Kinetic Study of General and Specific Catalysis of Hydrolysis of Para-Substituted *N*-Benzoylimidazoles and of *N*-Acetylimidazole in Imidazole Buffers[1]

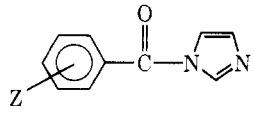
Waldemar Palaitis[2] and Edward R. Thornton*

Contribution from the Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19174, and the Department of Science and Mathematics, Mount Saint Mary's College, Emmitsburg, Maryland 21727. *Received August 19, 1974*

Abstract: Rates of hydrolysis of *p*-nitrobenzoylimidazole have been measured in H_2O and D_2O imidazole buffers, and rates of hydrolysis of *p*-chloro- and *p*-methylbenzoylimidazole and acetylimidazole have been measured in H_2O imidazole buffers at 25°, ionic strength = 0.5 *M*. Rate terms and isotope effects were obtained with very good precision for water, lyoxide, lyonium ion, and imidazole catalysis. A term for imidazolium ion catalysis was shown to be present for acetylimidazole and probably for *p*-methylbenzoylimidazole substrates. Hammett ρ values, followed by the *p*-nitrobenzoylimidazole D_2O solvent isotope effects, k_H/k_D, for the various catalytic terms are as follows: k_0, 1.414 ± 0.002, 2.36 ± 0.05; k_{LO^-}, 1.47 ± 0.08, $(1.03 \pm 0.02)^{-1}$; $k_{L_3O^+}$, 1.00 ± 0.03, $(1.33 \pm 0.04)^{-1}$; k_{GB}, 1.24 ± 0.03, 2.83 ± 0.04. It is concluded that these reactions probably all involve general-base catalyzed rate-determining nucleophilic attack by water at the carbonyl carbon atom by an unusual mechanism in which the proton does not appear to move in the reaction coordinate motion (imaginary vibration) of the transition state including the imidazole catalysis term not previously correlated with the other terms. It is also shown that the term for imidazolium ion catalysis actually involves the kinetically equivalent imidazole general-base catalysis of hydrolysis of acetylimidazolium ion.

While the demonstrated intermediacy of *N*-acetylimidazole in the imidazole-catalyzed hydrolysis of *p*-nitrophenyl acetate[3,4] clearly showed a propensity on the part of imidazole for nucleophilic catalysis, the finding that *N*-acetylimidazole is itself hydrolyzed in part through imidazole[5,6] and imidazolium ion[6] catalysis established imidazole as an efficient general acid–base catalyst as well. Such a mechanism was recognized as an important possibility for catalysis by the enzyme chymotrypsin, which requires an intact imidazole group in the active site, at His-57, for catalytic activity, as well as by other serine proteases, and general catalysis by this imidazole moiety is believed to be involved in both the acylation and deacylation of the serine hydroxyl group.[7-10]

In previous work, Klinman studied hydrolysis and imidazole-catalyzed hydrolysis of substituted benzoylimidazoles (**1**), BI, in a range of pH around 7.[11] In this range, several



1

catalytic terms contribute to the rate, and it was noted that accurate data for imidazole catalytic terms required further data, which we now report. BI have also been studied at high pH, where only the hydroxide term is important, and at low pH, where the reaction is hydrolysis of the benzoylimidazolium ion, BIH^+.[12-15] An indication of a catalytic term dependent on (imidazole) × (hydroxide) was found,[11] thus implicating a total of six terms in the observed rate constant, as shown in eq 1, where L = H or D, Im stands for imidazole, ImL^+, for imidazolium ion, and k_{obsd} is the observed pseudo-first-order rate constant.

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

The uncatalyzed and GB-catalyzed hydrolyses were of particular interest with regard to solvolysis and enzyme mechanisms. Two approaches to further mechanistic analysis were therefore taken. First, the study reported herein would examine the BI over a wider pL range than previously,[11] using more precise instrumentation and including as a check point acetylimidazole, which had been studied extensively by others.[5,9,15] Second, a study of *N*-benzoyl-*N'*-methylimidazolium ions would permit evaluation of water and imidazole catalytic terms in a kinetically simpler system expected to have only three catalytic terms in all. The work on this simpler system[1a] provided the key to evaluation of the BI data,[1c] as well as a simple, unified mechanism for essentially all catalytic terms, involving general-base catalyzed hydrolysis of rate-determining nucleophilic attack by water, with one proton partly transferred to the general base at the transition state but not being transferred as part of the reaction coordinate motion.

The presently reported work provides (1) a precise evaluation of the catalytic terms for the BI, (2) a demonstration that the k_{ImB} term of eq 1 results from inhibition by sodium nitrate or some similar effect, showing up only at high pL and low (Im), and is not a significant catalytic term, (3) an evaluation of the significance of the k_{GA} term for the substrates studied, and (4) a set of isotope effects in heavy water with good precision for the substrate *p*-nitrobenzoylimidazole. The larger pL range and the use of a logarithmic weighting procedure in the least-squares fits[1a] give catalytic terms which differ significantly from those reported previously,[11] with much lower standard deviations, and the isotope effects and substituent effects appear to support the suggested mechanism.

Experimental Section

The materials and procedures were generally the same as reported by Klinman,[11] with some differences, developed in the present work.[1c] Many of these differences have already been recorded by Choi so that only matters not appearing in that work[1a] are reported here.[1c]

Deuterium Oxide, >99.7% purity, was purchased from General Dynamics Corp. This reagent was boiled for 10 min and cooled in a dry N_2 atmosphere. Analysis by nmr using the method of standard additions showed the H atom fraction after boiling to be 0.13%.

Acetonitrile-d_3, >99% deuterated, was purchased from Stohler Isotope Chemicals and was distilled from phosphorus pentoxide before use as solvent for *p*-nitrobenzoylimidazole in the kinetic runs in D_2O.

***N*-Acetylimidazole** was prepared by the method of Boyer,[16] mp 102–103° (lit.[16] 101.5–102.5°); λ_{max} 247 nm (H_2O-imidazole

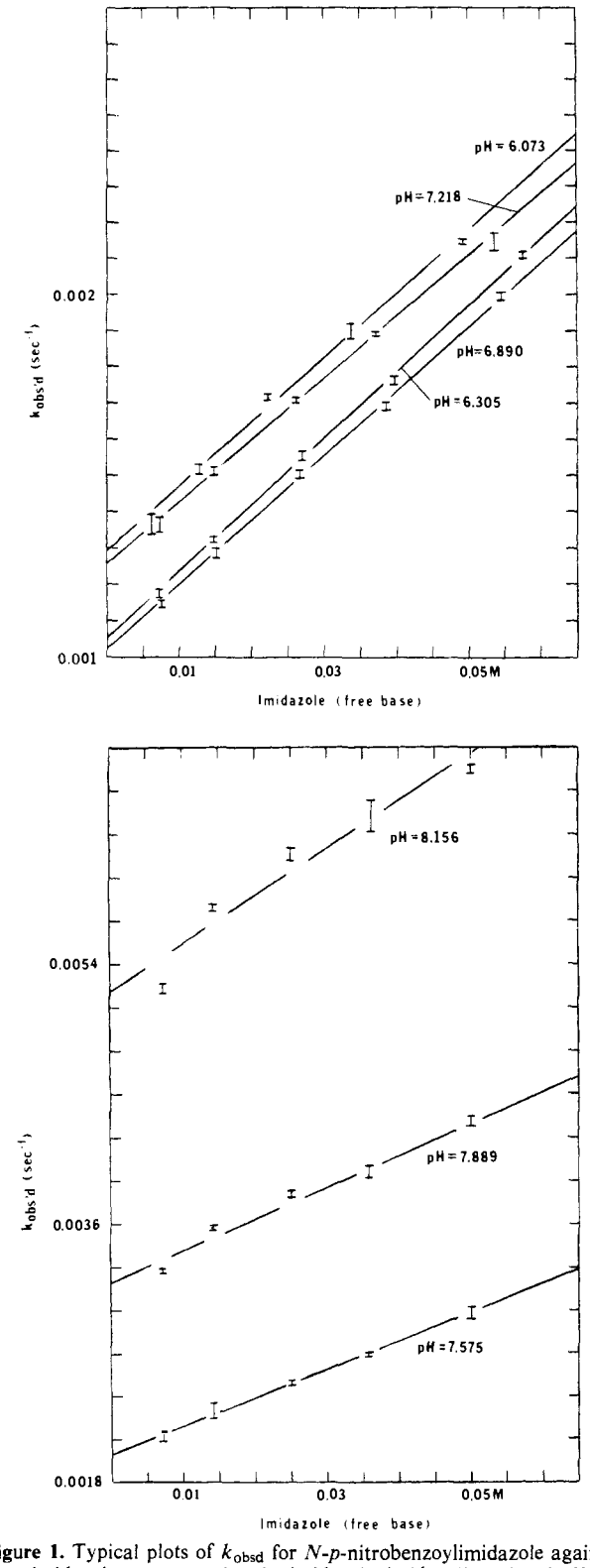


Figure 1. Typical plots of k_{obsd} for *N*-*p*-nitrobenzoylimidazole against free imidazole concentration in imidazole-imidazolium ion buffers, 25.03°, ionic strength = 0.5 *M*.

buffer) [lit.[17] 245 nm (H_2O)].

Buffers. Seven stock buffers of varying pL, each of about 0.05 *M* imidazole free base and 0.5 *M* ionic strength (adjusted to this value with $NaNO_3$), were separately diluted with 0.5 *M* $NaNO_3$ solution to yield seven series, each comprised of five buffer concentrations at a given pL. Deviations from the stock buffer pL observed upon dilution were corrected.[1a] The H_2O buffers were at pH 6.073, 6.305, 6.890, 7.218, 7.575, 7.889, 8.156; the D_2O buffers were at pD 6.640, 7.003, 7.329, 7.630, 7.921, 8.388, 8.710.

Kinetic Measurements. The Cary Model 16 spectrophotometer was used with 1-cm silica cells for *p*-nitrobenzoylimidazole, with 0.5-cm silica cells for the other two BI, and with special 1-cm silica cells fitted with inserts to reduce the path length to 1 mm for acetylimidazole. The latter cells still contained 2 ml of buffer solution and were satisfactory, while fixed 1-mm path-length cells, volume 0.3 ml, were found to be unsatisfactory primarily because of time losses during initial stages of kinetic runs resulting from efforts to eliminate air bubbles at the glass-buffer interface.

Absorption measurements were made generally at 1% reaction intervals, generally up to 90% completion. It was found necessary to replace the ultraviolet source lamp when a minimum sensitivity of 4 could no longer be obtained. Absorption measurements were made at the following wavelengths: *p*-NO_2BI, 260; *p*-ClBI, 252; *p*-CH_3BI, 252; acetylimidazole, 247 nm.

Observed rate constants, k_{obsd}, were calculated with the least-squares computer program, LSK1N1,[18] modified for use on the IBM 360 computer.[1c] The generalized least-squares program of Lietzke[19] modified for the IBM 360 and with logarithmic weighting of $a_{L_3O^+}$ and a_{LO^-} was used to fit the k_{obsd} values to appropriate forms of eq 1. Triplicate k_{obsd} determinations in a given buffer gave agreement within about ±1%.

Results

Plots of k_{obsd} *vs.* (imidazole) shown for *p*-NO_2BI in Figure 1, which are representative of all the substrates studied, confirm the applicability at least in the pH range 6.073-7.575 and pD range 6.640-7.921 of eq 2, *i.e.*, omitting k_{ImB}

$$k_{obsd} = k_0 + k_{LO^-}a_{LO^-} + k_{L_3O^+}a_{L_3O^+} + k_{GB}(Im) + k_{GA}(ImL^+) \quad (2)$$

from eq 1. The curvature seen in Figure 1 for pH 7.890 and 8.156 is not predicted by eq 1 or 2. A close examination of these and the similar plots for the other substrates studied shows consistently that such curvature is restricted to the region of low buffer concentration (but adequate buffer capacity). It is in this region that the contribution of sodium nitrate to the ionic strength is maximal, and an explanation for the curvature may lie in the observation that sodium salts depress the rate of *N*-acetylimidazole hydrolysis under some conditions.[15] A similar effect was seen by Choi,[1a] who used potassium chloride rather than sodium nitrate, but it is reported that potassium and sodium, as well as chloride and nitrate, have very similar effects.[15] Since the nature of the observed departure from linearity is such that it alone may be sufficient to account for all the k_{ImB} catalytic terms previously suggested, the data were recalculated with the omission of the offending buffers. Table I shows the results for *p*-NO_2BI in D_2O using eq 1-3 with just the omission of of-

$$k_{obsd} = k_0 + k_{LO^-}a_{LO^-} + k_{L_3O^+}a_{L_3O^+} + k_{GB}(Im) \quad (3)$$

fending buffers and with the entire data for the two uppermost pD values omitted. Similar results for other substrates lead us to reject eq 1 in all cases. In H_2O, use of eq 2 gives values of k_{GA} within their standard deviation of fit from zero for *p*-NO_2BI and *p*-ClBI; therefore, only eq 3 is included. Data for both eq 2 and 3 are given for *p*-CH_3BI and acetylimidazole, where k_{GA} appears to be significant. The best fit in terms of standard deviations is given by eq 3 in the case of *p*-CH_3BI, however; the fits to eq 2 and 3 are more nearly equivalent in the case of acetylimidazole.

Generally, the data with the omission of only the offending buffers are probably best, but the results with omission of the two highest pL values entirely are almost as good. Since the latter procedure is probably more justifiable in view of the fact that we do not have absolute proof of the reason for the curvature at these two highest pL values, rate constants obtained with omission of the two highest pL values entirely are used below.

Hammett ρ values[20] and solvent isotope effects for H_2O *vs.* D_2O in the case of *p*-NO_2BI are presented in Table II.

If the intercepts of plots of k_{obsd} *vs.* buffer concentration

Table I. Least-Squares Rate Constants for Hydrolysis and Imidazole-Catalyzed Hydrolysis of BI (1) and Acetylimidazole, 25.03°, Ionic Strength = 0.50 M ($NaNO_3$)[a]

Substrate	Runs[b]	10^5k_0, sec^{-1}	$10^{-2}k_{LO^-}$, l. mol^{-1} sec^{-1}	$10^{-2}k_{L_3O^+}$, l. mol^{-1} sec^{-1}	10^4k_{GB}, l. mol^{-1} sec^{-1}	10^6k_{GA}, l. mol^{-1} sec^{-1}	$10^{-2}k_{ImB}$, $l.^2$ mol^{-2} sec^{-1}
p-NO_2BI	100[c]	25.5 ± 0.4	37.7 ± 0.4	10.01 ± 0.21	64.6 ± 0.7		
(D_2O)	120[d]	25.9 ± 0.3	37.1 ± 0.2	9.78 ± 0.17	64.7 ± 0.8		
	120[d]	26.3 ± 0.4	37.1 ± 0.2	9.28 ± 0.34	63.2 ± 1.2	45 ± 25	
	120[d]	25.3 ± 0.6	38.8 ± 0.7	9.76 ± 0.42	65.8 ± 1.8	14 ± 30	−43 ± 18
	139[e]	31.6 ± 1.2	34.7 ± 1.0	7.34 ± 0.82	52.1 ± 3.8	190 ± 54	51 ± 31
p-NO_2BI	99[f]	60.3 ± 1.0	36.5 ± 0.4	7.50 ± 0.14	183 ± 2		
(H_2O)	127[g]	61.8 ± 0.7	35.4 ± 0.2	7.29 ± 0.12	183 ± 2		
p-ClBI	80[f]	10.0 ± 0.2	4.96 ± 0.07	2.02 ± 0.03	36.2 ± 0.4		
(H_2O)	98[h]	9.64 ± 0.15	5.15 ± 0.03	2.07 ± 0.02	36.4 ± 0.4		
p-CH_3BI	77[f]	2.81 ± 0.10	1.49 ± 0.04	0.824 ± 0.024	11.8 ± 0.3	12 ± 7	
(H_2O)	98[g]	2.78 ± 0.07	1.49 ± 0.02	0.830 ± 0.020	12.0 ± 0.3	10 ± 6	
	77[f]	2.74 ± 0.09	1.49 ± 0.04	0.856 ± 0.016	12.2 ± 0.2		
	98[g]	2.72 ± 0.07	1.48 ± 0.02	0.856 ± 0.012	12.3 ± 0.2		
N-Acetylimidazole	76[f]	5.70 ± 0.13	3.33 ± 0.04	2.82 ± 0.04	24.0 ± 0.4	78 ± 10	
(H_2O)	94[h]	5.91 ± 0.14	3.17 ± 0.05	2.80 ± 0.04	24.5 ± 0.5	68 ± 14	
	76[f]	5.32 ± 0.16	3.30 ± 0.06	3.03 ± 0.03	26.1 ± 0.4		
	94[h]	5.61 ± 0.14	3.15 ± 0.03	2.98 ± 0.03	26.3 ± 0.4		

[a] The rate constants in this table were obtained by fitting k_{obsd} values, each determined in a separate kinetic run, to eq 1 (all six rate constant columns filled), eq 2 (k_{ImB} empty), or eq 3 (k_{GA} and k_{ImB} empty); standard deviations of fit are also given. The first row of catalytic terms given for each substrate is the one used in subsequent calculations and discussion. [b] Number of separate kinetic runs (k_{obsd} values) used in the least-squares fit to derive the rate constants given in the row. [c] pD 6.640–7.921 data used. [d] pD 6.640–8.170 data used, but with omission of the two lowest imidazole concentrations at pD 8.388 and the three lowest imidazole concentrations at pD 8.710. [e] pD 6.640–8.710 data used, with no omissions. [f] pH 6.073–7.575 data used. [g] pH 6.073–8.156 data used, but with omission of the lowest imidazole concentration at pH 7.889 and the two lowest imidazole concentrations at pH 8.156. [h] pH 6.073–8.156 data used, but with omission of the two lowest imidazole concentrations at pH 7.889 and the two lowest imidazole concentrations at pH 8.156.

Table II. Hammett ρ Values for BI (1) in H_2O and D_2O Solvent Isotope Effects for p-NO_2BI, 25.03°, Ionic Strength = 0.50 M ($NaNO_3$)[a]

Catalytic constant	ρ	k_H/k_D
k_0	1.414 ± 0.002	2.36 ± 0.05
k_{LO^-}	1.47 ± 0.08	$(1.03 \pm 0.02)^{-1}$
$k_{L_3O^+}$	1.00 ± 0.03	$(1.33 \pm 0.04)^{-1}$
k_{GB}	1.24 ± 0.03	2.83 ± 0.04

[a] The ρ value for k_{GA} could not be calculated since only p-CH_3BI had a statistically significant value.

are plotted against a_{LO^-}, it is found that good straight lines are obtained if the intercepts at the two higher pL values are taken to be the ones resulting from linear extrapolation of points for the higher imidazole concentrations only (*cf.* Table I for specification of which imidazole concentrations were omitted). Strong curvature in these plots results if a curvilinear extrapolation is used to estimate the intercepts at the two higher pL values.

Discussion

It will be noted that the recognition of curvature problems at the higher pL values has led to a data analysis which gives excellent precision in the rate constants for the catalytic terms appearing in eq 3. Further, the plots of intercept against a_{LO^-} indicate rather strongly that the data at low imidazole concentrations at the two highest pL values are "wrong," *i.e.*, subject to some specific inhibition effect, perhaps by sodium and nitrate ions present to maintain the ionic strength. In turn, this recognition shows that the k_{ImB} term of eq 1 is not present within the limits of the data.

The k_{GA} term may be of significance in cases where the carbonyl group is not already substituted by electron-deficient groups. It is likely that this term involves the kinetically equivalent imidazole-catalyzed hydrolysis of protonated substrate (protonated on the second nitrogen atom of the imidazole moiety) by analogy with $k_{L_3O^+}$, where comparisons with N-acyl-N'-methylimidazolium ions have established this type of mechanism.[1a,5,21,22] This conclusion is strongly supported in the case of acetylimidazole, where the k_{GA} term can be compared with available data for N-acetyl-N'-methylimidazolium ion at 25°, ionic strength = 0.2. The N-methylimidazole-catalyzed hydrolysis of the latter ion was found to have a rate constant k'_{GB} = 0.30 l. mol^{-1} sec^{-1}.[21] It is easily shown that the rate constant k''_{GB} for the imidazole-catalyzed hydrolysis of N-acetylimidazolium ion, making the assumption that the entire k_{GA} term found really corresponds to the kinetically equivalnt k''_{GB}, is given by k_{GA} times the ratio of the acidity constant of acetylimidazolium ion to that of imidazolium ion. At ionic strength 1.0 and 25°, this ratio is $1.38 \times 10^{-4}/6.16 \times 10^{-8}$,[23,24] and since it is a ratio, this should be a good approximation for ionic strength = 0.5. Using k_{GA} from Table I, k''_{GB} = 0.18 l. mol^{-1} sec^{-1}. Thus, the entire k_{GA} term is accounted for by the N-acetyl-N'-methylimidazolium ion model; indeed, k'_{GB} is nearly twice k''_{GB}, as has previously been found for the $k_{L_3O^+}$ terms.[1a,5,21,22] The k''_{GB} term is experimentally difficult to evaluate,[6] but we believe the above arguments establish its existence and mechanistic form as general-base catalyzed hydrolysis of the protonated acetylimidazole. This comparison was made by Oakenfull and Jencks,[23] but only in their Figure 7; the corresponding rate constant (k''_{GB}) was not reported in their tables.[22,23]

In the previous study, isotope effects were found to be virtually independent of substituent.[1a] Since isotope effects should not change a great deal between 15 and 25°, the isotope effect (Table II) for p-NO_2BI may be compared with those previously reported for p-ClBI, BI, and p-CH_3BI at 15°. There was strong curvature in Hammett plots for the previous data,[1a] believed to be an artifact arising from the limited range of substituents which could be studied. The present data (Table II) provide much more precise ρ values which, however, can probably be justifiably compared with the ρ values derived from the previous work.

For the rate term k_0, the ρ value is quite similar to the 15° value (1.35), and the isotope effect is almost exactly equal to the mean of the 15° isotope effects for k_0. For the rate term k_{LO^-}, ρ is somewhat higher, though still by a small amount, than the 15° value (1.32), and the isotope effect is similar to those at 15° (1.06^{-1}, 1.03^{-1}, 1.13^{-1}) for

k_{LO^-}. For the rate term $k_{L_3O^+}$, the ρ value is very close to the 15° value (0.99), and the isotope effect is almost exactly equal to the mean of the 15° isotope effects for $k_{L_3O^+}$.

The interpretation of the 15° data[1a] suggested that the mechanism involves general-base catalyzed rate-determining nucleophilic attack upon the BI for k_0 (water acting as base) and k_{LO^-}, upon BIL^+ (water acting as base) for $k_{L_3O^+}$ (actually k''_0, kinetically equivalent), and upon BMI^+ (*N*-benzoyl-*N'*-methylimidazolium ions) for k'_0 (water acting as base) and k'_{GB} (*N*-methylimidazole acting as base). The k_0 term was shown to involve little contribution from the kinetically equivalent k''_{LO^-}, *i.e.*, attack of lyoxide on BIL^+, since the lyoxide term, k'_{LO^-}, for BMI^+ was about 14 times smaller than the calculated k''_{LO^-}. The similarity of isotope effects and substituent effects for k_0 and other terms then strongly suggests that this term is general-base catalyzed by water.[1a] The k''_0 term, however, was about two times smaller than the k'_0 term for BMI^+, demonstrating that k''_0 accounts for this entire rate term with little, if any, contribution from the kinetically equivalent $k_{L_3O^+}$.[1a]

The data on isotope and substituent effects suggested that the general-base mechanism of the previous paragraph was common to all the catalytic terms and was of an unusual type, in that the proton was partly transferred from the nucleophilic water molecule to the general base *but* was not actually moving in the reaction coordinate motion (imaginary vibration) of the rate-determining transition state.[1a,25] General-base catalysis normally involves transfer of a proton to the base in a single reaction step, with the proton moving extensively as part of the reaction coordinate motion, thus developing primary H/D isotope effects. The small isotope effects observed and their lack of substituent or base dependence suggested that they are not primary isotope effects. Therefore, a mechanism with the proton partly transferred to the base but not moving in the reaction coordinate motion was suggested, and the (secondary) isotope effect was then supposed to arise mainly from this partly transferred proton, as has been shown by kinetic studies in H_2O–D_2O mixtures to be the case in the intramolecular general-base catalysis by carboxylate anion of hydrolysis of *O*-dichloroacetylsalicyclic anion.[25] This mechanism in turn requires a tortuously curved reaction path, difficult to fit onto a suitable energy surface, or else an intermediate with a "partly-transferred" proton, or else a pair of consecutive transition states (with perpendicular reaction coordinate motions),[1a] all which are considered to be unusual mechanistic phenomena.

The close correlation of data on k_0, k_{LO^-}, and $k_{L_3O^+}$ at 15 and 25° supports the conclusion that these terms at 25° follow the same mechanistic path as suggested for 15°. We consider this agreement, especially the isotope effects at a higher temperature and for a new para substituent, to provide additional support of the previous interpretation involving a nearly constant transition-state structure for all substituents and general bases.

With the above correlations in mind, the new data provide a probable interpretation for k_{GB}, a term which was not determined at 15°. In particular, the isotope effect is close to those for k'_{GB} for the *N*-methylimidazole-catalyzed hydrolysis of BMI^+ (2.70, 2.69, 2.98) and strongly suggests that k_{GB} does proceed by rate-determining nucleophilic attack by water upon BI, general-base catalyzed by imidazole, and proceeding through the unusual type of transition state described previously. The ρ value seems somewhat low, however, compared with those for k_0 and k_{LO^-}, yet one might have expected it to fall between those for the weaker base water and the stronger base LO^-. This difference also appears to be associated with a somewhat higher isotope effect for imidazole catalysis than for water or LO^-. For the BMI^+, the ρ values are not directly comparable, but one does find lower ρ or ρ^+ values for k'_{GB} than for k'_0 and similarly larger isotope effects.[1a] The k'_{LO^-} term cannot be compared since it is proceeding by a mechanism involving direct nucleophilic attack by lyoxide on BMI^+.[1a] Perhaps these observations indicate incursion of a reaction coordinate involving a small degree of proton motion.

Since k'_{GB} appears to involve this general-base mechanism, it seems certain that the k''_{GB} term also involves the same mechanism, and thus that imidazolium ion catalysis is actually the kinetically equivalent imidazole general-base catalyzed rate-determining nucleophilic attack by water upon the acylimidazolium ion, by the unusual mechanism proposed for other terms.

In sum, the present work (1) provides correlation of 25 and 15° data, suggesting similar mechanisms, (2) demonstrates that k_{GA} for acetylimidazole really corresponds to the kinetically equivalent imidazole general-base catalyzed hydrolysis of acetylimidazolium ion, and (3) provides accurate data for imidazole-catalyzed hydrolysis of benzoylimidazoles, showing that this term probably proceeds by an unusual general-base catalysis mechanism.

Acknowledgments. Support by the National Aeronautics and Space Administration, the U.S. Public Health Service, and the National Science Foundation is gratefully acknowledged. The liberal use of computer facilities of the University of Pennsylvania and of Mount Saint Mary's College and the programming assistance of Mr. William E. O'Toole of Mount Saint Mary's College are also gratefully acknowledged.

References and Notes

(1) (a) Previous paper: M. Choi and E. R. Thornton, *J. Amer. Chem. Soc.*, **96**, 1428 (1974). (b) Supported in part by the National Aeronautics and Space Administration, in part by the U.S. Public Health Service through Grant AM-12,258, and in part by the National Science Foundation through Grant GP-34,491X. (c) For further details, *cf.* W. Palaitis, Ph.D. Dissertation, University of Pennsylvania, 1969.
(2) NASA Trainee, University of Pennsylvania, 1967–1969.
(3) T. C. Bruice and G. L. Schmir, *J. Amer. Chem. Soc.*, **79**, 1663 (1957).
(4) M. L. Bender and B. W. Turnquest, *J. Amer. Chem. Soc.*, **79**, 1656 (1957).
(5) W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1272, 1280 (1959).
(6) T. H. Fife, *J. Amer. Chem. Soc.*, **87**, 4597 (1965).
(7) M. L. Bender, "Mechanisms of Homogeneous Catalysis from Protons to Proteins," Wiley-Interscience, New York, N.Y., 1971.
(8) (a) J. Kraut in "The Enzymes," Vol. III, 3rd ed, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1971, Chapter 5; (b) D. M. Blow, *ibid.*, Chapter 6; (c) G. P. Hess, *ibid.*, Chapter 7.
(9) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N.Y., 1969.
(10) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. 1, W. A. Benjamin, New York, N.Y., 1966.
(11) J. P. Klinman and E. R. Thornton, *J. Amer. Chem. Soc.*, **90**, 4390 (1968).
(12) J. A. Fee and T. H. Fife, *J. Org. Chem.*, **31**, 2343 (1966).
(13) J. A. Fee and T. H. Fife, *J. Phys. Chem.*, **70**, 3268 (1966).
(14) M. Caplow and W. P. Jencks, *Biochemistry*, **1**, 883 (1962).
(15) S. Marburg and W. P. Jencks, *J. Amer. Chem. Soc.*, **84**, 232 (1962).
(16) J. H. Boyer, *J. Amer. Chem. Soc.*, **74**, 6274 (1952).
(17) Reference 9, p 67.
(18) 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.
(19) M. H. Lietzke, "A Generalized Least Squares Program for the IBM 7090 Computer," ORNL-3259, Office of Technical Services, U.S. Dept. of Commerce, Washington, D.C. 20025.
(20) L. P. Hammett, "Physical Organic Chemistry," 2nd ed, McGraw-Hill, New York, N.Y., 1970.
(21) R. Wolfenden and W. P. Jencks, *J. Amer. Chem. Soc.*, **83**, 4390 (1961).
(22) D. G. Oakenfull, K. Salvesen, and W. P. Jencks, *J. Amer. Chem. Soc.*, **93**, 188 (1971).
(23) D. G. Oakenfull and W. P. Jencks, *J. Amer. Chem. Soc.*, **93**, 178 (1971).
(24) W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, **90**, 2622 (1968).
(25) S. S. Minor and R. L. Schowen, *J. Amer. Chem. Soc.*, **95**, 2279 (1973).