Z-D-Glu-OCH_2Ph, 65706-99-2; $F_3C(CF_2)_9CH_2CH_2OH$, 865-86-1; $Me(CH_2)_{17}OH$, 112-92-5; (Z)-$[Me(CH_2)_{14}CO_2CH_2CH_2]_2NCOCH{=}CHCO_2H$, 82797-95-3; H-Cys-OH, 52-90-4; $Me(CH_2)_{17}NH_2$, 124-30-1; (R)-$H_2NCH_2CH_2CH(NH_2)CO_2H$, 26908-94-1; (S)-$H_2NCH_2CH_2CH(NH_2)CO_2H$, 1758-80-1; $Me(CH_2)_{11}OH$, 112-53-8; Z-Asn-$[(CH_2)_{17}Me]OCH_2Ph$, 118355-82-1; Z-Gln$[(CH_2)_{17}Me]OCH_2Ph$, 118355-83-2; $Me(CH_2)_{15}CHBrCO_2H$, 142-94-9; 5α-cholestan-3β-ol, 80-97-7; palmitic acid succinimidyl ester, 14464-31-4.

Direct O-Acylation of Small Molecules Containing CO_2^----HN←HO Units by a Distorted Amide: Enhancement of Amine Basicity by a Pendant Carboxylate in a Serine Protease Mimic

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Abstract: The kinetics of the reaction of two series of amino alcohols (ethanol amines and 2-hydroxymethylimidazoles) with a distorted amide were studied as models for the acylation of the serine proteases. The pH/log k_2^{max} profiles plateau above the amine pK_a, indicating the basic form is active. In all cases, the reactions proceed by initial O-acylation to produce esters. With primary or secondary ethanolamines, subsequent O→N acyl transfer occurs to give amides. In each series, a Brønsted relationship exists relating the increasing amine pK_a with an increasing second-order rate constant for O-acylation. Amino alcohols containing a pendant carboxylate (e.g., serine and the 4(5)-[α,α-dimethylacetic acid] derivative of 2-hydroxymethylimidazole) fit on the Brønsted plots exactly, which suggests the amine pK_a controls the reactivity. The role of the carboxylate is to enhance the amine basicity by electrostatic/inductive means. This is particularly effective in solvents of reduced polarity (40% and 80% v/v, $EtOH/H_2O$) since the above effects counteract the normal drop in amine pK_a exhibited by the other amino alcohols that do not possess the CO_2^-. Both series exhibit low kinetic solvent isotope effects of between 1 and 2. In the imidazole alcohol series, as the amine pK_a increases the kinetic solvent isotope effect tends to 1.0. This is discussed in terms of a possible involvement of direct nucleophilic attack of an ammonium alkoxide zwitterion on **1**.

The serine proteases (SPases) comprise a large class of enzymes with active sites containing a $AspCO_2^-$---His imidazole---SerOH triad.[1] During the course of all SPase-catalyzed amide and ester hydrolyses, the SerOH group becomes transiently acylated. Although this suggests common hydrolytic pathways, continuing studies with various members of this class indicate subtle substrate-dependent diversities. For example, evidence exists that unnatural ester substrates such as phenyl benzoates[2a] and *p*-nitrophenyl acetate[2b] (pNPA) may be hydrolyzed by chymotrypsin via routes that involve first N-acylation of the His-57 imidazole followed by rapid acyl transfer to the Ser-195 hydroxyl. In addition, recent reports[3] using proton-inventory analysis indicate several SPases employ mechanisms that recruit 2 or more protons in flight for specific substrates and 1 proton in flight for nonspecific substrates.

Central to the issue of the function of the triad is the role of the $AspCO_2^-$ unit in influencing acylation of the SerOH. Two main possibilities have been discussed[4] at much length in the literature. These are stylized in Scheme I wherein the carboxylate acts either as a general base (path a) or to electrostatically enhance the imidazole basicity (path b). Recent evidence indicates that the $AspCO_2^-$---H^+-Im-His hydrogen bond is maintained in enzymes inhibited with species approximating the initial tetrahedral intermediate,[4,5] perhaps supporting path b. Even so, the issue has not been generally resolved, particularly since the proton inventory studies[3] leave open the possibility that the extent of involvement of the carboxylate may be substrate dependent. Nevertheless, the wide-spread occurrence of this catalytic triad suggests a considerable mechanistic advantage to the enzymes that employ it.

If such an arrangement leads to obvious acceleration, it is surprising how few studies have addressed the ability of a triad to facilitate O-acylation of a small molecule. A number of reports deal with the reaction of amino alcohols with esters[6] or reactive amides.[7] A smaller number deal with the interaction of imidazole and CO_2^- during acyl transfer from esters to H_2O,[8] although these

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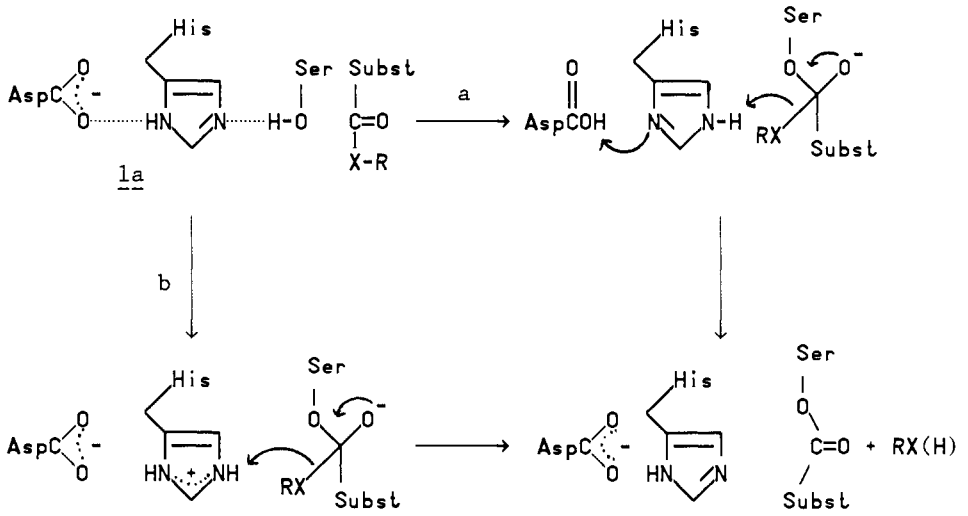
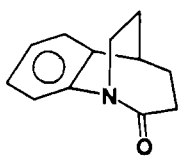
0002-7863/89/1511-1445$01.50/0

Scheme I

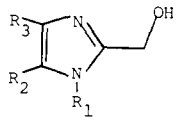


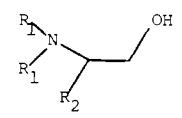
are more properly models for the deacylation of SPases. Apparently, our preliminary report[7c] and those of Bender et al.[9] are the only published ones of a model employing the triad in O-acylation, in the latter case by *m*- and *p-tert*-butylphenylacetate.

Recently we reported the synthesis and kinetic study of the hydrolysis of anilide **1**[10] in which nucleophilic attack is activated by N—C=O distortion. That species shows an unusual reactivity toward bifunctional nucleophiles, including dicarboxylic acids[11] and amino alcohols.[7b] In the latter study, it was demonstrated that 2-(hydroxymethyl)imidazoles (**2a,b**) and other β-hydroxy amines (e.g., **3a**) were directly O-acylated by **1** with intramolecular assistance by the amine. Since that process bears a formal similarity to the O-acylation of the SPases,[12] and the general structures of **2** and **3** allow further elaboration, we have extended the study to include some β-hydroxy amines that also bear a CO_2^- unit. Herein we report the syntheses of the imidazole alcohol derivatives **2c–g** and the kinetics of O-acylation of these and certain serine derivative (**3b,c**) by **1** in an effort to assess the role of a pendant carboxylate.



1





2a $R_1=R_2=R_3=H$
b $R_1=CH_3$; $R_2=R_3=H$
c $R_1=H$; $R_2=R_3=CH_3$
d $R_1=R_3=H$; $R_2=C(CH_3)_2CO_2H$
e $R_1=R_3=H$; $R_2=C(CH_3)_2CO_2CH_3$
f $R_1=R_3=H$; $R_2=C(CH_3)_2CO_2H$; $OH=OCH_3$
g $R_1=R_3=H$; $R_2=C(CH_3)_2CN$

3a $R_1=CH_3$; $R_2=H$
b $R_1=H$; $R_2=CO_2H$
c $R_1=H$; $R_2=CO_2Et$
d $R_1=H$; $R_2=CO_2H$; $OH=OCH_3$

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(12) The overwhelming majority of studies with amino alcohols as models[6] for the SPases have employed *p*-nitrophenyl esters as substrates. It is important to note that in most of these studies where the amine was imidazole, an initial N-acylated derivative was detected, which subsequently underwent N to O acyl transfer.

Experimental Section

a. General. All melting points are uncorrected. ^1H NMR spectra were recorded with a Bruker WP-80 spectrometer. Mass spectra were recorded on an AEI MS-50 high-resolution mass spectrometer. THF was distilled from sodium benzophenone ketyl. All butyllithium reactions were carried out under Ar with use of standard syringe techniques.

b. Syntheses. Amide **1** was synthesized as previously described.[10] Compounds **2d,e** were prepared from 4(5)-(cyanomethyl)imidazole[13] as described below.

1-(1-Ethoxyethyl)-4-(cyanomethyl)imidazole (4). Finely divided 4-(5)-(cyanomethyl)imidazole[13] (10.7 g, 100 mmol) was suspended in 200 mL of THF and cooled to −40 °C. To this was added 100 mmol of *n*-BuLi in hexane over a period of 0.5 h with vigorous stirring.

After an additional 30 min at −40 °C, 1-chloroethyl ethyl ether[14] (12.5 mL, 11.9 g, 110 mmol) was added over a 5-min period. The resulting solution was stirred at room temperature overnight after which 50 mL of saturated Na_2CO_3 was added. The organic layer was separated and the aqueous phase extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and the volatiles subsequently removed by rotary evaporation to yield 18 g of a brown oil. This was purified by Kugelrohr distillation (0.1 Torr, 90 °C) to yield 14.5 g (81%) of product which, by NMR, was >95% pure and used without further purification. NMR ($CDCl_3$) δ 7.60 (s, 1 H), 7.12 (s, 1 H), 5.30 (q, 1 H), 3.75 (s, 2 H), 3.35 (m, 2 H), 1.65 (d, 3 H), 1.20 (t, 3 H); IR (film) 2240 cm^{-1}; mass spectrum, *m/z* 179.1056 (M^+, calcd for $C_9H_{14}N_3O$, 179.1058).

1-(1-Ethoxyethyl)-4-(1-cyano-1-methylethyl)imidazole (5). This material was prepared by the stepwise methylation of **4**. To a solution of **4** (9.0 g, 50 mmol) in 100 mL of THF at −45 °C was added 50 mmol of nBuLi in hexane over 10 min. After 30 min, 3.1 mL of CH_3I (7.1 g, 50 mmol) was added and the solution warmed to −20 °C. It was then again cooled to −45 °C and the above sequence repeated. After being warmed to room temperature, the solution was worked up as described for **4**, yielding a brown oil that was purified by Kugelrohr distillation (0.1 Torr, 100 °C). The resulting colorless oil (8.0 g, 78%) was sufficiently pure (>95% by NMR) for the following steps. NMR ($CDCl_3$) δ 7.62 (s, 1 H), 7.08 (s, 1 H), 5.35 (q, 1 H), 3.42 (m, 2 H), 1.73 (s, 6 H), 1.64 (d, 3 H), 1.20 (t, 3 H); IR (film) 2240 cm^{-1}; mass spectrum, *m/z* 207.1375 (calcd for $C_{11}H_{17}N_3O$ 207.1372).

1-(1-Ethoxyethyl)-4-(1-cyano-1-methylethyl)imidazole-2-carbaldehyde (6). To a cooled (−45 °C) solution of **5** (5.25 g, 25 mmol) in 50 mL of THF was added 26 mmol of nBuLi in hexane. The solution was stirred 15 min and then 2.5 mL of distilled DMF added after which the mixture was allowed to come to room temperature overnight. On workup (as described for **4**) the aldehyde was obtained as a yellow oil containing some residual DMF (~90% pure by NMR) but no further purification was done. NMR ($CDCl_3$) δ 12.76 (s, 1 H), 7.12 (s, 1 H), 5.85 (q, 1 H), 3.55 (m, 2 H), 1.75 (s, 6 H), 1.65 (d, 3 H), 1.22 (t, 3 H); IR (film) 2240, 1695 cm^{-1}.

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1-(1-Ethoxyethyl)-4-(1-cyano-1-methylethyl)-2-(hydroxymethyl)imidzole (7). To the crude aldehyde **6** was directly added 50 mL of CH_3OH and the solution was cooled to 10 °C. Sodium borohydride (0.95 g, 25 mmol) was added to it in small portions over 5 min. After the mixture was stirred for 4 h at room temperature, most of the CH_3OH was removed under reduced pressure to yield a semisolid to which 10 mL of H_2O was added. This was followed by dropwise addition of 6 N HCl until the pH was 2–2.5 (pH paper). The acidic solution was stirred for 2 min followed quickly by neutralization with excess solid Na_2CO_3. It was then extracted with $CHCl_3$ (3 × 30 mL) and the combined extracts were dried over Na_2SO_4. After removal of $CHCl_3$ under reduced pressure, the resulting oil was stored under vacuum (0.1 Torr) for 4 h at 30–35 °C to ensure the removal of residual DMF (carried over from the preparation of **6**). The semisolid so obtained was dissolved in warm ether and alcohol **7** began to precipitate. The precipitation was completed by storing the flask in a freezer compartment for 2 h. A heavy solid material was obtained by filtration and washed with a minimum amount of cold ether. After drying, 3.9 g of the product **7** was obtained (68% from **5**): mp 108 °C; NMR ($CDCl_3$) δ 7.05 (s, 1 H), 6.05 (br s, 1 H), 5.65 (q, 1 H), 4.72 (s, 2 H), 3.45 (m, 2 H), 1.68 (s, 6 H), 1.60 (d, 3 H), 1.20 (t, 3 H); IR (Nujol) 3200, 3140, 2240 cm^{-1}; mass spectrum, m/z 237.1480 (calcd for $C_{12}H_{19}N_3O$ 237.1477).

4(5)-(1-Carboxy-1-methylethyl)-2-(hydroxymethyl)imidazole (2d). Acid hydrolysis of 2.0 g of **7** accompanied by deprotection was effected by overnight reflux in 20 mL of 6 N HCl. The resulting brown solution was diluted with 10 mL of H_2O and decolorized by boiling with 1 g of charcoal followed by filtration through a 3-mm bed of charcoal. H_2O was removed from the nearly colorless filtrate and the solid HCl salt of **2d** was dissolved in 20 mL of H_2O. Lithium hydroxide (solid) was added until the solution pH was 10 after which it was boiled until the evolution of NH_3 ceased (30–40 min). The volume was then restored to 20 mL by addition of H_2O, and the hot solution was once again decolorized with 1 g of charcoal. The pH of the resulting colorless filtrate was adjusted to 5.2 by dropwise addition of 2 N HCl. The solution was concentrated by removal of H_2O (~15 mL) until shiny crystals of **2d** began to appear. After the mixture was stored in a freezer overnight, followed by filtration, 0.95 g (61%) of crystalline **2d** was obtained: mp 210–211 °C; NMR (D_2O) δ 7.12 (s, 1 H), 4.8 (s, 2 H), 1.40 (s, 6 H); IR (Nujol) 3300, 3150, 2700–2450, 1630, 1560 cm^{-1}; mass spectrum, m/z 184.0849 (calcd for $C_8H_{12}N_2O_3$ 184.0848). Anal. Calcd for $C_8H_{12}N_2O_3$: C, 52.15; H, 6.51; N, 15.21. Found: C, 51.69; H, 6.56; N, 15.21.

4,5-Dimethyl-1-(1,1-diethoxymethyl)imidazole (8) was prepared by refluxing 23 g (0.24 mol) of 4,5-dimethylimidzole (**9**)[15] with 150 mL (0.9 mol) of triethyl orthoformate and 1.0 g of *p*-toluenesulfonic acid and removing the HOEt as it was formed according to the method of Brown and Curtis.[16] After workup[16] and distillation from Na_2CO_3 at 75–85 °C (0.025 Torr) was obtained 25 g (52%) of the title compound as a clear oil: NMR ($CDCl_3$) δ 1.22 (t, 6 H), 2.13 (s, 3 H), 2.17 (s, 3 H), 3.57 (q, 4 H), 5.90 (s, 1 H), 7.57 (s, 1 H); IR ($CHCl_3$ cast), 2980, 1600, 1440, 1320, 1280, 1102, 1067 cm^{-1}; mass spectrum, m/z 198.1380 (calcd for $C_{10}H_{18}N_2O_2$ 198.1368).

4,5-Dimethylimidazole-2-carbaldehyde (10). To 90 mL of dry THF was added 5.0 g (0.025 mol) of 4,5-dimethyl-1-(1,1-diethoxymethyl)-imidazole and the solution was then cooled to −40 °C. To this was slowly added 11.5 mL of 2.37 M nBuLi in hexane and the solution stirred for 30 min at −40 °C following which 2.1 mL (0.027 mol) of DMF was added. After the solution was stirred overnight at room temperature it was quenched with 20 mL of H_2O and enough acid to bring the pH to 4. The mixture was stirred for 30 min to effect removal of the bis(ethoxymethyl) blocking group[16] and then neutralized with $NaHCO_3$. The organics were extracted with 4 × 100 mL of $CHCl_3$ and the combined extracts dried over $MgSO_4$. Removal of the volatiles yielded ~85% of the aldehyde as a yellow oil which was immediately reduced. NMR ($CDCl_3$) δ 2.25 (s, 6 H), 9.45 (s, 1 H), 11.0 (br, 1 H); mass spectrum, m/z 124.0639 (calcd for $C_6H_8N_2O$: 124.0637).

4,5-Dimethyl-2-(hydroxymethyl)imidazole (2c). To 3 g (0.025 mol) of 4,5-dimethylimidazole-2-carbaldehyde was added 60 mL of dry methanol and 2.4 g of $NaBH_4$. The mixture was stirred overnight and then filtered and the solvent removed under vacuum. The product yellow solid was recrystallized from $EtOH/Et_2O$ to give 2.6 g (83%) of **2c**: mp 183–185 °C; NMR ($CDCl_3$) δ 2.25 (s, 6 H), 4.90 (s, 2 H); IR ($CHCl_3$ cast) 3100–2800, 1665, 1461, 1377, 1055, 868 cm^{-1}; mass spectrum, m/z 126.0795 (calcd for $C_6H_{10}N_2O$, 126.0793). Anal. Calcd for $C_6H_{10}N_2O$: C, 57.10; H, 7.99; N, 22.22. Found: C, 56.91; H, 7.94; N, 22.03.

4(5)-(1-Cyano-1-methylethyl)-2-(hydroxymethyl)imidazole (2g). This material was prepared by selective hydrolysis of 1.4 g of **7** in 20 mL of a 1:1 $HOAc/H_2O$ solution heated at reflux for 2 h. The solvents were removed under reduced pressure and the solid residue left under vacuum (0.1 Torr) for 6 h. The product was then dissolved in boiling H_2O (30 mL) and decolorized with 1 g of charcoal. The clear filtrate upon cooling deposited colorless platelets. After 1 h of refrigeration, the mixture was filtered to yield 860 mg (89%) of **2g**: mp 198–199 °C; NMR (D_2O/DCl) δ 7.40 (s, 1 H), 4.85 (s, 2 H), 1.72 (s, 6 H); IR (Nujol) 3160, 3120, 2720–2640, 2240 cm^{-1}. Anal. Calcd for $C_8H_{11}N_3O$: C, 58.16; H, 6.71; N, 25.44. Found: C, 57.80; H, 6.59; N, 25.36.

4(5)-(1-Carboxy-1-methylethyl)-2-(methoxymethyl)imidazole (2f). Alcohol **7** (475 mg, 2 mmol) was dissolved in 10 mL of dry THF containing 460 mg (2 mmol) of suspended silver oxide and 250 μL (570 mg, 4 mmol) of CH_3I. The mixture was stirred 3 days at room temperature by which time ~80% of the alcohol was methylated (by NMR). The resulting ether was purified by chromatography over neutral alumina with $CHCl_3$ as an eluent to yield 306 mg (61%) of the cyano ether. The ether was directly hydrolyzed in 6 N HCl as described for **2d** from **7** to yield 180 mg (79%) of **2f**: mp 204–205 °C; NMR (D_2O) 7.25 (s, 1 H), 4.55 (s, 2 H), 3.32 (s, 3 H), 1.48 (s, 6 H); IR (Nujol) 2700–2450, 1630, 1560 cm^{-1}; mass spectrum, m/z 198.1000 (calcd for $C_9H_{14}N_2O_3$ 198.1000). Anal. Calcd for $C_9H_{14}N_2O_3$: C, 54.55; H, 7.07; N, 14.14. Found: C, 54.05; H, 7.22; N, 14.14. Serine (**3b**) and *O*-methylserine (**3d**) were commercial materials (Sigma) and used as supplied. Ethyl serinate (**3c**) was prepared by refluxing serine in absolute ethanol saturated with HCl gas for 4–5 h: the product exhibited the expected spectral properties.

c. Kinetics. Rates of reaction of **1** in the presence of **2** and **3** were monitored in duplicate by UV methods with various concentrations of **2** or **3** as its own buffer, μ = 0.2 (KCl), and T = 25.0 °C by observing the rate of appearance of the ring-opened product aniline at 250 or 291 nm. The detailed instrumentation and methodology is analogous to that described.[7b] Stock solutions were prepared by diluting a known weight of compound and KCl to 1 or 10 mL in a volumetric flask to obtain the desired molarity and the pH adjusted with concentrated KOH or 6 N HCl. For the serine compounds (**3**) 10-mL solutions were prepared at 3 or 4 concentrations for each pH. Three milliliters of the latter solutions were added to a 1.0-cm quartz cuvette and the reactions initiated by injecting 10–20 μL of **1** (0.026 M) in CH_3CN. Reactions were followed to at least 4 half-lives. For the imidazole alcohols (**2**) only 1 mL of solution was prepared at each of 3–5 concentrations due to limitations of the availability of material. Four-hundred microliters of these solutions were placed in a 1-mL vial and 10–20 μL of **1** (0.026 M) in CH_3CN injected. The mixed solution was immediately transferred to a 1 mm path length quartz cell and absorbance vs time data collected at 291 nm for at least 4 half-times. Duplicate determinations were done. Observed pseudo-first-order rate constants (k_{obsd}) were obtained by fitting the absorbance vs time data to a standard exponential model with a nonlinear least-squares (NLLSQ) treatment. Second-order rate constants at each pH (k_2^{obsd}) were calculated from the slopes of k_{obsd} vs [alcoholamine] by linear regression. pH readings were obtained with a Radiometer GK24028 combination electrode and a Radiometer PHA943B pH meter standardized with Fisher certified buffers (pH 4.0, 7.0, 10.0). pH measurements were made before and after a kinetic run: no deviations greater than 0.05 units were found.

In the mixed solvent studies the solutions were assembled as above except for the addition of 40% or 80% (v/v) ethanol. The measured pH values were corrected by subtracting 0.09 or 0.20 units respectively according to a published procedure.[17] Solvent kinetic isotope studies with **2a–d,g** and **1** were performed analogously to those described above with the exception that D_2O was employed and the pH adjusted with concentrated NaOD or DCl. pD readings were made with the above electrode/meter setup and corrected according to pD = (pH reading) + 0.4.[18]

Potentiometric titrations were performed at 25 °C in a jacketed 10-mL cell. The pH was measured with use of the above mentioned electrode/meter in conjunction with a Radiometer TTT2 titrator module. The pH was recorded as a function of added 0.02 N NaOH (or 0.002 N NaOH/ethanol) delivered by a Radiometer ABU12 autoburette. A 5-mL stock solution containing 12–20 mg of alcohol amine with 0.025 mequiv of added HCl was made in H_2O (or 40, 80% v:v $EtOH/H_2O$) and 1.0 mL of this solution added to the jacketed cell along with 6 mL of H_2O, or mixed solvent. The data were analyzed by a computer determination of the inflection point to give the pK_a values. Reported pK_a values are averages of three determinations.

d. Product Studies. The various alcohol amines (1 × 10^{-4} mol) were dissolved in 0.4 mL of D_2O and the pH adjusted to ~1 unit above the pK_a in D_2O by the addition of small aliquots of 6 N DCl or NaOD. One

(15) Brederick, H.; Theilig, G. *Chem. Ber.* **1953**, *86*, 88.
(16) Curtis, N. J.; Brown, R. S. *J. Org. Chem.* **1980**, *45*, 4058.
(17) Bates, R. G.; Paabo, M.; Robinson, R. A. *J. Phys. Chem.* **1963**, *67*, 1833.
(18) (a) Fife, T. H.; Bruice, T. C. *J. Chem. Phys.* **1961**, *65*, 1079. (b) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.

Table I. Second-Order Rate Constants (k_2^{max}) and Kinetic pK_a Values for the Attack of β-Hydroxyamines **3a–d** on Amide **1**[a]

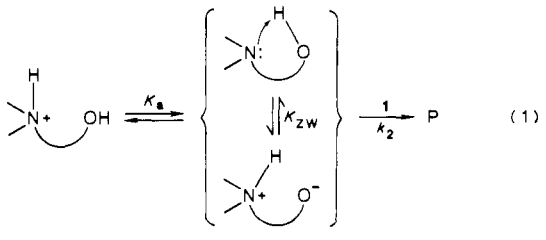
compd	pK_a thermodynamic[b]	pK_a kinetic[c]	k_2^{max},[c] M^{-1} s^{-1}
N,*N*-dimethylethanolamine (**3a**)	9.35 (0.05)	9.38 (0.02)	(2.49 (0.1)) × 10^{-1} (2.40 (0.1) × 10^{-1})[e]
serine (**3b**)	9.21[d]	9.18 (0.03)	1.52 (0.16) × 10^{-1} (1.24 (0.02) × 10^{-1})[e]
ethyl serinate (**3c**)	7.15 (0.01)	7.10 (0.04)	1.05 (0.09) × 10^{-2}
O-methylserine (**3d**)	9.05 (0.02)		2.45 (0.34) × 10^{-3}[f]

[a] T = 25 °C, μ = 0.2 (KCl), k_2^{obsd} values determined from slopes of plots of k_{obsd} vs [**3**] at 0.05–0.2 M. [b] Titrated at μ = 0.2 (KCl), T = 25 °C, unless otherwise stated. Values in brackets are the error of the mean of duplicate titrations. [c] From fits of k_2^{obsd} vs [H^+] according to eq 2. Values in brackets are standard deviations from NLLSQ fit. [d] From ref 20, μ = 0.15, T = 25 °C. [e] k_2^{max} in D_2O, μ = 0.2, T = 25 °C. [f] On the basis of $k_2^{obsd} = k_2^{CO_2} + k_2^{max}K_a/(K_a + [H^+])$. K_a fixed at the titrimetric value.

equivalent (20 mg) of amide **1** was then dissolved in 0.4 mL of CD_3CN and the two solutions mixed in an NMR tube. The tubes were kept at 25 °C and the ^1H NMR spectra periodically monitored until the reactions were complete (1–4 days). After this time the solvents were evaporated under vacuum and the residues analyzed by mass spectrometry and IR spectroscopy. In the case of the imidazoles, the products were the expected esters formed by nucleophilic attack of the alcohol on the acyl group of **1** as implied by a 0.5–1.0 δ downfield shift of the ImCH_2–O resonances and typical ester IR carbonyl frequencies of ~1730 cm^{-1}. In the case of the serine derivatives the final products invariably were ser-NH-acylated amides as identified by typical amide C=O IR frequencies (~1650 cm^{-1}) and no downfield SerCH_2-OH ^1H NMR chemical shifts. The data are given in Tables S1 and S2 (supplementary material). (*N*,*N*-Dimethylamino)ethanol **3a**, in a previous publication,[7b] has been shown to yield the corresponding ester.

Results and Discussion

Werber and Shalitin, in a careful early study,[6b] concluded tertiary amino alcohols were O-acylated by active ester acylating agents. Similarly, Page and Jencks[7a] found that ethanolamine and quinuclidinol reacted with acetylimidazole about 5-fold more rapidly than amines of comparable basicity that do not contain a hydroxyl group. Our preliminary study[7b] indicated that the decomposition of **1** is markedly accelerated by species containing a β-hydroxy amine (e.g., *N*,*N*-dimethylethanolamine, the buffers TRICENE, TRIS, HEPES, BISTRIS, **2a**, and **2b**). For each, only the basic form with neutral N is active. However, triethylamine, a much more basic material (pK_a 10.75[19]), is inactive toward **1** and *N*-methylimidazole (pK_a 7.56[20]) is ~30-fold less reactive than **2a** or **2b**. The enhanced reactivity of the β-OH amines was attributed[7b] to a cooperative interaction between N and OH (as in eq 1) which leads to direct O-acylation. Solvent kinetic isotope effects with **2a** and **2b** suggested an intramolecular



(1)

general base pathway, but the relatively low magnitude (k_{H_2O}/k_{D_2O} = 1.8 and 1.6, respectively[7b]) allows the possibility that both the neutral and zwitterionic forms (eq 1) are reactive, with the latter exhibiting a lower solvent isotope effect than the former.

The same general phenomenon of O-acylation appears true for the amino alcohols here as evidenced by control experiments (vide infra) and product studies (see Experimental Section). In Figure 1 are plots of log k_2^{obsd} vs pH for the attack of serine (**3b**) and imidazole alcohol **2d** which exemplify the kinetic behavior showing plateaued activity above the pK_a of N. The primary second-order

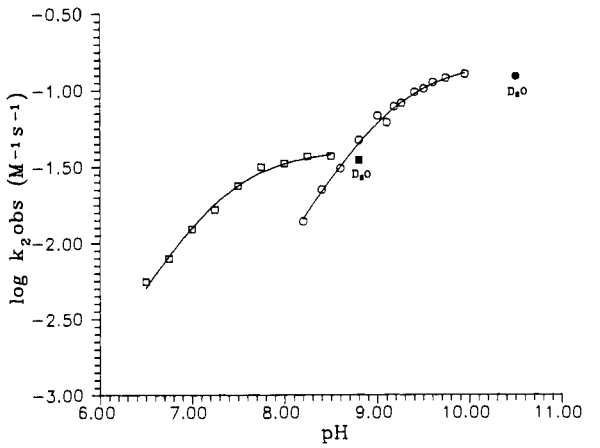


Figure 1. Plot of k_2^{obsd} vs pH for attack of **2d** (□) and serine (○) on amide **1** in H_2O at T = 25 °C, μ = 0.2 (KCl). Filled symbols, k_2^{max} in D_2O.

Table II. Second-Order Rate Constants (k_2^{max}) and Kinetic pK_a Values for the Attack of Imidazoles **2a–g** on Amide **1**[a]

compd	pK_a thermodynamic[b]	pK_a kinetic[c]	k_2^{max},[c] M^{-1} s^{-1}
2a	6.73[d]	6.77 (0.02)	2.66 (0.02) × 10^{-2} (1.45 × 10^{-2})[e]
2b	6.93 (0.05)	6.92 (0.08)	2.45 (0.12) × 10^{-2} (1.50 × 10^{-2})[e]
2c	7.88 (0.03) (8.50 (0.02)) D_2O	7.88 (0.08)	1.20 (0.13) × 10^{-1} (1.1 (0.05) × 10^{-1})[e]
2d	7.38 (0.05) (7.82 (0.01)) D_2O	7.35 (0.03)	4.06 (0.5) × 10^{-2} (3.85 (0.07) × 10^{-2})[e]
2e	6.18 (0.02)	*f*	*f*
2-methyl-imidazole	7.56	7.44 (0.04)	9.53 (0.35) × 10^{-4}
2f	7.04 (0.02)		5.90 (0.45) × 10^{-3}
2g	4.76 (0.02) (5.25 (0.03)) D_2O		8.30 (0.23) × 10^{-4}[g] (4.10 (0.24) × 10^{-5})[e,g]

[a] T = 25 °C, μ = 0.2 (KCl), k_2^{obsd} values determined from plots of k_{obsd} vs [Im] from 0.05 to 0.2 M. [b] Titrated at μ = 0.2 (KCl); bracketed values are errors from triplicate measurements. [c] From fits of k_2^{obsd} vs [H^+] according to eq 3. Values in brackets are standard deviations from the NLLSQ fit. [d] Eiki, T.; Kawada, S.; Matsushima, K.; Mori, M.; Tagaki, W. *Chem. Lett.* **1980**, 997. [e] D_2O. [f] Insoluble in H_2O at concentrations required for kinetics. [g] Determined at pH (pD) 7.0 in 0.1 M MOPS buffer, μ = 0.2 (KCl), T = 25 °C.

rate constant (k_2^{obsd}) data at various pH values for each amino alcohol (Tables S3–S13, supplementary material) were fit to the expression in eq 2 by standard NLLSQ procedures that give the

$$k_2^{obsd} = k_2^{max}K_a/(K_a + [H^+]) \quad (2)$$

kinetic pK_a and k_2^{max} values reported in Tables I and II. When the previously reported log k_2^{max} values for the buffers[7b] are statistically corrected for the number of β-OH groups (3 for TRIS and TRICENE, 5 for BISTRIS) and plotted against their pK_a values the Brønsted plot given in Figure 2 is obtained (slope β

(19) Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solution*; Butterworths: London, 1965.
(20) Quoted in: Grimmett, M. R. *Comprehensive Heterocyclic Chemistry*; Pergamon: Elmsford, NY, 1984; Vol. 4, p 384.

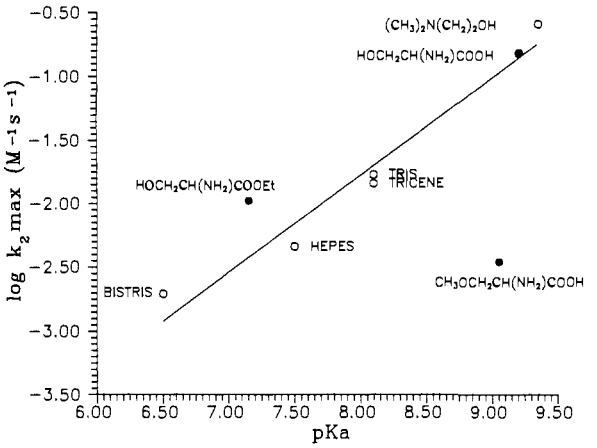


Figure 2. Brønsted plot of pK_a values for various buffers and serine derivatives against their respective k_2^{max} values (corrected for the number of CH_2OH groups) reacting with amide **1** in H_2O at $T = 25$ °C, $\mu = 0.2$ (KCl).

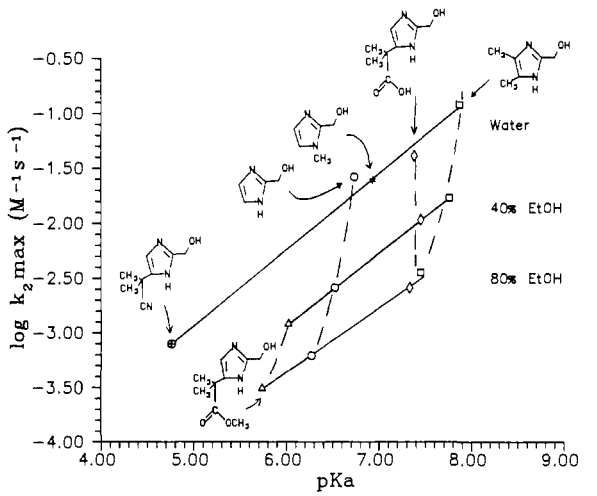
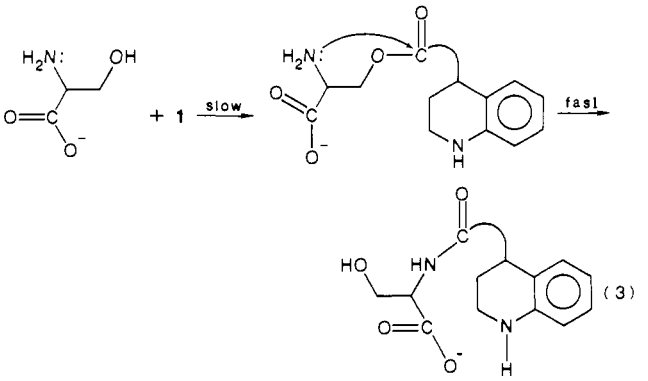


Figure 3. Brønsted plot of pK_a^{Im} values for imidazole alcohols against their respective k_2^{max} values reacting with amide **1** in H_2O, 40% and 80% EtOH/H_2O. $T = 25$ °C, $\mu = 0.2$ (KCl).

= 0.75 (0.1), intercept −7.8 (0.2), $r = 0.950$, 5 data). In Figure 3 are the Brønsted plots for the reaction of the imidazole alcohols with **1** in H_2O, 40% and 80% (v/v) EtOH/H_2O. In these cases the β values are relatively constant (0.69 (0.05), 0.66 (0.03), 0.60 (0.02), respectively) although diminished solvent polarity gives a reduction in the k_2^{max} values as expected (intercepts −6.33 (0.31), −6.87 (0.01), −6.93 (0.02), respectively). (The data used to construct the lower two lines in Figure 3 are given in Table S14, supplementary material.)

The Brønsted lines in Figures 2 and 3 pertain to the influence of the amine basicity in effecting O-acylation by **1**. This benchmark allows one to assess the role of a pendant CO_2^- unit in influencing that process. When the k_2^{max} values for **3b** and **2d** are placed on the Brønsted plots in Figures 2 and 3, respectively, they lie essentially on the lines. Therefore it is apparent that the carboxylate group in these two systems is incapable of providing another mechanism for O-acylation other than one simply dependent upon amine basicity. A fundamentally different or enhanced pathway such as CO_2^- acting as a general base (on NH) in concert with an amine---HO interaction would be expected to produce an upward deviation from the lines.

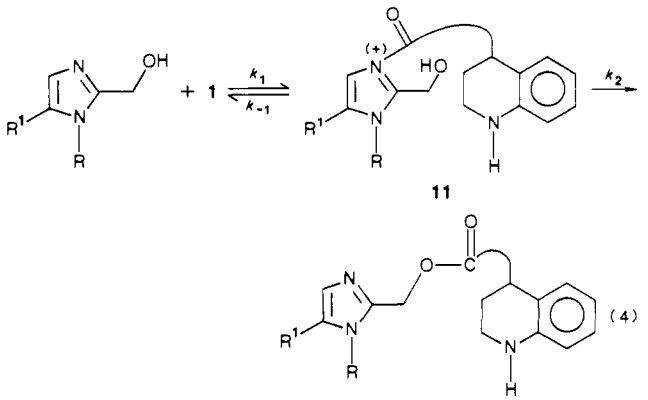
Inspection of the data for the derivatives in Figures 2 and 3 illustrates that all three functional groups play a role. In the case of the serine derivatives (Figure 2), the eventual products are amides produced by N-acylation of the primary amine by **1**. Nevertheless, the route by which the amide product is formed from serine must have involved initial O-acylation followed by rapid intramolecular acyl transfer to the NH_2 group as in eq 3.[21] This is evidenced by the fact that O-methylserine (**3d**) reacts ~60-fold



slower with **1** than does serine even though the pK_a values are similar. Direct N-acylation of serine of **3d** would be anticipated to be simply dependent upon the amino pK_a irrespective of whether the β-oxygen is present as OH or OCH_3. Finally we note that when O–N acyl transfer is inhibited (e.g., N,N-dimethylethanolamine) the kinetic datum fits the Brønsted line in Figure 2 but the product is the ester.[7b]

In this system the free carboxylate enhances the N pK_a as can be judged from the comparison of ethylserinate (**3c**) and serine. For the former, a pK_a reduction of ~2 units is observed along with a corresponding loss of activity. Ethyl serinate (from Figure 2) lies slightly above the Brønsted line which may signify that both direct and indirect N-acylation occur concurrently although at present we cannot assess the relative amounts of each pathway unambiguously.

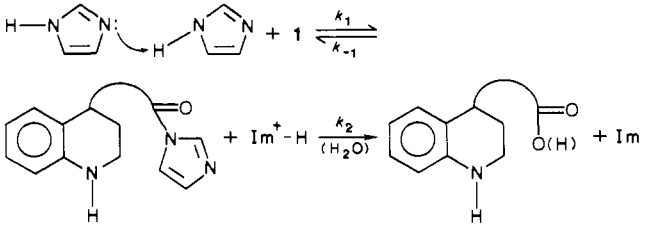
Imidazole Derivatives. Reaction of the imidazole alcohols with **1** produces ester products as evidenced by IR, ^1H NMR, and mass spectrometry (supplementary material). This finding is a necessary consequence of direct OH attack but, as stated by us[7b] and Werber and Shalitin,[6b] does not necessarily rule out N attack (since that process should also depend upon imidazole pK_a) followed by competitive N→O acyl transfer as in eq 4. In an earlier study[10] we had observed that the free base of imidazole facilitates the



hydrolysis of **1** by pathways that are uni- and bimolecular (in imidazole). This has also been observed for imidazole and certain substituted phenyl esters[22] and is interpreted as involving nucleophilic attack of one imidazole promoted by a second as in eq 5. With amide **1**, the intermediate acyl imidazole (**12**) is hydrolyzed by H_2O in competition with its reversion to **1**. Therefore it might be envisioned that the process depicted in eq 4 occurs with **2a–g** with the pendant hydroxymethyl group acting to rapidly intramolecularly deplete the acylimidazole (ium) intermediate (**11**) in competition with reversion to **1**. Hydrolysis of **1** facilitated by diacids capable of forming cyclic anhydrides has been shown to

(21) Greenhalgh (Greenhalgh, R.; Heggie, R. M.; Weinberger, M. A. *Can. J. Chem.* **1963**, *41*, 1662) have observed that above pH 8, the O to N acetyl transfer in ethanolamine is rapid.

(22) (a) Bruice, T. C.; Benkovic, S. J. *J. Am. Chem. Soc.* **1964**, *86*, 418. (b) Caplow, M.; Jencks, W. P. *Biochemistry*, **1962**, *1*, 773.

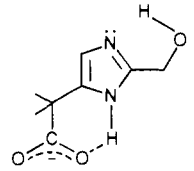


12 (5)

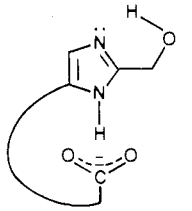
occur by an analogous process.[11] This would also account for the enhanced reactivity of the (hydroxymethyl)imidazoles relative to imidazole alone. While one cannot unambiguously rule this out in general, steric arguments for 4,5-dimethyl-2-(hydroxymethyl)imidazole (**2c**) render it unlikely. It is well-known that alkylation of C-2 decreases the nucleophilicity of imidazole toward active esters such as *p*-nitrophenyl acetate[23] and typically these imidazoles lie below Brønsted plots for N-acylation of non-C-2 substituted imidazoles by pNPA. A similar encumbrance to N nucleophilic attack is expected for a 2-CH_2OH group in **2a–g**, but these compounds are more reactive than imidazoles of comparable basicity containing no CH_2OH group. Furthermore, 4,5-dimethylation as in **2c** should strongly diminish N nucleophilicity for steric reasons if eq 4 represented the dominant pathway for **2a–g** reacting with **1**. The fact that **2c** lies on the same Brønsted line defined by less encumbered derivatives of **2** therefore suggests that direct O-acylation occurs.

Solvent Studies with 2a-g. A solvent study in which the dielectric constant[24] of the medium is reduced by the addition of HOEt was undertaken to address certain questions. First, ester **2e** has a limited solubility in H_2O: sufficient to obtain a reliable titrimetric pK_a but insufficient for kinetic determinations since the reactivity with **1** is low which obviates comparison with acid **2d**. Second, it is expected that a reduction of the medium polarity[24] will enforce a stronger H-bonding and electrostatic association of the carboxylate and imidazole. Finally, it is known that CO_2H pK_a values of α-amino acids increase with the mole fraction of alcohol,[25] by $\sim$1.5 units in passing from 0 to 80% EtOH.[25a] By contrast, the NH^+ pK_a values of those amino acids is scarcely affected by the less polar environment presumably due to zwitterionic stabilization in the ammonium carboxylate which offsets the normal reduction of basicity of amines observed in less polar media.[25b]

The phenomena discussed above should also raise the CO_2H pK_a in **2d** thereby enhancing the effectiveness of the carboxylate to act as a general base, and if that is important, it may perhaps switch on an additional catalytic benefit not accessible to the other imidazole alcohols that lack a CO_2^-. An additional consequence of enhancing the basicity of CO_2^- by a medium effect is that it may, in part, compensate for what might be a structurally imposed non-optimal alignment of the CO_2^----H-N in the sense described by Gandour[26] (see **13** and **14**).

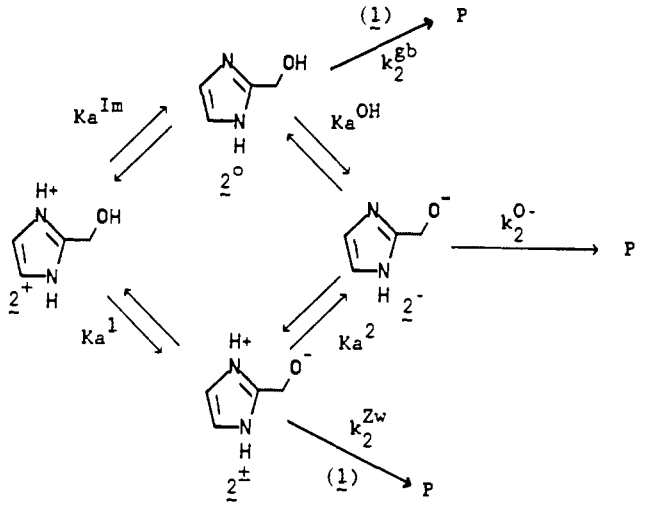


13
non-optimal
anti alignment



14
hypothetical optimally
syn aligned carboxylate

Scheme II



Given in Table S14 are k_2^{max} and titrimetric pK_a values for various derivatives of **2** in 40 and 80% (v/v) EtOH/H_2O with the Brønsted plots being illustrated in Figure 3. The fact that **2d** in all media fits precisely on the line again indicates that the imidazole pK_a dictates the activity and whatever influence the CO_2^- exerts produces no deviation from the activity expected on the basis of the Brønsted relationship.

Since neither serine nor **2d** lies above its respective Brønsted line, at first glance one might suggest that the carboxylate group is of no positive benefit in O-acylation. However, the above solvent study indicates the situation is more subtle. In passing from H_2O to media of increased EtOH content, the activity of all the imidazole alcohols is reduced as expected, and there is a noticeable drop in the pK_a value of those materials with no carboxylate. This is visualized by the curved dashed lines in Figure 3. However, the electrostatic and/or H-bonding stabilization in the zwitterionic form of **2d** counteracts the general medium-induced reduction in N-basicity[25] thereby enhancing the activity in relation to that of **2a–c,e**. On the basis of the figure, one might envision that in media of even lower polarity, the activity of **2d** will exceed that of **2c** despite the fact that in water the latter is the most reactive of the series.

Solvent Kinetic Isotope Effects. Given in column 4 of Tables I and II are the k_2^{max} values for **3a,b** and **2a–d,g** in D_2O. In all cases the values of 1–2 are generally lower than normal ones of 2–3 expected for general-base catalyzed acyl transfer to oxygen.[27] However, low values are not inconsistent for that process since examples exist which also show small solvent isotope effects.[27,28] Analysis of solvent isotope effects is complex[27,29] and small values have been explained by invoking non-linear N---H-O geometries, asymmetric transition states, and solvation effects, any of which could be operative in the reaction of **2** or **3** with **1**.

The geometry of the 5-membered ring required for intramolecular general base assisted acylation of the oxygens in **2** and **3** is not particularly favorable for the formation of a strong H-

(23) (a) Bender, M. L. *Mechansims of Homogeneous Catalysis From Protons to Proteins*; Wiley-Interscience: New York, 1971; pp 147–176. (b) Bruice, T. C.; Schmir, G. L. *J. Am. Chem. Soc.* **1958**, *80*, 148. (c) Street, J. P.; Skorey, K. I.; Brown, R. S.; Ball, R. G. *J. Am. Chem. Soc.* **1985**, *107*, 7669.

(24) For the dependence of medium dielectric constant on percent EtOH see: Åkerlöf, G. *J. Am. Chem. Soc.* **1932**, *54*, 4125.

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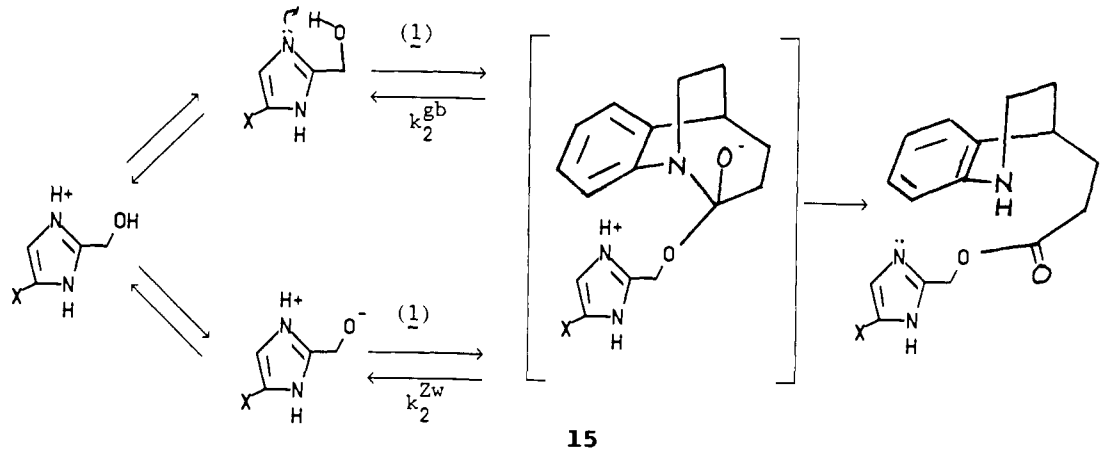
(27) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; pp 243–281.

(28) (a) Jencks, W. P.; Carriuolo, J. *J. Am. Chem. Soc.* **1960**, *82*, 675. (b) Bruice, T. C.; Benkovic, S. J. *Ibid.* **1964**, *86*, 418.

(29) For a discussion of factors influencing solvent kinetic isotope effects see ref 27 and: (a) Melander, L.; Saunders, W. H., Jr. *Reaction Rates of Isotopic Molecules*; Wiley-Interscience: New York, 1980; pp 202–224 and 42. (b) Schowen, R. L. *Prog. Phys. Org. Chem.* **1972**, *9*, 275–332. (c) More O'Ferrall, R. A. In *Proton Transfer Reactions*; Calden, E. F., Gold, V., Eds.; Wiley: New York, 1975; p 201.

Scheme III



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bond.[30] However, all other factors being equal, as the HO group attacks the C=O group of amide **1**, the acidity of the attached hydrogen will increase, favoring stronger H-bonding at the transition site.[30b] For intramolecular proton transfer between two centers separated by very few atoms, bridging H_2O molecules have been implicated.[31] Since the transfer of more than one proton in a transition state would be reflected in a bowl-shaped proton inventory plot,[32] the k_2^{max} value for the attack of *N,N*-dimethylethanolamine (**3a**) on **1** was determined in 50% D_2O/H_2O. The experimental value (2.51 (0.10) $\times 10^{-1}$ M^{-1} s^{-1}) is essentially the same as in pure H_2O and D_2O given in column 4 of Table I and provides no indication of the anticipated downward bowing (in the plot of k_2^{max} vs mole fraction of D_2O) expected if a water molecule was acting as a bridge during the acylation process.

On the other hand, the diminution of $k_2^{max}(H_2O)/k_2^{max}(D_2O)$ from 2 → 1 as the pK_a^{Im} value increases in passing from **2a** → **2c** suggests a nucleophilic route may also be operative. While nucleophilic attack of amines on *p*NPA generally proceeds with solvent isotope effects of 1.0–1.1,[33] the arguments presented above for serine and **2c** rule out that process. Rather, it is possible that O-acylation by **1** of a zwitterionic form occurs as in Scheme II. We have already established the dominance of zwitterionic pathways for the attack of 2-(methylthio)imidazoles on *p*NPA between pH 6.5 and 9.[23c,34] Such a role has also been suggested by Page and Jencks[7a] to explain the enhanced reactivity of 3-quinuclidinol toward *N*-acetylimidazole.

Given in eq 6 is the expression relating k_2^{obsd} and $[H^+]$ for the imidazole alcohols based on the terms defined in Scheme II with K_{ZW} being defined as $[2^{\pm}]/[2^0]$. Below pH 9, the amount of

$$k_2^{obsd} = \frac{(k_2^{gb}K_a^{Im} + k_2^{ZW}K_{ZW}K_a^{Im})[H^+] + k_2^{O^-}K_a^{Im}K_a^{OH}}{[H^+]^2 + (K_a^{Im} + K_a^{Im}K_{ZW})[H^+] + K_a^{Im}K_a^{OH}} \quad (6)$$

alkoxide 2^- is low and no kinetic involvement of that species is in evidence from the pH vs log k_2^{obsd} profiles for **2a–d,g**. Thus the second term of the numerator and the third term can be omitted and the plateau region above pK_a^{Im} is then comprised of two terms, one for the general base route and one for the zwitterion, i.e., $k_2^{max} = k_2^{gb} + k_2^{ZW}K_{ZW}$.

In general it is expected that the nucleophilicity of the zwitterionic alkoxide will be much higher than the general base assisted alcohol attack on **1**,[35] but it should exhibit lower solvent isotope effects than a general base process.[29] However, the overall contribution attributable to the zwitterion is damped by low values of K_{ZW}. In one limit, when K_{ZW} is very low, then $k_2^{max} \simeq k_2^{gb}$ and solvent isotope effects >1 are to be expected. On the other hand, since $K_{ZW} = [2^{\pm}]/[2^0] = K_a^1/K_a^{Im}$, then as the imidazole basicity increases, so too does the amount of zwitterion with a net effect of lowering the observed solvent isotope effect since $k_2^{ZW}K_{ZW}$ becomes appreciable. Although we cannot be certain of this explanation, the Brønsted line relating N basicity and k_2^{max} for O-acylation may be a reflection of increasing contributions of zwitterionic materials at higher pK_a^{Im}.[37]

Conclusions

Amide **1** is unique in that structural distortion increases its susceptibility to attack by certain dicarboxylic acids[11] and β-amino alcohols,[7b] including those studied here. From the above, the latter reaction involves initial O-acylation of the amino alcohol facilitated by the basic form of the intramolecular amine as in Scheme III. For the serine derivatives that contain a primary amino group, the final product is a serinamide formed by O→N acyl transfer from the initially produced ester. When a tertiary amine forms part of the β-amino alcohol (*N,N*-dimethylethanolamine) or when a stable amide cannot subsequently be obtained (i.e., acyl imidazole), the final products are esters. From that scheme, the role of the amino function may be to act as a general base in promoting O-attack, and/or as an imidazolium ion that electrostatically and inductively allows a greater amount of a nucleophilic zwitterionic form. Also, it may be envisioned that the nascent imidazolium ion in **15** assists the breakdown of the tetrahedral intermediate by intramolecular general acid catalysis.

The purpose of the study was to assess the involvement of an anionic pendant carboxylate in influencing the above acylation in an effort to reproduce in a simple system some role for the triad which may be of relevance to the SPases. With serine or **2d**, the carboxylate simply acts to enhance the pK_a of the amine (NH^+) thereby making it a better base and more capable of assisting O-acylation. The effect is particularly pronounced in solvents of lower polarity since the zwitterionic nature of **16** tends to counteract the general medium induced drop in basicity of the other imidazole alcohols.

(30) (a) For a compendium of reviews on H-bonding see: March, J. *Advanced Organic Chemistry*; 3rd ed.; Wiley-Interscience: New York, 1985; pp 71–74. (b) For a review of the relationship between H-bond strength and acid–base properties see ref 5 in ref 30a.

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(32) (a) Schowen, R. L. *Prog. Phys. Org. Chem.* **1972**, *9*, 275. (b) Kresge, A. J. *Pure Appl. Chem.* **1964**, *8*, 243. (c) Schowen, R. L.; Schowen, K. B. J. *Methods Enzymol.* **1982**, *87*, 551. (d) Alvarez, F. J.; Schowen, R. L. In *Isotopes in Organic Chemistry*; Buncel, E., Lee, C. C., Eds.; Elsevier: Amsterdam, 1987; Vol. 9, pp 1–60.

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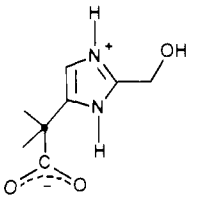
(35) Attack of cholinate anion on **1** proceeds with a k_2^{ZW} of 85 M^{-1} s^{-1} at 25 °C, μ = 0.2,[7b] and choline has a pK_a^{OH} of 12.8.[36]

(36) Haberfield, P.; Pessin, J. *J. Am. Chem. Soc.* **1982**, *104*, 6191.

(37) The proposed involvement of a $SerO^-$–HisIm–H^+ ionic pair has been discussed[38] as a possible pathway for acylation of the serine proteases but was downplayed because of the disparity in pK_a values for imidazole and SerOH.[39]

(38) Bruice, T. C.; Benkovic, S. *Bioorganic Mechanisms*; W. A. Benjamin, Inc.: New York, 1966; Vol. 1, pp 249–250.

(39) On the basis of a value of 13.6 for pK_a^{OH} of *N*-acetylserinamide: Bruice, T. C.; Bruno, J. J.; Brandon, N. E. *Biochemistry* **1962**, *7*, 1.



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The study also shows that the carboxylate in **2d**, although not optimally aligned with the imidazole in the sense described by Gandour,[26] enhances the imidazole basicity by 1.2 pK_a units relative to comparison ester **2e**. For imidazolium ionizations wherein an intramolecular carboxylate is *syn* oriented, reference has been made to large ΔpK_a values in comparison with a corresponding imidazolium ester[40a] or other comparison materials[40b] containing an *anti* disposed carboxylate- - -imidazolium couple. However, the data herein indicate that a *syn* orientation is not required for a large ΔpK_a. Moreover, we note that large ΔpK_a values are conditional upon the nature of the amine (e.g., pK_a^{NH} serine–ethyl serinate = 2.06, Table I) and solvation (e.g., pK_a^{Im}-H^+ **2d–2e** is 7.45 - 6.02 = 1.43 in 40% EtOH/H_2O and 7.33 - 5.74 = 1.59 in 80% EtOH/H_2O). A *syn* orientation of the carboxylate may be of evolutionary advantage to the enzymes that employ it not only for the basicity arguments[26,41] but also because that arrangement places the center of (-)-charge in the carboxylate closer to the imidazole H–N thereby optimizing the electrostatic interaction.

To term this system a model for the acylation of the SPases invites comparison with the enzyme which may not be justified given the unorthodox geometry of the acylating agent and perhaps non-optimal alignment of the functional groups in serine or **2d**. Furthermore, even with the enzymes the mechanism of acylation shows subtle substrate dependent diversities so that in some cases the Asp CO_2^- may act electrostatically and in others it may act as a bonafide general base.[3] What is certain from the above study is a demonstration of a role for carboxylate that enhances the amine basicity and allows the latter to more effectively influence direct CH_2OH acylation. This may be viewed as a small molecule precedent for a similar role suggested to occur in the SPases.[4]

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Supplementary Material Available: Tables of k_2^{obsd} and k_2^{max} values for the serine derivatives and **2a–f** reacting with **1**, k_2^{max} data used in the calculation of Brønsted lines in Figure 3, and product IR, NMR, and MS data (14 pages). Ordering information is given on any current masthead page.

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(41) The basicity argument requires that a proton be transfered from the proximal imidazole N–H to carboxylate concurrent with the distal N: acting as a general base on SerOH. This is thermodynamically favorable only if the normal pK_a values of imidazolium and the carboxylic acid be reversed in the enzyme active site as the transition state for the acylation is approached.[4]

Stereochemistry and Mechanism of the Biosynthesis of Leukotriene A_4 from 5(*S*)-Hydroperoxy-6(*E*),8,11,14(*Z*)-eicosatetraenoic Acid. Evidence for an Organoiron Intermediate

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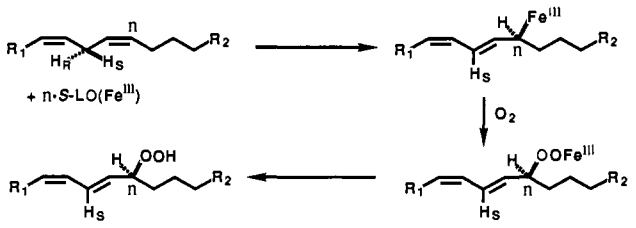
Abstract: The pathway of biosynthesis of leukotriene A_4 (LTA_4, **2**) from 5(*S*)-hydroperoxy-6(*E*),8,11,14(*Z*)-eicosatetraenoic acid (5-*S*-HPETE, **1**) has been explored by the comparative study of (*S*)- and (*R*)-lipoxygenase (LO) enzymes as catalysts. The purified LO from potato, an *S*-lipoxygenase, converts (anaerobically) **1** to **2** (determined as the characteristic hydrolysis mixture of two epimeric 5,6-diols and two epimeric 5,12-diols), as previously reported by Samuelsson et al. However, the 8-*R*-LO from the coral *Plexaura homomalla* transforms **1** (anaerobically) into 6-*epi*-LTA_4 (**6**). The observed divergence of stereopathways agrees with predictions based on the intermediacy of organoiron intermediates in enzymic lipoxygenation (Scheme I) and detailed in Schemes II and III. Further evidence for the intervention of such intermediates has been obtained by trapping experiments under pure O_2 at pressures of 1–60 atm. Under O_2 pressure **1** is converted by the potato LO to a new product, the bis(hydroperoxide) **7**, whereas the coral LO converts **1** to the diastereomeric bis(hydroperoxide) **9**.

The biosynthesis of the physiologically and medically important leukotrienes[1] from arachidonate is initiated by two processes which are also of great mechanistic interest: (1) the conversion of arachidonate by a (5*S*)-lipoxygenase (5-LO) to 5(*S*)-hydroperoxy-6(*E*),8,11,14(*Z*)-eicosatetraenoic acid (5-*S*-HPETE, **1**), and (2) the transformation of 5-*S*-HPETE to leukotriene A_4 (LTA_4, **2**).[1] Evidence has been obtained that the 5-LO, either from murine mast cells[2] or from potato,[3,4] catalyzes the conversion of arachidonate to 5-*S*-HPETE *and also of 5-S-HPETE to* LTA_4. Further, it has been reported that the enyzmic transformation of

Scheme I



(1) See: *The Leukotrienes, Chemistry and Biology*, Chakrin, L. W., Bailey, D. M., Eds.; Academic Press: New York, 1984.

(2) Shimizu, T.; Izumi, T.; Seyama, Y.; Tadokoro, K.; Rådmark, O.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4175–4179. Because LTA_4 undergoes rapid hydrolysis in neutral aqueous media, it was not isolated from incubation experiments but was analyzed as the mixture of 5,6- and 5,12-diols which results from hydrolysis.

(3) Shimizu, T.; Rådmark, O.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 689–693.

0002-7863/89/1511-1452$01.50/0