Synthesis and Evaluation of a Model for the So-Called "Charge-Relay" System of the Serine Esterases

Gary A. Rogers[1] and Thomas C. Bruice*

Contribution from the Department of Chemistry, University of California, Santa Barbara, California 93106. Received September 22, 1973

Abstract: The synthesis, acid–base properties, and hydrolytic reactions of model compounds for the Asp-His-Ser triad of the serine esterases are presented. The phenyl acetates VII, VIII, and IX [4(5)-methyl-5(4)-dimethylacetic acid derivatives of O-acetyl esters of unsubstituted and substituted 2-(2'-hydroxyphenyl)imidazoles] were found to hydrolyze via the same mechanistic pathways previously determined for the esters without the carboxyl function. That is, while both imidazolyl general acid and general base assisted H_2O attack at the ester bond are observed, no O→N acetyl transfer to the imidazole anion was detected. Evidence presented shows a threefold rate enhancement, attributable to the carboxyl group, and associated with the imidazolyl general base assisted H_2O attack upon esters VII and IX. When IX is transferred to an aprotic solvent (CH_3CN) containing but 6% H_2O, no enhancement of the catalytic role of either the imidazolyl or the carboxyl group is observed. The acid–base properties of the phenols IV, V, and VI (phenolic hydrolysis products of VII, VIII, and IX) in both H_2O and aqueous–organic mixed solvents of lower dielectric show that there is no inversion of the carboxyl and imidazolyl base strengths. Even in 96% dioxane, $pK_{ImH^+} \gg pK_{COOH}$. These findings are discussed in light of what has been proposed for the pK_a values of the catalytic triad of amino acid residues at the active site of the serine esterases.

The most studied enzyme of a group of enzymes called the serine esterases is α-chymotrypsin. Others in this group include trypsin, thrombin, and subtilisin. The term serine esterase derives from the fact that this class of enzymes contain at their active site a serine hydroxyl group which exhibits unusual reactivity to agents such as diisopropyl fluorophosphonate. X-Ray crystallography has provided the tertiary structure of chymotrypsin,[2] trypsin,[3] and subtilisin.[4] Although the amino acid sequences for chymotrypsin and subtilisin are quite dissimilar, the catalytic apparatus of their active sites are composed of the same functional groups arranged in approximately the same geometrical relationship. Such invariance in active site structure implies identity of mechanism. In this study we are concerned with the role of a triad of functional groups consisting, for chymotrypsin, of the carboxyl group of Asp-102 which is in hydrogen bonding distance of the imidazolyl group of His-57 which in turn is in hydrogen bonding distance of the hydroxyl group of Ser-195.[5] The geometrical arrangement of the triad Asp-His-Ser in the serine esterases has been referred to as the charge-relay system. For normal esters and amides the nucleophilic center is the oxygen of the serine moiety[6] though for certain highly reactive esters the imidazole nitrogen may be the primary nucleophilic center.[7] Serine oxygen attack is thought to be assisted by general base removal of the H-O-serine hydrogen by imidazole. General base catalysis of the transesterification of esters is a well-established phenomenon.[8] In the pro-

posed charge-relay mechanism for the serine esterases the carboxyl anion of an aspartic acid moiety functions as a second general base in concert with the histidine imidazolyl group. The envisioned mechanism of the charge relay in the acylation and deacylation steps for ester hydrolysis is shown in A. Precedent for a tandem general base mechanism does exist in the literature. Thus, the reaction of imidazole with p-CH₃O and p-CH₃C₆H₄OC(O)CH₃ is bimolecular in imidazole and possesses a deuterium solvent isotope effect (k^{H_2O}/k^{D_2O}) of 2.2 in accord with the mechanism of B.[9]

In considering the established serine esterase triad of $-COO^- \cdots IM \cdots HOR$ two questions come to mind. First, what are the acid–base properties and how are they influenced by the dielectric constant of their surroundings (i.e., can an alteration of the dielectric constant of the medium invert the pK_a values of $-IMH^+$ and $-COOH$, etc.)? Second, can the inherently weaker COO^- base serve a useful kinetic role as a tandem general

A

B
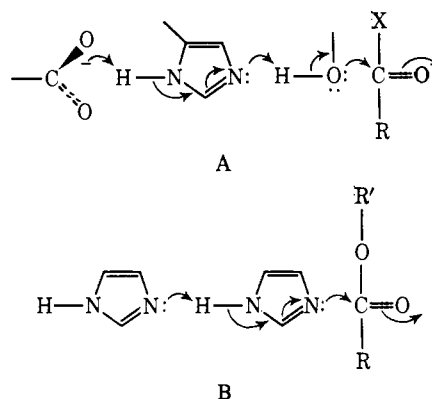
base to assist proton removal by a more strongly basic imidazole group? To answer these specific questions the triad, without and with substrate, must be isolated

(1) A portion of the material submitted by G. A. Rogers in partial fulfillment of the requirements for the Ph.D. in Chemistry.

(2) D. M. Blow, Biochem. J., 112, 261 (1969).

(3) R. M. Stroud and M. Krieger, Abstracts, 9th International Congress of Biochemistry, Stockholm, July 1973, Paper 25a-2.

(4) C. S. Wright, R. A. Alden, and J. Kraut, Nature (London), 221, 235 (1969).

(5) D. M. Blow, J. J. Birktoff, and B. S. Hartley, Nature (London), 221, 337 (1969).

(6) For a comprehensive review of the chemical evidence which establish the role of the serine hydroxyl and histidine imidazole groups in the mechanism of chymotrypsin action, see T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, New York, N. Y., 1966, Chapter 2.

(7) C. D. Hubbard and J. F. Kirsch, Biochemistry, 11, 2483 (1972).

(8) W. P. Jencks and J. Carriulo, J. Amer. Chem. Soc., 83, 1743 (1961).

(9) T. C. Bruice and S. J. Benkovic, J. Amer. Chem. Soc., 86, 418 (1964).

Scheme I
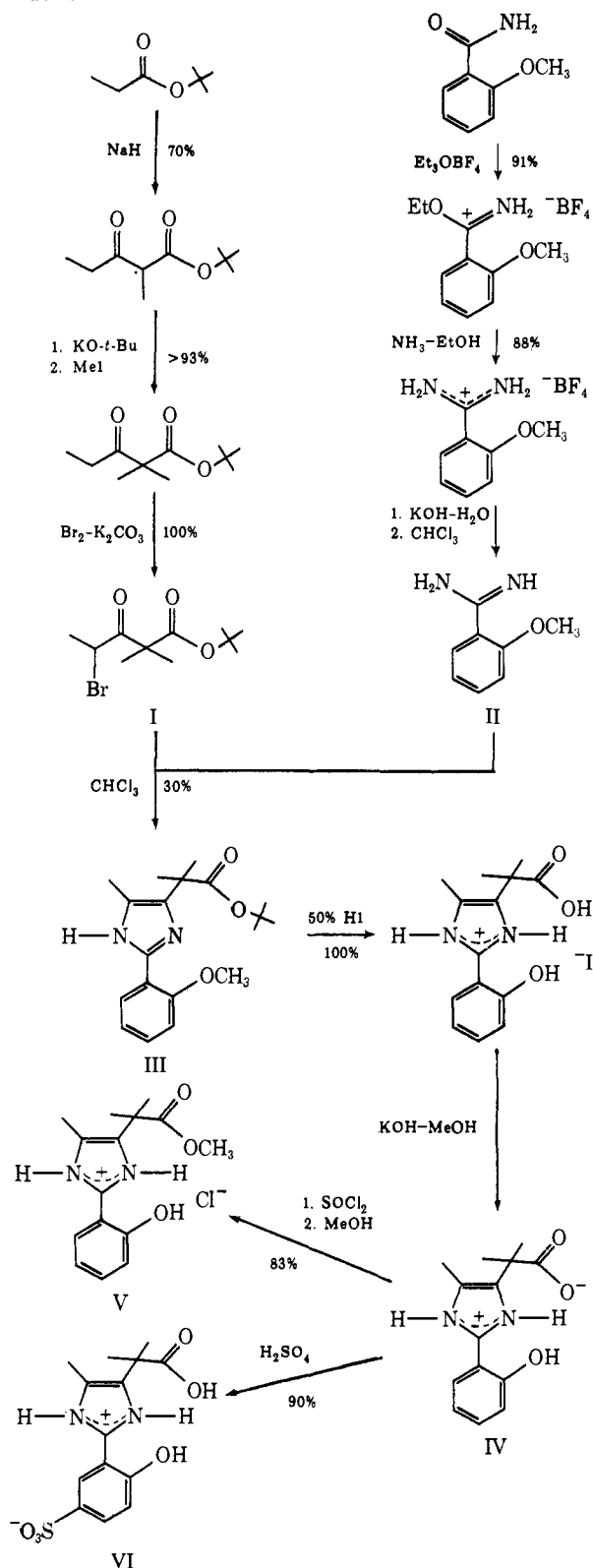
from other features of the active site (oxyanion hole,[10] etc.). This is best accomplished through study of a synthetic model. We report herein studies of the acid–base properties of compounds IV, V, and VI and the results of kinetic studies of the hydrolysis of their phenolic acetyl esters (VII, VIII, and IX, respectively). The synthetic sequences employed to obtain IV, V, and VI are provided in Scheme I.

(10) J. D. Robertas, J. Kraut, R. A. Alden, and J. Birktof, *Biochemistry*, 11, 4293 (1972).

Experimental Section

Materials. Potassium chloride was reagent grade and used without further purification. All water used in kinetic runs was deionized and double glass distilled.

tert-Butyl 4-Bromo-2,2-dimethylpropioacetate (I). To *tert*-butyl propionate (120.7 g, 0.928 mol, K and K) over a 2.5-hr period there was added, with constant stirring under anhydrous conditions, 40.5 g (0.96 mol) of NaH (57% oil dispersion). When addition was complete the temperature was increased from 65 to 120°. The reaction mixture was heated for an additional 18 hr, cooled, and dissolved in an ether–water mixture. After the two layers were separated, the water layer was extracted three times with ether and the combined ether fractions were dried (CaCl₂), filtered through MgSO₄, and concentrated on a rotary evaporator and the resulting yellow oil placed in the freezer in a graduated cylinder. The white mineral oil (from the NaH dispersion) separated to the top of the cylinder and was removed. The remaining yellow oil was distilled through a claison head at 0.5–0.75 mm. Colorless oil was collected from 38 to 46°: yield of *tert*-butyl 2-methylpropioacetate = 60.5 g (0.325 mol); ir (liquid film) 1730 (CO_2R), 1710 (C=O), and 1365 cm^{-1} (*tert*-butyl); nmr (neat) δ 1.00 (t,3,J = 7 Hz, CH_3CH_2), 1.18 (d,3,J = 7 Hz, CH_3CH), 1.45 (s, 9), 2.58 (q,2,J = 7 Hz, CH_2CH_3), and 3.48 ppm (q,1,J = 7 Hz, $CHCH_3$).

Potassium *tert*-butoxide (24.1 g, 0.215 mol) was dissolved in *ca.* 150 ml of *t*-BuOH, followed by the addition of 40 g (0.215 mol) of *tert*-butyl 2-methylpropioacetate. After a few minutes the solution turned into a solid white mass. Methyl iodide (33.8 g, 0.238 mol) was added and the solution was stirred at ambient temperature for 24 hr followed by reflux for 5 hr. The solution was filtered and the precipitated KI was washed with *t*-BuOH and Et_2O (three times). Solvent was removed on a rotary evaporator to yield 39.87 g (0.20 mol) of pale yellow oil: ir (liquid film) 1735 (CO_2R), 1705 (C=O), and 1365 cm^{-1} (*tert*-butyl); nmr (neat) δ 1.00 (t,3,J = 7 Hz, CH_3-CH_2), 1.25 (s,6), 1.43 (s,9), 2.46 (q,2,J = 7 Hz, CH_2CH_3). The nmr showed no significant impurities and the *tert*-butyl 2,2-dimethylpropioacetate was used without further purification. A sample was distilled under reduced pressure for analysis.

Anal. Calcd for $C_{11}H_{20}O_3$: C, 65.97; H, 10.07. Found: C, 66.08; H, 9.72.

tert-Butyl 2,2-dimethylpropioacetate (10 g, 0.05 mol) was dissolved in 70 ml of dioxane (freshly distilled from Na) which contained 7.0 g of K_2CO_3. This mixture was stirred and titrated with Br_2, taking up in slight excess over 1 equiv. When the Br_2 color persisted for several minutes, the reaction mixture was filtered through $NaHCO_3$ and concentrated on a rotary evaporator to give a yellow oil. The oil was dissolved in ether and the ethereal solution was extracted three times with H_2O to give a colorless solution. The ethereal solution was dried (CaCl₂), filtered through MgSO₄, and concentrated *in vacuo* yielding I quantitatively: ir (liquid film) 1745 (CO_2R), 1715 (C=O), and 1365 cm^{-1} (*tert*-butyl); nmr (CDCl₃) δ 1.37 (s,3), 1.47 (s,9), 1.52 (s,3), 1.75 (d,3,J = 7 Hz, CH_3CHBr), and 4.68 ppm (q,1,J = 7 Hz, $CHBrCH_3$).

2-Methoxybenzamidine (II) was prepared from 2-methoxybenzamide by the procedure of Weintraub.[11] 2-Methoxybenzamide was prepared from salicylamide (Sigma) in good yield by methylating with dimethyl sulfate in acetone over K_2CO_3. Crystallization from benzene afforded white crystals: mp 126–126.5° (lit.[12] 127.5°). 2-Methoxybenzamide was converted into the ethyl imidate fluoroborate salt (using Et₃OBF₄) in 91% yield. The salt was nonhygroscopic with mp 144–146°; ir (KBr) 3290 (=NH₂⁺), 3180 (=NH₂⁺), and 1670 cm^{-1} (–C=N⁺). The amidine free base (II) was obtained in 88% yield *via* treatment of the imidate with NH₃/EtOH followed by extraction from strongly alkaline aqueous solution with CHCl₃: ir (Nujol) 1630 cm^{-1} (N=C–N); nmr (CDCl₃) δ 3.84 (s,3), 6.54 (s, 1.5), and 6.8–7.7 ppm (m,4). Upon addition of trifluoroacetic acid to the nmr sample, the peak at 6.54 ppm shifted downfield to 6.90 ppm and increased in intensity (identifying it as the amidinium proton peak). Interestingly, the aromatic resonances were identical before and after protonation of the amidine. The picrate salt was prepared in EtOH: mp 235–237°; nmr (DMSO-d_6) δ 3.93 (s,3), 7.0–7.9 (m,4), 8.71 (s,2,ArH), 8.87 (s,2, NH), 9.20 (s,2, NH).

Anal. Calcd for $C_{14}H_{13}N_5O_8$: C, 44.33; H, 3.45; N, 18.47. Found: C, 44.49; H, 3.53; N, 18.52.

(11) L. Weintraub, S. Oles, and N. Kalish, *J. Org. Chem.*, 33, 1679 (1968).
(12) H. Lindemann and W. Schultheis, *Justus Liebigs Ann. Chem.*, 451, 255 (1930).

2-(2'-Methoxyphenyl)-4(5)-methyl-5(4)-(tert-butyl 2'',2''-dimethylacetate)imidazole (III). tert-Butyl 4-bromo-2,2-dimethylpropioacetate (I) (6.93 g, 24.8 mmol) was dissolved in ca. 50 ml of benzene and 7.5 g (50 mmol) of 2-methoxybenzamidine (II) was added. When the mixture was heated to reflux, extensive foaming occurred. Enough $CHCl_3$ was added to reduce foaming and the solution was refluxed for 10 hr, cooled, and filtered. A white solid (5 g) was identified as the amidine hydrobromide by ir and nmr, mp 149.5–150°. The filtrate was concentrated to an oil and then diluted with 10 ml of CH_2Cl_2 and 40 ml of petroleum ether. A yellow, amorphous solid precipitated which was composed mostly of 2-methoxybenzamide. The solution was decanted from the amide and crystallization began within minutes. The first crop yielded 0.6 g of white crystals, while subsequent crops were yellow. Recrystallization from acetonitrile (slowly at −5°) afforded large white crystals of III in 33% yield (based on I): mp 136–137°; ir (KBr) 3380 (NH), 1720 (CO_2R), and 1365 cm⁻¹ (tert-butyl); nmr ($CDCl_3$) δ 1.43 (s,9), 1.60 (s,6), 2.33 (s,3), 4.00 (s,3), 6.8–7.3 (m,3), and 8.3 ppm (m, 1); mass spectrum (70 eV) m/e 330 (parent peak).

Anal. Calcd for $C_{19}H_{26}N_2O_3$: C, 69.06; H, 7.93; N, 8.48. Found: C, 69.06; H, 7.69; N, 8.47.

2-(2'-Hydroxyphenyl)-4(5)-methyl-5(4)-(2'',2''-dimethylacetate)-imidazolium (IV). The deblocking of III was carried out in the dark in refluxing 50% aqueous HI for 20 hr. The colorless solution was cooled to 0° and the white solid was collected by filtration. Yield of white crystals (after drying *in vacuo* over KOH) was 98%: mp 218–220° dec; ir (KBr) 1725 (CO_2H), 1690 (CO_2H chelated), 1640 (ImH⁺), and 1505 (C=C); nmr (D_2O) δ 1.83 (s,6), 2.47 (s,3), and 6.9–7.7 ppm (m,4). The hydriodide was converted into the neutral zwitterion (IV) by dissolving in MeOH followed by precipitation with 5 N KOH (aqueous) to an end point determined by uv. The white precipitate was collected by filtration in >93% yield: mp 261–263° dec; ir (KBr) 1635 (ImH⁺), 1605 (C=C), and 1555 cm⁻¹ (CO_2^-); pK_a ($H_2O/1$ M KCl) = 3.18 (CO_2H), 6.93 (ImH⁺), and 9.81 (PhOH).

2-(2'-Acetoxyphenyl)-4(5)-methyl-5(4)-(2'',2''-dimethylacetic acid)imidazolium Chloride (VII). The acetate ester of IV was prepared by the addition of a twofold excess of acetyl chloride to 80 mg of the zwitterion in 7 ml of dry CH_2Cl_2 protected with a $CaCl_2$ drying tube. Within 5 min of the addition of ca. 60 mg of pyridine (dried over KOH) all of the zwitterion dissolved. After 10 hr the solution was diluted with anhydrous Et_2O and gassed with dry HCl. After the solvent was removed *in vacuo*, the resulting oil was taken up in 6–7 ml of hot CH_2Cl_2 and placed in the freezer. After 2 days a small amount of crystals (VII) was collected and washed with Et_2O: mp 187–189° dec; uv spectra showed some of the crystals to be contaminated by the starting material (IV); ir (KBr) 1770 (CO_2R), 1730 (CO_2H), 1640 (ImH⁺), and 1185 cm⁻¹ (CO).

2-(2'-Hydroxyphenyl)-4(5)-methyl-5(4)-(methyl 2'',2''-dimethylacetate)imidazolium Chloride (V). The methyl ester of IV was prepared by the slow addition of 0.5 g to 5 ml of $SOCl_2$ at 50°. The solution was maintained at 50° for an additional 10 min, the excess $SOCl_2$ was removed *in vacuo*, and the remaining oil was dissolved in benzene. The benzene was also removed *in vacuo* and the process repeated with $CHCl_3$. The oil was dissolved in MeOH and refluxed for 1 hr. Removal of excess MeOH left a white solid which after recrystallization from CH_3CN–MeOH amounted to 0.51 g (83%) of pure methyl ester hydrochloride V with mp 196–198°; ir (KBr) 1725 (CO_2R), 1640 (ImH⁺), 1255 (CO), and 1235 cm⁻¹ (CO); pK_a ($H_2O/1$ M KCl) = 6.17 (ImH⁺) and 9.55 (PhOH).

Anal. Calcd for $C_{15}H_{19}N_2O_3Cl$: C, 57.97; H, 6.16; Cl, 11.41. Found: C, 58.01; H, 6.09; Cl, 11.22.

2-(2'-Acetoxyphenyl)-4(5)-methyl-5(4)-(methyl 2'',2''-dimethylacetate)imidazolium Chloride (VIII). Acetylation of V [via the neutral base form obtained from water; ir (KBr) 1710 cm⁻¹ (CO_2R)] was accomplished with an excess of acetyl chloride in dry pyridine. After 10 hr the solution was concentrated *in vacuo* and the resulting oil redissolved in dry $CHCl_3$. Addition of anhydrous Et_2O to the solution and gassing with dry HCl caused the precipitation of a white solid (collected by filtration) whose ir [(KBr) 3450 (PhOH), 1770 (CO_2Ph), 1745 (CO_2CH_3), and 1640 cm⁻¹ (ImH⁺)] shows starting phenol (V) as an impurity. Crystallization from $CHCl_3$–Et_2O gave crystals VIII with mp 182–188° and an ir which still exhibits phenolic absorbances.

2-(2'-Hydroxy-5'-sulfophenyl)-4(5)-methyl-5(4)-(2'',2''-dimethylacetic acid)imidazolium (VI). Sulfonation of IV was conducted by slowly adding 15 ml of cold concentrated H_2SO_4 to 1.85 g (0.12 mmol) of IV held at 0°. After 1.5 hr at ambient temperature and 12 hr at 5° the reaction was quenched by slow addition of ice-

water until precipitation was complete. The precipitate was collected by filtration and redissolved in aqueous KOH. The solution was heated to 60° and acidified with concentrated HCl until cloudy (pH 5). Voluminous precipitation of feathery needles occurred upon cooling. The solution was further cooled to 5° and 1.97 g (73%) of the potassium salt of VI was obtained. Further acidification to pH 1 yielded 570 mg (17%) of the neutral zwitterion (VI): mp (VI) >360°; (K⁺ salt) >170° dec; uv ($H_2O/1$ M KCl at pH 5) λ 310 nm (10.4), 273 (11.5), 285 sh (10.3), and 233 (14.9); ir (VI) (KBr) 1710 (CO_2H), 1640 (ImH⁺), 1170 (SO_3^-), 1040 (SO_3^-), and 600 cm⁻¹ (SO_3^-); (K⁺ salt) (KBr) 1645 (ImH⁺), 1620 sh (CO_2^-), and 1035 cm⁻¹ (SO_3^-); nmr (K⁺ salt) (DMSO-d_6) δ 1.57 (s, 6), 2.28 (s, 3), 6.89 (d,1,J = 8.5 Hz), 7.59 (q,1,J = 8.5 Hz, J = 2 Hz), and 8.25 ppm (d,1,J = 2 Hz); pK_a ($H_2O/0.1$ M KCl) = 3.4 (CO_2H), 6.0 (ImH⁺), and 9.6 (PhOH).

Anal. Calcd for $C_{14}H_{13}N_2O_6SK \cdot H_2O$: C, 42.40; H, 4.32; N, 7.07; S, 8.09. The same sample submitted to two laboratories gave the following results: C, 42.52; H, 4.21; and C, 41.48; H, 4.20; N, 6.93; S, 7.85.

2-(2'-Acetoxy-5'-sulfophenyl)-4(5)-methyl-5(4)-(2'',2''-dimethylacetic acid)imidazolium (IX). Acetylation of the neutral zwitterion VI was accomplished on 102.7 mg (0.302 mmol) suspended in dry $CHCl_3$ employing 10 equiv of pyridine (dried over KOH) and 5 equiv of freshly distilled acetyl chloride. The acetyl chloride was added during a 6-hr interval. After 18 hr the suspension was yellow and after 38 hr, 109 mg (94.5%) of the phenyl acetate IX was collected by filtration and washed with $CHCl_3$ and Et_2O: mp >265° turns yellow, decomposing without melting; uv (H_2O at pH 5) λ 275 nm; ir (KBr) 1780 (COOPh), 1730 (CO_2H), 1645 (ImH⁺), 1045, and 655 cm⁻¹ (SO_3^-); nmr (DMSO-d_6) δ 1.65 (s,6), 2.28 (s,3), 2.38 (s,3), and 7.2–8.1 ppm (m,3); pK_a ($H_2O/1$ M KCl, 3°) = 3.18 (CO_2H) and 6.6 (ImH⁺).

Anal. Calcd for $C_{16}H_{18}N_2O_7S$: C, 50.25; H, 4.74; S, 8.39. Found: C, 50.47; H, 4.60; S, 8.41.

Apparatus. All spectrophotometric kinetic measurements were made on either a Gilford Model 2000 spectrophotometer (<pH 3) equipped with four thermospacers through which water at 30 ± 0.1° was circulated, a Durrum-Gibson Model 13001 stopped-flow spectrophotometer equipped with a Kel-F cell and valve block through which water was circulated at 30 ± 0.2°, or a spectrophotometric titration apparatus designed around a Cary 15 spectrophotometer and Radiometer autotitrator[13] through which water was circulated at 30 ± 0.1°. Ultraviolet spectra were recorded on the Cary 15 at 30°. pH measurements at 30 ± 0.1° were taken with a Radiometer Model 26 pH meter equipped with a Metrohm EA 125 combined glass-calomel electrode. Infrared spectra were recorded using a Perkin-Elmer 137 sodium chloride spectrophotometer. Pmr spectra were recorded on a Varian T-60 spectrophotometer, using TMS or DSS (in H_2O) as an internal standard. Melting points were measured on a Mel-temp and are uncorrected.

Kinetics. All kinetic measurements (unless specifically stated) were in aqueous solutions at 30° with μ = 1.0 with KCl. All substrates were readily soluble in water which allowed their addition as solids to kinetic solutions. Ester VII was initially dissolved at pH 5–6 and a scan was recorded to check for the presence of pyridinium hydrochloride which cocrystallized with the ester during the synthetic work-up. If any pyridine could be visually detected, that sample of substrate was discarded. This occurred only once. Hydrolysis was followed by the production of phenol (IV) between 310 and 330 nm. Repetitive scans at pH 9.80 showed tight isosbestic points at 307.5 and 255 nm. The hydrolysis of the phenyl ester VIII was complicated at pH's ≥ 9.50 by the presence of a second, slower reaction exhibiting an isosbestic point at 310 nm (pH 9.50). The appearance of product (V) was followed at either the isosbestic wavelength for the second reaction (310 nm) or in the absence of a significant subsequent reaction (pH <9) at 310–340 nm. For the hydrolysis of IX the appearance of product (VI) was monitored between 305 and 351 nm or the disappearance of ester at 245–255 nm. Repetitive scans recorded during hydrolysis between pH's 8.00–9.75 all showed tight isosbestic points throughout the reaction.

A limited number of hydrolytic reactions of esters IX and Xb were also conducted in 94% acetonitrile–H_2O (v/v) with μ = 0.10 with tetraethylammonium bromide (Eastman). All such reactions were carried out in the Cary 15 cell[13] with pH held constant with 1% tetrapropylammonium hydroxide (Eastman) in 90% CH_3CN–H_2O. All pH measurements are uncorrected for the

---

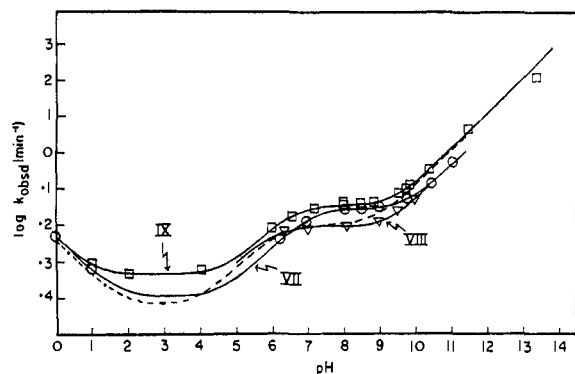(13) T. C. Bruice and J. R. Maley, *Anal. Biochem.*, **34**, 275 (1970).

Figure 1. Plots of log k_{obsd} *vs.* pH for esters VII (O), VIII (∇), IX ($\square$), and Xa (—) in H_2O ($\mu = 1.0$ with KCl) at 30°. The points are experimental and the lines theoretical employing eq 1 and 2 and the rate constants recorded in Table IV. All rates are spectrophotometric.

Table I. λ_{max} (nm) for Esters[a] in H_2O (1 M KCl)

Ester	1	2	3
VII		268	285
VIII			270
IX	268[b]	275	295

[a] $[Ester]_T = 1 + 2 + 3 = [CO_2H, ImH^+] + [CO_2^-, ImH^+] + [CO_2^-, Im]$. [b] 3°.

Table II. λ_{max} (nm) for Phenolic Products[a]

Phenol	1	2	3	4
III[b]	303, 270 (283)			
IV	306, 269 (281)	306, 271 (284)	319 (310, 340), 284, 272, (235)	329, 285, 274, (238)
V	306, 269 (281)		306 (316), 280 (271)	329, 282, 272, (236)
VI	308, 271 (283), 232	310, 273 (285), 233	330, 285, 274, 249	332 (343), 280, 252 (237)

[a] $[Phenol]_T = 1 + 2 + 3 + 4 = [CO_2H, ImH^+, PhOH] + [CO_2^-, ImH^+, PhOH] + [CO_2^-, Im, PhOH] + [CO_2^-, Im, PhO^-]$. Numbers in parentheses indicate shoulders. [b] Precipitation occurs upon ionization.

dependency of hydrogen ion activity upon solvent composition since a comparison of the reactivity of the two esters under identical conditions was the primary goal of the experiments.

Reactions were usually followed to completion and the pseudo-first-order rate constants (k_{obsd}) were obtained from least-squares analysis of plots of ln $(OD_\infty - OD_0)/(OD_\infty - OD_t)$ *vs.* time. Some of the slower rates were followed to only 4-5 half-lives, in which case k_{obsd} was calculated by the method of Guggenheim.[14] Pseudo-first-order plots for all substrates were linear to >2 half-lives. 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. Spectrophotometric titration of the free carboxyl of IV whose ionization produces a 10% increase in OD at 307 nm between pH 1.95 and 4.62 was used to show that the second, slower reaction which occurs during the hydrolysis of the diester VIII was hydrolysis of the methyl ester. It was also determined by the same procedure that at lower pH's (<9), no significant amount of free carboxylic acid was produced from VIII during the hydrolysis of the phenyl ester.

Spectral Characteristics of Esters and Phenolic Products. All esters showed only one transition for each ionic species in solution [H_2O $\mu = 1.0$ (KCl)·] at the wavelengths listed in Table I. The λ_{max} (nm) for the various ionic species of phenols IV-VI and the methyl ether III are tabulated in Table II. The uv spectra of IV-VI closely resemble those of the related phenols possessing hydrogens at the 4 and 5 positions of the imidazolyl ring.[15] Added complexity arises, however, due to spectral changes associated with ionization of the carboxyl groups of IV and VI and the associated

(14) E. A. Guggenheim, *Phil. Mag.*, **2**, 538 (1926).
(15) G. A. Rogers and T. C. Bruice, *J. Amer. Chem. Soc.*, **96**, 2463 (1974).

isosbestic points (see spectra of Figure 5). Spectral data in H_2O were impossible to obtain for III in the imidazole free base form due to its insolubility.

pK_a Determinations. Spectrophotometric titration of IV, V, VI, and IX indicated that where applicable, removal of the first proton was associated with one set of isosbestic points, and removal of the second and third protons each with a different set of isosbestic points. Thus in each case pK_{a_1} could be determined *via* spectrophotometric titration at an isosbestic wavelength associated with pK_{a_2}, and *vice versa*. The wavelengths used were: IV, pK_{CO_2H} at 283.5 nm, pK_{ImH^+} at 351.6 nm, pK_{PhOH} at 247.8 nm; V, pK_{ImH^+} at 255.7 nm, pK_{PhOH} at 240.5 nm; VI, pK_{CO_2H} at 320.5 nm, pK_{ImH^+} at 351 nm, pK_{PhOH} at 322 nm; and IX (3°), pK_{CO_2H} at 287.5 nm, pK_{ImH^+} at 330 nm. The pK_a values so determined are tabulated in Table III along with those for Xa and Xb.

Effect of Solvent Composition upon pK_a. Basically two types of experiments were performed. The spectrophotometric titration of VI (1.22×10^{-5} M) was carried out in aqueous dioxane solutions ranging from 0 to 83% (v/v) dioxane and with ionic strength held constant at 0.1 by the addition of tetramethylammonium bromide. In the second set of experiments VI was titrated in 80 and 96% dioxane without control of ionic strength (*i.e.*, $\mu = 10^{-5}$-10^{-3}). All dioxane was freshly distilled from Na. pH meter readings were corrected for the effects of solvent composition as high as 83% dioxane-H_2O according to the data of Van Uitert and Fernelius.[16] The pK_a values reported in 96% dioxane-H_2O, although uncorrected, would still retain the same relative values were electrode corrections applied (*i.e.*, pK_{COOH} − pK_{ImH^+} is independent of correction).

Results and Discussion

Effect of Carboxyl Ionization on Rates of Deacylation

Table III. pK_a Values of Phenolic Products[a] in H_2O (1 M KCl)

Phenol	pK_{COOH}	pK_{ImH^+}	pK_{PhOH}
IV	3.18	6.93	9.81
V		6.17	9.55
VI[b]	3.4	6.0	9.60
IX	3.18	6.6	
Xa		6.5	9.32
Xb		5.7	8.32

[a] Also ester IX at 3°. [b] pK_a's determined in H_2O with $\mu = 0.1$ (KCl).

in H_2O. At constant pH all spectrophotometrically determined rate constants (k_{obsd}) were found to be pseudo first order. For pH values ≤ 2 lyate species provided buffer capacity while at pH values >2 the Cary 15 pH-Stat cell assembly[13] was employed. Plotted in Figure 1 are the values of k_{obsd} (in H_2O) *vs.* the constant pH at which k_{obsd} was measured for esters VII-IX and ester Xa.[15] The points of Figure 1 are experimental and the curves were manually iterated and computer drawn to the points using eq 1 for VIII and eq 2

$$k_{obsd} = [k_3 K_{app_1}/(a_H + K_{app_1})] + k_4 a_{OH} \qquad (1)$$

$$k_{obsd} = k_1 a_H + k_2 +$$
$$[k_3 K_{app_1}/(a_H + K_{app_1})] + k_4 a_{OH} \qquad (2)$$

(16) L. G. Van Uitert and W. G. Fernelius, *J. Amer. Chem. Soc.*, **76**, 5887 (1954).

Table IV. Hydrolytic Rate Constants and Apparent pK_a's Determined from the Best Fit of Eq 1 and 2 to the Experimental Points of Figure 1[a]

	VII	VIII	IX	Xa	Xb
k_1, M^{-1} min^{-1}	5.00×10^{-3}		4.70×10^{-3}	4.16×10^{-3}	4.10×10^{-3}
k_2, min^{-1}	1.0×10^{-4}		4.2×10^{-4}	6.0×10^{-5}	3.6×10^{-4}
k_3, min^{-1}	2.75×10^{-2}	9.50×10^{-3}	3.60×10^{-2}	1.00×10^{-2}	1.20×10^{-2}
k_4, M^{-1} min^{-1}	2.80×10^2	3.20×10^2	8.80×10^2	8.00×10^2	
pK_{app}	7.05	6.05	6.60	6.19	5.65

[a] Also listed are data from ref 15 for esters Xa and Xb.

VII, R_1 = H; R_2 = H
VIII, R_1 = CH$_3$; R_2 = H
IX, R_1 = H; R_2 = SO$_3^-$

Xa, R = H
b, R = SO$_3^-$

Figure 2. Plots of log k_{obsd} vs. pH calculated assuming no kinetic involvement of the carboxylic acid of IX in hydrolysis (top profile, from Figure 1) and assuming k_0 for EH$_2$ (Scheme II) negligible.
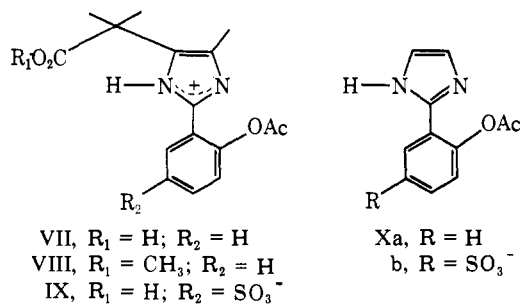
for VII, IX, and Xa and the derived constants of Table IV.

From the obvious similarity of the pH–log k_{obsd} profiles of VII, VIII, and IX to ester Xa (Figure 1) it may be concluded that VII–IX hydrolyze *via* the same mechanistic pathways elucidated in ref 15 for 2-(2'-acetoxyphenyl)imidazole (Xa) which does not possess a carboxyl function. The established mechanisms are illustrated here in Scheme II for IX. Equation 3 re-

Scheme II
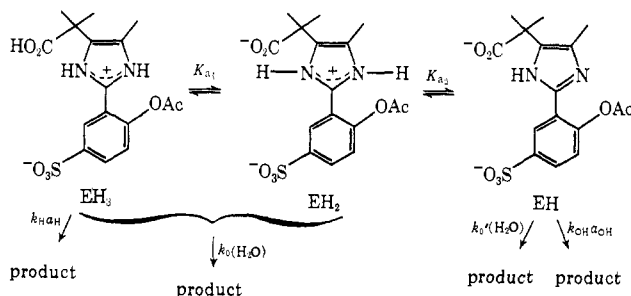
$$k_{obsd} = \frac{a_H{}^3(k_H a_H + k_0) + k_0 a_H{}^2 K_{a_1} + K_{a_1} K_{a_2}(k_0' a_H + k_{OH} K_w)}{a_H(a_H{}^2 + a_H K_{a_1} + K_{a_1} K_{a_2})}$$

(3)

lates k_{obsd} to hydrogen ion activity and is derived from a material balance in EH$_3$ + EH$_2$ + EH and assumed acid–base equilibria in these species. The justification for equating EH$_3$ with EH$_2$ in the k_0 pathway can be seen in Figure 2. The top profile is that for ester IX (Figure 1) assuming k_0 to be identical for EH$_3$ and EH$_2$ while the lower curve was calculated ignoring k_0 for EH$_2$. The data clearly indicate that the carboxyl ionization is not kinetically important for the k_0 pathway (but not necessarily the k_0' pathway) and therefore rules out the participation of the carboxyl group in the associated mechanism.

In the previous paper[15] the various modes of catalysis exhibited by the imidazolyl group of Xa,b and other derivatives were fully investigated. The catalytic mechanisms of hydrolysis were found to be those of XIa, b, and c. The importance of each mechanism is de-
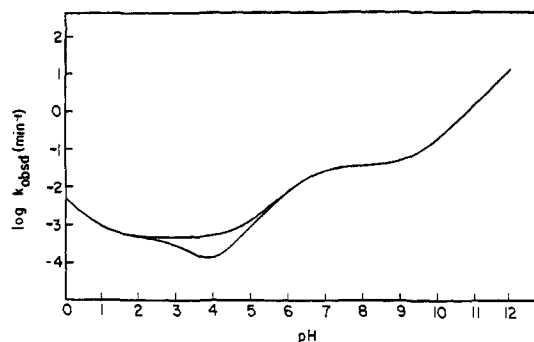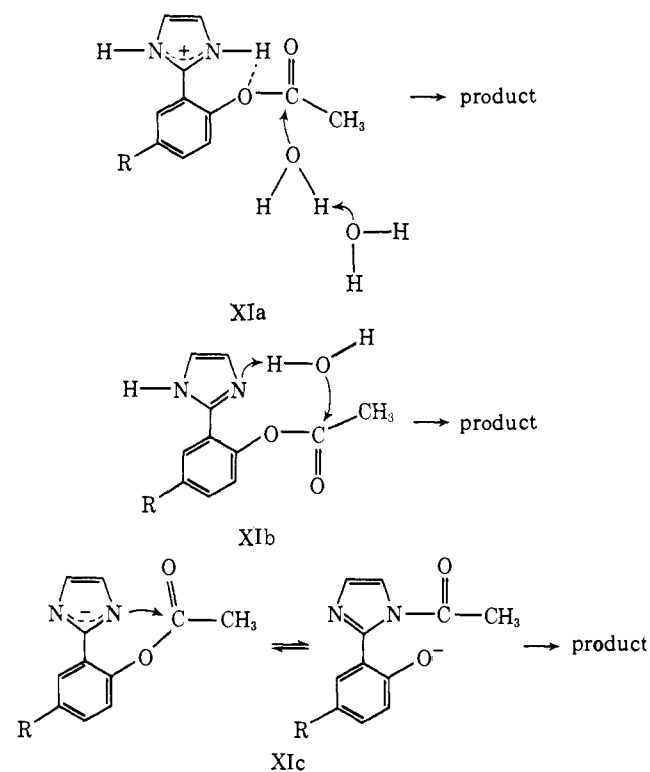

XIa

XIb

XIc

pendent on the pH and the nature of the substituent group R. For esters VII and IX, k_0 (Scheme II) is associated with the general acid mechanism of XIa and k_0' (including VIII) with the general base mechanism of XIb. None appear to hydrolyze *via* the nucleophilic mechanism XIc. It was calculated that the *o*-imidazolyl group provided *ca*. 10^4-fold rate enhancement in ester hydrolysis *via* mechanism XIb. In considering mechanism XIb (pathway k_0' of Scheme II) we have an imidazole removing a proton from H$_2$O concerted with the latter's attack upon an ester carbonyl group as is the case in the deacylation step of the serine esterases.
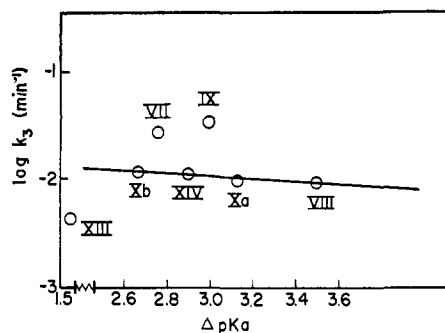
Figure 3. Plot of log k_3 vs. ΔpK_a (pK_{PhOH} − pK_{app}). (Table IV for esters VII–Xb and ref 15, Table III for esters XIII and XIV.)

Figure 4. Plots of log k_{obsd} vs. pH for esters IX (O) and Xb (□) in 94% CH_3CN-H_2O (v/v) (μ = 0.1 with Me_4NBr) at 30°. The points are experimental and the lines theoretical employing eq 4 (see text for discussion of rate constants). All rates are spectrophotometric.

Incorporation of a carboxylate function juxtaposed to the imidazolyl NH (esters VII and IX) should provide an accurate assessment of the role of a second tandem general base in promoting hydrolysis via the imidazolyl group as envisaged in XII.[17]

XII
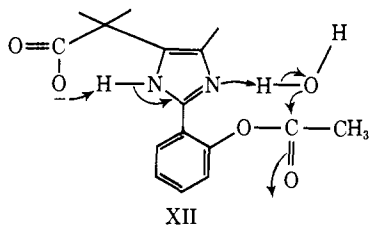
A comparison of the rate constants associated with the general base mode of catalysis (k_3, Table IV) for the esters of our previous study[15] which lack the carboxylic acid functional group with those of this study establishes that the carboxyl anion provides no appreciable assistance. A better appreciation of the influence of a neighboring carboxyl group can be obtained from a plot (Figure 3) of log k_3 vs. the difference in pK_a of the leaving phenols and kinetically apparent pK_a of the catalytic imidazole group. The deviation of the point associated with XIII from the best line is

XIII   XIV
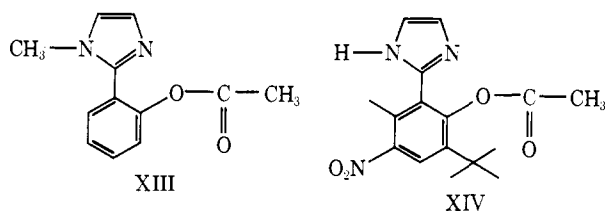
understandable in view of its unique N-methylimidazole structure. Esters XIV, VIII, and Xa, b all are devoid

(17) The structures IV, VI, VII, and IX have in common an α-[4(5)-imidazole]acetic acid moiety with α-gem-dimethyl substituents. gem-Dialkyl substitution has been established via kinetic and thermodynamic measurements to result in the juxtaposition of the terminal groups in an alicyclic system [T. C. Bruice and W. M. Bradbury, J. Amer. Chem. Soc., 87, 4846, 4851 (1965)]—in the present case imidazole nitrogen and carboxyl oxygen. The additional methyl substitution on the 4(5) position of the imidazole ring enhances the gem-dialkyl effect and locks the ends of the alicyclic system (imidazole NH and carboxyl oxygen) into close proximity and greatly restricts freedom of rotation. This device has been termed by Cohen [S. Milstein and L. A. Cohen, J. Amer. Chem. Soc., 94, 9158 (1972); R. T. Borchardt and L. A. Cohen, ibid., 94, 9166, 9175 (1972)], its inventor and exploiter, as the trialkyl lock. The employment of the trialkyl lock in the structures of this study ensures that the carboxyl oxygen remains in hydrogen bonding distance of the imidazolyl N–H bond. The only free rotation is about the bond of XII connecting the imidazole and benzene rings. We tacitly assume that the presence or absence of the remote carboxyl anion group does not influence the rotamer distribution about the bond connecting the imidazole and benzene rings.
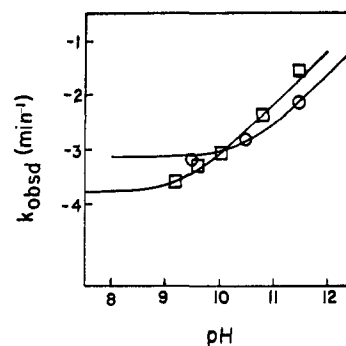
of a neighboring carboxylate group and possess nearly equal rate constants. The two positive deviations (esters VII and IX) in spite of the greater basicities of their phenolic leaving groups (pK_{PhOH} of Table III) show enhanced rates of deacylation. This demonstrates that the introduction of the hydrogen bonding carboxylate does enhance catalysis by the neighboring imidazole. *However, this enhancement is but threefold. From the standpoint of enzymatic catalysis this is, of course, negligible.*

Effect of Carboxyl Ionization on Rates of Deacylation in Acetonitrile Containing 3.32 M H_2O. Plotted in Figure 4 are the values of k_{obsd} [in 94% CH_3CN-H_2O (v/v)] for esters IX and Xb vs. the constant pH meter reading at which k_{obsd} was measured. The points of Figure 4 are experimental and the curves were manually iterated and computer drawn to the points using eq 4

$$k_{obsd} = k_3' + k_4'a_{OH} \qquad (4)$$

and the derived constants k_3' = 7.2 × 10^{-4} min^{-1} (IX) and 1.6 × 10^{-4} min^{-1} (Xb). Meaningful values cannot be assigned to k_4' since the autoprotolysis constant of water (K_w) was not determined for this solvent system. However, our interest lies only in the plateau rates associated with k_3' which is independent of K_w. In particular, the ratio of plateau rates in 94% acetonitrile-H_2O (v/v) for IX vs. Xb (4.5) is to be compared with the same ratio of rates (k_3 of Table IV) in H_2O (3.0). Thus, transferring from H_2O to a solvent of limiting H_2O concentration has virtually no effect upon the catalytic role of the carboxylate group in the intramolecular reactions. An additional interesting point can be made. If the pseudo-first-order rate constants (k_3 and k_3') are adjusted to second-order rate constants (k_{H_2O} and k_{CH_3CN}) by dividing by the concentration of H_2O in both solvent systems (53.4[18] and 3.3 M) then it is found that the second-order rate constants (Table V) reveal a very low sensitivity (3–5-fold decrease H_2O → CH_3CN) of hydrolysis rates to solvent composition. Since a H_2O molecule is an integral part of mechanism XIb it was not possible to employ a completely aprotic media. In any case the milieu of the serine esterase triad does not resemble an aprotic solvent so much as a hydrogen bonding media of low dielectric constant.

(18) B. Holmquist and T. C. Bruice, J. Amer. Chem. Soc., 91, 2993 (1969).

Table V. Second-Order Rate Constants (k_{H_2O} and k_{CH_3CN}) for Hydrolysis of Ester IX and Xb in H_2O and 94% Acetonitrile

Ester	$k_{H_2O} \times 10^4$, M^{-1} min^{-1}	$k_{CH_3CN} \times 10^4$, M^{-1} min^{-1}
IX	6.75	2.17
Xb	2.25	0.48

Acid-Base Equilibria. Considerable interest has and will be displayed in the acid-base chemistry of the $-COO^- \cdots IM \cdots HO-$ triad. A shortcoming of simple model systems as a means of assessing features of enzyme catalysis is the present impossibility of creating for a small molecule the microscopic heterogeneous milieu of the active site.[19] This simplification, which may not be trivial, applies to the present study. This consideration aside, it might be anticipated that pK_a values for VI and their perturbation by solvent change would be predictable from what is known about molecules capable of existing in a zwitterionic state.[20] We have found this to be so.

Careful examination of the titrimetric spectra of VI (Figure 5) reveals the intricate spectral changes and isosbestic points which allow the discrete assignment (Experimental Section) of pK_a to each functional group (Table VI). As an example, the large absorbance increase near 250 nm as the pH is increased is due solely to the ionization of the imidazolyl group, being bounded by isosbestic points at 247 and 253 nm associated with ionization of the carboxyl and phenolic groups, respectively. The certainty with which discrete pK_a values have been assigned to each of the three functional groups of IV and VI rests not only on uv spectral interpretation, but also upon kinetic parameters and the results of chemical modification. The esterification of the carboxyl group of IV by known procedures to give the methyl ester V (see Experimental Section; structure confirmed by ir and elemental analysis) results in the disappearance of the spectrophotometrically titratable group with $pK_a = 3.18$. This unequivocally identifies the carboxylate moiety as the ionizable group uniquely associated with this pK_a. Corroborating this finding is a comparison of the pK_a's of Xa,b with IV and VI (Table III). Here again, the ionization near pH 3 is absent in those compounds lacking the carboxylic acid.

The assignment of the observed pK_a's between 6 and 7 to the microscopic ionizations of the imidazole groups (Table III) is most convincingly verified in the case of IX where the phenolic OH has been blocked by an acetyl group. The titratable group with $pK_a = 6.6$ (3°) agrees excellently with the kinetically determined $pK_{app} = 6.6$ (Table IV, 30°) for the catalytic group aiding ester hydrolysis. A similar close agreement is observed in ref 15 between the titrimetric pK_a values in the region 6-7 and the kinetically apparent pK values determined for ester hydrolysis.

Ionization of the carboxylic acid of VI (Figure 5) is associated with isosbestic points at 239, 247, and 272 nm (H_2O) and a marked change in the phenolic transition near 310 nm, the latter being quite useful in product analysis (Experimental Section). The ab-

(19) T. C. Bruice, *Enzymes*, 3rd Ed., 2, 217 (1970).
(20) (a) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides as Ions and Dipolar Ions," Reinhold, New York, N. Y., 1943; (b) J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press, New York, N. Y., 1958.
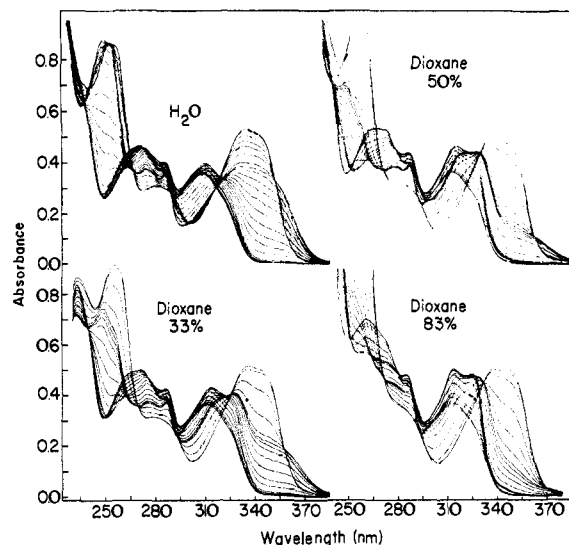

Figure 5. Uv spectra of VI recorded at various pH's in the region 2-13 in the solvents indicated ($\mu = 0.1$ with Me$_4$NBr) at 30°.

sorbance near 310 nm is present when the imidazolyl function is ortho to a methoxy or hydroxy group and is not seen when the imidazolyl group is para to the hydroxyl or ortho to an acetylated hydroxyl group (Table II of ref 15). Arguments have been presented that the 310-nm transition arises when the imidazolyl ring lies in the same plane as the benzene ring and that hydrogen bonding of the imidazole hydrogen with the phenolic oxygen is a factor in maintaining this planarity.[15] The substantial perturbation of the extinction coefficient of the transition at ca. 310 nm upon ionization of the carboxyl group may then be attributed to one or both of the following factors: (1) the placement of a point charge in close proximity to a chromophoric group; and (2) an increase in the hydrogen bonding of the carboxyl anion with its neighboring imidazolium group as compared to the like hydrogen bonding of the undissociated carboxyl group (XV $\leftrightarrows$ XVI). Sup-

XV   XVI
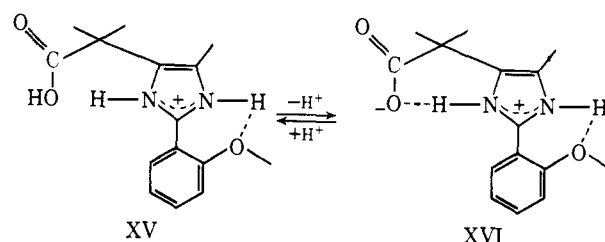
porting the influence of charge interaction is the observation of Donovan, Laskowski, and Scheraga[21] that for histidine, where the uv chromophore is the imidazole ring, there is a larger change in difference spectra (reference solution pH <1) upon ionization of the amino group than of the imidazole ring itself. That a hydrogen bond should be expected between the carboxyl group and imidazolium ion is attested to by the fact that internal hydrogen bonding has been definitely established for the zwitterion of o-(N,N-dimethylamino)benzoic acid (XVII). Temperature-jump spectrophotometric measurements have shown that the rate of reaction of HO^- with the bridged proton of

(21) J. W. Donovan, M. Laskowski, and H. A. Scheraga, *J. Amer. Chem. Soc.*, 83, 2686 (1961).
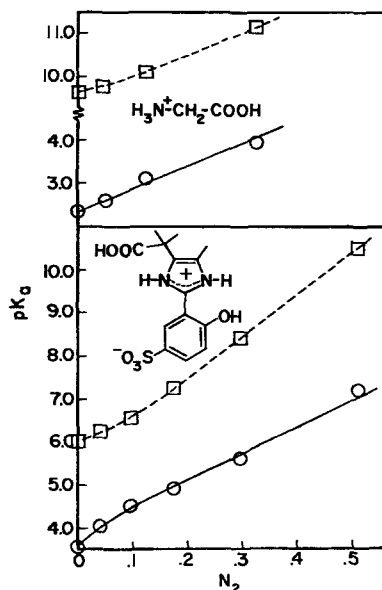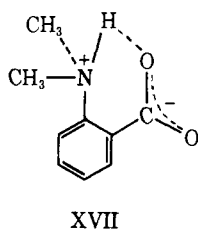
Figure 6. Plots of pK_a vs. mole fraction dioxane (N_2) for dioxane–H_2O mixtures in the range 0–83% (v/v) for VI (Table VI, μ = 0.1) and in the range 0–70% (w/w) for glycine [data of H. S. Harned and C. M. Birdsall, *J. Amer. Chem. Soc.*, **65**, 54 (1943); **65**, 1117 (1943).

XVII

XVII is at least 10^3 slower than the diffusion-controlled value[22] observed for anilinium ions. An examination of the magnitude of the low pH changes at 310 nm as the percentage of dioxane is increased reveals (Figure 5) that the perturbation of the electronic transition increases as the dielectric constant decreases. Both dipole–dipole interaction and hydrogen bonding would be expected to increase as the mole per cent of H_2O and the dielectric constant are decreased.

The ability to determine with accuracy the pK_{COOH}, pK_{ImH^+}, and pK_{PhOH} values of VI has in turn allowed an evaluation of what might be expected of the acid–base properties of the serine esterase triad. The solvent dependencies of the pK_a values of VI are provided in Table VI. Inspection of Table VI reveals that the relatively small positive values of ΔpK_{COOH} and ΔpK_{ImH^+} on transfer from water to solvent of lower dielectric constant are very similar to that expected for the ionization of a charged molecule to form a cation and a zwitterion. Thus, transfer of glycine from H_2O to 70% dioxane–H_2O is accompanied by an increase of pK_{COOH} by 1.61 units while $pK_{NH_3^+}$ is increased by 1.51.[23] This similarity is more graphically represented in Figure 6 where values of pK_a in solvents of varying mole fraction dioxane are plotted for glycine and pK_{COOH} and pK_{ImH^+} of VI. In contrast, transfer of acetic acid from

Table VI. Dependence of pK_a^a upon Solvent Composition for VI

% dioxane (v/v)	μ^b	CO_2H	ImH^+	PhOH
0	0.1	3.57	6.00	9.20
16.6	0.1	4.05	6.25	9.85
33.2	0.1	4.50	6.55	10.45
49.8	0.1	4.90	7.25	11.20
66.4	0.1	5.62	8.44	12.12
83	0.1	7.2	10.5	14.0
80^c	~0	7.0	11.0	e
96	~0	$(2.7)^d$	$(9.1)^d$	$(\geq 12)^d$

^{a} Unless specifically stated otherwise, pK_a values have been corrected for solvent composition according to the data in ref 16. ^{b} For μ = 0.1 constant ionic strength was maintained by the addition of tetramethylammonium bromide. For μ = ~0 no salts were added except those necessary for titration. ^{c} For purpose of correction μ assumed to be 0.001. ^{d} No activity correction was made to the pH meter readings. The recorded "pH" values are incorrect but the ΔpK_a values are correct. ^{e} Not determined.

H_2O to 82% dioxane–H_2O (30°) results in an increase of pK_{COOH} by 5.77[23] units and like transfer of imidazole results in an actual small decrease in pK_a.[24] There can be no doubt that the acid–base properties of the hydrogen bonded triad present in VI behave, on lowering the dielectric of the media, as would be predicted on the basis of classical electropotential considerations. The very slight effect of the carboxyl ionization on the rate constant for mechanism XIb with ester IX is attributable to the fact that the basicity of the COO^- group is much less than that of the imidazolyl group. A kinetically significant tandem general base catalysis (charge relay) would not be expected.

Large rate enhancements have been reported for reaction of imidazole with a phenyl ester on addition of a carboxylic acid anion when nearly anhydrous acetonitrile or toluene is employed.[25] These results have been offered in support of the charge-relay hypothesis. These systems may be criticized on the following basis. In an absolutely apolar–aprotic solvent the basicity of the carboxylic acid would considerably exceed that of the imidazole since the two are partially free of each other to diffuse through the solvent. Thus, a general catalysis as seen in B (introduction) is possible. Secondly, the reaction of imidazole with ester involves formation of dipolar transition states from a neutral ground state. In acetonitrile or toluene the reaction would be virtually forbidden unless a counterion (carboxyl anion) were added. Lastly, the rates for these reactions are less than or only marginally greater than that for imidazoles alone in aqueous solution. The addition of the carboxyl anion merely raises the rates to values between that in an apolar–aprotic solvent and that in a dipolar–protic solvent.

In summary, studies of the dielectric dependence of the kinetic and acid–base properties of a $-COO^-\cdots$ $IM\cdots HOR$ triad (XII) reveal that for the serine esterases there is not to be expected: (1) a kinetically significant charge relay; (2) a pK_a arising from the triad as a whole; or (3) an inversion of the pK_a values of the COOH and IMH^+ groups (*i.e.*, $pK_{COOH} > pK_{ImH^+}$). If any of these attributes are evidenced by the serine

(22) M. Eigen and E. M. Eyring, *J. Amer. Chem. Soc.*, **84**, 3254 (1962).
(23) From values collected by H. S. Harned and B. B. Owen, "Physical Chemistry of Electrolyte Solutions," 3rd ed, Reinhold, New York, N. Y., 1958, p 756, Table 15-6-2A.

(24) L. Michaelis and M. Mizutani, *Z. Phys. Chem.*, **116**, 135 (1925).
(25) (a) G. Wallerberg, J. Boger, and P. Haake, *J. Amer. Chem. Soc.*, **93**, 4938 (1971); (b) F. M. Menger and A. C. Vitale, *ibid.*, **95**, 4931 (1973).

esterases[26] they must be ascribed to little understood factors not duplicated in the model studies (e.g., the hetero-

geneous and ordered surroundings of the triad functional groups at the active site[19]).

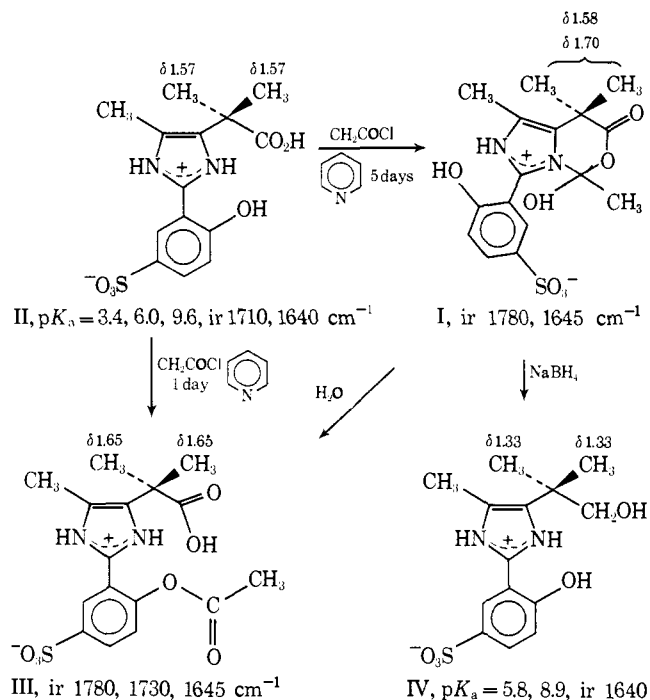# The Mechanisms of Acyl Group Transfer from a Tetrahedral Intermediate

Gary A. Rogers[1] and Thomas C. Bruice*

Contribution from the Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106. Received September 22, 1973

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

Scheme I

II, pK_a = 3.4, 6.0, 9.6, ir 1710, 1640 cm^{-1}

I, ir 1780, 1645 cm^{-1}

III, ir 1780, 1730, 1645 cm^{-1}

IV, pK_a = 5.8, 8.9, ir 1640

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.

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).