Integrating both sides, we get

$$\ln Z = \frac{k_1\tau}{\beta - \gamma} \ln \frac{(1 + \beta e^{-1/\tau})^\gamma}{(1 + \gamma e^{-1/\tau})^\beta} + \text{constant} \quad (A.15)$$

At $t = 0$

$$\ln Z = \frac{k_1\tau}{\beta - \gamma} \ln \frac{(1 + \beta)^\gamma}{(1 + \gamma)^\beta} + \text{constant} \quad (A.16)$$

Exponentiating, we get

$$Z_{t=0} = C\left[\frac{(1 + \beta)^\gamma}{(1 + \gamma)^\beta}\right]^{k_1\tau/(\beta-\gamma)} \quad (A.17)$$

Since, at $t = 0$, [irreversible adduct] $= 0 = P_0 + Z_{t=0}$, we get

$$C = -P_0\left[\frac{(1 + \gamma)^\beta}{(1 + \beta)^\gamma}\right]^{k_1\tau/(\beta-\gamma)} \quad (A.18)$$

At $t = \infty$, [irreversible adduct] $= P_0 + C$; hence,

$$[\text{irreversible adduct}]_{t=\infty} = P_0\left\{1 - \left[\frac{(1 + \gamma)^\beta}{(1 + \beta)^\gamma}\right]^{k_1\tau/(\beta-\gamma)}\right\} \quad (A.19)$$

Substituting back and setting $\phi = [\text{irreversible adduct}]_{t=\infty}/P_0$, get eq 4.

For the limit $K_x \gg X_0$, the denominator in eq A.11 becomes $K_x(K_d + S_0e^{-1/\tau})$. The corresponding homogeneous differential equation for second-order attack of SP is then

$$(e^{2t/\tau} + \beta e^{t/\tau})Z' + k_1\beta\gamma Z = 0 \quad (A.20)$$

whence the solution for the extent of modification becomes

$$\phi = 1 - e^{k_1\gamma\tau}(1 + \beta)^{k_1\beta\gamma\tau} \quad (A.21)$$

Registry No. $MeC(O)CH_2CO_2Et$, 141-97-9; $NaBH_4$, 16940-66-2; $NaBH_3CN$, 25895-60-7; acetoacetate decarboxylase, 9025-03-0; cyclopentanone, 120-92-3; cyclohexanone, 108-94-1.

Supplementary Material Available: Tables of individual session scores for Figure 2 and for control animals (2 pages). Ordering information is given on any current masthead page.

Biomimetic Models for Cysteine Proteases. 1. Intramolecular Imidazole Catalysis of Thiol Ester Solvolysis: A Model for the Deacylation Step

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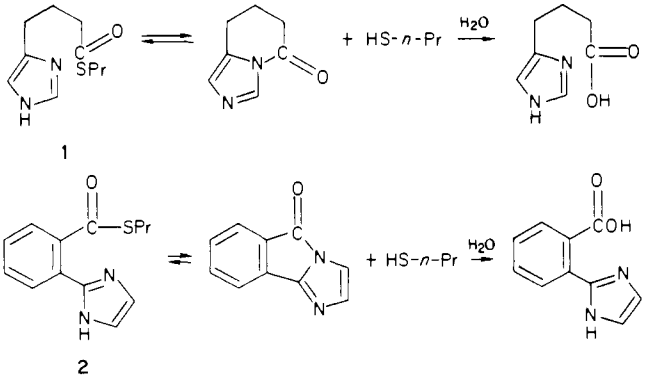
Abstract: The solvolysis of 2-[2-(acetylthio)phenyl]-4,5-dimethylimidazole (**3**) has been studied in ethanol–water solution as a model for the thiol ester intermediates occurring during the catalytic cycle of cysteine proteases. Additionally the solvolyses of *N*-acetyl-4,5-dimethyl-2-phenylimidazole (**4**) and benzenethiolacetate (**5**) were studied as reference compounds. At neutrality, a plateau in the pH–log k_{obsd} profile for solvolysis of **3** is seen due to the participation of imidazole. Evidence is given that the mechanism in this pH region is intramolecular general-base-catalyzed solvent attack on the thiol ester by imidazole. At pHs greater than 10, specific-base-catalyzed solvolysis of the thiol ester becomes predominant. There is no kinetic importance of an S-to-N acyl transfer during the course of solvolysis. The relevance of these results to the chemical mechanism of cysteine proteases is discussed.

I. Introduction

The catalytic cycle of the cysteine proteases, of which papain is the most widely studied example, is known to proceed through an intermediate thiol ester.[1] Present in the active site[2] of these enzymes is a histidylimidazole residue which presumably is catalytically involved with both the acylation and deacylation steps. The role of imidazole in the deacylation step has often been suggested[3] as that of a general base in assisting delivery of an attacking water molecule. Support for the involvement of imidazole comes mainly from the large solvent deuterium isotope effect measured for the enzymatic deacylation reaction[1d,e,4] and more recent spectrophotometric and NMR titration experiments of Shafer et al.[5] The only well-documented chemical precedent of this mechanism with a thiol ester is the intermolecular general-base-catalyzed hydrolysis of ethyl trifluorothioacetate.[6] It is

Scheme I



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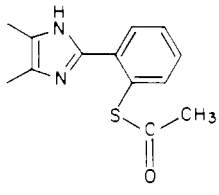
significant, however, that this is the only thiol ester studied for which an uncatalyzed "water" reaction is observed, that for other thiol esters being too slow to measure.

Aminolysis of thiol esters is generally first order in amine, but second-order terms are seen for reactions of hydroxylamine, hydrazine, and morpholine with γ-valerothiollacetone, indicating an additional general-base mechanism, at least in aminolysis.[7]

Previous model studies involving intramolecular imidazole catalysis of thiol ester hydrolysis have failed to show a general-base role for the imidazole unit.[8] Bruice[8a] has studied *S*-*n*-propyl γ-(4-imidazolyl)thiolbutyrate (**1**) and Fife and DeMark[8] have studied *S*-*n*-propyl *o*-(2-imidazolyl)thiolbenzoate (**2**).

The reaction of both compounds proceeds by a rapid intramolecular nucleophilic attack of imidazole displacing thiol(ate) and forming an imidazolelactam intermediate which subsequently hydrolyses as in Scheme I. The significance of these studies with respect to the enzymatic hydrolysis is increased by the fact that a large deuterium isotope effect exists for hydrolysis of acetylimidazole.[9] Thus, rate-limiting hydrolysis of a rapidly produced acylimidazole intermediate could then account for the observed enzymatic D_2O effect. Moreover, Fife and DeMark[8d] have shown that in a sterically compressed system (**2**), the intramolecular nucleophilic attack is general-base-catalysed and shows a solvent kinetic isotope effect, a finding which may be germane to the situation existing in the enzymatic process.[8e]

The major drawback of these models in relation to the situation with cysteine proteases is that the normally favorable back reaction of acyl transfer to sulfur is severely limited in the reaction of **1** and **2**, a problem of which the authors were aware. This is a result of the fact that the important functional groups, thiol and imidazole, are not retained as part of the same molecule as is the situation that exists in the enzymatic case. Indeed when enough external thiol was added to the reaction mixture containing **1**, the hydrolysis was completely inhibited due to a bimolecular back reaction.[8a] A unimolecular analogy of this situation has been shown to exist by McDonald et al.[10] who showed that *N*-(*n*-propyl)-2-mercaptomethyl)benzamide expels propylamine to form a thiol lactone. To cast light on the possibly important back reaction as it pertains to the cysteine proteases, we have prepared 2-[2-(acetylthio)phenyl]-4,5-dimethylimidazole (**3**) and studied its solvolysis as a model for an acyl-enzyme intermediate in which, following hydrolysis, the imidazole and mercaptan functionalities are retained as part of the same molecule. For comparison, we



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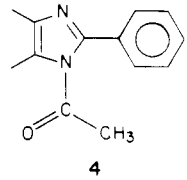
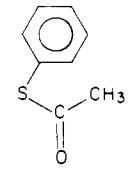


4



5

have also studied the hydrolysis of the simpler N and S acyl derivatives **4** and **5**. We report evidence here that the imidazole in **3** does indeed facilitate the hydrolysis of the thiol ester by acting as what is best analyzed as a general base. This appears to be the first such example of this mechanism in the hydrolysis of a thiol ester containing a proximal imidazole.

II. Experimental Section

(a) Materials. Sodium acetate, succinic acid, disodium phosphate, boric acid, and sodium carbonate were all reagent grade. Water was triply glass distilled. 95% Ethanol was used as supplied. Me_2SO was distilled from CaH_2 and stored over molecular sieves. D_2O (Merck, Sharp & Dome Canada) was 99.7% isotopically pure. Mercaptoethanol was redistilled under N_2.

2-[2-(Acetylthio)phenyl]-4,5-dimethylimidazole (3). *S*-Benzylthiosalicylamide[11] (mp 157–158 °C) was reduced with a 10/3 molar excess of borane for 15 h in refluxing THF by the method of Brown and Heim.[12] Workup[12] gave a 90% crude yield of 2-(benzylthio)benzylamine as an oil: ^{1}H NMR ($CDCl_3$) δ 1.5 (s, 2 H), 3.85 (s, 2 H), 4.10 (s, 2 H), 7.1–7.4 H (m, 9 H). Without further purification, 12.1 g of the amine was condensed with 8.5 g of ethyl oximinoacetoacetate in 200 mL of acetonitrile by the general method of Veronese et al.[13] The mixture was stirred at room temperature for 24 h followed by 24 h at reflux. The product, 2-[2-(benzylthio)phenyl]-4-carbethoxy-5-methylimidazole was recrystallized from acetonitrile to yield 10.6 g (57%) of a colorless solid: mp 128.5–129.5 °C (uncorrected); ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3 H), 2.55 (s, 3 H), 3.98 (s, 2 H), 4.40 (q, 2 H), 7–7.6 (m, 8 H), 8.3 (d of d, 1 H); mass spectrum, *m/e* [M^+] calcd for ($C_{20}H_{20}N_2O_2S$) 352.1247, found 352.1237.

This product was reduced to 2-[2-(benzylthio)phenyl]-4,5-dimethylimidazole with an excess of borane in refluxing THF for 24 h. The solution was made strongly acidic with aqueous HCl, and the THF was distilled, allowing the temperature to reach 100 °C. The aqueous residue was basified (pH 8–9), the product extracted into CH_2Cl_2, and the organic solution dried over $MgSO_4$. From 7.2 g of the ester, 5 g of crude product was obtained. The crude material was dissolved in a minimum amount of CH_2Cl_2 and triturated with a small amount of CCl_4 which caused 2.72 g (42%) of the imidazole to precipitate as a white powder: mp 129–143 °C. The mother liquor was treated with picric acid and the yellow mass twice recrystallized from boiling 80% ethanol, yielding 1.7 g of the imidazolyl picrate: mp 176–178 °C. The free imidazole was obtained by continuous extraction of picric acid into benzene from an aqueous HCl–acetone solution of the picrate (overall yield of the free base was 63%). ^{1}H NMR (free base, $CDCl_3$) δ 2.18 (s, 6 H), 3.94 (s, 2 H), 7.–7.5 (m, 8 H), 8.1–8.3 (m, 1 H); H NMR (picrate Me_2SO-d_6) δ 2.25 (s, 6 H), 4.25 (s, 2 H), 7.25–7.75 (m, 9 H), 8.60 (s, 2 H); mass spectrum, *m/e* [M^+] calcd for ($C_{18}H_{18}N_2S$) 294.1192, found 294.1185.

To a solution of 2.74 g of 2-[2-(benzylthio)phenyl]-4,5-dimethylimidazole in 40 mL of dry THF and 200 mL of liquid ammonia was added 0.6 g of Na metal. The blue solution was held $^1/_2$ h at −86 °C and then quenched by the addition of solid NH_4Cl. Ammonia was evaporated after which time dilute HCl was added. The mercaptan residue was oxidized to the disulfide with excess iodine in CH_2Cl_2, extracted into aqueous acid, and then the aqueous extract was basified to pH 9 which yielded 0.7 g (37%) of the precipitated disulfide. The disulfide was quantitatively reduced by excess triphenylphosphine in 10% aqueous methanol, 0.1 N in HCl.[14] Water was added and the aqueous phase extracted with CH_2Cl_2. The water phase was then evaporated under vacuum to yield 0.85 g (100%) of 2-(2-mercaptophenyl)-4,5-dimethylimidazole HCl salt. The crude material was dissolved in 0.005 N ethanolic HCl and refrigerated below 0 °C to give yellow crystals. This material sublimes readily under vacuum and heat but decomposes when exposed to air: ^{1}H NMR (Me_2SO-d_6 + CF_3CO_2D) δ 2.25 (s, 6 H), 7.4–7.8 (m, 4 H); mass spectrum, *m/e* [M^+] calcd for $C_{11}H_{12}N_2S$ 204.0722, found 204.0711. Anal. Calcd for $C_{11}H_{13}N_2SCl$: C, 54.89; H, 5.44; N, 11.64. Found: C, 54.50; H, 5.55; N, 11.45.

The mercaptan imidazole HCl salt was dissolved in hot acetic anhydride and upon cooling 2-[2-(acetylthio)phenyl]-4,5-dimethylimidazole HCl salt crystallized. This was filtered, washed with hexane, and dried under vacuum at 100 °C: mp 209–211 °C; ^{1}H NMR (CD_3OD) δ 2.30 (s, 6 H), 2.35 (s, 3 H), 7.68 (s, 4 H); mass spectrum, *m/e* [M^+] calcd for $C_{13}H_{14}N_2OS$ 246.0828, found 246.0828. Anal. Calcd for $C_{13}H_{15}N_2OSCl$: C, 55.21; H, 5.35; N, 9.91. Found: C, 54.93; H, 5.38; N, 9.85.

***N*-Acetyl-4,5-dimethyl-2-phenylimidazole (4).** 4-Carbethoxy-5-methyl-2-phenylimidazole,[13] 5.2 g, was refluxed with 2.5 g of LAH in

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Table I. Rate Constants for Solvolysis of **3–5**[a]

compd	k_1(HOR),[b] s^{-1}	k_2(HOR),[b] s^{-1}	$k_3(^-OR)$,[b] s^{-1} M^{-1}	pK_a[c]	soln[d]
3		$4.14 \pm 0.2 \times 10^{-3}$	124 ± 12	6.69 ± 0.03	EtOH–H_2O
3		$1.44 \pm 0.3 \times 10^{-3}$	56 ± 10	6.91 ± 0.03	H_2O
4	$\simeq 1 \times 10^{-3}$	$\simeq 7 \times 10^{-5}$	$\simeq 40$	4.6 ± 0.4	EtOH–H_2O
5			10.0 ± 0.5		EtOH–H_2O

[a] Measured at 37.6 ± 0.3 °C. Rate constants in mixed solvent represent combined terms of ethanol and water. [b] k_1 = solvent attack on the protonated species, k_2 = solvent attack on the neutral species, k_3 = RO^- attack on the neutral species. [c] Kinetically determined pK_a's. [d] A 40:60 volume to volume mixture of 95% ethanol and water, ≃33 wt % ethanol in water, $pK_w = 14.6$.[21]

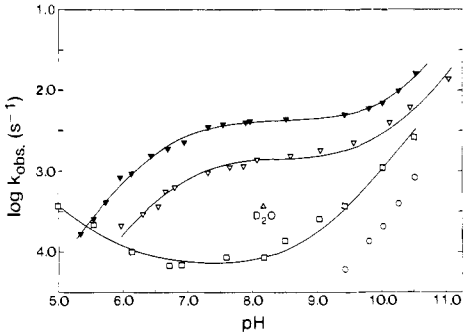

Figure 1. Plot of observed pseudo-first-order rate constants vs. pH at 37.6 ± 0.3 °C; **3** in ethanolic buffer (▼); **3** in aqueous buffer (▽); **4** in ethanolic buffer, □; **5** in ethanolic buffer (○). (△) represents the hydrolysis of **3** in D_2O solution at pD(H) = 8.2. (For actual conditions, see Experimental Section.)

THF for 48 h. The solution was acidified with HCl, the THF removed, and the aqueous residue basified and extracted into CH_2Cl_2 to give 2.2 g 57%) of crude 4,5-dimethyl-2-phenylimidazole. This was sublimed according to the procedure of Cornforth and Huang:[15] mp 236–240 °C [lit.[15] 241 °C]; ^1H NMR ($CDCl_3$) δ 2.15 (s, 6 H), 7.2–7.4 (m, 3 H), 7.8–8.0 (m, 2 H).

The imidazole (0.851 g) was reacted with 0.194 g of acetyl chloride in 40 mL of dry THF for $^1/_2$ h, 50 mL of pentane added, and the precipitated dimethylphenylimidazole hydrochloride filtered off. The filtrate was evaporated to give the *N*-acetyl-dimethylphenylimidazole which was used immediately without further purification: ^1H NMR ($CDCl_3$) δ 2.1 (s, 3 H), 2.18 (s, 3 H), 2.30 (s, 3 H), 7.1–7.5 (m, 5 H); IR (film on NaCl) 1730 cm^{-1}.

Benzenethiolacetate (5). Benzenethiol was acetylated with acetic anhydride and the product distilled under nitrogen, the middle fraction being collected: bp 222–223 °C (uncorrected) [lit.[16] 228–230 °C].

(b) Buffers. Ethanolic buffers were prepared in a 40/60 (v/v) mixture of 95% ethanol and water, making a 33 wt % ethanol solution. Only oxygen-acid buffers (acetate, succinate, phosphate, borate, and carbonate) were used, and the pH was determined by subtracting 0.09 unit from the meter reading according to Bates et al.[17] Buffers were 0.1 M (except for those in D_2O experiment and for the study of **4**) at various ionic strengths. No variation in rate with ionic strength was detected nor was there buffer catalysis except for **4**. Aqueous buffers were 0.1 M, μ = 0.3 (NaCl), except for the buffer used in the following D_2O experiments.

(c) D_2O Experiment. Matching 0.01 M phosphate buffers were prepared in water and 99.7% D_2O. pD was determined by adding to the meter reading +0.4 unit.[17b] These buffers correspond to the points at pH 8.06 and pD 8.19 in Figure 1.

(d) Kinetics. Kinetic data were obtained by observing the rate of change of absorbance of buffered solutions of **3** (350 or 390 nm), **4** (290 nm), and **5** (280 nm) at the designated wavelengths by using a Cary Model 210 spectrophotometer interfaced as previously described.[18] Temperature was maintained at 37.6 ± 0.3 °C by means of an external Colora Model NB-ELE circulating water bath. Solutions (3 mL of buffer in 1-cm quartz cuvettes) were equilibrated for 20 min prior to initiation of a run. Buffers for **3** and **5** were degassed by bubbling argon through the solutions which were then transferred to argon-flushed septum-sealed cells via syringe. Stock solutions of 0.1 M **3**, **4**, or **5** were prepared in Me_2SO, the solution for **3** containing a trace of trifluoroacetic acid to retard decomposition. These solutions were frozen when stored between runs and periodically replaced. Reactions were begun by injecting 5 mL of a stock solution into the cuvettes containing buffer. Observed pseudo-first-order rate constants were obtained by fitting the absorbance vs. time data to a standard exponential equation via a nonlinear least-squares program.[18]

pH was measured by using separate glass and saturated calomel electrodes (Fischer) and a Fischer Model 825 MP meter. pH was measured before and after runs. No deviation greater than 0.03 unit was found.

Table II. Second-Order Kinetic Constants for 2-Mercaptoethanol Attack on **3** and **5** at 37.6 °C in 33% Ethanol/H_2O

compd	pH	k_{obsd}^{RSH},[a] M^{-1} s^{-1}	$\Delta\log k_{obsd}^{RSH}/\Delta pH$
3	7.50	1.22 ± 0.01	
			0.66 ± 0.02
3	8.37	4.58 ± 0.04	
5	7.50	0.162 ± 0.001	
			1.01 ± 0.02
5	8.31	1.06 ± 0.02	

[a] $k_{obsd}^{RSH} = (k_{obsd}(\text{with thiol}) - k_{obsd}(\text{blank}))/[RSH]$.

III. Results

(a) Product Studies. Solvolysis of **3** was followed by ^1H NMR in CD_3OD by adding solid sodium carbonate to a 0.11 M sample of **3**/HCl. Over a 10-min period, the ($SCOCH_3$) methyl singlet at δ 3.35 diminished and was replaced by a singlet at δ 2.02 appropriate for CD_3OCOCH_3. Corresponding changes were also noted in the aromatic region with the final spectrum being that of 2-(4,5-dimethylimidazol-2-yl)benzenethiol. The initial colorless solution became yellow due to formation of the thiolate anion. During the course of solvolysis, no signals appropriate for the *N*-acetyl isomer **6** (Scheme II) were observed. UV spectra determined with solutions used to determine k_{obsd} of **3** at the completion of the reaction were identical with those of the product thiol in the same buffer.

(b) Kinetic Studies. Kinetic reactions of **3** were determined in ethanol/H_2O buffers to correlate with acylation studies utilizing the free mercaptan[18b] and to alleviate precipitation problems. Generally the buffer strength was kept at 0.1 M, but no evidence of buffer catalysis or ionic strength effects was apparent. For the sake of comparison, the hydrolysis of **3** was also determined in aqueous buffers, the k_{obsd} values being ~3–4-fold less at a given pH than in the organic buffers.

The increased solvolytic rate for **3** observed in ethanol/H_2O buffers over that found in H_2O can be ascribed to the greater nucleophilicity of ethanol (ethoxide) over H_2O (hydroxide).[19] We note that similar rate enhancements in the deacylation rate of acylpapains have been observed with methanol and ethanol cosolvents.[20] In the case of *p*-nitrophenylthiolacetate, ethoxide is ~10-fold more nucleophilic than hydroxide.[19] In the pH-independent region at pH 8.2, a solvent isotope effect of $k_{H_2O}/k_{D_2O} = 3.75$ is seen for the hydrolysis of **3** in aqueous buffer.

Plotted in Figure 1 are log k_{obsd}/pH profiles for the solvolysis of **3–5**. Values for **4**, which showed buffer catalysis as is usual for *N*-acylimidazole hydrolyses,[5b,9,19] were extrapolated to zero [buffer] which accounts for some of the scatter in the plot.

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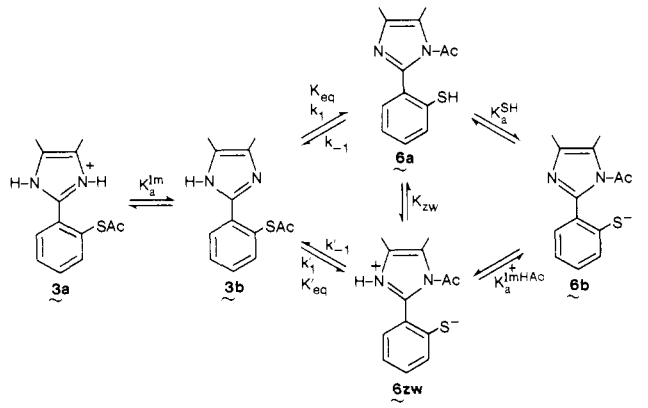
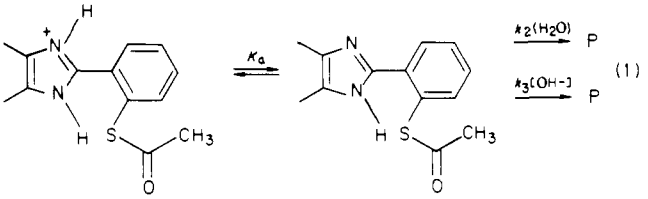
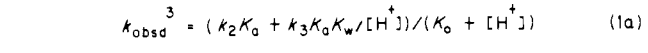
Scheme II



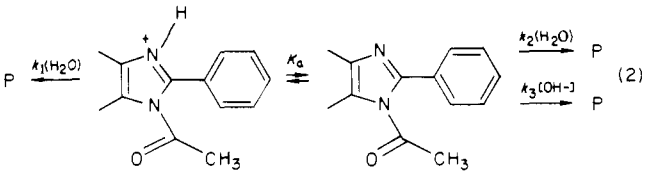
Also shown in Figure 1 as the solid lines are computer fits of the data for **3** and **4** to eq 1a and 2a, respectively. The latter



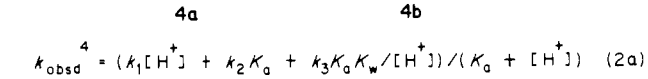
(1)



(1a)



(2)



(2a)

two equations correspond to the simplest interpretation of the mechanisms for solvolysis of **3** and **4**, each possessing a single pH-dependent ionization. The calculated rate constants and apparent pK_a's obtained from the computer fits are presented in Table I. The value of k_3 given in Table I for OR^- attack on benzenethiolacetate (**5**) was obtained from the slope of a plot of k_{obsd} for **5** vs. total [RO^-] using a pK_w of 14.6 in 33% ethanolic H_2O.[21] Hence, values obtained in the ethanol/H_2O medium represent combined rate constants for the specific reactions of ethanol (ethoxide) and H_2O (OH^-).

Second-order rate constants for the reaction of 2-mercaptoethanol with **3** and **5** were measured in ethanol/H_2O solutions at two pH values and the slope of the log k_{obsd} attributable only to RSH attack vs. pH is given in Table II.

IV. Discussion

The solvolysis of **3** is characterized by a log k_{obsd} vs. pH profile showing a plateau between pH 7.5 and 10. We believe that the pH-independent region and solvent isotope effect (k_{H_2O}/k_{D_2O} = 3.75 at pH 8.2) can be most simply accounted for by the reaction of species **3a** given in eq 1 where $k_2(H_2O)$ represents a general-base-assisted delivery of solvent to the thiol ester by the closely positioned imidazole. There are, however, several other kinetically equivalent schemes which could be envisioned which involve S to N acyl transfer akin to that seen previously in hydrolyses of imidazole-containing thiol esters.[8a,c,d] While such acyl transfer may occur, we believe it can be ruled out as a solvolytically important event by the following rationale.

Prior to hydrolysis, **3** exists in several forms, the relative concentrations of the predominant ones being controlled by the values of K_a^{Im}, K_a^{SH}, K_{eq}, and K_{Zw} as in Scheme II.[22] The fractional amount (α) of each species is therefore pH-dependent as is given in eq 3–7. It is important to note that at a given pH, species **3b**,

$$\alpha\mathbf{3a} = 1/X \tag{3}$$

$$\alpha\mathbf{3b} = (K_a^{Im}/[H^+])/X \tag{4}$$

$$\alpha\mathbf{6a} = (K_a^{Im}K_{eq}/[H^+])/X \tag{5}$$

$$\alpha\mathbf{6b} = (K_a^{Im}K_{eq}K_a^{SH}/[H^+]^2)/X \tag{6}$$

$$\alpha\mathbf{6}_{Zw} = (K_a^{Im}K_{eq}K_{Zw}/[H^+])/X \tag{7}$$

where $X = (1 + K_a^{Im}/[H^+] + (K_a^{Im}K_{eq})/[H^+] + (K_a^{Im}K_{eq}K_{Zw})/[H^+] + (K_a^{Im}K_{eq}K_a^{SH})/[H^+]^2)$

6a, and $\mathbf{6}_{Zw}$ have the same relative proportions which is governed by the values of K_{eq}, K_{eq}', and K_{Zw}.[22a] A priori any or all the species given in Scheme II could be solvolytically important, but the broad plateau seen in Figure 1 can be accounted for by only a limited number of possibilities. In the first, the forward rate constants for S → N transfer ($k_1 + k_1'$) could be rate-limiting and independent of pH. This would require that the rate of solvent capture of (**6a** + $\mathbf{6}_{Zw}$) and/or **6b** can be much greater than k_{-1}. However both k_1 and k_{-1} are expected to be larger than the rate constants for H_2O attack on **6a** or **6b** (we will discuss the situation for $\mathbf{6}_{Zw}$ below). Bruice[8a] has reported that the rate constant for cyclization of **1** is 9×10^{-2} s^{-1}, while Fife and DeMark[8d] have reported that at pH 7, the value for **2** is $\sim 2 \times 10^{-2}$ s^{-1} and increases linearly with pH. Since **1** has more rotational degrees of freedom than does **3** and **2** is a benzoate derivative, it is expected that intramolecular S-to-N acyl transfer in **3** is much faster. Furthermore, Hupe and Jencks[19] have determined that the equilibrium constant for intermolecular acyl transfer between imidazole and benzenethiol favors benzenethiolacetate by a factor of 1000. For his-

(21) Hepler, L. G.; Wooley, E. M. *J. Phys. Chem.* **1970**, *74*, 3908–3913.

(22) (a) In Scheme II, K_{eq} = [**6a**]/[**3b**]; K_{eq}' = [$\mathbf{6}_{Zw}$]/[**3b**]; K_{Zw} = [$\mathbf{6}_{Zw}$]/[**6a**]. Since it can be shown that $K_{Zw} = K_a^{Im}{}^{+HAc}/K_a^{SH}$, it follows that if reasonable values for the latter two microscopic K_a's were known, the relative proportion of K_{eq}' to K_{eq} could be evaluated. Based on the pK_a of the *N*-acetylimidazolium ion of 3.6[9] and the expected substituent and stabilizing electrostatic effects, we estimate p$K_a^{Im^+HAc}$ to be ~5–5.5. We have evaluated the microscopic imidazolium pK_a of the zwitterion of **9** at 9.0 ± 0.1.[18b] N-Acetylation should reduce the pK_a by ~3.4 units as it does in imidazole; hence the p$K_a^{Im^+HAc}$ value of 5.0–5.5 seems quite reasonable. In addition, pK_a^{SH} of **6a** should be 5.5–6 based on the reported values for aromatic thiols.[19] Although these estimates may be subject to some error, the two values are likely to be quite close such that K_{Zw} should fall in the range of 10^{-1}–10^{1} which means that the values of K_{eq}' and K_{eq} will be within a factor of 10. (b) It has been appropriately suggested by a referee that a trapping experiment similar to that used by Heller et al.[8c] employing Ellman's reagent (5,5′-dithiobis(2-nitrobenzoic acid) or DTNB) might be used to determine the value of k_1 and hence K_{eq}. We have determined at pH 8.0 that the pseudo-first-order rate constant for trapping (k_{trap}) of **3** is linearly dependent upon [DTNB] at least up to 2×10^{-2} M. Since $k_{trap} = k_1k_2$ [DTNB]/($k_{-1} + k_2$[DTNB]) = 0.13 s^{-1} at [DTNB] = 2×10^{-2} M and the k_2 for thiolate trapping of the reagent is probably $\sim 10^5$ M^{-1} s^{-1} (see Semenow-Garwood, D.; Garwood, D. C. *J. Org. Chem.* **1972**, *37*, 3804–3810; Whitesides, G. M.; Lilburn, J. E.; Szajewski, R. P. *J. Org. Chem.* **1977**, *42*, 332–338) an upper limit of $\sim 7 \times 10^{-5}$ can be placed on K_{eq}. We point out however that since k_{trap} shows no sign of leveling off with increasing [DTNB], the limit for K_{eq} is probably at least 1 order of magnitude smaller which agrees nicely with our calculated value. Additionally, since $k_{trap} \gtrsim 0.13$ s^{-1}, it is clear that k_1 must be substantially greater than this value. (c) We have determined that the microscopic pK_a for SH ionization of N-protonated **9** is ~3.9 ± 0.1, this low value being caused by the electrostatic stabilization afforded in the zwitterionic form.[18b] The reduction in thiol pK_a should also be evident in the N-acetylated species $\mathbf{6}_{Zw}$ as well. It is therefore expected that such a thiolate ion will be even less effective at acting as a general base in this situation. We must be careful to point out that the arguments presented do not absolutely rule out the intermediacy of $\mathbf{6}_{Zw}$ as the catalytically viable species; they simply place it in considerable doubt. A referee has suggested that study of the corresponding *N*-methyl analogue of **3** will be informative. However, this species has available to it a situation analogous to that presented in Scheme II with the exception that **6a** and **6b** cannot be formed. Since *N*-methyl **3b** and $\mathbf{6}_{Zw}$ can still be formed, study of this analogue will not resolve the kinetic ambiguity. At present, we do not know of an experiment which will unambiguously resolve this question.

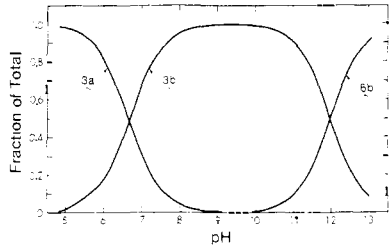


Figure 2. Fractional amount of each species of eq 3 as a function of pH for $K_{eq} = 10^{-6}$, $pK_a^{SH} = 6$, $pK_a^{Im} = 6.69$. The pH dependence of the fraction of **6a** is a bell-shaped curve with the maximum value of 9.96×10^{-7} at pH 9.3.

tidine-containing S-acylated peptides, Klotz and co-workers[8c] have placed an upper limit of 4.4×10^{-3} on the intramolecular S-to-N acyl transfer equilibrium constant. Assuming these results can be more or less accurately transposed to the **3b** ⇄ **6a** situation should yield a K_{eq} (**6a**/**3b**) $\leq 10^{-3}$ (although we believe the true value is far less).[22b] Should the observed plateau rate constant of 10^{-3} s^{-1} be attributable to a rate-limiting k_1, then the pseudo-first-order rate constants for hydrolysis of **6a,b** must be at least 100 times faster than k_{-1}, or ~ 100 s^{-1}. This value for the unassisted solvolysis of the putative **6a,b** is some 10^5–10^6-fold faster than k_{obsd} for the solvolysis of **4** from pH 7.5–10.

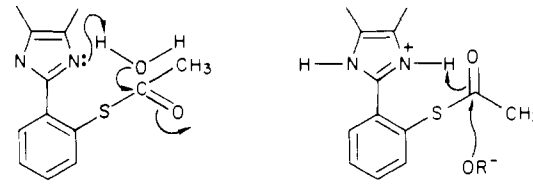
The latter can be taken as a model for the N-acylated derivatives (**6**) and although one might invoke special catalytic general-base role(s) for the aromatic S(H) unit, such a role appears to be unprecedented. Finally, the observed rate increase for disappearance of **3** in ethanol/H_2O vs. H_2O (Table I) is not easily explained by a scheme in which k_1 (or k'_1) is rate-limiting.[22b] Taken together, the above arguments suggest that the origin of the plateau region does not depend upon the S–N acyl transfer.

It might be expected that $\mathbf{6}_{Zw}$ is rapidly enough hydrolyzed to account for the pH-independent k_{obsd} value of **3**. To cast light on this possibility requires some estimation of the magnitude of K'_{eq}.[22a] Given appropriate values for pK_a^{Im}, pK_a^{SH}, K_{eq}, and K_{Zw},[22] the fraction (α) of **3a,b** and **6a,b**$_{Zw}$ can be calculated as a function of pH according to eq 3–7. The first apparent pK_a of 6.9 in H_2O is appropriate for pK_a^{Im}, and the decrease to 6.7 in passing to a medium of 33% ethanol/H_2O is consistent with this interpretation. Since pK_a^{SH} can be reasonably estimated at 5.5–6,[22a] K_{eq} can be treated as a variable to obtain the fractional amounts of **3** and **6** required to explain the pH/log k_{obsd} profile in Figure 1. Given that $K_{Zw} \sim 10^{-1}$–10^{1},[22a] it is important to realize that the relative concentrations of **6a** and $\mathbf{6}_{Zw}$ as well as K'_{eq} and K_{eq} differ by no more than a factor of 10. Shown in Figure 2 is the profile for the fractional amount of these species as a function of pH when $K_{eq} = 10^{-6}$ [22b] Given this value and the fact that $pK_a^{SH} < pK_a^{Im}$, it is seen that at all pH values below 10–10.5 (which is the limit given in Figure 1) species **3a** and **3b** are dominant. The pH dependence of [**6a**] and [$\mathbf{6}_{Zw}$] is also bell-shaped like that of **3b** with the maximum computed fractional value of **6a** being $\sim 10^{-6}$. Hence, the maximum $\alpha\mathbf{6}_{Zw}$ is 10^{-5}. Now in order to have the zwitterionic form being responsible for the observed rate of decomposition of **3** requires that the aromatic S^- be capable of catalyzing the delivery of H_2O to the protonated *N*-acylimidazole unit (for which protonated **4** is a good model, Table I) by $\sim 10^5$-fold. Since neither thiols nor thiolate anions appear to behave as general bases, we feel the putative activity for $\mathbf{6}_{Zw}$ is unreasonably large.[22c]

Finally, we wish to point out that in order to explain the pH/log k_{obsd} profile by the species given in Scheme II, it is absolutely necessary to have $K_{eq} \leq 10^{-6}$. Raising the value shifts to lower pH the point at which **6b** becomes dominant, but **6a** and $\mathbf{6}_{Zw}$ are never important species. An NMR experiment conducted under highly basic conditions (Na_2CO_3/CD_3OD, where large amounts of **6b** should accumulate if $K_{eq} > 10^{-5}$) failed to detect any amount of N-acetylated derivative, which again supports the low value for K_{eq}.

All the above arguments stress the importance of the back reaction, k_{-1} in Scheme II. Molecular models suggest that the S(H) unit in **6** is more ideally poised for a nucleophilic capture of the N-acyl group than in the systems reported by Klotz et al.[8c] for which a value of $K_{eq} \leq 4 \times 10^{-3}$ is reported.

Given that **3a** and **3b** are sufficient to explain the pH/log k_{obsd} profile, the solvolytic process reduces to a simplified mechanism given in eq 1. The plateau region from pH 7.5 to 10 can be now explained in terms of two kinetically indistinguishable processes, imidazole acting as a general base as in **7** or imidazolium acting as a general acid in promoting the attack of $OH^-(OR^-)$ as in **8**.



7 **8**

Both mechanisms could be consistent with the observed solvent isotope effect. At sufficiently high pH, the mechanism changes to specific base attack on the deprotonated thiol ester ($k_3[OR^-]$, eq 1). Should the general-acid-catalyzed reaction be dominant for **3a**, it requires that the calculated second-order rate constant for OR^- attack as in **8** be $\sim 3.2 \times 10^5$ M^{-1} s^{-1}, which represents a 2000-fold increase over OR^- attack on the deprotonated thiol ester **3b** (k_3, Table I).[23] It seems reasonable to expect that such catalysis if present would be observable in the attack of other anionic nucleophiles as well. To test this expectation, the attack of 2-mercaptoethanol on **3** and **5** was examined. Nucleophilic reactions of thiols on C=O units are not subject to general-base catalysis and in all known cases proceed via the attack of RS^-.[24,25] Furthermore, the pK_a of 2-mercaptoethanol at 9.61 (in H_2O)[19] is sufficiently high that nucleophilic attack of thiolate (and not breakdown of the tetrahedral intermediate) would be rate limiting.[19] The slope of a plot of log of the second-order rate constant of total thiol vs. pH for the attack of 2-mercaptoethanol on **5** is unity (Table II), while that observed for **3** is 0.63. These data indicate that mercaptoethanol serves as a nucleophile toward **5** strictly according to [RS^-]. However, the thiolate anion is slightly more active toward **3** at lower pH than is expected, suggesting that some form of rate enhancement is operative, likely via the involvement of **3a**. However, when examined more carefully,[26] **3a** is calculated to react only 10-fold more rapidly than is **3b** when attacked by RS^-. We feel that the calculated rate enhancement, although quite real, is too small for a dominant general-acid pathway akin to that in **8** and probably is attributable to a simple electrostatic effect whereby **3a** is activated to anionic attack relative to **3b** or a leaving group effect.[19] If one accepts this argument, then the plateau region evident from pH 7.5 to 10 in the solvolysis of **3** is most easily accounted for by a general-base role for imidazole in the hydrolysis of an adjacent thiol ester.

This result is the same as the mechanism reported by Rogers and Bruice[28] for a series of closely related oxygen–ester analogues. Although there has been controversy whether the active site group in papain which is important in controlling deacylation is actually imidazole[5] or carboxylate,[27] recent arguments have tended toward the involvement of imidazole,[3,5] consistent with the fact that the apparent pK_a for this group decreases in the presence of added organic cosolvent.[1e] Nevertheless, the hydrolysis of thioaspirin (the carboxyic acid analogue of **3**) exhibits a pH-independent

(23) $k_{obsd} = (k'K_w + k_3K_aK_w/[H^+])/(K_a + [H^+])$ where $k' = k_{OH^-}$ on **3a**. k_2 from eq 1a $= k'K_w/K_a$.

(24) Lienhard, G. E.; Jencks, W. P. *J. Am. Chem. Soc.* **1966**, *88*, 3982–3995.

(25) Fersht, A. R. *J. Am. Chem. Soc.* **1971**, *93*, 3504–3515.

(26) $k^{RSH}_{obsd_2}/k^{RSH}_{obsd_1} = [(k_3[K_a/(K_a + [H^+]_2)] + k'[[H^+]_2/(K_a + [H^+]_2)])/(k_3[K_a/(K_a + [H^+]_1)] + k'[[H^+]_1/(K_a + [H^+]_1)])]$ $([RS^-]_2/[RS^-]_1)$ using $pK_a^{Im} = 6.69$, $pH_1 = 7.50$, $pH_2 = 8.37$, and values from Table II, $k'/k_3 = 8.9$.

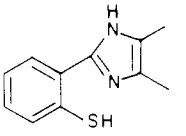
(27) (a) Bender, M. L.; Brubacher, L. J. *J. Am. Chem. Soc.* **1966**, *88*, 5880–5889. (b) Lucas, E. C.; Williams, A. *Biochemistry* **1969**, *8*, 5225–5135. (c) Zannis, V. I.; Kirsch, J. F. *Biochemistry* **1978**, *17*, 2669–2674.

(28) Rogers, G. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1974**, *96*, 2463–2472.

region, having a reported k_{obsd} of $7.0 \pm 3.0 \times 10^{-6}$ s^{-1}.[29] By way of comparison, the k_{obsd} value for hydrolysis of **3** in the plateau region is 1.44×10^{-3} s^{-1}. The acceleration of 100–200-fold is quite reasonable for general-base catalysis of imidazole over a similar role for carboxylate in such hydrolyses[27a] and argues against a nucleophilic role for either species.

If our results can be accurately transposed to the case of the deacylation of papain, it is likely that the active site imidazole can indeed function as a general base. It is somewhat difficult to determine the magnitude of the rate enhancement to thiol ester hydrolysis which is attributable to the intramolecular general-base role for imidazole since the H_2O term for thiol esters is undetectably small. Some estimate for the rate enhancement in **3** relative to the attack of OH^- can be made by extrapolating the $k_3(OH^-)$ term (Table I) for **3** to a given pH. Hence at pH 7–8, the imidazole unit affords some 10^3 acceleration over the pseudo-first-order rate constant for attack of OH^- on **3**. The overall rate of deacylation for **3** is quite slow relative to that for the fastest deacylations of various acylpapains (3–46 s^{-1})[3c] but compared favorably with that reported for *trans*-cinnamoylpapain ($k_{deacylation} = 3.68 \times 10^{-3}$ s^{-1}).[27a] It might be that the slow deacylation of **3** is a consequence of a nonideal fit of H_2O between the imidazole N and acyl C=O group, a problem which might be overcome by a suitable structural change.[30] However, insofar as we are aware, the hydrolysis of **3** represents the only well-defined chemical precedent for imidazole acting as an intramolecular general base in promoting the hydrolysis of a thiol ester. The observed solvent isotope effect of 3.75 for **3** is consistent with this interpretation and compares favorably with those observed for the deacylation of α-*N*-benzoyl-L-arginylpapain and *trans*-cinnamoylpapain (2.75 and 3.35, respectively).[1d,27a]

Finally is of note that **3** and its free mercaptan hydrolysis product **9** were designed as models for the acylated and free forms



9

of the active site of papain. Accordingly, **9** does indeed act as a true nucleophilic catalyst in promoting the hydrolysis of *p*-nitrophenylacetate.[18b,30]

Acknowledgment. We gratefully acknowledge the financial support of the Alberta Heritage Foundation for Medical Research for a postdoctoral fellowship to J.P.S., the University of Alberta, and the Natural Sciences and Engineering Research Council of Canada. We also gratefully acknowledge E. Feschuk (University of Alberta) for instrument servicing.

Registry No. 3, 97878-91-6; **3**·HCl, 97879-00-0; **3** (mercaptan), 97878-97-2; **3** (disulfide), 97878-98-3; **4**, 97878-92-7; **5**, 934-87-2; *S*-benzylthiosalicylamide, 54705-18-9; 2-(benzylthio)benzylamine, 97878-93-8; ethyl oximinoacetoacetate, 5447-76-7; (2-(benzylthio)phenyl)-4-carbethoxy-5-methylimidazole, 97878-94-9; 2-(2-(benzylthio)phenyl)-4,5-dimethylimidazole, 97878-95-0; 2-(2-(benzylthio)phenyl)-4,5-dimethylimidazole picrate, 97878-96 1; 2-(2-mercaptophenyl)-4,5-dimethylimidazole hydrochloride, 97878-99-4; 4-carbethoxy-5-methyl-2-phenylimidazole, 77335-93-4; 4,5-dimethyl-2-phenylimidazole, 13682-20-7; 5,5′-dithiobis(2-nitrobenzoic acid), 74959-07-2; cysteine protease, 37353-41-6; benzenethiol, 108-98-5; 2-mercaptoethanol, 60-24-2.

(29) (a) Schonbaum, G. R.; Bender, M. L. *J. Am. Chem. Soc.* **1960**, *82*, 1900–1904. (b) The reported k_{obsd} given in ref 29a was determined at 25 °C. The value extrapolated to 37 °C would likely be $\sim 1.4 \times 10^{-5}$ s^{-1}.

(30) Street, J. P.; Skorey, K.; Brown, R. S. *J. Am. Chem. Soc.*, in press.

Halomethylenes: Effects of Halogen Substitution on Absolute Heats of Formation

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Contribution from the Center for Chemical Physics, National Bureau of Standards, Gaithersburg, Maryland 20899, and the Department of Chemistry, University of Maryland Baltimore County, Catonsville, Maryland 21228. *Received April 22, 1985*

Abstract: New values for the heats of formation of CF_2, CCl_2, CClF, CFH, and CClH have been derived from estimations of the thermochemistry of the reaction(s) $CXYH^+ + B \rightarrow CXY + BH^+$ where X and Y are F and/or Cl and B is a molecule for which an absolute value of the gas-phase basicity (or proton affinity) is available. The experiments, carried out in an ion cyclotron resonance spectrometer, lead to the following values for the heats of formation (in kcal/mol) of the ground-state singlet carbenes: CF_2, -49 ± 3; CCl_2, 39 ± 3; CFCl, -2 ± 7; CFH, 26 ± 3; CClH, 71 ± 5. The value for CF_2 is lower by about 5 kcal/mol than the previously accepted value, but in good agreement with values derived from previous "bracketing" results and also in agreement with values derived from the observed threshold energies for ionic dissociation processes. The value for CCl_2 is significantly lower than the 1976 value of 47 ± 3 kcal/mol recommended by S. W. Benson, but in good agreement with an earlier value (40 ± 5 kcal/mol) recommended by this author and with more recent experimental results on the onset energy for the formation of Cl_2^- from CCl_4. The values for CFH, CClH, and CFCl are all approximately equal to the averages of the heats of formation of the corresponding CX_2 and CY_2 species, in agreement with assumptions made in previous estimates of these quantities. Values for the C–X bond energies in the halomethylenes, the heats of formation of the corresponding CXY^+ ions, and the ionization potentials of the CXY species are derived from the results. From the most recent calculations of the energy differences between the ground-state singlet halomethylenes and the first triplet state, values for the heats of formation of the triplet halomethylenes are obtained; an analysis of trends in these values indicates that $^3CF_2$ is substantially destabilized.

There has been considerable interest in carbene chemistry in recent years.[2,3] Theoretical[3] and experimental[2] examinations of the properties of halogenated carbenes have provided information about the nature of the ground and first excited electronic states,

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