esterases[26] they must be ascribed to little understood factors not duplicated in the model studies (*e.g.*, the heterogeneous and ordered surroundings of the triad functional groups at the active site[19]).

(26) M. W. Hunkapiller, S. H. Smallcombe, D. R. Whitaker, and J. H. Richards, *Biochemistry*, 12, 4732 (1973).

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The Mechanisms of Acyl Group Transfer from a Tetrahedral Intermediate

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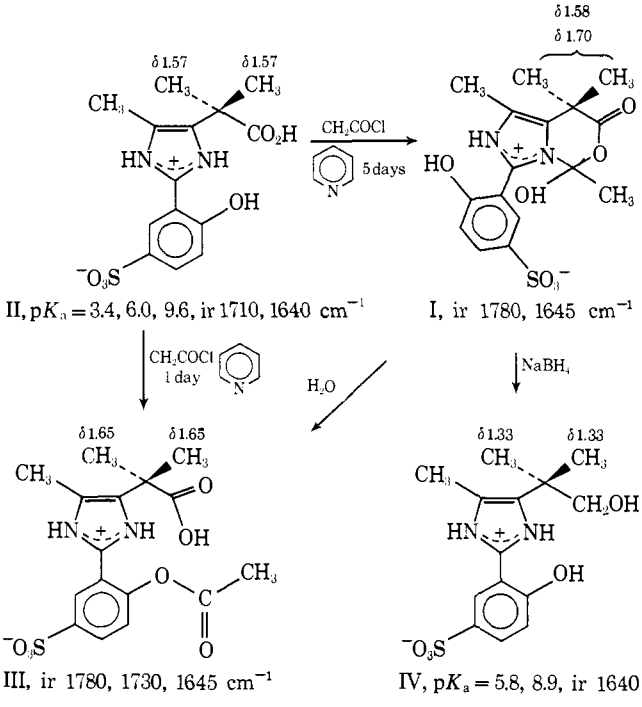
Abstract: The first isolation and unequivocal characterization of a tetrahedral intermediate in an acetyl transfer reaction are reported. The structure elucidation was achieved *via* observations involving the compounds' ir and nmr spectra, borohydride reduction product, and intra- and intermolecular reactions in solution. In H_2O at all pH's studied (0–12.4), the tetrahedral intermediate (I) rearranges quantitatively to the phenyl acetate (III) which subsequently hydrolyzes to the phenol (II). The proposed mechanism of intramolecular acetyl group transfer to the phenol group (I → III) is one involving the intermediate formation of an *N*-acetylimidazole. In contrast, intermolecular attack at the carbonyl carbon of the acylal-type tetrahedral intermediate can be shown to account for the rate acceleration of acetyl group transfer in the presence of nitrogen bases.

The synthesis and structural elucidation of the tetrahedral intermediate I (Scheme I) were described in a recent communication.[2] The specific experimental details are contained herein (see Experimental Section). The present manuscript deals with the kinetics and mechanisms of conversion of I to its hydrolytic product II *via* the phenyl ester III and to amides of II (*i.e.*, II′) *via* the amides of III (*i.e.*, III′) in the presence of nitrogen base species. Compound I may be considered to be a hemiacylal tetrahedral intermediate formed along the reaction path in an acetyl transfer from a carboxyl moiety to an imidazolyl group. The isolation of I has provided us with the opportunity to examine the reactivity of such a tetrahedral intermediate held in juxtaposition to a reactive hydroxyl function. This situation may well arise in the case of acyl transferase enzymes in which the primary nucleophilic center is a carboxyl group.

Scheme I



Experimental Section

Materials. Potassium chloride, potassium acetate, formic acid, ethyl formate, potassium phosphate (mono- and dibasic), and Tris were reagent grade and used without further purification. Chloroacetic acid (Matheson Co.), betaine hydrochloride (J. T. Baker), imidazole (Aldrich), methoxylamine hydrochloride (Eastman), hydroxylamine hydrochloride (Fisher), hydrazine hydrochloride (MCB), trifluoroethylamine hydrochloride (Pierce), glycine ethyl ester hydrochloride (Aldrich), semicarbazide hydrochloride (Aldrich), and ethylamine hydrochloride (Eastman) were recrystallized and dried under vacuum. Morpholine and *N*-methylimidazole (both Aldrich) were distilled. The preparations of 2-(2′-hydroxy-5′-sulfophenyl-4(5)-methyl-5(4)-(2″,2″-dimethylacetic acid)imidazolium zwitterion (II) and 2-(2′-acetoxy-5′-sulfophenyl)-4(5)-methyl-5(4)-(2″,2″-dimethylacetic acid)imidazolium zwitterion (III) have been reported in a previous paper.[3] Acetyl-d_3 chloride was prepared from acetic acid-d_4 (Diaprep) by the method of Brown.[4]

Tetrahedral Adduct (I). Acetylation of II yields the isomeric tetrahedral compound (I) and the phenyl ester (III). Dependent upon conditions, I and III are formed to the apparent total exclusion of each other. Solubility characteristics appear to be the driving force which favors one or the other. To 10 ml of dry $CHCl_3$ was added 128 mg (0.376 mmol) of finely crushed II. After the suspension was reduced to 8 ml by distillation, it was stirred (under a $CaCl_2$ drying tube) for several hours until particle size was minimal.

(1) A portion of the material submitted by G. A. Rogers in partial fulfillment of the requirements for the Ph.D. in Chemistry, University of California at Santa Barbara.

(2) G. A. Rogers and T. C. Bruice, *J. Amer. Chem. Soc.*, **95**, 4452 (1973).

(3) G. A. Rogers and T. C. Bruice, *J. Amer. Chem. Soc.*, **96**, 2473 (1974).

(4) H. C. Brown, *J. Amer. Chem. Soc.*, **60**, 1325 (1938).

Freshly distilled acetyl chloride (or acetyl-d_3 chloride) (2 equiv) and pyridine (dried over KOH) (7 equiv) were added with no apparent change. Stirring was continued and 8 equiv of acetyl chloride and 7 equiv of pyridine were added over a 5-day period. (If this time period is shortened, lower yields result.) The orange suspension was filtered and 110 mg (0.288 mmol) of white tetrahedral adduct (I) was collected and washed with $CHCl_3$ and Et_2O. After 2 weeks an additional 10.35 mg of I was collected to provide an overall yield of 83.5%. This compound has been prepared seven times (≥50% yield) with no evidence (uv or ir) of contamination by the isomeric III. When heated, I begins to decompose at 160°, turning dark red-brown, without melting, by 330°: uv (H_2O–1 *M* KCl, pH 4) 310, 322 (sh), and 291 nm; ir (KBr) 1780 (CO_2R), 1645 (ImH^+), 1180 (SO_3^-), and 1035 cm^{-1} (SO_3^-); nmr (C_5D_5N) δ 1.58 (s, 3), 1.70 (s, 3), 1.82 (s, 3) (this peak is not present in the sample prepared with CD_3COCl), 2.36 (s, 3), 7.19 (d, 1, J = 8.4 Hz), 8.41 (q, 1, J = 8.4 Hz, J = 2.2 Hz), and 9.13 ppm (d, 1, J = 2.2 Hz).

Anal. Calcd for $C_{16}H_{18}N_2O_7S$: C, 50.25; H, 4.74; N, 7.33; S, 8.39. Found: C, 50.53; H, 4.60; N, 7.05; S, 8.30.

2-(2′-Hydroxy-5′-sulfophenyl)-4(5)-methyl-5(4)-(2″,2″-dimethylethanol)imidazolium Zwitterion (IV). A 110-mg (0.288 mmol) sample of I was suspended in 8 ml of DMF (distilled from CaH_2 and stored over molecular sieves); 6 hydride equiv (16.3 mg) of $NaBH_4$ was added with stirring and within 30 sec all compound dissolved to give a clear, colorless solution which was stirred at ambient temperature for 2 days followed by 15 min at 100°. Excess borohydride was decomposed with aqueous HCl and the DMF was removed by extraction with $CHCl_3$. Concentration of the aqueous phase (<35°) to 5 ml (after adding enough NH_4OH to make the solution basic) initiated crystallization of inorganic material. After cooling to −5° for 3 hr the inorganic crystals were collected by filtration and discarded. The aqueous solution was further concentrated to 2 ml and placed on a column containing 15 g of Sephadex G-15. Elution was carried out using *ca.* 8×10^{-3} *M* NH_4OH (pH 10.6) and the eluent was monitored *via* uv (330 nm) and $AgNO_3$. The first 20 ml contained a small amount (<1%) of II (by uv titration of CO_2H). The subsequent fractions contained both Cl^- and the desired product IV and finally just IV. Concentration of these fractions gave 61.44 mg (65.5% yield) of white microneedles of the alcohol IV: mp >340° dec; ir (KBr) 1640 (ImH^+) and 1035 (SO_3^-); nmr (D_2O–KOD) δ 1.33 (s, 6), 2.32 (s, 3), 3.65 (s, 2), 6.77 (d, 1, J = 8.5 Hz), 7.52 (q, 1, J = 8.5 Hz, J = 2.5 Hz), and 8.15 ppm (d, 1, J = 2.5 Hz); pK_a (H_2O–1 *M* KCl) = 5.89 (ImH^+) and 8.9 (PhOH).

Anal. Calcd for $C_{14}H_{18}N_2O_5S$: C, 51.52; H, 5.56; N, 8.59; S, 9.83. Found: C, 51.45; H, 5.51; N, 8.61; S, 9.73.

When II and III were subjected to the same reduction conditions, no alcohol IV was obtained. If monitored by uv, II exhibited no reaction and III was reduced slowly to II (three pK_a's identical with authentic II).

Apparatus. Nmr spectra of I were recorded on a Varian HA-100. All other apparatus has been described in previous papers.[3,5]

Kinetics. All kinetic measurements were in aqueous solutions (except where specifically stated) at 30° and with μ = 1.0 with KCl. All reactions carried out in the absence of external buffers were followed spectrophotometrically in a pH-Stat cell assembly designed for a Cary 15 spectrophotometer.[6] Buffered reactions carried out within the buffer range of the buffer were followed using a Gilford 2000 spectrophotometer, while those outside the buffer range were also conducted in the Cary cell. Reactions in the alkaline region were followed using a Durrum-Gibson Model 13001 stopped-flow spectrophotometer.

Addition of I to kinetic solutions as a solid was necessary because of its extremely low solubility in common organic solvents (except pyridine). In order to facilitate dissolution (at pH ≤5.67), an estimated amount of I was first suspended in 10 μl of MeOH (organic solvents produce a very fine dispersion of I). The suspension was dissolved by the addition of 1 ml of water (1 *M* KCl) and this solution was then added to the Cary cell which contained *ca.* 20 ml of aqueous 1 *M* KCl. For reactions which were run above pH 6, a fine suspension of I in acetonitrile (MCB, Spectroquality) was added directly to the kinetic solutions. Reactions which were followed *via* stopped-flow spectrophotometry (pH >9.7) were initiated by mixing equal volumes of aqueous solutions; one containing phosphate or carbonate buffer (0.05–0.38 *M*) at the appropriate pH and the other, I (*ca.* 5×10^{-5} *M*), in phosphate buffer (5×10^{-4} *M*) at pH 6.65 (pH at which I has maximum stability). In separate experiments, each high pH buffer was mixed with an equal volume of the dilute phosphate buffer and the pH was measured at 30°.

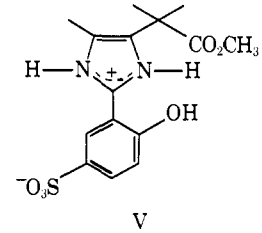
At pH values ≤5.67 in the absence of external buffers, the disappearance of I was monitored at 320 nm. At pH's >6 the reaction of I is kinetically biphasic. The first phase of the reaction was monitored at a convenient, previously determined (by repetitive scan) isosbestic wavelength of the second phase.[7] All reactions were followed to completion and the pseudo-first-order rate constants (k_{obsd}) were obtained from least-squares analysis of plots of $\ln (OD_\infty - OD_0)/(OD_\infty - OD_i)$ *vs.* time. All actual computations were carried out using an Olivetti-Underwood Programma 101, a Hewlett-Packard 9820A, or an IBM 360 Model 75 computer employing programs written in this laboratory.

Product Analysis. The reaction of I at pH <5 in the absence of external buffers gives a relatively stable product with a single absorbance maximum in the uv at 275 nm (pH 4). This λ_{max} is identical with that exhibited by authentic III at the same pH. Furthermore, if the pH is raised to 9.55 at the completion of the reaction at pH 4, a subsequent reaction occurs which gives II as the product (by spectrophotometric determination of the three pK_a's) and exhibits isosbestic points identical with the hydrolysis reaction of authentic III at the same pH. At pH >6 where disappearance of I as monitored at 320 nm is biphasic, the rate of hydrolysis of the initially formed phenyl acetate (III) becomes significant compared to the rate of its formation and accounts for the second phase. The reaction of I at pH 12.4 and 3° can also be shown to give III as the exclusive initial product (by the same procedure).

Deuterium solvent isotope effects were determined for the rearrangement I → III in 99.8% deuterium oxide (Stohler Isotope Chemicals) with μ = 1.0 with KCl using the previously described pH-Stat assembly to maintain constant pH. The pD values were taken as the pH meter readings plus the proper correction (0.39) at 30°[8] which was verified for the electrode employed (Metrohm EA 125).

pK_a Determinations. The titrimetric pK_a's of II (and III) have been determined previously[3] by procedures discussed therein. In the present study the pK_a's of IV and V were determined employing the same procedures. The wavelengths used were: IV, pK_{ImH^+} at 249 or 253 nm; pK_{PhOH} at 220.5 nm; V, pK_{ImH^+} at 354 nm, and pK_{PhOH} at 307 nm. The values thus determined are tabulated in Table I along with those previously determined for II and III.

Reaction of I with MeO^- in MeOH. The reaction was initiated by addition of <0.1 mg of sodium methoxide to 3 ml of a 5×10^{-5} *M* solution of I in anhydrous MeOH (distilled from Mg). The reaction was characterized by isosbestic points at 320, 280, and 225 nm and the spectrum of the product exhibited absorbances at 325 (340 sh), 276 (284 sh), and 250 nm. In comparison with the spectra of II *vs.* III[9] (allowing for solvent differences), the product resembled a phenoxide rather than a phenyl ester. The complete elucidation of the product structure was conducted in the following manner. At the completion of the methanolic reaction, the methanol was removed at ambient temperature (*in vacuo*). The residue was dissolved in 1 ml of 1 *M* HCl and the solution was immediately added to 20 ml of 1 *M* KCl in the Cary 15 cell (3.3-cm path length). Spectrophotometric titration revealed only two pK_a's between pH 1.61 and 10.29. These correspond to pK_{ImH^+} and pK_{PhOH} (Table I) for compound V. To confirm the esterification of the carboxylic



V

acid, the pH was raised to 12.3 and the subsequent hydrolysis was followed by repetitive scanning in the uv. The hydrolytic reaction exhibited isosbestic points at 283, 267, and 258 nm and k_{obsd} =

(5) G. A. Rogers and T. C. Bruice, *J. Amer. Chem. Soc.*, **96**, 2463 (1974).

(6) J. R. Maley and T. C. Bruice, *Anal. Biochem.*, **34**, 275 (1970).

(7) *E.g.*, between pH 10.50 and 12.12 the second phase of the reaction exhibited isosbestic points at 316.5–317, 271 ± 0.5, and 220.5–221 nm on a Cary 15 spectrophotometer.

(8) T. H. Fife and T. C. Bruice, *J. Phys. Chem.*, **65**, 1079 (1961).

(9) Compounds VI and IX, respectively, of ref 3.

Scheme II

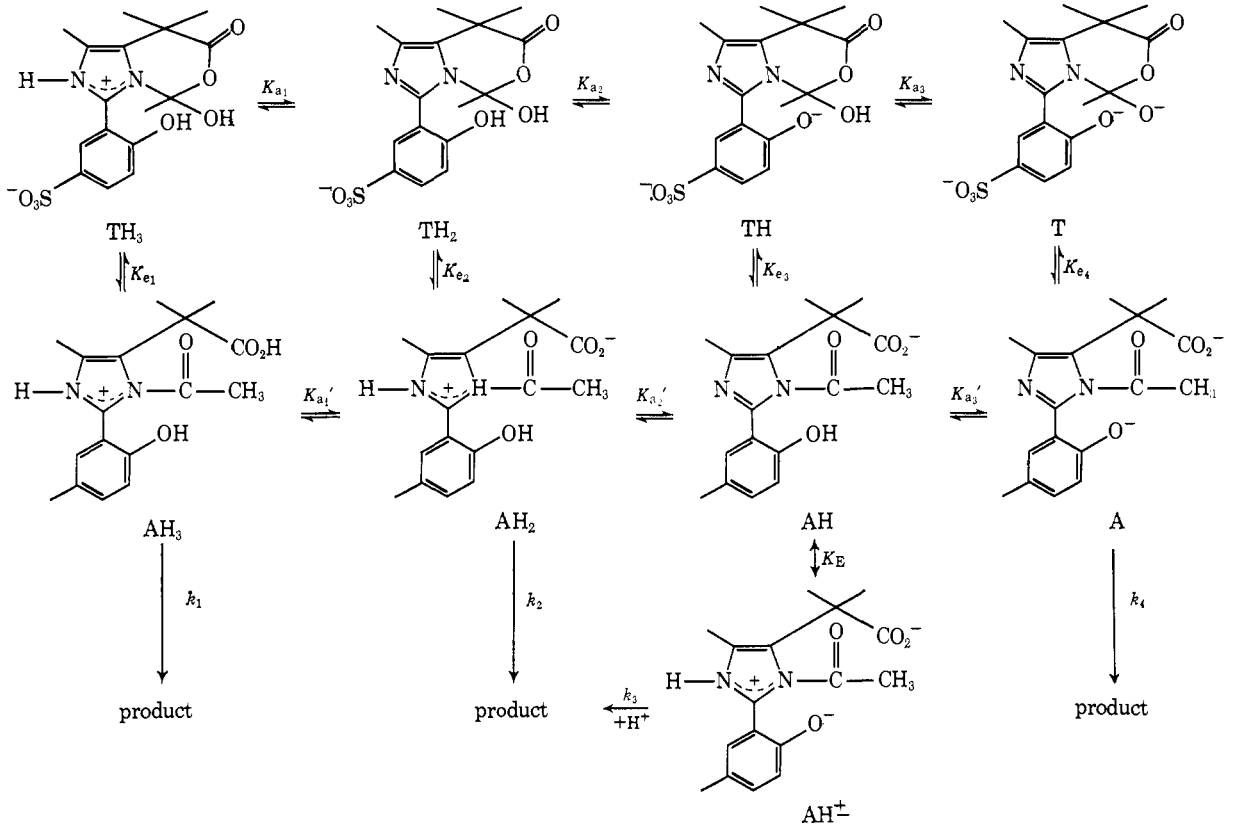


Table I. pK_a Values of II–V in H_2O (1 *M* KCl)

Compd	pK_{COOH}	pK_{ImH^+}	pK_{PhOH}
II	3.4	6.0	9.60
III	3.18[a]	6.6	
IV		5.89	8.9
V		5.4	8.6–8.7

[a] T = 3°; all others at 30°.

0.13 min^{-1}. This rate of hydrolysis is only threefold faster than that of the authentic methyl ester lacking the sulfonate group.[10] After hydrolysis was complete, spectrophotometric titration of the product gave spectra and pK_a's in excellent agreement with II.

Reaction of I with Oxygen and Nitrogen Bases. The rate of disappearance of I was determined in the presence of a number of aliphatic amines and carboxylic acids as well as phosphate buffers. Experiments were not performed over a wide pH range which would allow the assignment of catalytic coefficients to each ionic species of I. All buffer-catalyzed reactions were simply carried out at pH 6.50 (except where specifically stated otherwise). In each case the reaction was found to involve rearrangement of I → III or an acyl derivative of III. Unfortunately, even at 3° the I → III rearrangement is too rapid to allow spectrophotometric titration of the pK_a's of I. However, if the phenolic pK_a of I is near that of II (9.60) and if the imidazolium pK_a is similar to that of an acetylimidazole (*ca.* 3.60[11]) then at pH 6.50 the predominant species of I should be TH_2 (Scheme II). Therefore, at best, the buffers are all reacting with only one species of the substrate, and at worst, if a substrate pK_a exists in the vicinity of pH 6.50, then each buffer will still be reacting with the same mixture of substrate ionic species.

The hydrolysis of III at pH 6.50 in the absence of buffers exhibits a convenient isosbestic point at 267.4 nm at which the rearrangement of I → III can be monitored without optical interference from the subsequent hydrolysis of III → II. All of the carboxylic acid and phosphate buffered rearrangements of I were followed at this wavelength. For the amine buffers (as will be shown) an amide of III (*i.e.*, III′) is formed so that the isosbestic wavelengths for the subsequent hydrolysis of III′ had to be determined individually and are listed in Table II which includes the experimental conditions for all buffer studies. In the cases of buffers 5, 7–9, 13, and 15 the isosbestic wavelengths were determined by uv repetitive scanning of I → III′ → II′ where the λ_{max} of III′ in all cases was 284 nm. For

Table II. Experimental Conditions[a] for Reactions of I with Oxygen and Nitrogen Bases

Buffer	pH range	No. of k_{obsd}	Wavelength, nm	Concn range, *M* (total buffer)
1. Acetate	1.65, 6.51	9	320, 268.4	0.01–0.3
2. Formate	1.65, 6.51	11	320, 269	0.01–0.3
3. Chloroacetate	6.51	6	268.5	0.01–0.3
4. Betaine	2.00, 6.51	6	320, 268.5	0.1–0.5
5. Phosphate	6.26–12.19	26	268.5, 275, 305.7	0.067–0.623
6. Semicarbazide[b]	6.50	5	275	0.1–0.4
7. Hydroxylamine[c]	6.50	8	274	5×10^{-4}–5×10^{-3}
8. Methoxylamine	6.50	5	275	0.1–0.4
9. Hydrazine	6.50	6	274	0.005–0.03
10. Tris	6.50	4	274.5	0.3–0.5
11. Trifluoroethylamine	6.50	6	275	0.1–0.5
12. Glycine ethyl ester	6.50	5	274.5	0.1–0.5
13. Ethylamine	6.50	4	273.8	0.15–0.5
14. Methoxyethylamine	6.50	6	275	0.1–0.5
15. Morpholine	6.50	9	313	0.1–1.0
16. Trimethylamine	6.50	4	268.5	0.2–0.5
17. Carbonate	9.70–10.60	15	272, 306	0.05–0.5

[a] All reactions at 30° with μ = 1.0 with KCl. [b] All buffers were used on the same day they were made up and stored at 5° until used. [c] Reactions were run on a stopped-flow spectrophotometer with I dissolved in 1.7×10^{-2} *M* phosphate buffer at pH 6.50.

(10) See compound V of ref 3.

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

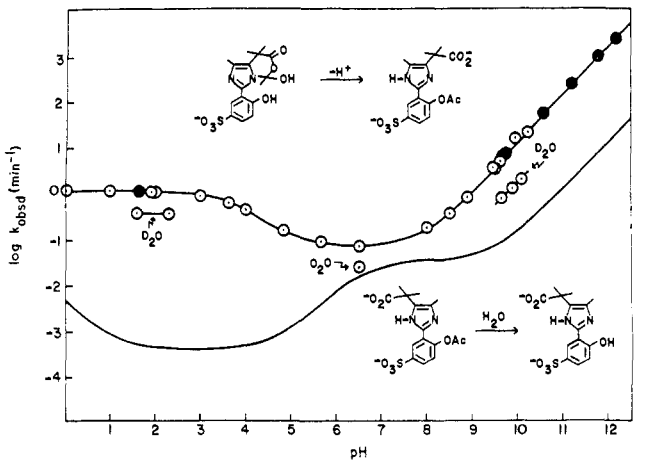


Figure 1. Plots of log k_{obsd} *vs.* pH for the rearrangement of tetrahedral compound I (○) and the hydrolysis of phenyl ester III (—). The points for I are experimental and the line theoretical employing eq 1 and the constants of Table III. The line for III was taken from ref 3: (○) either lyate species buffering or the use of the Cary 15 pH-Stat assembly; (●) k_{obsd} values obtained by extrapolation of buffer dilution plots to zero buffer concentration. All rates are spectrophotometric.

the remainder of the amines (except trimethylamine, which could be followed near the "spontaneous" isosbestic wavelength at 268.5 nm) a wavelength was used which, by trial and error, gave the most stable absorbance reading at t_∞ for I → III′ (always 274.4 ± 0.6 nm).

In addition to the buffers listed in Table II, imidazole (0.02 *M*, pH 6.50) was also studied *via* uv repetitive scanning for its ability to catalyze I → III. In fact, imidazole represents a more complex reaction which possesses two metastable intermediates. Repetitive scans of III under the same conditions showed an intermediate whose wavelength of maximum change during hydrolysis was *ca.* 245 nm, but was essentially undetectable at $\lambda > 280$ nm.

Rearrangement of I in DMF; Added Nucleophiles. In dry DMF (distilled from CaH_2 and stored over molecular sieves) as well as in DMSO and pyridine, the tetrahedral adduct (I) was stable for several days at least. The addition of triethylamine to a solution of I in DMSO did not catalyze the rearrangement nor did it alter the spectrum significantly. The rearrangement of I in DMF with imidazole (0.05 *M*) exhibited a $t_{1/2} > 1$ day. However, addition of *ca.* 40 μl of 1 *M* HCl (aqueous) to a solution of I (*ca.* 10^{-5} *M*) in 3 ml of DMSO catalyzed the rearrangement to III within minutes. Furthermore, sodium azide and methyl hydrazine (0.05 and 0.08 *M*, respectively) in DMF catalyze I → III′ with $t_{1/2}$ of minutes and seconds. Product spectra (uv) from the reaction of I → III′ → II′ in the latter two solutions were slightly different ($\Delta\lambda_{max}$ = 3 nm) from the product spectra from authentic III → II in the same solutions. Also, dilution of 3 ml of DMF solution containing the above mentioned products to 20 ml with 1 *M* aqueous KCl and titration *vs.* pH showed large differences in the isosbestic points associated with various pK_a's. While the product of III → II exhibits spectra *vs.* pH consistent with pure II, the product(s) of I → III′ → II′ exhibit(s) spectra indicative of more than one compound and significantly different from pure II.

Reaction of I with $HONH_2$ in H_2O. When I (7.7×10^{-6} *M*) was allowed to rearrange in water (1 *M* KCl) in the presence of 7×10^{-6} *M* $HONH_2$ at pH 6.51 (Cary 15/pH-Stat assembly), a large deviation from first-order kinetics was observed during the first 2 half-lives. For the 4th and 5th half-lives a first-order plot of the data was linear with $k_{obsd}' = 6.45 \times 10^{-2}$ min^{-1}. After completion of the first reaction the pH was raised to 8.10 and a second reaction was repetitively scanned until completion (*ca.* 3 hr). The pH was then lowered to 6.60 and a second aliquot of I was added. The subsequent rearrangement exhibited much less deviation from first-order kinetics than did the first aliquot.

Results and Discussion

Lyate Species Catalysis. At constant pH, and in the presence of a great excess of buffer over tetrahedral adduct (I) or when the Cary pH-Stat was employed, all spectrophotometrically determined rate constants (unless stated otherwise) for I → III were found to be pseudo first order (k_{obsd}). For those experiments employing buffers, extrapolation of plots of k_{obsd} *vs.* buffer concentration to zero buffer provided as intercepts the values of the pseudo-first-order rate constants for non-buffer-catalyzed reactions, and as slopes the values of the apparent second-order rate constants for buffer-catalyzed reactions.

Plotted in Figure 1 are the values of log k_{obsd} at zero buffer concentration *vs.* the constant pH (pD) at which k_{obsd} was measured for I in water and deuterium oxide. The points are experimental and the curve was manually iterated and computer drawn to the experimental points using eq 1 and employing the derived constants in Table III. Also included in Figure 1 and Table III are

Table III. Hydrolytic Rate Constants and Apparent pK_a's of I and III

	I	III
k_1'	1.05 min^{-1}	4.70×10^{-3} M^{-1} min^{-1}
k_2', min^{-1}	7.30×10^{-2}	4.2×10^{-4}
k_3', min^{-1}	13	3.60×10^{-2}
k_4', M^{-1} min^{-1}	$\geq 7.5 \times 10^4$	8.80×10^2
pK_{app_1}	3.75	6.60
pK_{app_2}	10.1	
pK_{app_3}	$\geq 13.7^a$	

[a] This apparent pK_a lies outside the kinetically accessible pH range and therefore uncertainty in k_4' results.

$$k_{obsd} = \frac{k_1' a_H}{a_H + K_{app_1}} + k_2' + \frac{k_3' K_{app_2}}{a_H + K_{app_2}} + \frac{k_4' K_{app_3}}{a_H + K_{app_3}} \quad (1)$$

the experimental results for the hydrolysis of III → II from a previous study.[3] The justification for the inclusion of pK_{app_2} into eq 1 comes directly from the data of the pH–log k_{obsd} profile for the rearrangement of I (Figure 1). The ascending limb of the profile in the alkaline region shows a small, but definite break at pH 10.1 where the slope of the line is >1. Since the rates of rearrangement in the vicinity of pH 10 are in the region accessible to both conventional and stopped-flow spectrophotometry, a certain amount of overlap was obtained in order to assure that the apparent break in the profile was not an artifact of changing measurement technique. The overlap and agreement of data collected on both types of spectrophotometers affirm the presence of a pK_{app} at pH 10.1.

Reaction rates in D_2O for I in three regions of the pH–log k_{obsd} profile are also plotted in Figure 1. From these values and those in H_2O at the corresponding pH's were calculated solvent deuterium kinetic isotope effects which are listed in Table IV. The ratio of the autoprotolysis constants of H_2O and D_2O [$K_W(H_2O)/K_W(D_2O)$] = 8.93[12] was used to adjust the isotope effect in the alkaline region to the condition $a_{OH} = a_{OD}$.

Of the several mechanistic schemes which could be devised to account for the profile for I (Figure 1), perhaps the most attractive is one which would postulate

(12) A. K. Covington, R. A. Robinson, and R. G. Bates, *J. Chem. Phys.*, 70, 3820 (1966).

Scheme III

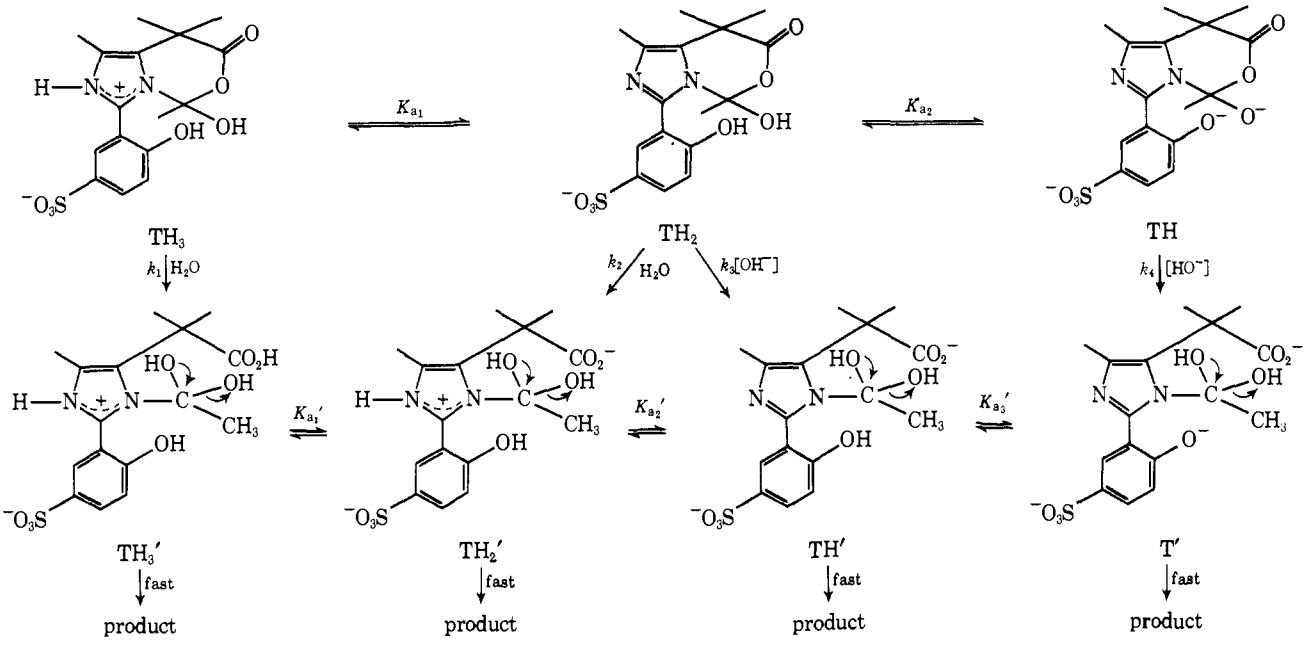


Table IV. Solvent Deuterium Isotope Effects for I

pH (range)	k^{H_2O}/k^{D_2O} [a]
1.59–2.31	3.0
6.51	2.9
9.65–10.10	0.82

[a] All reactions were run in the Cary 15/pH-Stat assembly in the absence of external buffers.

attack of phenol or phenolate upon an acetylimidazole intermediate as illustrated in Scheme II. Equation 2

$$k_{obsd} = \frac{K_{a_1}K_{e_2}[a_H{}^2(k_1a_H + k_2K_{a_1}') + K_{a_1}'K_{a_3}'(k_3a_H + k_4K_{a_2}')]}{K_{a_1}'[a_H{}^2(a_H + K_{a_1}) + K_{a_1}K_{a_2}(a_H + K_{a_3})]} \quad (2)$$

relates k_{obsd} to the hydrogen ion activity and is derived from a material balance in $TH_3 + TH_2 + TH + T$ and other indicated equilibrated intermediates. Equation 2 is kinetically equivalent to eq 1 and therefore Scheme II and eq 2 satisfy the experimental profile of Figure 1. The solvent deuterium kinetic isotope effects for I → III (3.0 and 2.9, Table IV) are in excellent agreement with those reported for the attack of water upon acetylimidazolium ion and acetylimidazole (2.5 and 2.7, respectively).[11] Unfortunately, without data on the magnitude of the equilibria constants relating the concentrations of the acetylimidazole intermediates to the concentrations of the ionic species of the tetrahedral intermediate (K_{e_1}–K_{e_4}), no assignment can be made relating the rate constants k_1–k_4 to the empirical constants k_1'–k_4' of eq 1. Strong precedent for $TH_2^{\pm}$ as the reactive intermediate at neutrality comes from the elegant studies of Jencks[13] on acetylimidazole and *N*-methylacetylimidazolium ion in which the plateau region for attack of phenols on acetylimidazole was shown to proceed through phenoxide attack on the protonated acetylimidazolium ion species.

(13) (a) W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1272 (1959); (b) R. Wolfenden and W. P. Jencks, *J. Amer. Chem. Soc.*, **83**, 4390 (1961); (c) J. Gerstein and W. P. Jencks, *ibid.*, **86**, 4655 (1964).

The second mechanism (Scheme III) which might seem plausible would have water and its conjugate base add covalently to the carbonyl carbon (evidence presented later will show this mode of catalysis operative in the case of nitrogen nucleophiles) or carry out a displacement upon the hemiacylal tetrahedral carbon. From a material balance in $TH_3 + TH_2 + TH$ and assumed acid–base equilibria the derived rate expression is provided in

$$k_{obsd} = \frac{a_H{}^2(k_1a_H + k_2K_{a_1}) + K_{a_1}K_w(k_3a_H + k_4K_{a_2})}{a_H{}^2(a_H + K_{a_1}) + a_HK_{a_1}K_{a_2}} \quad (3)$$

While not of the same mathematical form as eq 2, eq 3 also may be employed to fit the pH–log k_{obsd} profile of Figure 1. As is seen in this scheme, a second tetrahedral intermediate must be included on the path to an acetylimidazole and eventually the phenyl acetate (III). It must be regarded as unlikely for the formed tetrahedral intermediates (TH_3' and TH_2') to expell hydroxide ion in preference to neutral imidazole ($\Delta pK = 8.85$) with the 100% fidelity necessary to account for the single phenyl acetate product. While general acid assistance by the neighboring phenol or carboxyl in expelling the hydroxyl group as neutral water is entirely possible in view of the substrate geometry, this mechanism could not be functional above the pK_a of the phenol. However, even at pH 12.4 the phenyl acetate III is formed as the initial product. Expulsion of imidazole in preference to hydroxide would give acetate and the phenol II, not III. In addition, molecular models of the formed intermediate were impossible to construct without introducing severe bond distortion in the carboxylic acid side chain.

Buffer Acceleration. In all instances where buffers were employed, rates of disappearance of I at constant pH were found to be linearly related to buffer concentration. No buffer acceleration was observed by acetic and formic acids at pH 1.65, by phosphate at pH 11.22–12.19, nor by carbonate at pH 10.60. Phosphate was employed at three pH values in the neutral pH plateau region and the buffer dilution plots are

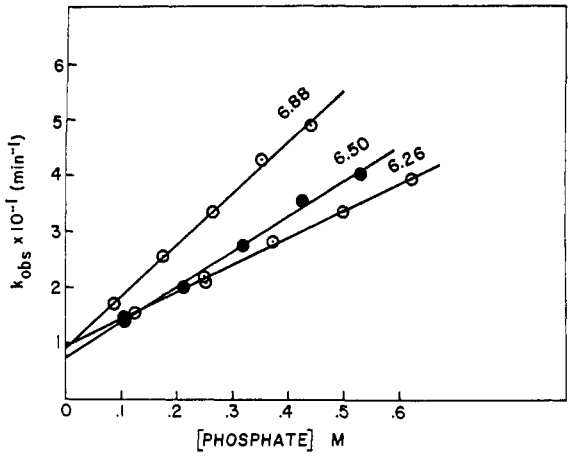


Figure 2. Plots of k_{obsd} *vs.* [phosphate]$_{total}$ for I → III at pH 6.88, 6.50, and 6.26; (●) buffer dilutions carried out at pH 6.50.

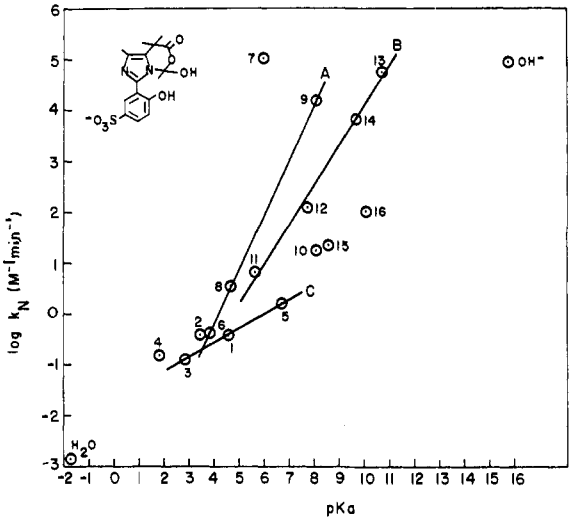


Figure 3. Brønsted plots of the log of the second-order rate constants (k_N) representing buffer-catalyzed rearrangement of I → III (or III′) *vs.* the pK_a of the buffer species. Numbers refer to the buffers of Table V. Line A connects nitrogen α-effect nucleophiles, line B primary amines, and line C anionic acetates and phosphate.

illustrated in Figure 2. The increase in the slope of the plots with increasing pH indicates the basic form of the buffer is involved in the reaction. The second-order rate constants (k_b and k_N) representing buffer acceleration are listed in Table V. The values of the second-order rate constants (k_N) are plotted against the respective basicity of buffer in Figure 3. Examination of Figure 3 reveals that line A (slope 1.0) correlates the α-effect[14] nucleophiles 6, 8, and 9 which all must react by nitrogen attack; line B (slope = 0.77) joins buffers 11–14 which are all primary amines with similar steric properties; and line C (slope 0.3) somewhat arbitrarily connects two anionic acetates (1 and 3) with phosphate (5). This division into diverse Brønsted families is anticipated if nucleophilic attack is involved with a portion of the bases.[15] The observance of converging Brønsted plots has been observed previously for a series of

(14) J. O. Edwards and R. G. Pearson, *J. Amer. Chem. Soc.*, **84**, 16, 3233 (1962).

(15) T. C. Bruice and R. Lapinski, *J. Amer. Chem. Soc.*, **80**, 2265 (1958).

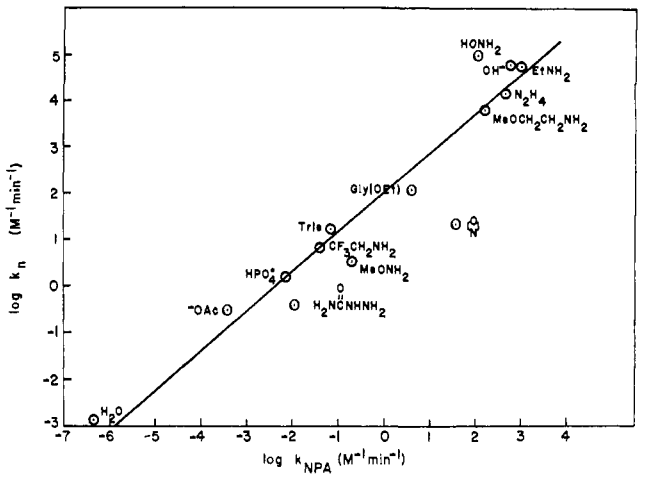


Figure 4. Plot of log of the second-order rate constants representing buffer-catalyzed rearrangement of I → III (or III′) (k_N) *vs.* log of the second-order rate constants for hydrolysis or aminolysis of *p*-nitrophenyl acetate.

Table V. Second-Order Rate Constants (k_N, M^{-1} min^{-1}) for Buffer Catalysis

Buffer	pK_a	k_b,[a] M^{-1} min^{-1}	k_N,[b] M^{-1} min^{-1}
1. Acetate	4.61[c]	0.393	0.393
2. Formate	3.49[d]	0.379	0.383
3. Chloroacetate	2.73[e]	0.131	0.131
4. Betaine	1.84[f]	0.154	0.154
5. Phosphate	6.7[g]	0.629	1.62
6. Semicarbazide	3.88[e]	0.416	0.417
7. Hydroxylamine	5.98[h]	7.93×10^4	1.03×10^5
8. Methoxylamine	4.68[e]	3.44	3.49
9. Hydrazine	8.10[h]	3.70×10^2	1.51×10^4
10. Tris	8.10[h]	0.44	18
11. Trifluoroethylamine	5.63[i]	5.92	6.72
12. Glycine ethyl ester	7.75[h]	6.57	1.23×10^2
13. Ethylamine	10.71[i]	3.45	5.6×10^4
14. Methoxyethylamine	9.72[j]	3.95	6.6×10^3
15. Morpholine	8.59[k]	0.181	22.4
16. Trimethylamine	10.08[l]	0.054	1.03×10^2

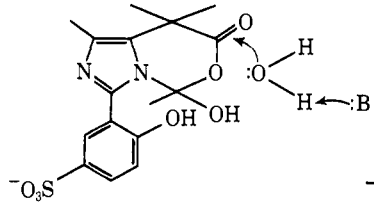
[a] Slopes of k_{obsd} *vs.* total buffer concentration at pH 6.50. [b] k_b divided by the fraction of buffer in the basic form. [c] B. Holmquist and T. C. Bruice, *J. Amer. Chem. Soc.*, **89**, 2327 (1967). [d] D. Auld and T. C. Bruice, *ibid.*, **89**, 2098 (1967). [e] At 25°; E. G. Sanders and W. P. Jencks, *ibid.*, **90**, 4377 (1968). [f] R. M. C. Dawson, *et al.*, "Data for Biochemical Research," Clarendon Press, Oxford, 1959. [g] Apparent pK_a from kinetic data of Figure 2. [h] B. Holmquist and T. C. Bruice, *J. Amer. Chem. Soc.*, **91**, 2993 (1969). [i] T. C. Bruice, A. Donzel, R. W. Huffman, and A. R. Butler, *ibid.*, **89**, 2106 (1967). [j] W. P. Jencks and Mary Gilchrist, *ibid.*, **90**, 2622 (1968). [k] T. C. Bruice and W. S. Chou, *ibid.*, **85**, 1659 (1963). [l] T. C. Bruice and S. J. Benkovic, *ibid.*, **85**, 1 (1963).

bases of different type[16] (nitrogen *vs.* oxygen nucleophiles, etc.) reacting with δ-thiolvalerolactone. In this instance a good correlation was observed when the logarithms of the second-order rate constants were plotted *vs.* the like constants for nucleophilic attack on *p*-nitrophenyl acetate. This treatment of the data tends to cancel out differences in nucleophilicities due to solvation of nucleophiles and steric factors and compares the nature of the transition states for the reaction of nucleophiles with two different esters. When this approach is applied to the data of Table V (k_N), a reasonably good correlation is obtained (Figure 4) with

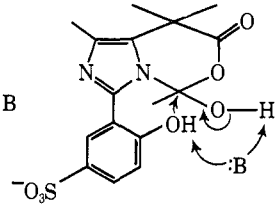
(16) (a) T. C. Bruice, J. J. Bruno, and Wei-Shin Chou, *J. Amer. Chem. Soc.*, **85**, 1659 (1963); (b) M. J. Gregory and T. C. Bruice, *ibid.*, **89**, 2121 (1967).

but a few exceptions. There is no particular pattern to the observed deviations from the line of Figure 4 and any discussion of the causes would be merely speculative. The excellent correlation for the primary amines and the slope of 0.86 indicates transition states exhibiting similar sensitivity to steric and electronic effects for reaction of nucleophiles with I and *p*-nitrophenyl acetate. It might be tenuously concluded, and more conclusive evidence will be presented, that the reaction of primary amines with I involves direct nucleophilic attack at the carbonyl carbon to provide an amide. These amides will be shown to be amides of III (and will be designated as III′).

The conclusion based upon the probable similarities in transition states between *p*-nitrophenyl acetate and the tetrahedral adduct (I) is most simply substantiated by the result obtained when hydroxylamine and I were reacted in equal concentrations in the pH-stated Cary 15 cell (Experimental Section). If hydroxylamine were acting as a general base either by assisting the attack of water (VIII) or by removing the proton from

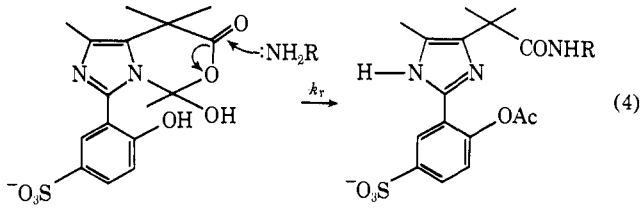


VIII



IX

the tetrahedral or phenolic OH (IX), then first-order kinetics would be observed early in the reaction with possible deviation later due to decrease in hydroxylamine concentration brought about by acetyl transfer from III. However, what was observed with a large deviation from first-order kinetics early in the reaction (1st and 2nd half-lives) and a transition to a first-order rate nearly identical with the "spontaneous" rearrangement of I at that pH. Thus, the first step (and the rate-determining one) in the amine reaction is the conversion of I to an amide of III (III′) as depicted in eq 4.

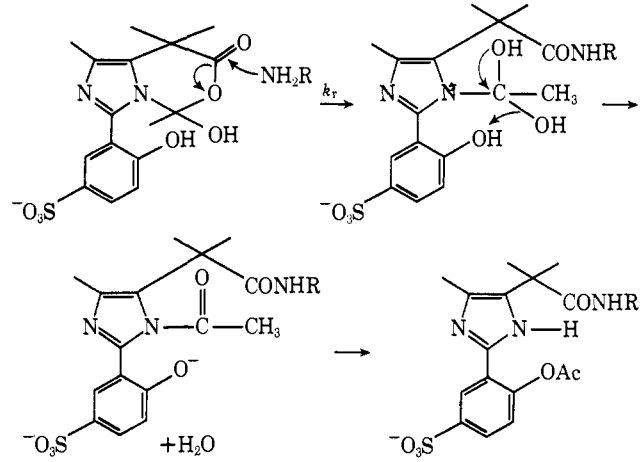


(4)

I III′

Choice of the carbonyl carbon over the tetrahedral carbon as the site for nucleophilic attack by amines is favored for the following reasons. (1) The attack of hydroxylamine upon the tetrahedral carbon with subsequent rearrangement to the phenyl acetate (III′) would necessitate that the amine be regenerated in a fast step in order for first-order kinetics to be observed. (2) The isosbestic wavelength for the second phase of the reaction I → III′ → II′ at pH 6.50 catalyzed either by lyate species or carboxylic acids and phosphate buffers is different from that obtained in the presence of amines. This shift is from 268.5 to 274.4 nm. The only explanation for this shift is that either the phenyl ester or phenol (or both), which are formed in the amine reactions, have spectra different from III or II (*i.e.*, I → III′ → II′). That the wavelength shift is not due to a change in solvent composition by inclusion of amine is evidenced by the fact that even at 5×10^{-3} *M* hydroxylamine, I rearranges to III′ (λ_{max} = 284 nm) and III′ hydrolyzes to II′ with an isosbestic point at 274 nm. Therefore, differences in spectra between III and III′ arise because of covalent addition of amines to I to give the amide–phenyl ester III′. If such an intermediate were formed in the acetate- or phosphate-catalyzed rearrangement of I to give an anhydride or acylphosphate, respectively, it would surely hydrolyze in a fast step to give III. Therefore by this method of product analysis such a reaction is indistinguishable from mechanism IX. (3) The reaction of I with methoxide in anhydrous MeOH to give the methyl ester of II (*i.e.*, V) strongly supports the carbonyl carbon of I as the site of attack by strong nucleophiles. The observance of first-order kinetics for the reaction I → V illustrates that CH_3O^-/CH_3OH possesses an enhanced reactivity compared to HO^-/H_2O toward both the phenyl ester and the carbonyl group of the tetrahedral intermediate. The greater reactivity of alkoxides *vs.* hydroxide toward phenyl acetates is well known.[17] (4) The conversion of the carboxyl group in the acetylimidazole intermediate of Scheme IV to an amide pre-

Scheme IV



vents the breakdown of this intermediate from being the rate-determining step as in Scheme III. That is, the partitioning of the acetylimidazole intermediate back to starting material (I) is blocked by chemically removing the carboxylate from the competition with the phenol. This mode of catalysis is also operative in the case of methoxide (but not hydroxide) attack at the carbonyl carbon.

The site for amine attack upon I as depicted in eq 4 would appear to lead to the same difficulties of leaving group expulsion noted in Scheme III. Experimentally, however, amines do carry out nucleophilic attack at the carbonyl group of the tetrahedral intermediate (I) to yield a phenyl ester. This requires explanation. If amine reacts with TH_3, then expulsion of a neutral imidazolyl species should be competitive with expulsion of HO^- leading to acetate plus II and not III′. However, amine attack on TH_2 with intramolecular general acid phenolic assistance of expulsion of HO^- from the intermediate tetrahedral intermediate (Scheme IV) would give III′. Though the phenolic hydroxyl group

(17) Footnote *j* of Table V.

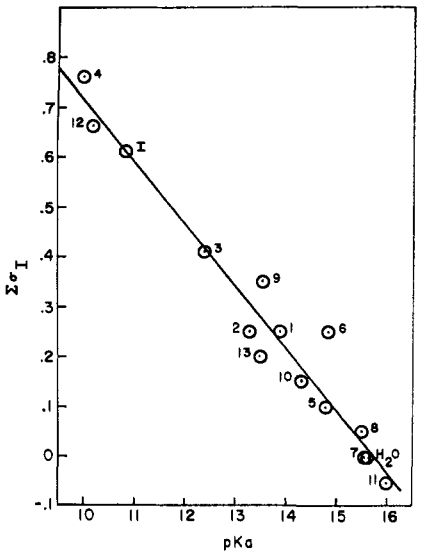
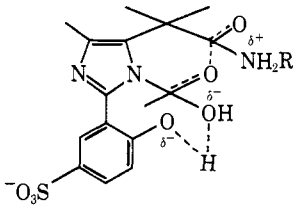


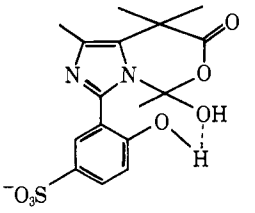
Figure 5. Plot of σ_I or $\Sigma\sigma_I$ *vs.* pK_a (from various sources) for a series of alcohols: (1) choline, (2) $HCH(OH)_2$, (3) CF_3CH_2OH, (4) $PhCCF_3(OH)_2$, (5) CH_2OHCH_2OH, (6) $MeO(CH_2)_2OH$, (7) MeOH, (8) $CH_2{=}CHCH_2OH$, (9) $HC{\equiv}CCH_2OH$), (10) $CHCl_2CH_2OH$, (11) EtOH, (12) $CF_3CH(OH)_2$, (13) $CH_3CH(OH)_2$. The tetrahedral compound I is also included.

is a weak acid, it is in pK_a intermediate to that for protonation of the tetrahedral OH group and the HO^- product and thus fulfills the requirement of a general acid for this process. Considering the facility with which the *gem*-diol intermediate of Scheme IV is expelled by amines of low basicity, it would not be unreasonable to propose that the expulsion of the tetrahedral OH catalyzed by the phenolic general acid is concerted with amine expulsion of the "lactone-like" oxyanion. Hence, in the transition state X, the oxygen



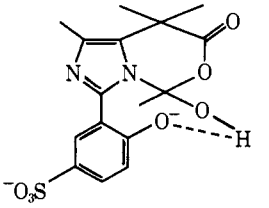
X

being displaced by the amine would already possess some carbonyl character. The mechanism of amine-catalyzed rearrangement of I → III′ closely mimics the type of intramolecular general catalysis often thought to function at the active site of many enzymes. That is, catalytic groups held in close proximity catalyze the expulsion of substrate functionalities which could not otherwise be expelled by groups of lower basicity. Strong evidence for ground-state hydrogen bonding between the tetrahedral hydroxyl of I and the phenolic group comes from the apparent perturbations of their two pK_a values. The difference in the titrimetric pK_a of the phenolic hydroxyl group [pK_{PhOH} = 9.50 (Table I)] and pK_{app} [pK_{a_2} = 10.1 (Scheme II)] is consistent with the weak hydrogen bond expected in structure XI.



XI

However, from a plot of $\Sigma\sigma_I$ *vs.* pK_a's of alcohols (Figure 5) (and estimating σ_I for the imidazolyl group of I at +0.27) one would expect the tetrahedral hydroxyl to possess a pK_a of *ca.* 11. The fact that pK_{a_3} of Scheme II is $\geq$13.7 indicates strong hydrogen bonding in the structure XII. Of course, ground-state hydrogen



XII

bonding may not be indicative of processes occurring in the transition state, but it does indicate that spatial arrangement of the catalytic groups does favor the mechanism of X.

Acknowledgment. This work was supported by a grant from the National Institutes of Health. We should like to thank Mr. Stanley Mathes for providing the HA-100 nmr spectrum of the tetrahedral adduct (I).