expected that this type of catalysis would be less sensitive to the leaving tendency of $-XR'$ than IM catalysis since the protonation of $-XR'$ would become of greater importance as the leaving tendency of this group decreased. However, in the chemical literature very few reactions are recorded for which this type of mechanism has been proposed: (H), the catalysis of the mutarotation of tetramethylglucose by α-pyridone and carboxylic acids[41]; (I), the catalysis of the hydrolysis of Sarin[35] and acid halides[42] by the monoanion of catechol; and (J), the solvolysis of phthalamic acid.[43]

Reaction H (which is not operative in aqueous solvents) has been proposed to be driven by the increase in acid or base strength of the catalyst when attack by one group has been initiated. In the case of reaction I there is no possibility of a tautomeric alteration of catalyst as with α-pyridone.

The substitution of a hydroxyl group into the 4- or 5-positions of benzimidazole and imidazole or the 2'-position of 4-phenylimidazole leads to compounds in which the electronic relationship of oxygen and nitrogen is as in 8-quinolinol (which is ineffective as a catalyst in reaction H). On the other hand, these compounds would be analogous to the catechol monoanion and, therefore, the possibility existed that they might catalyze the hydrolysis of *p*-NPA at a rate greater than that expected of the imidazole and phenolic functions separately. Nevertheless, this was not evident for compounds 7, 12, 14, 17, 20 and 6 (Table II, Fig. 4). Because of insolubility, a reliable rate constant for the reaction of 2-(2'-hydroxyphenyl)-benzimidazole with *p*-NPA could not be obtained though it is certain that the value is expectedly small. Attempts to determine k_2' for the reaction of phenyl acetate with 4-hydroxybenzimidazole were unsuccessful because a suitable portion of the spectra could not be found for following this reaction and the autotitrator was not stable enough for prolonged measurements. It was not possible, therefore, to determine whether the sensitivity to the leaving tendency of $-OPh$ is less for this catalyst. The inability of the hydroxyl group to participate in a bifunctional catalysis, as in (J), probably reflects the lack of hydrogen bonding ability of *p*-NP^- which exists as such at *p*H 8.0.

Evidence has been presented that the hydroxyl group of serine may be involved in the reaction of *p*-NPA with chymotrypsin.[44,45] The compound 4-hydroxymethylimidazole possesses an aliphatic hydroxyl group in position adjacent to an amidine nitrogen. However, k_2' for the reaction of this compound with *p*-NPA is as calculated from equation 15. Also, the 4-hydroxymethylimidazole catalysis is no less sensitive to the leaving tendency of the phenol. Thus, the value of k_2' (*p*H 8.0) for the reaction of this substance with phenyl acetate was found to be 0.030 l. mole^{-1} min.$^{-1}$ and, therefore, $\rho \sim 1.8$. By inspection of Table II it is also

TABLE VI

THE CATALYSIS OF *p*-NPA HYDROLYSIS BY PURINES [T = 30°, *p*H 8.0][a]

Substituent	*p*Ka'	k_2', l. mole^{-1} min.$^{-1}$
6-$SeCH_3$		14.3
6-SCH_3	8.74[b]	12.9 ± 0.9[a]
6-OCH_3	2.21; 9.16[b]	8.4 ± .7[a]
6-CH_3	2.6; 9.02[b]	9.1 ± .2[a]
6-NH_2	4.2; 9.8[b]	8.6 ± .8[a]
6-Cl	7.8[b]	7.7
6-OH	1.98; 8.94; 12.02[b]	6.0 ± .2[a]
6-SH	7.77, 10.84[c]	3.2 ± .1[a]
6-SeH	7.33[c]	12.2
Adenosine	3.45, 12.5[d]	0.34 ± .02

[a] The average and deviation of two experiments. [b] A. Albert, *J. Chem. Soc.*, 2060 (1954). [c] H. G. Mautner, THIS JOURNAL, **78**, 5292 (1956). [d] J. Davoll, *J. Chem. Soc.*, 967 (1948). [e] Solvent, ionic strength, etc., same as expts. of Table II.

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

(37) Calculated from the data of reference 36 by the equation $\rho = 1/\sigma \log k/k_0$, where k_0 is k_1 of the Michaelis equation for the hydrolysis of phenyl acetate, k is the like constant for a phenyl acetate with a *m*- or *p*-substituent of electronic displacement σ.

(38) T. M. Lowry, *J. Chem. Soc.*, **127**, 1371 (1925).

(39) See for example K. J. Laidler, *Disc. Faraday Soc.*, **20**, 83 (1955).

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

(41) C. G. Swain and J. F. Brown, THIS JOURNAL, **74**, 2538 (1952).

(42) J. W. Churchill, M. Lapkin, F. Martinez and J. H. Zaslowsky, Paper 17 of the Organic Division, 132nd National Meeting of the American Chemical Society, New York, N. Y., September 8 to 13, 1957.

(43) M. L. Bender, THIS JOURNAL, **79**, 1258 (1957).

(44) H. Gutfreund and J. M. Sturtevant, *Biochem. J.*, **63**, 656 (1956). See also: N. K. Schaffer, S. C. May and W. H. Summerson, *J. Biol. Chem.*, **202**, 67 (1953); R. A. Oosterbaan, P. 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).

(45) It has recently been shown [H. B. Henbest and B. J. Lovell, *J. Chem. Soc.*, 1965 (1957)] that, in the case of monoesters of 1,3-diols of cyclohexanes, when the hydroxyl and ester groups are held close together by their molecular environment hydrolysis of the ester is facilitated. These experimental results lend some support to the electrophilic participation of an aliphatic hydroxyl group in ester hydrolysis.

seen that the protonated amino group of histamine gives no assistance in the catalysis of *p*-NPA hydrolysis and k_2' for the reaction of histamine with phenyl acetate (*p*H 8.0) is 0.044 l. mole^{-1} min.$^{-1}$ ($\rho \sim 1.7$).

The Catalysis of *p*-NPA Hydrolysis by Purines and Pyrimidines.—Just as the catalytic property of the imidazole ring is retained in the benzimidazoles, it would also be expected to be present in the pyrimidoimidazoles (purines). To be able to separate the catalysis due to the pyrimido ring from that of the imidazole ring we have studied the catalysis of hydrolysis of *p*-NPA by both purines and pyrimidines (Table VI). At *p*H 8.0 the pyrimidines uracil, cytosine and cytidine proved to be non-catalytic up to 2×10^{-4} *M*, a concentration at which the purines exhibited easily measurable rate constants.

Little can be made of the data of Table VI and there seems to be no relation of pK_a' to k_2'. It can be noted, however, that the k_2' values are in the range found for the benzimidazoles and that ribosidation of adenine markedly decreases k_2' and the 6-$SeCH_3$, 6-SCH_3 and 6-OCH_3 substituted purines have greater rates than the 6-Se, 6-S and 6-O purines.

Acknowledgments.—We wish to express our appreciation to Dr. M. L. Bender for the use of preprints, and particularly our gratitude to Dr. F. M. Richards for instruction in the use of the Radiometer autotitrator. This work was supported by grants from the Rockefeller Foundation and from the Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

NEW HAVEN, CONNECTICUT

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

3-Benzoyl-4-piperidones

BY EARLE VAN HEYNINGEN

RECEIVED AUGUST 22, 1957

The products of Mannich reactions between acetophenones and β-alkylaminopropionic esters were cyclized to 3-benzoyl-4-piperidones.

Although there are many known substituted 4-piperidones, a search of the literature has failed to reveal any 3-benzoyl derivatives. This paper reports the synthesis of several of these compounds.

It was assumed by analogy with the ring closure of bis-(β-carbalkoxyethyl)-amines[1] to 3-carbalkoxy-4-piperidones that β-(β-benzoylethylamino)-propionic esters could be cyclized to the desired 3-benzoyl-4-piperidones. With this in mind a search was made for an adequate synthesis of the precursory esters.

The first method investigated was the addition of methyl β-methylamino-α-methylpropionate (II) to phenyl vinyl ketone (I). This condensation yielded the desired aminoester III in quite satisfactory yield (63.5%). As was expected this ester could be cyclized by sodium hydride in ether to a 3-benzoyl-4-piperidone (IV). An attempt was made to extend this method to the synthesis of the *p*-anisyl analog, but the attempt was unsuccessful since the pyrolysis of β-dimethylamino-*p*-methoxypropiophenone hydrochloride yielded the requisite *p*-anisyl vinyl ketone only in trace amounts. As a consequence, this approach to analogs of III was abandoned in favor of a search for a more general method.

A general method was developed that was characterized by simplicity which to some degree compensated for the moderate yields of VII obtained by its use. A Mannich reaction between acetophenone or its derivatives V and a substituted β-alkylaminopropionic ester VI gave the desired ketoaminoester VII. Some of these intermediates could be purified by distillation in vacuum to give analytically pure material with only very slight

(1) S. M. McElvain and K. Rorig, THIS JOURNAL, **70**, 1820 (1948).

CHART I

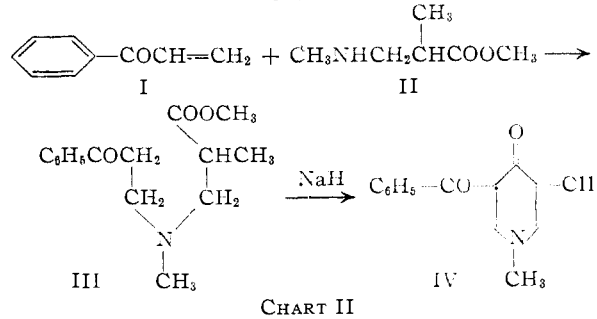
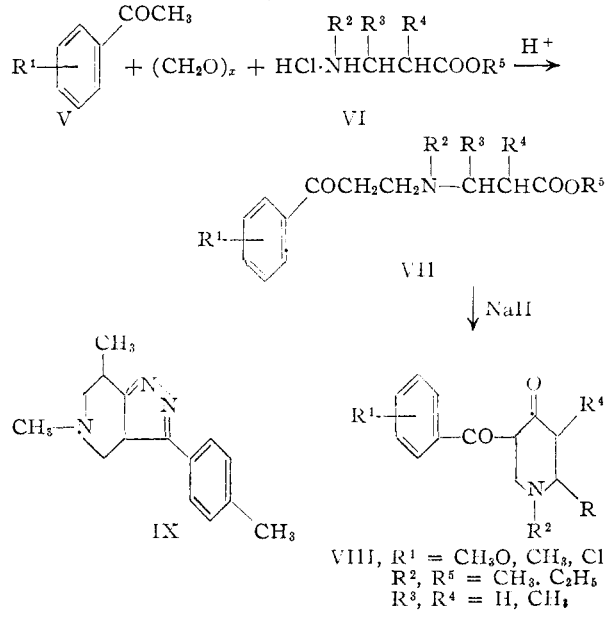


CHART II



VIII, R^1 = CH_3O, CH_3, Cl
R^2, R^5 = CH_3, C_2H_5
R^3, R^4 = H, CH_3