Solvolysis Mechanisms. A Kinetic Study of the Hydrolysis and Imidazole-Catalyzed Hydrolysis of *p*-Methyl-, *p*-Chloro-, and *p*-Nitrobenzoylimidazole in H_2O and of *p*-Nitrobenzoylimidazole in D_2O[1-3]

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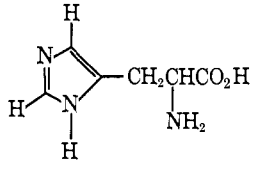
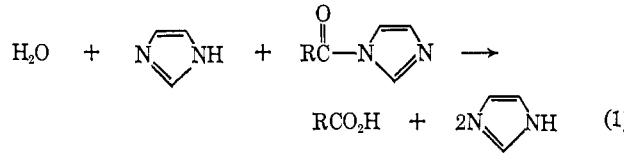
Abstract: The kinetics of the hydrolysis of *p*-methyl-, *p*-chloro-, and *p*-nitrobenzoylimidazole in H_2O–imidazole buffer solutions and of *p*-nitrobenzoylimidazole in D_2O–imidazole buffer solutions was studied at 25.07 ± 0.05° and μ = 0.0400 ± 0.0006 *M*. The rates of hydrolysis were first order in acylimidazole concentration. A linear dependence of the observed rate constants on the imidazole free base concentration was demonstrated for each compound at each pL (L = H or D), giving intercept k_{L_2O} and slope k_{ImT}/Im. The pL dependence of k_{L_2O} in the pL regions studied (pH 6.9–7.8, pD 7.5–8.1) is given by $k_{L_2O} = k_1 + k_2[LO^-]$. The isotope effects observed for k_1 and k_2 in the reaction of *p*-nitrobenzoylimidazole were $k_1(H_2O)/k_1(D_2O) = 5.57 \pm 0.51$; $k_2(D_2O)/k_2(H_2O) = 1.47 \pm 0.16$. From Hammett plots for k_1 and k_2, ρ = 1.41 and 1.48, respectively. The imidazole catalysis appears to be pL dependent, although experimental scatter and the limited pL range presently available preclude precise analysis. The most consistent treatment of the data includes three terms, proportional to imidazole, to imidazolium, and to the product of imidazole and lyoxide concentrations. Estimates of these three imidazole-dependent terms were not made from k_{ImT}/Im, but by the (statistically less biased) direct fit of experimentally observed rate constants to the complete, five-term expression including k_1, k_2, and the three imidazole-dependent terms, using a least-squares computer program. In studies of imidazole catalysis, it seems unsafe to assume dependence entirely on free imidazole even in the region of neutrality, unless measurements are made at several pL values.

Study of the acid and base catalysis of chemical reactions, especially of carboxylic acid derivatives, has been undertaken with renewed vigor in the past few years because of the realization that a key part of the catalytic action of many enzymes is acid and/or base catalysis involving functional groups which are part of the protein structure.[5] Since the amino acid histidine has an imidazole side chain, catalysis by imidazole groups is a realistic possibility upon an enzyme. The



most likely reaction of imidazole with a carboxylic acid derivative is nucleophilic addition to the carbonyl carbon atom,[5] followed by formation of an acylimidazole, the latter compounds being rapidly hydrolyzed in aqueous solution. Imidazole is also known to catalyze hydrolysis by a general base mechanism, the clearest example being imidazole-catalyzed hydrolysis of acylimidazoles[6] (eq 1), for here nucleophilic attack by imidazole leads to no net reaction.



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As a step in obtaining precise substituent effects upon the catalytic terms in hydrolysis of acylimidazoles as well as kinetic isotope effects for H_2O *vs.* D_2O, we have investigated the solvolysis of *p*-methyl-, *p*-chloro-, and *p*-nitrobenzoylimidazole in H_2O–imidazole buffers and of *p*-nitrobenzoylimidazole in D_2O–imidazole buffers. Initially, we assumed that imidazole catalysis would be pH independent,[6] but our data, obtained at pH values near 7, indicate a complex pH dependence, possibly involving three imidazole-dependent terms as shown in eq 2, where k_{obsd} is the observed pseudo-first-order

$$k_{obsd} = k_1 + k_2[LO^-] + k_{Im}[Im] + k_{ImA}[ImL^+] + k_{ImB}[Im][LO^-] \quad (2)$$

rate constant at constant pL and imidazole concentration (L = H or D, depending on whether the solvent is H_2O or D_2O). We can now report k_1 and k_2 values with good precision, along with the total imidazole terms. Since experimental scatter and the limited pH range so far studied make it impossible to estimate k_{Im}, k_{ImA}, and k_{ImB} with acceptable precision,[3] we mention these terms only briefly, deferring discussion until further work[7] provides definitive values.

For acetylimidazole, the imidazole-catalyzed part of the rate is independent of pH,[6] *i.e.*, k_{ImA} and k_{ImB} are small. For other aliphatic N-acylimidazoles, it has been found that there is a k_{ImA} as well as k_{Im} term.[8]

(1) Previous paper in this series: G. J. Frisone and E. R. Thornton, *J. Am. Chem. Soc.*, 90, 1211 (1968).

(2) Supported in part by the National Science Foundation (Grant GP-6047).

(3) For further details, *cf.* J. P. Klinman, Ph.D. Dissertation in Chemistry, University of Pennsylvania, 1966; submitted to University Microfilms.

(4) National Institutes of Health Predoctoral Fellow, 1964–1966; National Science Foundation Summer Research Fellow, 1964.

(5) For reviews of the extensive literature in this area, see T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. 1, W. A. Benjamin, Inc., New York, N. Y., 1966; M. L. Bender, *Chem. Rev.*, 60, 53 (1960); S. L. Johnson, *Advan. Phys. Org. Chem.*, 5, 237 (1967).

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The k_2 term for five substituted benzoylimidazoles has been measured in H_2O at 30°,[9] giving results which are reasonable in comparison with our results at 25°. Kinetic studies of acylimidazole hydrolysis at low pH have been carried out, giving values for a $k_3[H_3O^+]$ term[9,10] which was not appreciable at the pH values we have studied. A term in $[LO^-]^2$ has been reported for hydrolysis of anilides,[11] and also terms in [glycine][HO^-] and [glycinate][HO^-].[11b] At the pH values we have studied, the $[LO^-]^2$ term was not appreciable; however, the latter two terms do provide a possible analogy for our k_{ImB} term (eq 2).

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected, as are boiling points. Elemental analyses were performed by Micro-Analysis, Inc., Wilmington, Del.

Imidazole. Imidazole was purchased from Sigma Chemical Corp. (grade II), and was recrystallized once from benzene, mp 89–90° (lit.[12] 89–90°).

Imidazolium Nitrate. This compound was prepared, according to the method of Dedichen,[13] by mixing 5 g (0.074 mol) of imidazole (Baker), which had been recrystallized twice from benzene, with 75 ml (0.074 mol) of 1 *M* nitric acid. The volume of the solution was reduced on a steam bath yield, 61% after one recrystallization from absolute ethanol–ether. Recrystallization to constant melting point gave mp 117–118° (lit.[13] 118°).

Sodium Nitrate. Sodium nitrate (Allied Chemical Co.) was used without further purification.

H_2O. Distilled protium oxide was redistilled from alkaline potassium permanganate. This doubly distilled water was boiled for 15 min and cooled in a carbon dioxide free atmosphere immediately before being used.

D_2O. Deuterium oxide, >99.7% pure, was purchased from General Dynamics Corp.

N-Benzoylimidazoles. All N-benzoylimidazoles were prepared according to a modification of the method of Caplow and Jencks.[10b]

N-*p*-Nitrobenzoylimidazole. Imidazole (Baker, 1.36 g, 0.02 mol) which had been recrystallized once from benzene was dissolved in approximately 250 ml of warm benzene, which had been freshly distilled from sodium. *p*-Nitrobenzoyl chloride (1.86 g, 0.01 mol) was added slowly to this solution. After having been stirred overnight at room temperature, the reaction mixture was filtered to remove imidazolium chloride, and the filtrate was freeze dried to remove benzene yield 70%. Two recrystallizations from cyclohexane gave N-*p*-nitrobenzoylimidazole, mp 120.5–122° (lit.[10b] 120–122.5°). An ultraviolet spectrum (taken in spectroquality acetonitrile on a Perkin-Elmer Model 202 spectrophotometer at 25°) gave λ_{max} 259.5 mμ (lit.[10b] 261 mμ (H_2O)).

N-*p*-Chlorobenzoylimidazole. *p*-Chlorobenzoyl chloride (Fisher, 1.75 g, 0.01 mol) was added to a solution of 1.36 g (0.02 mol) of imidazole (Baker), which had been recrystallized once from benzene, in approximately 200 ml of benzene. The reaction mixture was stirred overnight at room temperature, then filtered and flash-evaporated (while immersed in a water bath whose temperature was kept close to room temperature) to remove the benzene. Recrystallization from petroleum ether (bp 74–104°) twice gave mp 85–86° (lit.[10b] 85–86.5°). An ultraviolet spectrum taken in spectroquality tetrahydrofuran showed λ_{max} 247 mμ (lit.[10b] 250 mμ (H_2O)).

N-*p*-Methylbenzoylimidazole. *p*-Toluoyl chloride (Research Organic Co., 1.41 g, 0.01 mol) was used in a procedure otherwise identical with that for N-*p*-chlorobenzoylimidazole. Two recrystallizations from petroleum ether (bp 74–104°) gave mp 70–70.8° (lit.[10b] 69–71°). An ultraviolet spectrum taken in spectroquality acetonitrile showed λ_{max} 253 mμ (lit.[10b] 253 mμ (H_2O)).

Acetonitrile. Spectroquality acetonitrile (Matheson Coleman and Bell) was distilled from phosphorus pentoxide before use, bp 82° (lit.[12] 81.6°).

Handling of Substituted Benzoylimidazoles. Since substituted benzoylimidazoles are hydrolyzed when exposed to the atmosphere for any length of time, a solution of each of these compounds in acetonitrile was prepared and enough material for a single kinetic run was stored in a sealed melting point capillary, the sealed capillaries being stored in the refrigerator.

Preparation of Buffers in H_2O and D_2O. A series of imidazole buffers was prepared in H_2O at four different pH values, and in D_2O at four different pD values.[3] At least four buffer solutions of varying concentrations of imidazole (free base) and imidazolium ion were prepared at each pH. The concentration of imidazole (free base) in these buffers was never less than 0.01 *M* and never greater than 0.06 *M*.

All buffers were prepared in a nitrogen-filled drybox. Before D_2O buffers were prepared, the humidity in the drybox was reduced to zero by circulating the drybox atmosphere through an Electro-Dryer. All glassware which was used in preparing the D_2O buffers was dried for at least 2 hr in a 220° oven. The compounds which comprised the buffers, imidazole, imidazolium nitrate, and sodium nitrate were dried in an Abderhalden drying apparatus for 2–3 hr at 56° before use.

Although imidazole has one exchangeable proton and imidazolium nitrate has two, these compounds were not preexchanged in D_2O. Calculations utilizing literature rate constants[14] indicate that the 24 hr or longer period which elapsed between the time the buffer solutions were prepared and the time they were used for kinetic studies would ensure complete exchange of all exchangeable protons. The maximum contamination of the D_2O purity which could have resulted from this exchange was calculated for pD 7.503 ([Im] = 0.03520, [ImD^+] = 0.03940) to be 0.10% H atoms.

A stock imidazole buffer solution was prepared by transferring weighed samples of imidazole and imidazolium nitrate to a volumetric flask and bringing the solution to the line. This stock solution, once its ionic strength had been adjusted to 0.0400 ± 0.0006 with sodium nitrate, was used as the most concentrated buffer solution. Three other buffers were prepared by dilution of the stock solution before its ionic strength had been corrected. The ionic strength of these three buffers was also adjusted to 0.0400 and their pH was made equal to that of the most concentrated buffer solution. From a knowledge of the total concentration of imidazole present as acid and base, the pH or pD of a solution, and the value of pK_H = 7.052[15] (at 24.98° and μ = 0.040 *M*), and of pK_D = 7.552[15] (at 24.98° and μ = 0.040 *M*), the exact concentrations of imidazole and imidazolium ion were calculated from the equation

$$\mathrm{pH} - \mathrm{p}K = \log\left(\frac{\mathrm{Im}}{\mathrm{Im(TOT)} - \mathrm{Im}}\right)$$

where Im = concentration of imidazole free base and Im(TOT) = concentration of imidazole plus imidazolium ion. The concentrations calculated in this way differed in nearly all cases by 1% or less from the amounts of imidazole and imidazolium nitrate actually added to the solution. The activity of LO^- was calculated from pL and K_{L_2O},[15] for use in evaluating the pL dependence of the rates (Tables I and III).

pH Adjustment. pH measurements were made with a Radiometer pH meter, Type 25, extended by a scale expander, Type pHA-925 (pH meter 25 SE). The electrodes used were a Radiometer G202B glass electrode and K401 calomel electrode. When the glass electrode was used in D_2O solutions, a correction factor of 0.398[15] was added to the scale reading to give the true pD. Standardization of the pH meter was effected by the use of two National Bureau of Standards buffers, which were prepared according to literature specifications.[16] A phosphate buffer (pH 6.865) and a Borax buffer (pH 9.180) were chosen since they bracketed the pH range in which this work was done. The accuracy of these buffers, when properly prepared, is reported to be ±0.005 pH unit.[16] Since the limits of the Radiometer pH meter 25 SE are supposed to

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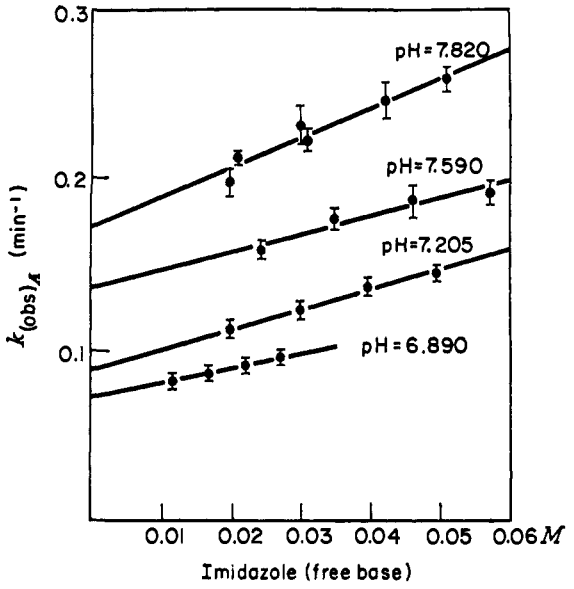


Figure 1. Relationship between average k_{obsd} and imidazole concentration for *p*-nitrobenzoylimidazole in H_2O, 25.07°, μ = 0.04 *M*.

be ±0.003 pH unit, pH readings were assumed to have an experimental error of ±0.005 pH unit.

The solution whose pH was being adjusted was pipetted into a jacketed electrode cell in the drybox. This cell was fitted with a ground-glass cap which at the time of titration was replaced (under a stream of N_2 for the D_2O buffers) with a ground-glass cover through which fit both electrodes. By circulation of water through the jacket of the electrode cell, temperature was maintained at 24.95 ± 0.02°. The electrode cell cover was relatively air tight; however, when D_2O buffer solutions were in the cell, the surrounding atmosphere was flushed with a steady stream of nitrogen. Also, in order not to expose D_2O buffer solutions unnecessarily to the atmosphere, after the pD of these solutions had been read and adjusted, the electrodes were detached from the meter and the electrode cell with the electrodes still intact was returned to the drybox. In the case of the H_2O buffer solutions, a ground cap, which had been flushed with nitrogen, was quickly inserted into the electrode cell as the cover with the electrodes was withdrawn. Once the electrodes had been detached from the pH meter, it was found necessary to restandardize the meter. When the pH of a buffer was too low, it was titrated with a solution of imidazole in H_2O or D_2O of known concentration,[3] while the pH was monitored. In the case of a buffer whose pH was too high, a weighed amount of solid imidazolium nitrate was added in slight excess, and the buffer solution was then back-titrated with the above-described imidazole solution.

Kinetic Measurements. Kinetic measurements were carried out using a Zeiss PMQII spectrophotometer in conjunction with an electric timer (Precision Scientific Co.), by following the decrease in absorption of the reactants with time. The kinetics was pseudo first order for all reactions studied; the rate constants obtained were linearly dependent on the imidazole concentration and were independent of the initial concentration of each benzoylimidazole for 1.5-fold changes in concentration. Since linear plots of $(A_t - A_0)$ *vs.* time were obtained in all cases, it was assumed that Beer's law was followed. Each kinetic run was followed at approximately 5% reaction intervals to two half-lives, and infinity points were read after ten half-lives.[3]

A temperature of 25.07 ± 0.05° was maintained by the use of a brass block into which both the reference and sample cell fit, and through which water was circulated by a Forma (Catalog No. 2095) constant temperature water bath equipped with a mercury thermoregulator (H. B. Instrument Co., Philadelphia, Pa., Catalog No. 7530). The temperature control in the sample cuvette was monitored with an iron-constantan thermocouple; the difference in potential between the solution in the cuvette and an ice–water bath was read on a Leeds and Northrup Potentiometer (Catalog No. 8686).

For each kinetic run 2 ml of the buffer solution under study was injected in the drybox into 3-ml reference and sample ultraviolet cells. The cells were capped with ground-glass stoppers or Teflon caps when H_2O buffers were being studied; rubber serum caps were used with D_2O buffers. After the cells had reached temperature equilibrium in the cell block (*ca.* 15 min), 3–6 μl of a solution of reactant in acetonitrile, as described above, was injected with a 10-μl syringe, giving initial optical densities of 0.3–0.9*A* (concentrations of reactants 1–6 × 10^{-5} *M*; acetonitrile 0.15–0.30% by volume of final solution). The following wavelengths were used for kinetics: *p*-nitro, 260; *p*-chloro, 252; *p*-methyl, 252 mμ (observed λ_{max} for products, 268, 244, 248 mμ, respectively).

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, which was written by De Tar[17] to calculate rate constants from first-order rate data. The program was designed to calculate the best value of the rate constant (*K*), the initial concentration (A_0), and the infinity concentration (A_{inf}) for a given set of data. For the calculations of the rate data in this study, however, A_{inf} was fixed at its experimentally observed value. It was hoped that variation of the infinity point, simultaneous with the variation of the other two parameters, would lead to more accurate values of the rate constants; since the infinity point is subtracted out of every experimental point, it is very important that it be known accurately. It was found, however, that in every case variation of the infinity point led to larger standard deviations in the rate constants and to much larger per cent deviations of rate constants from their average values. This phenomenon is understood statistically, since it is the squared deviations of A_{obsd} and A_{calcd} that are minimized and not any deviation in *K*.

Results

There is a relationship between the average values of the observed rate constants, k_{obsd}, and the imidazole concentration (free base), at any one pH, for all of the reactions studied. Typical results are illustrated graphically in Figure 1. It can be seen that k_{obsd} is linearly dependent upon the first power of the imidazole concentration. The slopes of the lines represent the contribution of kinetic terms containing the imidazole concentration to the over-all rate constant, referred to as k_{ImT}/Im. The intercepts of the lines, k_{L_2O}, represent all nonimidazole-containing rate terms. The values for the intercepts and slopes were obtained by a linear least-squares calculation;[18] the results are shown in Table I.

Table I. Values of k_{ImT}/Im and k_{L_2O} as a Function of pL (25.07°, μ = 0.04 *M*)

Compd	pL	k_{ImT}/Im, l. mol^{-1} min^{-1} (slope)	Std dev	k_{L_2O}, min^{-1} (intercept)	Std dev	10^6[LO$^-$], *M*
p-NO_2	6.890	0.815	0.189	0.0721	0.0011	0.0776
(H_2O)	7.205	1.10	0.146	0.0898	0.0016	0.163
	7.590	1.01	0.129	0.137	0.0016	0.389
	7.820	1.71	0.188	0.172	0.0022	0.667
p-Cl	6.890	0.256	0.0341	0.0116	0.00019	0.0776
	7.205	0.235	0.020	0.0135	0.00023	0.163
	7.590	0.211	0.012	0.0211	0.00015	0.389
	7.820	0.319	0.026	0.0263	0.00024	0.667
p-CH_3	6.890	0.0779	0.0078	0.00344	0.000048	0.0776
	7.205	0.0892	0.0093	0.00356	0.000064	0.163
	7.590	0.0670	0.0045	0.00591	0.000056	0.389
	7.820	0.102	0.006	0.00715	0.000070	0.667
p-NO_2	7.503	0.906	0.143	0.0227	0.0011	0.0446
(D_2O)	7.800	0.425	0.0512	0.0341	0.00063	0.0881
	7.950	0.476	0.0829	0.0409	0.0011	0.125
	8.111	0.356	0.0891	0.0574	0.00071	0.180

(17) D. F. De Tar and C. E. De Tar, "Computer Program LSKIN1," Department of Chemistry and Institute of Molecular Biophysics, The Florida State University, Gainesville, Fla.

(18) Program kindly supplied by P. Smith, University of Pennsylvania.

Evaluation of k_{L_2O}. The values of k_{L_2O} increase in a regular fashion with increasing pH, in contrast to the more complicated relationship between pH and k_{ImT}/Im. The relationship between k_{L_2O} and lyoxide ion activity is quite linear, *i.e.*, there is no appreciable $k_3(H_3O^+)$ term. The nonimidazole rate constant, k_{L_2O}, can therefore be expressed by eq 3. In contrast,

$$k_{L_2O} = k_1 + k_2[LO^-] \quad (3)$$

for other acylimidazoles in a similar pH range, an acid term occurs along with k_1 and k_2[6] or instead of k_2.[8]

The relationship between k_{L_2O} and the lyoxide ion activity was treated by the linear least-squares computer program.[18] The resulting values of k_1 and k_2, and their standard deviations, are given in Table II.

Table II. Values of k_1 and k_2 Obtained from the Relationship between k_{L_2O} and $[LO^-]$ (25.07°, μ = 0.04 *M*)

Compd	k_1, min^{-1}	Std dev	$10^{-6}k_2$, l. mol^{-1} min^{-1}	Std dev $\times 10^{-6}$
p-NO_2 (H_2O)	0.0624	0.0035	0.171	0.015
p-Cl	0.00981	0.00055	0.0257	0.0024
p-CH_3	0.00282	0.00022	0.00677	0.00095
p-NO_2 (D_2O)	0.0112	0.0008	0.252	0.015

Hammett plots of k_1 and k_2 give ρ = 1.41 and 1.48, respectively. A ρ value of 1.4 has been reported for the k_2 term only (determined at high pH) at 30°.[9] For *p*-nitrobenzoylimidazole, the isotope effects are found to be $k_1(H_2O)/k_1(D_2O)$ = 5.57 ± 0.51 and $k_2(D_2O)/k_2(H_2O)$ = 1.47 ± 0.16.

Discussion

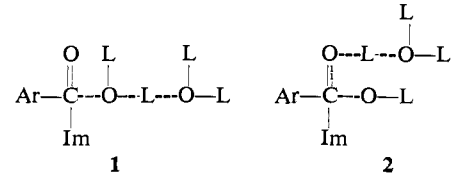
The inverse isotope effect for k_2 indicates a mechanism involving nucleophilic attack of LO^- on the acylimidazole carbonyl carbon atom, since DO^- is a stronger base than HO^- by a factor of *ca.* 2.12[15] at 25°. Partial O- - -C bond formation should therefore produce an inverse secondary isotope effect between 1.0 and 2.12. Similar inverse isotope effects have been noted in the basic hydrolysis of esters.[19] With a relatively poor leaving group, the hydroxide term in hydrolysis of N-methyltrifluoroacetanilide is found to have an isotope effect k_H/k_D = 3.3 at 25.5°,[11d] presumably resulting from a change to leaving group expulsion as the rate-determining step, rather than nucleophilic attack.

We believe the isotope effect on k_1 and the fact that ρ is nearly the same for k_1 as for k_2 rule out several otherwise reasonable mechanisms for the water reaction. Mechanisms involving only water molecules or involving equal numbers of L_3O^+ and LO^- ions are kinetically allowed. The isotope effect, $k_1(H_2O)/k_1(D_2O)$ = 5.6, requires either a primary isotope effect, *i.e.*, that some L atom move considerably in the reaction coordinate motion of the transition state, or that (neglecting the unlikely possibility that two or more L_3O^+–LO^- pairs participate) both L_3O^+ and LO^- are present in the transition state with their charges essentially unneutralized (giving expected maximum secondary isotope effect contributions of about 3.0 and 2.12, respectively, for completely unneutralized charges, or a total effect of 6.4, consistent with the observed 5.6).

The isotope effect, therefore, rules out two of the simplest possibilities for the rate-determining transition state: simple nucleophilic attack by water on the carbonyl carbon atom, analogous to the $LO^-(k_2)$ mechanism already discussed, and nucleophilic attack by lyoxide on acylimidazolium ion. In each case, it is not possible that the secondary isotope effects involved could be large enough. For the former, nearly complete charge formation at the transition state ($C-O^+H_2$) would give only *ca.* $3.0^{2/3}$ = 2.1. For the latter, even a maximum lyoxide isotope effect of 2.12 would require *ca.* 2.3 for the secondary isotope effect on protonation of acylimidazole, and this effect shoud be considerably less than 1.5.

In a somewhat more tentative vein, we believe the near equality of ρ values for k_1 and k_2 indicates that the water mechanism, too, involves rate-determining nucleophilic attack on the carbonyl carbon atom.

Five mechanisms consistent with these conclusions suggest themselves: (1) a water molecule acting as a general base to remove a proton from another water molecule acting as a nucleophile; (2) a lyonium ion acting as a general acid at the carbonyl oxygen atom with a lyoxide ion acting as a nucleophile; (3) a lyonium ion acting as a general acid at the 3-nitrogen atom of the imidazole group with a lyoxide ion acting as a nucleophile; (4) a water molecule acting as a general acid at the carbonyl oxygen atom with another water molecule acting as a nucleophile; and (5) a water molecule acting as a general acid at the 3-nitrogen atom of the imidazole group with another water molecule acting as a nucleophile. We disfavor 4 and 5, suspecting water to be an insufficiently good general acid. Catalysis at the imidazole group would appear to be ineffective if nucleophilic addition is rate determining, making 3 and 5 unlikely.[20] Mechanisms 1 and 2, the transition states for which are shown schematically as **1** and **2**, respectively,



1 **2**

remain as likely possibilities. Further distinction of these mechanisms will hopefully be provided by substituent effects on isotope effects and by isotope effects for D_2O–H_2O mixtures.

Mechanisms **1** and **2** are both consistent with the observed kinetic isotope effect whether there be a primary isotope effect contribution or not (see estimated maximum secondary isotope effect above). In fact, various extreme types of "proton transfer" transition states are possible,[3,21,22] including: concerted, with the proton bound in a stable potential; concerted, unstable potential; nonconcerted, stable potential; nonconcerted, unstable potential. It has been con-

(19) T. C. Bruice, T. H. Fife, J. J. Bruno, and P. Benkovic, *J. Am. Chem. Soc.*, **84**, 3012 (1962).

(20) A SN2-type mechanism for both k_1 and k_2 is not ruled out, though we consider it very likely that a tetrahedral intermediate is actually on the most favorable reaction path. If the mechanism were SN2-like, type 3 would become an attractive mechanistic possibility.

(21) C. G. Swain, D. A. Kuhn, and R. L. Schowen, *J. Am. Chem. Soc.*, **87**, 1553 (1965).

(22) E. R. Thornton, *ibid.*, **89**, 2915 (1967).

Table III. Values for the Individual Rate Constants Contributing to k_{obsd} Obtained by a Five-Parameter Treatment of the Data (eq 2)[a]

Term	*p*-Me	*p*-Cl	$(p\text{-}NO_2)_H$	$(p\text{-}NO_2)_D$
k_1, min^{-1}	0.00262 ± 0.00024	0.00968 ± 0.00073	0.0636 ± 0.0082	0.0109 ± 0.0042
$10^{-6}k_2$, l. mol^{-1} min^{-1}	0.00715 ± 0.00053	0.0257 ± 0.0016	0.159 ± 0.017	0.257 ± 0.036
$10^{-6}k_{ImB}$, $l.^2$ mol^{-2} min^{-1}	0.0592 ± 0.0159	0.242 ± 0.048	1.28 ± 0.46	2.10 ± 0.85
k_{ImA}, l. mol^{-1} min^{-1}	0.0282 ± 0.0086	0.0749 ± 0.026	−0.162 ± 0.247	0.947 ± 0.142
k_{Im_1} l. mol^{-1} min^{-1}	0.0504 ± 0.00734	0.130 ± 0.022	0.878 ± 0.218	−0.278 ± 0.143
No. of individual k_{obsd}	51	59	77	56

[a] 25.07°, μ = 0.04 *M*.

cluded for the present type of mechanism, *i.e.*, proton transfer between oxygen and oxygen, that the proton will always be in a stable potential at the transition state and be transferred in a very rapid prior or subsequent step.[21] The experimental exploration of this conclusion for acylimidazole hydrolysis is an extremely interesting problem.

Attempted Analysis of k_{ImT}/Im. Table I shows that the slopes of k_{obsd} *vs.* free imidazole concentration are not equal at all four pH values investigated, as would be expected for imidazole-catalyzed hydrolysis alone. Neither are the slopes linear in lyoxide activity, though experimental scatter is relatively large; they appear to pass through a minimum. In order to produce results which were as unbiased as possible,[3] we made a least-squares computer fit[23] of our observed rate constants to eq 2. The k_{ImB} term seems rather clearly required by all reactions except the one in D_2O, because the slope at highest pH was significantly higher than the others. Since there were over 50 actual values of k_{obsd} for each reaction, the five parameters were calculated with fair internal precision. In view of the uncertainties present, we give the results in Table III but will not attempt to make mechanistic interpretations. It will be noted that the values of k_1 and k_2 are quite similar to those in Table II and that the imidazole terms seem reasonable in comparing one compound with another, except for two negative values presumably representing rate constants near zero.

Another alternative is that the apparent changes of k_{ImT}/Im with pH result from experimental uncertainty, and the imidazole catalysis is really pH independent. On this hypothesis, the values of k_{Im}, in l. mol^{-1} min^{-1}, with standard deviations, would be: *p*-CH_3, 0.084 ± 0.015; *p*-Cl, 0.255 ± 0.047; *p*-$NO_2(H_2O)$, 1.16 ± 0.39; and *p*-$NO_2(D_2O)$, 0.54 ± 0.25. Clearly, more data are required.[7]

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(23) M. H. Lietzke, "A Generalized Least-Squares Program for the IBM 7090 Computer," ORNL-3259, Office of Technical Services, U. S. Department of Commerce, Washington, 25, D.C.