A Kinetic Study of the Hydrolysis of Substituted *N*-Benzoylimidazoles and *N*-Benzoyl-*N'*-methylimidazolium Ions in Light and Heavy Water. Hydrogen Bridging without Rate-Determining Proton Transfer as a Mechanism of General Base CatalyzedHydrolysis and a Model for Enzymic Charge-Relay[1]

Myung-un Choi and Edward R. Thornton*

Contribution from the Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19174. *Received October 1, 1973*

Abstract: Rates and kinetic solvent isotope effects for the title substrates have been studied at 15°. Isotope effects k_{H_2O}/k_{D_2O} on the order of 2.5, essentially independent of substituent, were found for the "uncatalyzed" hydrolysis of both substrates, as well as benzoylimidazolium ions, and for the methylimidazole-catalyzed hydrolysis of benzoylmethylimidazolium ions. The lyoxide term for the latter has inverse isotope effects k_{DO^-}/k_{HO^-} of 1.2–1.4, larger for more electron-supplying substituents, as expected for simple rate-determining nucleophilic attack on the carbonyl carbon atom. The lyoxide term for the benzoylimidazoles has isotope effects near 1.0. It is concluded that general base catalyzed hydrolysis, with water, methylimidazole, or lyoxide as general base as the case may require, is the probable mechanism for all terms studied (except the one case of simple nucleophilic attack), and that this mechanism consists of rate-determining nucleophilic attack by water with one proton partly transferred to the general base but not being transferred as part of the reaction coordinate motion.

Involvement of the imidazole group of histidine in enzymic catalysis, as in the case of His-57 of chymotrypsin, is indicated by a great deal of evidence.[2,3] Acylenzyme intermediates are formed, esterified at serine in the case of serine proteases, and reaction is completed by hydrolysis of the acylenzyme, probably general base (GB) catalyzed by an imidazole group. A useful way of isolating the GB mechanism for detailed study in nonenzymic reactions is to use as substrates acylimidazoles, in which case nucleophilic catalysis by imidazole becomes an identity reaction and cannot interfere with hydrolysis by other mechanisms.[4,5] Extensive subsequent studies of hydrolysis[6,7] and other nucleophilic reactions[8-10] of acylimidazoles have established neutral, hydroxide and GB, and hydronium and general acid (GA) catalytic terms, the latter having been shown for acetylimidazole to be primarily the kinetically equivalent uncatalyzed and GB catalyzed nucleophilic displacements on acetylimidazolium ion in the case of water and other weak nucleophiles, but not in the case of ammonia.[5,8,9]

Hydrolysis of acylimidazoles in aqueous imidazole buffers therefore follows eq 1, where L = H or D, Im

$$k_{obsd} = k_0 + k_{LO^-}(LO^-) + k_{L_3O^+}(L_3O^+) + k_{GB}(Im) + k_{GA}(ImL^+) \quad (1)$$

stands for imidazole, ImL^+, for imidazolium ion, and k_{obsd} is the observed pseudo-first-order rate constant,[11] while *N*-acyl-*N'*-methylimidazolium ions follow eq 2.

$$k'_{obsd} = k'_0 + k'_{LO^-}(LO^-) + k'_{GB}(MeIm) \quad (2)$$

The uncatalyzed and GB catalyzed hydrolyses are particularly interesting mechanistically and as possible enzyme mechanisms, but there are many possibilities for rate-determining steps, resulting from stepwise mechanisms, proton transfers, and multiple catalytic sites.[1a,2-10,12,13] The first step in a program of further mechanistic analysis would be to examine substituent effects and the substituent dependence of isotope effects for various catalytic terms. The results would indicate mechanistic similarities or differences between the different catalytic terms, and variations of isotope effects with substituent would be expected for any term which involved a mechanism not having a single rate-determining step, since the proportional contribution of multiple rate-determining steps would be expected to change with substituent. Therefore, we have studied the hydrolysis of substituted benzoylimidazoles (BI), **1**, and the hydrolysis and *N*-methylimidazole-catalyzed hydrolysis of substituted *N*-benzoyl-*N'*-methylimidazolium ions (BMI$^+$), **2**. Imidazole catalysis of the BI was not studied in this work, since the five terms in eq 1 present problems of precision, and we wished to see what the ultimate precision in such studies might be.

(1) (a) Previous paper: J. P. Klinman and E. R. Thornton, *J. Amer. Chem. Soc.*, **90**, 4390 (1968). (b) Supported in part by the U. S. Public Health Service through Grant AM-12,258, and in part by the National Science Foundation through Grants GP-22,803 and GP-34,491X. (c) For further details, *cf.* M.-u. Choi, Ph.D. Dissertation in Chemistry, University of Pennsylvania, 1973.

(2) M. L. Bender, "Mechanisms of Homogeneous Catalysis from Protons to Proteins," Wiley-Interscience, New York, N. Y., 1971.

(3) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969.

(4) W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1272, 1280 (1959).

(5) R. Wolfenden and W. P. Jencks, *J. Amer. Chem. Soc.*, **83**, 4390 (1961).

(6) T. H. Fife, *J. Amer. Chem. Soc.*, **87**, 4597 (1965).

(7) J. A. Fee and T. H. Fife, *J. Org. Chem.*, **31**, 2343 (1966).

(8) D. G. Oakenfull and W. P. Jencks, *J. Amer. Chem. Soc.*, **93**, 178 (1971).

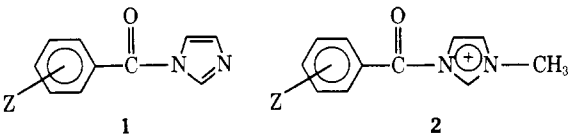
(9) D. G. Oakenfull, K. Salvesen, and W. P. Jencks, *J. Amer. Chem. Soc.*, **93**, 188 (1971).

(10) M. I. Page and W. P. Jencks, *J. Amer. Chem. Soc.*, **94**, 8818, 8828 (1972).

(11) A term proportional to (Im)(LO$^-$), previously believed present,[1a] must be considered unlikely, based on further experiments: W. Palaitis, University of Pennsylvania, unpublished results.

(12) S. L. Johnson, *Advan. Phys. Org. Chem.*, **5**, 237 (1967).

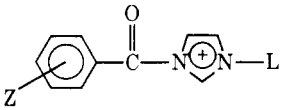
(13) R. L. Schowen, *Progr. Phys. Org. Chem.*, **9**, 275 (1972).



1 2

Precision in isotope effects of *ca.* 5% or better has been attained, except in the case of the most interesting—but most difficult, being subject to specific effects—term, k'_{GB} of eq 2, which has *ca.* 10% precision. These isotope effects are quite constant with substituent, indicating that we are probably dealing with a unique rate-determining step for each catalytic term, and that we can consider the isotope effects to be characteristic of the mechanistic type under study in each case, *i.e.*, not composed of contributions from two or more partially rate-determining steps.

Coupled with other GB data on acetylimidazole, our isotope effects have led us to conclude that *addition* to **1** or **2**, or to **3** in the case of $k_{L_3O^+}$ of eq 1, is in each case the most satisfactory mechanism. In the case of



3

k'_{LO^-}, the mechanism appears to be simple nucleophilic addition, but in the cases of k_0, k'_0, $k_{L_3O^+}$, k'_{GB}, and even k_{LO^-}, the mechanism appears to be GB catalyzed addition of water. The most satisfactory mechanism for GB catalyzed addition of water seems to be one involving a strong hydrogen bond between nucleophilic water and GB, but with a rate-determining transition state which does not involve hydrogen transfer as part of the reaction coordinate, which is instead mainly O···C bond making (HAR).[13,14] Since the nucleophilic oxygen atom has unshared electrons, it is possible—and probable—for a more-basic unshared pair to be used for nucleophilic bond formation, thus permitting O···H bond-breaking to be independent of O···C bond-making, so that proton transfer need not be part of the reaction coordinate motion.

Results

Values of k_{L_2O} for hydrolysis of BI at several pL were obtained by extrapolation of k_{obsd} to zero buffer concentration using a linear least-squares fit, and are given in Table I. A nonlinear least-squares fit of k_{L_2O} to the first three terms of eq 1 gave the three individual rate constants (Table II), with a typical fit illustrated in Figure 1.

Values of k'_{L_2O} and k'_{cat} for hydrolysis of BMI^+ at several pL were obtained by a linear least-squares fit of k_{obsd} against free *N*-methylimidazole (MI) concentration in MI/MIL^+ buffers and are given in Table III. A typical plot of such data is shown in Figure 2. Linear least-squares fits of k'_{L_2O} to the first two terms of eq 2 gave relatively bad results, and it was noted that the problem resided in the point at highest pL's being "too low" in all cases. In retrospect, a slight curvature could be seen in plots of k_{obsd} *vs.* (MI); curved extrapolation of such plots was found to give intercepts which were no longer "too low" in the subsequent fit. However, in view of the uncertainties in such a procedure, it was more satisfactory simply to omit the highest pL values of k'_{L_2O} and derive k'_0 and k'_{LO^-} values from the four lower pL points (Figure 2).

No such procedure could help with the problem of

(14) This type of mechanism has recently been suggested for intramolecular GB catalysis by carboxylate anion in hydrolysis of *O*-dichloroacetylsalicylic acid anion (k_{H_2O}/k_{D_2O} = 2.17): S. S. Minor and R. L. Schowen, *J. Amer. Chem. Soc.*, **95**, 2279 (1973).

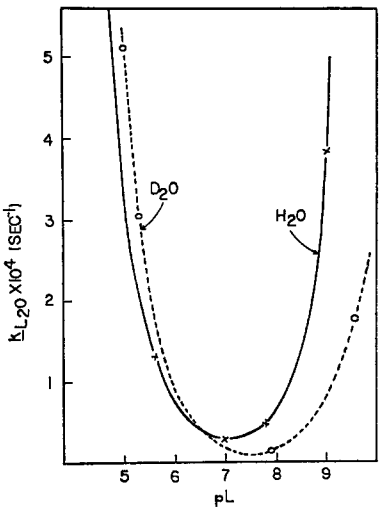


Figure 1. pL–rate profiles for k_{L_2O} (*p*-methylbenzoylimidazole), 15.08°, μ = 0.5 *M*. The points are experimental; the curves are derived from the least-squares rate constants k_0, $k_{L_3O^+}$, and k_{LO^-}.

Table I. Values of k_{L_2O} (Intercept) Obtained by Linear Least-Squares Fit of Observed Pseudo-First-Order Rate Constants for BI (**1**) in Light and Heavy Water Against Total Buffer Concentration, 15.08°, Ionic Strength = 0.5 *M* (KCl Added)[a]

p-Z	pH	k_{H_2O}, sec^{-1} [b]	pD	k_{D_2O}, sec^{-1} [b]
Cl	4.783	$2.01 \pm 0.01 \times 10^{-3}$	5.106	$1.34 \pm 0.01 \times 10^{-3}$
	5.628	$3.54 \pm 0.07 \times 10^{-4}$	5.333	$7.72 \pm 0.03 \times 10^{-4}$
	7.001	$7.75 \pm 0.14 \times 10^{-5}$	7.956	$3.86 \pm 0.03 \times 10^{-5}$
	7.828	$1.50 \pm 0.02 \times 10^{-4}$	9.548	$6.38 \pm 0.08 \times 10^{-4}$
	8.936	$1.30 \pm 0.01 \times 10^{-3}$		
H	4.783	$1.50 \pm 0.02 \times 10^{-3}$	5.106	$9.20 \pm 0.09 \times 10^{-4}$
	5.628	$2.39 \pm 0.07 \times 10^{-4}$	5.333	$5.92 \pm 0.01 \times 10^{-4}$
	7.001	$4.59 \pm 0.02 \times 10^{-5}$	7.956	$2.19 \pm 0.02 \times 10^{-5}$
	7.828	$7.49 \pm 0.38 \times 10^{-5}$	9.548	$3.29 \pm 0.02 \times 10^{-4}$
	8.936	$6.70 \pm 0.03 \times 10^{-4}$		
CH_3	4.783	$8.33 \pm 0.02 \times 10^{-3}$	5.106	$5.10 \pm 0.01 \times 10^{-4}$
	5.628	$1.26 \pm 0.03 \times 10^{-4}$	5.333	$3.06 \pm 0.02 \times 10^{-4}$
	7.001	$2.42 \pm 0.01 \times 10^{-5}$	7.956	$1.15 \pm 0.02 \times 10^{-5}$
	7.828	$4.38 \pm 0.48 \times 10^{-5}$	9.548	$1.79 \pm 0.03 \times 10^{-4}$
	8.936	$3.89 \pm 0.04 \times 10^{-4}$		

[a] Buffers: pH 4.783, 5.628, pD 5.106, 5.333 used acetate–acetic acid; pD 7.001, pD 7.956 used imidazole–imidazolium; pH 7.828, 8.936, pD 9.548 used tris(hydroxymethyl)aminomethane. A five-fold concentration range was used, generally with three concentrations, *ca.* 0.02–0.1 *M* (total buffer), and three replicate runs at each buffer concentration. [b] Rate constants, with standard deviations from least-squares fit; should be read as $(2.01 \pm 0.01) \times 10^{-3}$, etc.

Table II. Hydrolysis Terms and Isotope Effects for BI (1), 15.08°, Ionic Strength = 0.5 *M* (KCl)[a]

p-Z	$10^5 k_0$, sec^{-1}	$10^{-2} k_{L_3O^+}$, l. mol^{-1} sec^{-1}	$10^{-2} k_{LO^-}$, l. mol^{-1} sec^{-1}
Cl			
(H_2O)	5.12 ± 0.27[b]	1.23 ± 0.03[b]	3.20 ± 0.10[b]
(D_2O)	2.13 ± 0.14	1.65 ± 0.04	3.03 ± 0.11
(H_2O)/(D_2O)[c]	(2.40 ± 0.16)	$(1.35 \pm 0.04)^{-1}$	(1.06 ± 0.04)
H			
(H_2O)	2.86 ± 0.19	0.893 ± 0.030	1.60 ± 0.07
(D_2O)	1.25 ± 0.04	1.18 ± 0.01	1.55 ± 0.03
(H_2O)/(D_2O)[c]	(2.28 ± 0.15)	$(1.32 \pm 0.04)^{-1}$	(1.03 ± 0.04)
CH_3			
(H_2O)	1.48 ± 0.06	0.486 ± 0.010	0.954 ± 0.024
(D_2O)	0.635 ± 0.001	0.644 ± 0.0003	0.847 ± 0.0005
(H_2O)/(D_2O)[c]	(2.33 ± 0.10)	$(1.32 \pm 0.03)^{-1}$	(1.13 ± 0.03)

[a] Essentially identical rate constants for H_2O resulted when only four or three pH values were used in the least-squares fit instead of five. [b] Standard deviation of rate constant derived from least-squares fit. Standard deviation of isotope effect derived from standard deviations of rate constants. [c] The numbers in these rows are actual values of isotope effects, not multiplied by powers of ten given in column headings.

Table III. Values of k'_{L_2O} (Intercept) and k'_{cat} (Slope) Obtained by Linear Least-Squares Fit of Observed Pseudo-First-Order Rate Constants for BMI^+ (2) in Light and Heavy Water Against Free *N*-Methylimidazole Concentration in MI/MIL^+ Buffers, 15.08°, Ionic Strength = 0.5 *M* (KCl Added)[a]

p-Z	pH	$10^2 k'_{H_2O}$, sec^{-1}	$10^1 k'_{cat}$, l. mol^{-1} sec^{-1}	pD	$10^2 k'_{D_2O}$, sec^{-1}	$10^2 k'_{cat}$, l. mol^{-1} sec^{-1}
H	6.421	1.50 ± 0.004	1.70 ± 0.06	6.892	0.625 ± 0.003	4.33 ± 0.19
	6.956	1.66 ± 0.007	1.59 ± 0.05	7.469	0.684 ± 0.004	6.15 ± 0.29
	7.493	2.05 ± 0.01	1.72 ± 0.08	8.020	0.881 ± 0.005	6.66 ± 0.32
	8.001	3.34 ± 0.02	1.60 ± 0.06	8.504	1.53 ± 0.006	5.92 ± 0.12
	8.525	7.08 ± 0.12	1.50 ± 0.08	9.089	3.43 ± 0.02	5.26 ± 0.11
CH_3	6.421	0.668 ± 0.002	0.822 ± 0.024	6.892	0.289 ± 0.002	1.94 ± 0.11
	6.956	0.762 ± 0.002	0.754 ± 0.013	7.469	0.310 ± 0.002	3.10 ± 0.13
	7.493	0.955 ± 0.002	0.830 ± 0.015	8.020	0.421 ± 0.001	3.13 ± 0.07
	8.001	1.64 ± 0.004	0.730 ± 0.010	8.504	0.750 ± 0.003	2.86 ± 0.07
	8.525	3.37 ± 0.04	0.696 ± 0.025	9.089	1.74 ± 0.012	2.32 ± 0.08
OCH_3	6.421	0.257 ± 0.003	0.364 ± 0.037	6.892	0.110 ± 0.001	0.885 ± 0.098
	6.956	0.290 ± 0.002	0.360 ± 0.014	7.469	0.128 ± 0.002	1.21 ± 0.13
	7.493	0.414 ± 0.001	0.322 ± 0.008	8.020	0.174 ± 0.001	1.46 ± 0.10
	8.001	0.716 ± 0.003	0.344 ± 0.007	8.504	0.372 ± 0.003	0.935 ± 0.062
	8.525	1.56 ± 0.01	0.332 ± 0.009	9.089	0.841 ± 0.007	1.02 ± 0.05

[a] Generally four buffer concentrations were studied at each pL, with free (MI) varying over a factor of 5. The highest free (MI) studied varied from 0.03 *M* at the lowest pL to 0.5 *M* at the highest pL. Standard deviations from least-squares fit are given. Generally three replicate runs were carried out at each buffer concentration.

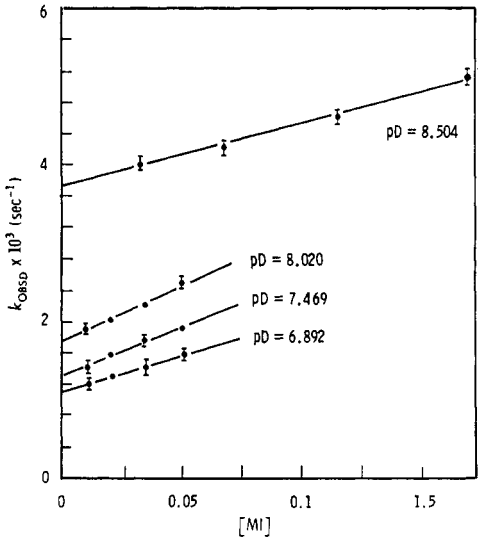


Figure 2. Typical plots of k_{obsd} for *N*-*p*-methoxybenzoyl-*N'*-methylimidazolium ion against free MI concentration in MI/MID^+ buffers in D_2O, 15.08°, μ = 0.5 *M*.

minor variations of k'_{cat} (k'_{GB} for the present case, eq 2) with pL, which appeared reproducible outside experimental error. No additional rate terms provide a simple explanation, since there appears to be a tendency toward a maximum at around neutrality, and it would appear that only some type of *special* salt or medium effects (at constant ionic strength) could explain the results. Such an effect, proportional to (MIL^+), for example, would give a nonlinear dependence of k_{obsd} on (MI) if it acted on k_{GB}, not observed significantly. On the other hand, an effect inhibiting k_0 would be linear in (MIL^+) and thus would show up in our data as an effect on k_{cat}, providing a possible explanation for the major variation, the decrease of k_{cat} at low pL. The effect is larger in D_2O, where there is not only a different medium, but also an effect which cannot be controlled, the tendency of the 2 position of imidazolium ions to undergo H–D exchange. Since any small secondary isotope effects which might result from this exchange could not be separated from observed isotope effects, these effects had to be ignored, although it is probable that the repeated exchange of buffer components used for D_2O (see Experimental Section) resulted in complete exchange at this position.[15] We are thus forced simply to average the values of k_{cat} at the different pL's; while this procedure gives a substantial standard deviation, the isotope effects are probably more reliable, since the effects tend to cancel in comparing

(15) J. L. Wong, University of Louisville, personal communication, 1969, gives a half-life on the order of 30 sec for this exchange in the 2 position of imidazole at 81° in the pH range 6–8.

Table IV. Water, Lyoxide, and *N*-Methylimidazole-Catalyzed Hydrolysis Terms and Isotope Effects for BMI^+ (2), 15.08°, Ionic Strength = 0.5 *M*(KCl)

p-Z	$10^3k'_0$, sec^{-1}	$10^{-4}k'_{LO^-}$, l. mol^{-1} sec^{-1}	$10^2k'_{GB}$, l. mol^{-1} sec^{-1}
H			
(H_2O)	14.7 ± 0.1[a]	4.15 ± 0.06[a]	16.2 ± 0.9[b]
(D_2O)	5.87 ± 0.05	5.08 ± 0.08	6.00 ± 0.51
(H_2O)/(D_2O)[c]	(2.51 ± 0.02)	$(1.22 \pm 0.02)^{-1}$	(2.70 ± 0.22)
CH_3			
(H_2O)	6.76 ± 0.08	2.02 ± 0.05	7.66 ± 0.60
(D_2O)	2.66 ± 0.04	2.61 ± 0.05	2.85 ± 0.34
(H_2O)/(D_2O)[c]	(2.54 ± 0.04)	$(1.29 \pm 0.03)^{-1}$	(2.69 ± 0.32)
OCH_3			
(H_2O)	2.53 ± 0.06	1.04 ± 0.04	3.44 ± 0.18
(D_2O)	0.970 ± 0.046	1.47 ± 0.04	1.16 ± 0.22
(H_2O)/(D_2O)[c]	(2.60 ± 0.13)	$(1.42 \pm 0.05)^{-1}$	(2.98 ± 0.60)

[a] Standard deviation of rate constant derived from least-squares fit. [b] Standard deviation of rate constant from mean of k'_{cat} at several pL values. [c] The numbers in these rows are actual values of isotope effects, not multiplied by powers of ten given in column heading. Standard deviation of isotope effect derived from standard deviations of rate constants; *cf.* E. B. Wilson, Jr., "An Introduction to Scientific Research," McGraw-Hill, New York, N. Y., 1952, p 273, eq 12.

H_2O and D_2O or in comparing different substituents. The values of k'_{cat} at pD 6.892 were, somewhat arbitrarily, omitted from the average, since they fell considerably below the values at the other four pD values for all three substrates, and the results for the BMI^+ are collected in Table IV.

Hammett ρ values could be derived from the substituent dependence of the various rate constants. Because of the very large amount of data necessary to evaluate the rate constants for each substituent, only three substituents were studied for BI and BMI^+, with only H and CH_3 in common, proceeding at reasonable rates for both BI and BMI^+ at 15°. As a result of concentrating on a common set of substituents, a narrow range was employed, and the Hammett plots exhibited curvature. Curvature of just this type has been observed previously[16] in the hydrolysis of BI in 0.1 *M* HCl; however, these plots "straighten out" with inclusion of more electron-withdrawing substituents. Comparisons within each series are nevertheless justifiable, since each rate term exhibits similar curvature. The ρ values (standard deviation) are: k_0, 1.35 (0.16); $k_{L_3O^+}$, 0.99 (0.26); k_{LO^-}, 1.32 (0.00); k'_0, 2.68 (0.58); k'_{LO^-}, 2.30 (0.36); k'_{GB}, 2.44 (0.42). Fits with σ^+ gave ρ^+ values: k'_0, 0.95 (0.05); k'_{LO^-}, 0.80 (0.09); k'_{GB}, 0.86 (0.08). Similar values were obtained for D_2O in each case.

Direct rate comparisons between corresponding terms in eq 1 and 2 could be made—the rate contribution k_0(BI) is kinetically equivalent to an expression of the form $k''_{LO^-}(BIL^+)(LO^-)$, and $k_{L_3O^+}(BI)(L_3O^+)$ to an expression of the form $k''_0(BIL^+)$. Using estimated values for the pK_a of BIL^+, k_0'' and k''_{LO^-} are compared with k'_0 and k'_{LO^-} for BMI^+ in Table V.

Table V. Comparison of Corresponding Terms in the Rate Expressions for BI (1) and BMI^+ (2), 15.08°, Ionic Strength = 0.5 *M*(KCl)

p-Z	$10^3k''_0$,[a] sec^{-1}	$10^3k'_0$, sec^{-1}	$10^{-5}k''_{LO^-}$,[b] l. mol^{-1} sec^{-1}	$10^{-5}k'_{LO^-}$, l. mol^{-1} sec^{-1}
H				
(H_2O)	7.23	14.7	5.15	0.415
(D_2O)	3.02	5.87	5.54	0.508
CH_3				
(H_2O)	3.94	6.76	2.67	0.202
(D_2O)	1.65	2.66	2.82	0.261

[a] $k''_0 = k_{L_3O^+}K_a^{BIL^+}$. $pK_a^{BIL^+}$ approximated by that of acetylimidazole, found to be 3.86 at 25° (μ = 1.0 *M*),[8] and using the Arrhenius relationship for imidazole (pK_a = 7.21 (25°) [W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, **90**, 2622 (1968)], 7.10 (30°),[6] μ = 1.0 *M*), giving ΔE = 9.09 kcal mol^{-1} and ΔpK_a (15–25°) = 0.231, to estimate the temperature dependence for the acylimidazole; thus $pK_a^{BIH^+} \cong 4.091$, $K_a^{BIH^+} \cong 8.1 \times 10^{-5}$ *M*. $K_a^{BID^+}$ estimated from isotope effect on pK_a of imidazole in D_2O (25°), ΔpK_a = 0.50 [L. Pentz and E. R. Thornton, *J. Amer. Chem. Soc.*, **89**, 6931 (1967)], $K_a^{BID^+} \cong 2.56 \times 10^{-5}$ *M*. [b] $k''_{LO^-} = k_0K_a^{BIL^+}/K_w$. For values of K_w, see Experimental Section. Using previous estimates[a] for $K_a^{BIL^+}$, $K_a^{BIH^+}/K_{H_2O} \cong 1.80 \times 10^{10}$ M^{-1}; $K_a^{BID^+}/K_{D_2O} \cong 4.44 \times 10^{10}$ M^{-1}.

Discussion

We first note that k'_0/k''_0 values moving down Table V are 2.0, 1.9, 1.7, and 1.6, respectively, while k''_{LO^-}/k'_{LO^-} values are 12, 11, 13, and 11. Since the values are near unity in the first case, *i.e.*, use of **2** as a model for the reactivity of **3** accounts for this entire catalytic term, the $k_{L_3O^+}$ term of eq 1 is shown to be predominantly the kinetically equivalent reaction of **3** with L_2O; this conclusion is further substantiated by the small change of the ratio with substituent, which would be unlikely if the L_3O^+ catalyzed mechanism were fast enough to contribute significantly along with the reaction of L_2O with **3**, because the relative contributions of the two mechanisms should change with substituent. Reactions of many nucleophiles with acetylimidazole and *N*-acetyl-*N'*-methylimidazolium ion, resulting in rate constants varying over a range of 10^9, have similarly shown agreement to within a factor of 2 for k''_0 and k_0 (25°), also interpreted as demonstrating involvement of the acetylimidazolium ion in these cases.[4,5,9] Likewise, a 14-fold discrepancy was observed for k''_{LO^-} *vs.* k'_{LO^-} in the acetyl case (25°),[4,5] similar to present observations, showing that the k_0 term of eq 1 does not involve reaction of **3** with lyoxide ion. Therefore, k_0, k_0', and k''_0 involve reaction with water of BI (**1**), BMI^+ (**2**), and BIL^+ (**3**), respectively.

Further similarities are indicated by the ρ values (as well as the curvatures), which are similar for k_0 and k_{LO^-}, and again for k'_0, k'_{LO^-}, and k'_{GB}. Previous ρ values for k_{HO^-} of 1.4[7] and 1.5,[1a] for k''_0 (directly measured with BI in 0.1 *M* HCl) of 1.7,[16] and for k_0 of 1.4[1a] have been recorded, the first at 30° and the rest at 25°. From the rate comparisons in Table V, it is clear that $k_{L_3O^+}$ does correspond to k''_0. Although the

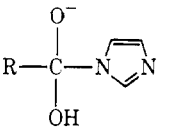
(16) M. Caplow and W. P. Jencks, *Biochemistry*, **1**, 883 (1962).

calculated ρ value for $k_{L_3O^+}$ is low, and although the involvement of methoxy as a substituent leads to the σ–σ^+ dichotomy, thus making comparison of BI and BMI^+ uncertain, the rate constant ratios for substituents H/CH_3 (Tables II and IV) are for BI 1.9, 1.8, and 1.7, and for BMI^+ 2.2, 2.0, and 2.1, again substantiating the close similarities of all the various catalytic terms.

Oakenfull and Jencks[8] have studied GB catalysis of hydrolysis of both acetylimidazole and acetylimidazolium ion and have found correlations with Brønsted β values of 0.55 and 0.34, respectively. With acetylimidazole, hydroxide falls on the extended Brønsted line, but water seems "too fast." On the other hand, water falls on the Brønsted line for acetylimidazolium ion, while hydroxide is "too fast." The existence of these Brønsted correlations, the close similarities noted above, and the close similarity of the isotope effects (Tables II and IV), (a) for the three substituents within each catalytic term, and (b) for k_0, k'_0, and k'_{GB} as well as k''_0 (estimated, Table V; calculations for *p*-Cl give 9.96 and 4.22×10^{-3} sec^{-1} in H_2O and D_2O), $(H_2O)/D_2O$ for *p*-Cl (2.36), *p*-H (2.39), and *p*-CH_3 (2.37), all suggest that the mechanisms involved are very similar for all the catalytic terms. The fact that Brønsted coefficients may not in certain cases reflect precisely the transition state structure[17,18] does not diminish the significance of these correlations in establishing mechanistic similarity or identity.

Numerous solvent isotope effects for hydrolysis and GB catalyzed hydrolysis of acyl derivatives fall in the range of 2–3, with some as high as 4.[12,13,19] The isotope effects for acetylimidazole hydrolysis are 2.7 and 2.5 in water (pH 7) and 0.02 *M* LCl, respectively, and 3.6 for imidazole-catalyzed hydrolysis, at 25°. Values of 2.6 for hydrolysis and *ca.* 4 for *N*-methylimidazole-catalyzed hydrolysis of *N*-acetyl-*N'*-methylimidazolium ion, at 25°, are recorded.[5] Isotope effects ranging from 2.3 to 2.8 are found for hydrolysis of *N*-alkanoylimidazoles in 0.1 *M* LCl at 30°.[7] General base catalysis of acetylimidazole hydrolysis by triethylenediamine has an isotope effect of 2.1 (25°).[8] These similarities suggest a commonality of mechanism, and the rather narrow range of isotope effects for various substrate structures is not expected for low primary isotope effects, which should be sensitive to structure even if there is a broad maximum in the region of higher isotope effects.[13]

Strong evidence exists that these reactions are GB catalyses of addition of water to the carbonyl group of the substrate.[8–10] Reactions of BMI^+ cannot reasonably involve catalysis of leaving group departure; therefore, the k''_0 mechanism does not involve such catalysis, either. The similarities detailed above suggest that k_0 and probably k_{LO^-} (which falls on the Brønsted plot) also do not involve catalysis of leaving group departure. Jencks and coworkers have presented a further, very persuasive case (based primarily on data for amine nucleophiles), which we cannot reiterate here, that weak nucleophiles having removable protons on the basic atom will react in GB catalyzed reactions with acetylimidazole and acetylimidazolium ion by a mechanism involving rate-determining addition of the nucleophile to the carbonyl, with GB acting to aid removal of the proton from the nucleophilic atom. They conclude that the tetrahedral intermediate thus formed (*e.g.*, in GB catalyzed hydrolysis, **4**) would eject imidazole extremely rapidly, possibly even being too unstable to



4

exist as a discrete intermediate—at least in the case of amine bases and *imidazolium* as leaving group. Considerable justification for this type of mechanism comes from symmetry considerations:[8–10] the question of which transition state type—nucleophilic attack or leaving group departure—is rate-determining should depend on the relative leaving group abilities of the nucleophile and the departing group, since, except for this relative difference, the reaction is the same in microscopic reverse as in the forward direction.

The question of *catalysis* of leaving group departure is distinct from the question of whether the tetrahedral intermediate may really correspond to a transition state—an antisymmetric stretching motion of which would be the reaction coordinate. This would correspond to an essentially tetrahedral version of an SN2 reaction, and it has been suggested that such a concerted displacement may occur with acetylimidazole, based on the observation of nearly equal *nucleophilic* Brønsted β values, near unity, for entering and leaving groups in such reactions.[20,21] Since the β value for the overall reaction is 1.7, however, we believe it is reasonable to assume that the observed broad range of "type II" mechanisms[20,21] reflects transition states closely resembling a tetrahedral intermediate with finite lifetime, which would have nearly the same β value whether formation or breakdown of the intermediate were rate determining. These β values could also be equal for a broad range of reactions if $\beta_{entering} = \beta_{leaving} \cong 1.7/2 = 0.85$, which is near the observed values. This constancy over a broad range is justifiable on the basis of Hammond's postulate,[22] if transition-state geometries all closely resemble the tetrahedral intermediate. Furthermore, some degree of cancellation of parallel effects on RP character (reactant-like: product-like character) is expected in such transition states, since the nucleophile or leaving group, whichever is *not* involved in the rate-determining reaction coordinate motion, is attached to a central carbon atom between a π-reacting bond (the C=O bond) and the σ-reacting bond of the carbonyl carbon atom to the nucleophile or leaving group.[23] Existence of a true tetrahedral intermediate seems likely, based on the general experience that structures having a good Lewis representation generally exist in that form (or as a resonance hybrid involving that form) as stable equilibrium nuclear geometries with finite lifetimes.

The above observations and interpretations lead us to suggest a unified mechanism for hydrolysis and GB

(17) F. G. Bordwell and W. J. Boyle, Jr., *J. Amer. Chem. Soc.*, **93**, 511 (1971).
(18) A. J. Kresge, *J. Amer. Chem. Soc.*, **92**, 3210 (1970).
(19) The complications mentioned previously[11] indicate that the values for *p*-nitrobenzoylimidazole are in the same range, for k_0, $\cong 2$, k_{GB}, $\cong 3$ (25°); the value of 5.6 estimated previously[18a] is too high.
(20) A. R. Fersht and W. P. Jencks, *J. Amer. Chem. Soc.*, **92**, 5442 (1970).
(21) W. P. Jencks, *Chem. Rev.*, **72**, 705 (1972).
(22) G. S. Hammond, *J. Amer. Chem. Soc.*, **77**, 334 (1955).
(23) E. R. Thornton, *J. Amer. Chem. Soc.*, **89**, 2915 (1967).

catalyzed hydrolysis of acetylimidazole derivatives. We shall discuss this mechanism in terms of transition-state theory, assuming thermally (Boltzmann) equilibrated systems.[24]

Coupling of proton motions with those of heavy atoms is generally avoided.[13] This generalization appears to be valid *even if the potential energy surface for nuclear motions is such that coupled motions appear to be favorable.* For example, HDO has essentially one O–H and one O–D stretching vibration, while H_2O (and D_2O) has coupled, symmetric and antisymmetric, stretches, even though isotopes move on the same, essentially identical potential energy surface. The fact is that nuclei of different masses do not follow the same normal coordinates, even on the same surface. Either we must examine these motions, and recognize that they do not precisely follow the valleys of the surface, or we must replot the surface using mass-weighted coordinates: but then H_2O and HDO will have somewhat different surfaces.

The mechanism of GB catalysis then presents a problem. In the present case, the substantial Brønsted β values indicate substantial proton transfer, yet clearly there is also (in fact, primarily) rate-determining attack of the nucleophile. We might therefore expect a concerted mechanism involving both heavy atom and proton motion in the transition state,[13,21] yet we see that this is unlikely because of the different masses. Also, the observed isotope effects seem difficult to explain as low, primary isotope effects, as discussed above. It is furthermore difficult to explain isotope effects in the range of 2–3 as secondary isotope effects arising from multiple causes when we note their similarity for catalytic terms of various types.

The most satisfactory explanation is a secondary effect contributed largely by *one* proton caught in a strong, approximately symmetrical (half-transferred) hydrogen bond at the transition state, so that the reaction coordinate motion is primarily nucleophilic attack of water on the carbonyl carbon atom. This kind of mechanism has been convincingly demonstrated for intramolecular GB catalysis by Schowen,[14] using H_2O–D_2O mixtures.

If proton transfer is not part of the reaction coordinate motion, and if formation of **4** is rate determining, one might expect that **4** would be preceded by another tetrahedral intermediate with the symmetrical hydrogen bond to the GB, which would then rapidly decompose into **4** by a transition state with a reaction coordinate consisting of H transfer to GB. While there is no evidence on this matter, occurring as it does after the suggested rate-determining step, and while it is possible to avoid such a seemingly unlikely situation as a strong, symmetrical hydrogen bond between oxygen and oxygen or nitrogen in an *intermediate* by suggesting a curved valley in the potential energy surface, we would like to suggest a further interesting possibility: coupling or tortuously curved valleys may be avoided by the hypothesis, generally not recognized as a possibility, of two *consecutive transition states.* In the present case, the first (rate-determining) transition state would be as suggested above.[14] The second transition state would result directly from the reaction coordinate motion of the first, giving a structure with a completed O–C bond and with H still "half-transferred;" the reaction coordinate motion of this new transition state structure would be H transfer. In this way, the heavy atom motions could remain essentially uncoupled from the H transfer. The second transition state would, it can be seen from its reaction coordinate motion, be the transition state for GB catalyzed removal of H from the tetrahedral intermediate $T^{\pm}$, formed by *uncatalyzed* addition of water to the carbonyl. The two consecutive transition states would avoid the probably very high-energy—if it exists at all—transition state for direct formation of the probably very unstable[8–10] intermediate $T^{\pm}$. Furthermore, the difficulty in superimposing a curved valley and a transition state for GB catalyzed removal of H from $T^{\pm}$, both in nearby regions of the energy surface, would be avoided.

The possibility of consecutive transition states arises from the orthogonality of the normal vibrations. The heavy-atom motion which is the reaction coordinate of the first transition state is simply a corresponding normal vibration (with positive restoring force) of the second transition state. The H transfer which is the reaction coordinate of the second transition state is simply a corresponding normal vibration of the first transition state. If one imagines a potential energy space in which the heavy-atom motion (approximately, O–C distance) is plotted in an energy-contour diagram against B–H distance (a measure of H transfer), then the two transition states appear as saddle points at right angles (Figure 3).

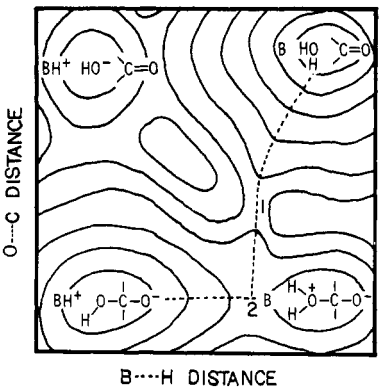


Figure 3. Schematic potential energy surface illustrating two consecutive transition states (**1** and **2**) along reaction pathway (---), with orthogonal reaction coordinates. Note that **2** breaks down equally into the intermediates at the lower right and left, but that equilibrium between these two intermediates is rapidly established relative to reversal through **1**.

(24) A distinction should be drawn between systems significantly "not at equilibrium," which is of course a property of products, and potentially of intermediates, in any rate process not yet at equilibrium, and systems in which "nonequilibrium solvation" and the like would be involved. In the former case, each *transition* state is in equilibrium with its immediate precursor, and unstable intermediates will be present in steady-state concentrations. In the latter case, lack of Boltzmann equilibrium of the transition state itself is suggested. While "nonequilibrium proton transfer" has been demonstrated, this is of the former type.[8] Although it is true that solvent molecules may not be able to "follow" fast reaction coordinate motions such as proton transfers, that fact is not a demonstration of the existence of Boltzmann nonequilibrium of transition-state populations, but merely of uncoupled motions of light and heavy nuclei[13] in the normal vibrations of a certain equilibrium nuclear geometry (which happens to be a transition state), a perfectly "respectable" equilibrium phenomenon. Therefore, Boltzmann nonequilibrium is highly suspect, and we feel probably does not occur in solution where there are very frequent, equilibrating collisions. It should also be emphasized that transition states are well-defined equilibrium nuclear geometries (zero forces on nuclei), and questions of whether "absolute rate theory" is valid in detail are only questions of how precisely the measured rate constants reflect the true structure of the transition state.

Amine–water hydrogen-bonded complexes have been shown to be explicitly involved in proton exchange reactions.[25] We suggest that the same type of mechanism applies for the analogous reactions of hydrogen-bonded complexes of water with the electrophilic acylimidazole derivatives (rather than, in the former case, the electrophilic hydronium ion). We rely on the similarity of isotope effects (Tables II and IV) and the Brønsted correlations.[8] Accordingly, the mechanisms k_0, k'_0, and $k_{L_3O^+}$ (k''_0) all involve a second water molecule as the GB catalyst for addition of water to the acyl group, k'_{GB} (and presumably k_{GB}) involves *N*-methylimidazole (or imidazole) as the GB catalyst, and k_{LO^-} involves LO^- as the GB catalyst. The distinction between k_{LO^-} and k'_{LO^-} is subtle, in that the two mechanisms, GB catalyzed water attack ($LO^- \cdots \overset{\frown}{LOL}$) and direct nucleophilic attack by lyoxide ($LOL \cdots \overset{\frown}{O-L}$), differ only in the position of a single proton within a hydrogen-bonded complex, in which proton transfer should be rapid; however, k'_{LO^-} appears to be best represented as direct nucleophilic attack, in contrast with k_{LO^-} and the others.

The Brønsted correlation does not provide evidence for k'_{LO^-}, since the correlation is for acetylimidazolium ion (not *N*-methyl), and the fact that the lyoxide term is "too fast" may reflect the occurrence of the kinetically equivalent reaction of water with acetylimidazole. The observed isotope effects on k'_{LO^-} are compelling evidence in this case, since they are inverse, are in the range expected for nucleophilic attack by lyoxide (deuteroxide being a stronger base than hydroxide by a factor of *ca.* 2),[13] and show a clear substituent dependence expected[22,23] for more product-like transition states with electron-releasing substituents.

Interpretation of k_{LO^-} as a GB mechanism is indicated by the fact that it falls on the Brønsted plot and by the very different isotope effects observed in comparison with k'_{LO^-}. Inverse isotope effects seem essentially required for simple nucleophilic attack. Diffusion-controlled LO^- attack, which should have a normal isotope effect in the range observed,[26] is ruled out as a mechanistic feature by the fact that it does not appear in the case of k'_{LO^-}, which would of necessity be faster than k_{LO^-}.

The GB mechanism not involving rate-determining proton transfer might be expected to lead to relatively constant isotope effects, as outlined above. The observed effects, *ca.* 2.3 for k_0, 2.5 for k'_0, 2.7 for k'_{GB}, and (estimated) 2.4 for k''_0 are remarkably similar. In this mechanism, assuming no secondary contribution from the second proton of the nucleophilic water molecule as a result of adjusting the O···C bond making to correspond to the B···L bond making,[14] there are two main factors which might lead to variations in isotope effects: the tightness or looseness of the B···L···O hydrogen bond, and the secondary isotope effects which might be contributed by isotopic substitution in B. It would be speculative to try to use these factors to explain in detail the observations, which we consider to differ by only small amounts. There may be an effect which would keep the secondary effect of water as GB lower than might be expected on the basis of the degree of proton transfer: for small species such as water, introduction of new bending vibrations in going to the transition state (favoring D over H) may lead other vibrational effects, in that these bends may be quite strong (have a substantial force constant, approaching product-like, already) at the transition state.[27] It is clear that the isotope effects on k_{LO^-} do not approach 2.3, even if one makes a correction (*ca.* $2^{1/2}$) for the extra secondary effect of a half-neutralized lyoxide ion (deuteroxide a stronger base than hydroxide). It is possible that the above extra effect of new bending vibrations, which ought to be especially important in the case of the diatomic lyoxide ion,[28] accounts for the discrepancy, or perhaps there is some special effect associated with the merging of the GB and direct nucleophilic attack mechanisms.

We have interpreted the k_0 mechanism as involving GB catalysis on the basis of isotope effect similarities. However, it will be recalled that this mechanism is "too fast" for the Brønsted correlation in the case of acetylimidazole. This discrepancy does not indicate the kinetically equivalent LO^- attack on acetylimidazolium ion, since that was "too fast" for the corresponding Brønsted plot also.[8] Possibly a *third* mechanism is indicated, but on the basis of experimental similarities, we believe that the mechanism is GB catalysis, noting also that there may be a special circumstance involving essentially complete O···C bond making at the transition state, which could influence the observed rate in this case, where the mechanism does not involve rate-determining proton transfer.

Clear answers to many of the above points about the exact origin of the isotope effects could be provided by studies in H_2O–D_2O mixtures;[13,14,29–31] however, a careful assessment of attainable precision would be essential. One interesting study of "water-catalyzed" reactions (of nitramide and acetic anhydride) in L_2O mixtures led to the conclusion that the transition states involved L_3O^+ catalysis (at carbonyl oxygen) of nucleophilic attack of LO^-, *i.e.*, involved proton positionings resembling to some partial extent L_3O^+ and LO^- (such studies can of course only define the proton characteristics, and not their location with respect to specific heavy atoms).[32] The transition state model we are proposing may also resemble these proton characteristics; there could be some L_3O^+ character in the GB water molecule, and some LO^- character in the nucleophilic water molecule, at the transition state. It seems possible that acetic anhydride, at least, is proceeding by the GB mechanism.

It is not unreasonable that chymotrypsin also uses this mechanism; $k_{H_2O}/k_{D_2O} = 2.4$ for hydrolysis (deacetylation) of acetylchymotrypsin.[33,34] In this case, the mechanism would consist of rate-determining GB catalysis, by the imidazole group of His-57, of the nucleo-

(25) E. Grunwald and E. K. Ralph, *Accounts Chem. Res.*, **4**, 107 (1971).
(26) R. E. Barnett and W. P. Jencks, *J. Amer. Chem. Soc.*, **91**, 6758 (1969).
(27) *Cf.* R. L. Schowen, *Progr. Phys. Org. Chem.*, **9**, 275 (1972), p 324.
(28) A. J. Kresge and Y. Chiang, *J. Amer. Chem. Soc.*, **91**, 1025 (1969).
(29) A. J. Kresge, *Pure Appl. Chem.*, **8**, 243 (1964).
(30) V. Gold, *Advan. Phys. Org. Chem.*, **7**, 259 (1969).
(31) E. K. Thornton and E. R. Thornton in "Isotope Effects in Chemical Reactions," C. J. Collins and N. S. Bowman, Ed., Van Nostrand-Reinhold, New York, N. Y., 1970, Chapter 4.
(32) B. D. Batts and V. Gold, *J. Chem. Soc. A*, 984 (1969).
(33) E. Pollock, J. L. Hogg, and R. L. Schowen, *J. Amer. Chem. Soc.*, **95**, 968 (1973).
(34) A. J. Kresge, *J. Amer. Chem. Soc.*, **95**, 3065 (1973).

philic attack of water on acetyl group, to form a tetrahedral intermediate analogous to **4** (but with Ser-195 as the leaving group rather than imidazole). If the analogy holds, then the rate-determining step would not involve proton transfer as part of the reaction coordinate motion, but instead would involve a symmetrical hydrogen bond and rate-determining nucleophilic attack by water.[14] Such a strong hydrogen bond may provide for a stable "charge-relay" system.

Experimental Section

Materials. Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. Ultraviolet spectra were taken on Perkin-Elmer Model 202 and Cary 16 spectrophotometers.

Acetic acid, sodium acetate, and acetic anhydride supplied by J. T. Baker Chem. Co. (Baker Grade) were used for acidic buffer solutions without further purification. Imidazole (Eastman White Label) and *N*-methylimidazole (Aldrich) were recrystallized or redistilled before use. Tris(hydroxymethyl)aminomethane (Aldrich, Ultrapure Grade 99.9 + %) was used for basic buffer solutions without further purification. *p*-Chlorobenzoyl chloride (Aldrich), benzoyl chloride (Matheson Coleman and Bell), *p*-methylbenzoyl chloride (Eastman White Label), and *p*-methoxybenzoyl chloride (Eastman White Label) were used in the procedures described below for the preparation of *N*-benzoylimidazoles and *N*-methyl-*N'*-benzoylimidazolium ions. Acetonitrile (Eastman, Spectrograde) was distilled from phosphorus pentoxide before use. Distilled protium oxide was redistilled from alkaline potassium permanganate and kept in a carbon dioxide free atmosphere. Deuterium oxide, 99.8% D, was purchased from Stohler Isotope Chemicals.

Amine Hydrochloride Salts. Imidazolium chloride, *N*-methylimidazolium chloride, and tris(hydroxymethyl)methylammonium chloride were prepared according to a modification of the method of Dedichen[35] by mixing an amount of the appropriate amine with an equivalent amount of 1 *N* hydrochloric acid. The resulting solutions were evaporated to dryness on a steam bath. The residues were recrystallized to constant melting point from absolute ethanol–ether and were stored over anhydrous $CaCl_2$ in a desiccator, except the very hygroscopic *N*-methylimidazolium chloride, which was kept over P_2O_5 in a vacuum desiccator.

***N*-Benzoylimidazoles** (*p*-Cl, *p*-H, and *p*-CH_3) were prepared essentially as described previously.[1a,36] In 200 ml of warm benzene, which was freshly distilled from sodium, was dissolved 0.02 mol of imidazole. To this solution was slowly added about 30 ml of a solution of 0.01 mol of substituted benzoyl chloride in benzene. After having been stirred overnight at room temperature, the reaction mixture was filtered to remove imidazolium chloride in a dry-nitrogen containing glove bag (I^2R Industries), and the filtrate was freeze dried to remove benzene. The crude products were recrystallized at least twice from cyclohexane or petroleum ether: mp 85–87° (lit.[16] 85–86.5°), λ_{max} 252 nm in H_2O (lit.[16] 250 nm) for *p*-chlorobenzoylimidazole; mp *ca.* 20° (lit.[37] 18–19°), λ_{max} 242 nm (lit.[16] 243 nm) for *N*-benzoylimidazole; mp 69–70.5° (lit.[16] 69–71°), λ_{max} 253 nm (lit.[16] 253 nm) for *N*-*p*-methylbenzoylimidazole.

***N*-Methyl-*N'*-benzoylimidazolium chlorides** (*p*-H, *p*-CH_3, and *p*-OCH_3) were prepared according to a modification of the method of Wolfenden and Jencks.[5] *N*-Methylimidazole (2.5 mmol) in 15 ml of anhydrous ether was dropped slowly into a solution (2.5 mmol) of a substitued benzoyl chloride in 15 ml of anhydrous ether at room temperature, with vigorous magnetic stirring, in a drybox. The white precipitate was filtered and washed with 150 ml of anhydrous ether. The ether slurry of the precipitate was transfered in a drybox to a desiccator containing P_2O_5, and the ether was removed by evacuation of the desiccator. The products were extremely hygroscopic white solids. *N*-Methyl-*N'*-benzoylimidazolium chloride had mp 29.5–31°, λ_{max} 245 nm in H_2O.

Anal. Calcd for $C_{11}H_{11}N_2OCl$: C, 59.32; H, 4.94; N, 12.58; Cl, 15.95. Found: C, 59.47; H, 5.20; N, 12.39; Cl, 15.84.

N-Methyl-*N'*-*p*-methylbenzoylimidazolium chloride had mp 90–92°, λ_{max} 257 nm in H_2O.

Anal. Calcd for $C_{12}H_{13}N_2OCl$: C, 60.89; H, 5.50; N, 11.84; Cl, 15.01. Found: C, 60.82; H, 5.58; N, 11.79; Cl, 15.04.

N-Methyl-*N'*-*p*-methoxybenzoylimidazolium chloride had mp 92–94°, λ_{max} 293 nm in H_2O.

Anal. Calcd for $C_{12}H_{13}N_2O_2Cl$: C, 57.03; H, 5.15; N, 11.09; Cl, 14.06. Found: C, 57.07; H, 5.20; N, 11.02; Cl, 14.11.

All reactants, *N*-benzoylimidazoles and *N*-methyl-*N'*-benzoylimidazolium chlorides, were dissolved in freshly distilled acetonitrile and stored in sealed melting point capillaries to prevent exposure to the atmosphere for any length of time.

Preparation of Buffers in H_2O and D_2O. All buffers were prepared essentially as described previously.[1a,36] Components of buffers in D_2O with exchangeable protons, imidazole, imidazolium chloride, tris(hydroxymethyl)aminomethane and its hydrochloride, and *N*-methylimidazolium chloride, were preexchanged three times in D_2O prior to being used in the preparation of buffers (dissolved in D_2O and evaporated down to very small volume), and recrystallized from an appropriate absolute organic solvent. Deuterated acetic acid was obtained directly from hydrolysis of acetic anhydride in D_2O at room temperature.[38] Calculation utilizing the rate constant for hydrolysis indicates that the 24 hr or longer period after preparation of the buffer solution would ensure complete hydrolysis. For each pL level studied, a series of three buffers varying in component concentration for kinetics of BI and of four buffers varying in *N*-methylimidazole concentration for BMI^+ was made up. A concentrated stock buffer about 0.5 *M* in one component was prepared, and diluted with 0.5 *M* KCl solution to yield the additional three or four buffers of a given series. All prepared buffer solutions were adjusted to ionic strength 0.5 with KCl.

pH Measurement and Adjustment. pH measurements were made with a Radiometer pH meter, Type 25, extended by a scale expander, Type pHA925, using a Radiometer G202B glass electrode and a K401 calomel electrode, and calibrated with National Bureau of Standards buffers. Four standard buffers of pH 3.999, 6.900, 7.448, and 9.276 at 15° were used.[39] When the glass electrode was used in D_2O buffers, a correction factor of 0.407[40,41] at 15° was added to the scale reading to give the true pD. The accuracy of the measured pL values is taken to be ±0.005 pL unit. The temperature of the water bath, Aminco (cat. no. 48605) equipped with a mercury thermoregulator (Aminco cat. no. 4-204), was maintained at 15.08 ± 0.05°.

A deviation from the pL of the stock buffer was observed for the diluted buffers. The pL of these diluted buffers was brought to that of the stock buffer by the addition of a known concentration of one component of the buffer, and the concentrations of components in buffers reflected these additions. The lyoxide ion activities of prepared buffers were calculated from water constants, $K_{H_2O} = 4.505 \times 10^{-15}$ [42] and $K_{D_2O} = 5.768 \times 10^{-16}$ [42] at 15°, which were not corrected for ionic strength (0.5).[43] Small salt effects are expected to be equal for H_2O and D_2O and thus cancel in the the computation of isotope effects.

Kinetic measurements were carried out using a Cary Model 16 spectrophotometer in conjunction with an electric timer (Precision Scientific Co.), by following the decrease in absorption of the reactants with time. Buffer was pipetted into silica cells (Beckman Instruments, Inc., 1 cm path length), which were capped with rubber serum caps in a nitrogen filled glove bag. After the temperature of the cell contents was equilibrated at least 30 min at 15.08°, an acetonitrile solution of the reactant being studied was injected by means of a 50-μl syringe. The volume per cent of acetonitrile in the resulting acetonitrile–buffer solution ranged from 0.2 to 0.4%, and the concentration of reactant in acetonitrile–buffer solution was *ca.* 5×10^{-5}–1.2×10^{-4} *M*. The following wave-

(35) G. Dedichen, *Ber.*, **39**, 1831 (1906).
(36) W. Palaitis, Ph.D. Dissertation in Chemistry, University of Pennsylvania, 1969.
(37) O. Gerngross, *Ber.*, **46**, 1908 (1913).
(38) V. Gold, *Trans. Faraday Soc.*, **44**, 506 (1948).
(39) R. G. Bates, *J. Res. Nat. Bur. Stand., Sect. A*, **66**, 179 (1962).
(40) A. K. Covington, M. Paabo, R. A. Robinson, and R. G. Bates, *Anal. Chem.*, **40**, 700 (1968).
(41) I. R. Bellodono and P. Beltrame, *J. Chem. Soc. B*, 620 (1969).
(42) A. K. Covington, R. A. Robinson, and R. G. Bates, *J. Phys. Chem.*, **70**, 3820 (1966).
(43) This correction cannot readily be applied, since it reflects a salt effect which cannot be independently measured. The present procedure simply defines hydroxide ion activity as the thermodynamic K_w/a_{H^+}, and thus any salt effect would amount simply to a multiplicative activity coefficient effect on k_{LO^-}. That is, activities rather than concentrations of L_3O^+ and of LO^- have been used in eq 1 and 2 for the evaluation of the rate constants.

lengths were used for kinetics: for BI, *p*-Cl, 260 nm; *p*-H, 250 nm; *p*-CH_3, 260 nm; for BMI^+, *p*-H, 250 nm; *p*-CH_3, 265 nm; *p*-OCH_3, 295 nm. Within experimental error the reaction was found to follow good pseudo-first-order kinetics for at least 4 half-lives.

A least-squares treatment of each set of kinetic data was carried out by the use of a modified version of the computer program LSKIN1.[44]

Acknowledgments. Support by the U. S. Public Health Service, the National Science Foundation, and the University of Pennsylvania Computer Center is gratefully acknowledged.

(44) D. F. De Tar and C. E. De Tar in "Computer Programs for Chemistry," Vol. I, D. F. De Tar, Ed., W. A. Benjamin, New York, N. Y., 1968, Chapter 6.

General Acid and General Base Catalysis of the Methoxyaminolysis of 1-Acetyl-1,2,4-triazole[1]

J. P. Fox and W. P. Jencks*

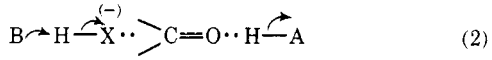
Contribution No. 934 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154. *Received October 2, 1973*

Abstract: Brønsted plots for general acid and for general base catalysis of the methoxyaminolysis of 1-acetyl-1,2,4-triazole are nonlinear, with equal limiting rate constants for strong acid and strong base catalysts. The data are consistent with mechanisms in which the rate-determining step is (I) a simple proton transfer that is diffusion controlled in the favorable direction or (II) diffusion-controlled encounter of an intermediate $T^{\pm}$ with the catalyst for strong catalysts and the breakdown of $T^{\pm}$ or T^0 for weak catalysts. Estimated p*K* values of the intermediate and structure–reactivity considerations favor mechanism II. The change from a mechanism that gives a linear Brønsted plot for kinetic general acid catalysis of the hydrazine–acetylimidazole reaction is consistent with the change in structure of the reactants. Rate constants for the reactions of acetyltriazole with water, buffers, and amines are reported and compared with those for the corresponding reactions of acetylimidazole.

We have been concerned with the problem of the driving force, detailed mechanism, and "concertedness" of general and specific acid–base catalysis of acyl transfer and related reactions. The experiments reported here were originally carried out in an attempt to detect concerted, bifunctional acid–base catalysis of an acyl transfer reaction, in which both an acid and a base catalyst are present in the transition state and give rise to a term in the rate law containing both of these species (eq 1). Examples of such catalysis in aqueous solution

$$v = k[\text{H–X}][\text{—C(=O)Y}][\text{HA}][\text{B}] \qquad (1)$$

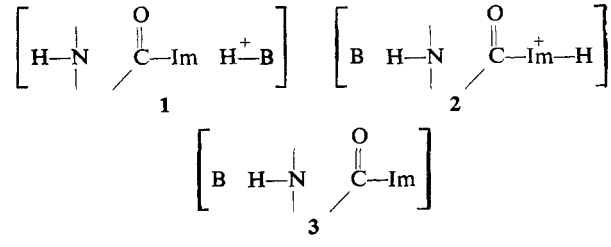
are rare or nonexistent and we suspected that one reason for this is that catalysis at one end of a system is likely to decrease the importance of catalysis at the other end. For example, a base that removes a proton from an attacking nucleophile H–X increases the effective basicity of the nucleophile (eq 2), and, since it is known that



(2)

carbonyl addition reactions are subject to concerted general acid catalysis only if the attacking nucleophile is a weak base, general base catalysis will tend to decrease the importance of general acid catalysis so that concerted acid–base catalysis becomes insignificant.[2]

Acetylimidazole and related compounds are useful models for studying acyl transfer reactions of amides because of their high reactivity and convenient ultraviolet absorption. By the use of *N*-methylacetylimidazolium ion as a model for acetylimidazolium ion, evidence has been obtained that general acid catalysis of the reaction of acetylimidazole with strongly basic amines involves proton donation to the leaving imidazole group (**1**), whereas this catalysis with weakly basic amines involves the kinetically equivalent general base catalysis of a reaction with the acetylimidazolium ion (**2**). Furthermore, it is known that the aminolysis of free acetylimidazole, with a much poorer leaving group, is subject to general base catalysis (**3**).[3] (The formulas **1**–**3** are shown only to suggest the relative locations of



the catalyst and reactants in the transition state, without any implication as to whether the formation or breakdown of a tetrahedral intermediate is rate determining or as to a detailed mechanism of catalysis.) We hoped to detect bifunctional acid and base catalysis of the reaction of 1-acetyl-1,2,4-triazole (**4**) with weakly basic amines on the basis of the following, rather naive, reasoning. General acid catalysis of leaving group expulsion from acetylimidazole is not observed with

(1) Supported by grants from the National Science Foundation (GB 5648) and the National Institute of Child Health and Human Development of the National Institutes of Health (HD 01247). J. F. was a Research Fellow of the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health (AM 36161).

(2) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, pp 196, 198.

(3) D. G. Oakenfull, K. Salvesen, and W. P. Jencks, *J. Amer. Chem. Soc.*, **93**, 188 (1971).