The Lactonization of 2′-Hydroxyhydrocinnamic Acid Amides: A Potential Prodrug for Amines

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The lactonization of two hydroxy amides—4-methoxyaniline 3-(2′-hydroxyphenyl)-3,3-dimethylpropionic acid amide (**2b**) and 4-methoxyaniline 3-(2′-hydroxy-4′,6′-dimethylphenyl)-3,3-dimethylpropionic acid amide (**3b**)—was studied over a pH range of 1–8. Due to the slowness of its reaction, a third hydroxy amide—4-methoxyaniline 3-(2′-hydroxyphenyl)propionic acid amide (**1b**)—was investigated only at pH values of 7.5 and 10. The lactonization of **2b** and **3b**, which was found to be subject to general catalysis by buffer components, was observed to be catalyzed concurrently but not concertedly by both the acidic and basic forms of the buffer. The buffer-independent pH rate profiles for the lactonization of **2b** and **3b** were found to obey the equation $k_o = k_{H^+}[H_3O^+] + k_{H_2O} + k_{OH^-}[OH^-]$, indicating the the reaction is also subject to specific catalysis by hydronium and hydroxide ions. A Brønsted analysis of the rate constants for buffer catalysis gave α and β values of 0.30 ± 0.02 and 0.54 ± 0.04, respectively, for **3b**. The rate constants for the accelerated lactonization of **1b** at 50, 70, and 90 °C and pH 10 were used to calculate values of 14.7 ± 0.8 kcal/mol and −9.5 ± 2.3 eu for the activation parameters, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, respectively. Comparison of the observed rates of lactonization at pH 7.5 and 30 °C for the three hydroxy amides allowed an estimate of the extent of rate enhancement provided by addition of a partial or total "trimethyl lock" for the hydroxy amide lactonization reaction under near physiological conditions. The order of reactivity of the three hydroxy amides was found to be **3b** ≫ **2b** > **1b** with rate enhancement factors of 2.5×10^4, 44, and 1, respectively. This study was begun with the objective of generating a hydroxy amide of very high reactivity at physiological pH for development into amine prodrug forms. **3b**, which exhibited a half-life of 65 s at pH 7.5, has been chosen for further development as an amine prodrug.

Introduction

Bioreversible derivatives of drugs, commonly referred to as prodrugs, have been shown to improve the physicochemical (e.g., solubility, lipophilicity) and biological (e.g., bioavailability) properties of many compounds.[1,2] However, a single chemical modification of a drug is not always sufficient to achieve the desired alteration in the physicochemical or biological properties of the drug. A commonly encountered problem is the creation a prodrug with adequate in vitro stability (e.g., good shelf life) which at the same time exhibits sufficient reactivity in vivo to regenerate the parent drug. One possible solution is to prepare a prodrug of a prodrug or a pro-prodrug,[3] as shown in eq 1. In this example, the prodrug itself is a chemically

$$\text{pro-prodrug} \xrightarrow[\text{step 1}]{\text{enzymatic}} \text{pro} + \text{prodrug} \xrightarrow[\text{step 2}]{\text{chemical}} \text{drug} \quad (1)$$

reactive species which rapidly undergoes chemical conversion to the parent drug under physiological conditions. However, this reactive prodrug is generated only after an enzymatic reaction on the chemically stable pro-prodrug.

In an effort to develop pro-prodrugs of amines, various investigators[4-6] have attempted to use derivatized hydroxy amides which would be converted enzymatically (step 1, Figure 1) to hydroxy amide intermediates capable of rapidly undergoing lactonization resulting in release of the amine-containing drug (step 2, Figure 1). For the pro-prodrug system depicted in Figure 1 to be successful, the intermediate hydroxy amide must be sufficiently reactive under physiological conditions to rapidly cyclize and release the amine. Under these circumstances, the rate-limiting step in the release of the drug becomes the enzymatic conversion of the pro-prodrug to the prodrug (step 1, Figure 1). Derivatives with this characteristic are said to contain an enzymic trigger. While hydroxy amide lactonization has been studied extensively in the past,[7-10] to our knowledge there are no examples of hydroxy amides which exhibit the reactivity at physiological pH and temperature necessary to make this pro-prodrug system for amines workable.

In a preliminary study[11] we have shown that the amide (**3b**, Figure 2) of 3-(2′-hydroxy-4′,6′-dimethylphenyl)-3,3-dimethylpropionic acid (**3a**, Figure 2) has the reactivity at physiological pH required to make the pro-prodrug system shown in Figure 1 feasible. Our approach was based on work described earlier by Milstien and Cohen[12] and Caswell and Schmir[13] who demonstrated that the methyl substitution known as the "trimethyl lock" (methyl groups at positions 3, 3, and 6′ of compound **3a**) facilitated the rate of lactonization of the molecule. It was observed that compound **3a** containing the trimethyl lock lactonized at a rate many orders of magnitude faster than its counterpart lacking these methyl groups (**1a**). A smaller rate enhancement was observed with the derivative (**2a**) possessing only the 3,3-dimethyl substitution. In our preliminary studies[11] with the hydroxy amides **1b**, **2b**, and **3b**, we observed similar rate enhancement effects in their lactonization, suggesting that **3a** might be a useful promoiety for amines.

In the present study we have provided a complete description of the synthesis of the hydroxy amides **1b**, **2b**, and **3b** and the kinetics of their lactonization.

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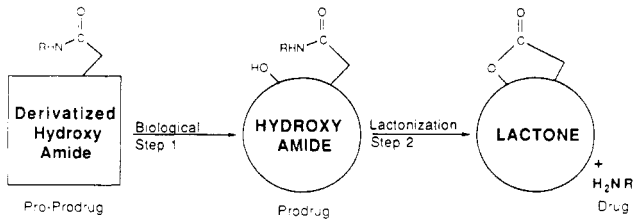

Figure 1. A schematic representation of derivatized hydroxy amides as examples of pro-prodrugs of amines which could exhibit good in vitro stability while at the same time possessing a good in vivo reconversion rate.

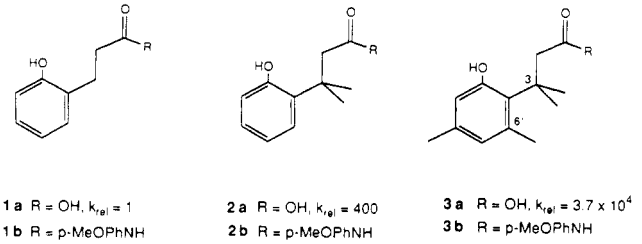

Figure 2. Compounds **1a**, **2a**, and **3a** are the previously studied[13] 2′-hydroxyhydrocinnamic acids and their relative rates of lactonization. Compounds **1b**, **2b**, and **3b** are the compounds investigated in this study.

Experimental Section

Synthesis. Melting points were determined on a Meltemp apparatus and are reported uncorrected. ^{1}H NMR spectra were recorded on $CDCl_3$ solutions with tetramethylsilane employed as an internal standard. Mass spectral analyses were conducted by the University of Kansas Mass Spectral Laboratory, and elemental analyses were determined by the University of Kansas Elemental Analysis Laboratory, Lawrence, KS. Column chromatography was performed with silica gel purchased from Aldrich Chemical Co. (70–270 mesh). Thin-layer chromatography was performed with TLC plates consisting of aluminum sheets precoated with silica gel 60 F_{254} which were purchased from EM Science, West Germany. $MgSO_4$ was employed as a drying agent in all syntheses.

3-(2′-(Benzyloxy)phenyl)propionic Acid (4). 2′-Hydroxycinnamic acid (10 g, 61 mmol) obtained from Aldrich Chemical Co. was dissolved in 200 mL of absolute ethanol. After addition of 0.5 g of a Pd/C catalyst, the hydrogenation was conducted for a period of 90 min, at which time TLC analysis (20:80, ethyl acetate–hexane) showed the reaction to be complete. The catalyst was removed by gravity filtration, and the ethanol was evaporated in vacuo, affording 10.26 g (99% yield) of 3-(2′-hydroxyphenyl)propionic acid: mp 83–85 °C; ^{1}H NMR ($CDCl_3$) δ 2.60–2.92 (4 H, dd, CH_2's), 5.16 (1 H, s, 2′-OH), 6.70–7.29 (4 H, m, Ar-H), 8.61 (1 H, s, COOH); UV λ_{max} (acetonitrile) 272 nm (ϵ = 1960).

3-(2′-Hydroxyphenyl)propionic acid (3.16 g, 19 mmol) was dissolved in 150 mL of dry acetone along with benzyl chloride (5.5 mL, 2.5 equiv), K_2CO_3 (10.5 g, 4 equiv), and KI (7.9 g, 2.5 equiv). The reaction was stirred at reflux for 12 h, at which time TLC analysis (20:80, ethyl acetate–hexane) showed the reaction to be complete. The reaction mixture was filtered, and the insoluble salts were subsequently washed two times with 75-mL portions of acetone. The combined acetone fractions were stirred with an equivalent volume of aqueous ammonium hydroxide (20%) for 3 h. This milieu was then extracted with 150 mL of ether. The ethereal layer was then washed with an equivalent volume of 0.1 N HCl to remove the benzylamine. Following drying, the ether was then removed under reduced pressure, resulting in a slightly yellow oil, which was purified by column chromatography (10:90, ethyl acetate–hexane). This resulted in a colorless oil (4.93 g, 75% yield), benzyl 3-(2′-(benzyloxy)-phenyl)propionate: bp dec 160 °C (5.5 mmHg); ^{1}H NMR ($CDCl_3$) δ 2.62–2.98 (4 H, dd, CH_2's), 5.00 (2 H, s, benzyl ester), 5.03 (2 H, s, benzyl ether), 6.45–7.12 (4 H, m, Ar-H), 7.23 (10 H, s, phenyl).

The benzyl propionate (4.93 g, 14.2 mmol) was dissolved in 75 mL of methanol and mixed with 75 mL of a 10% NaOH aqueous solution. This mixture was stirred for 12 h, and then the methanol was removed under vacuum. The remaining aqueous solution was washed with 75 mL of ether to remove unwanted neutral compounds. The aqueous solution was then acidified by dropwise addition of a 10% HCl solution until precipitate formation. This precipitate was removed by extraction with 50 mL of ether (two times), and the ether was dried. After filtering off the desiccant, the ether was removed under reduced pressure, leaving compound 4 (2.92 g, 80% yield): mp 55–57 °C; ^{1}H NMR ($CDCl_3$) δ 2.62–3.00 (4 H, dd, CH_2's), 5.10 (2 H, s, Ar-CH_2), 6.80–7.45 (4 H, m, Ar-H), 7.32 (5 H, s, phenyl), 8.91 (1 H, s, COOH); UV λ_{max} (acetonitrile) 271 nm (ϵ = 1730); MS (EI) m/e 256 (M), 239 (M – OH), 165 (M – benzyl), 91 (benzyl). Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 75.00; H, 6.01.

4-Methoxyaniline 3-(2′-(Benzyloxy)phenyl)propionic Acid Amide (5). Compound 4 (0.5 g, 1.95 mmol) was dissolved in 50 mL of dry tetrahydrofuran along with 285 μL of thionyl chloride (2 equiv), and the reaction mixture was heated to reflux for 4 h. The solvent and excess thionyl chloride were then removed under reduced pressure, affording an orange oil. This acid chloride was not purified and was used directly in the next step of the reaction. The oil was dissolved in 50 mL of dry THF along with anisidine (0.96 g, 4 equiv), and this mixture was stirred at room temperature for 12 h. At this point, TLC analysis (50:50, ethyl acetate–hexane) showed the reaction to be complete. The product was purified on a silica gel column (50:50, ethyl acetate–hexane), and the resulting compound was recrystallized from acetone and hexane to give a white crystalline solid (0.45 g, 64% overall yield from 4): mp 125–127 °C; ^{1}H NMR ($CDCl_3$) δ 2.61–3.04 (4 H, dd, CH_2's), 3.73 (3 H, s, CH_3O), 5.05 (2 H, s, Ar-CH_2), 5.53 (1 H, s, NH), 6.65–6.95 (4 H, m, Ar-H), 7.08–7.25 (4 H, dd, amide Ar-H), 7.37 (5 H, s, phenyl); UV λ_{max} (acetonitrile) 250 nm (ϵ = 15 600); MS (EI) m/e 361 (M), 270 (M – benzyl), 239 (M – benzyl and methoxy), 91 (benzyl). Anal. Calcd for $C_{23}H_{23}NO_3$: C, 76.43; H, 6.41; N, 3.88. Found: C, 76.33; H, 6.36; N, 4.06.

4-Methoxyaniline 3-(2′-Hydroxyphenyl)propionic Acid Amide (1b). Compound 5 (0.2 g, 0.55 mmol) was dissolved in 40 mL of an ethanolic suspension of 0.04 g of Pd/C catalyst. The reaction mixture was exposed to H_2 gas for a period of 8 h, after which TLC analysis (60:40, ethyl acetate–hexane) of the reaction mixture showed the reaction to be complete. The catalyst was filtered off, and the solvent was removed from the filtrate under reduced pressure. The residue was purified on a silica gel column (60:40, ethyl acetate–hexane). This purification afforded a white solid (0.14 g, 94% yield): mp 125–127 °C ^{1}H NMR ($CDCl_3$) δ 2.60–2.95 (4 H, dd, CH_2's), 3.73 (3 H, s, CH_3O), 5.32 (1 H, s, OH), 5.58 (1 H, s, NH), 6.70–6.92 (4 H, m, Ar-H), 6.98–7.32 (4 H, dd, amide Ar-H). Anal. Calcd for $C_{16}H_{17}NO_3$: C, 70.83; H, 6.32; N, 5.16. Found: C, 70.97; H, 6.17; N, 5.32.

4,4-Dimethyl-3,4-dihydrocoumarin (6). 3,3-Dimethylacrylic acid (20 g, 0.2 mol) was stirred in 60 mL of dry methanol along with 1 mL of concentrated sulfuric acid at room temperature for 72 h. The reaction mixture was then poured onto 100 mL of water. This mixture was then extracted three times with 100 mL of ether, and the ether fractions were combined. The ethereal layer was washed two times with 100 mL of 5% $NaHCO_3$ solution and with 100 mL of a saturated NaCl solution and then dried. Following filtration of the desiccant, the ether was removed under reduced pressure. Distillation of the resulting liquid afforded a colorless liquid (9.8 g, 43% yield): bp 135–137 °C (lit.[14] bp 97–99 °C (0.2 mmHg)).

Phenol (8.24 g, 87.6 mmol), methyl 3,3-dimethylacrylate (10 g, 87.6 mmol), and 4.9 mL of concentrated sulfuric acid were dissolved in 150 mL of benzene, and the mixture was heated to reflux for 90 min. The reaction mixture was then washed with 200 mL of water, 100 mL of 5% $NaHCO_3$ solution (two times), and 100 mL of saturated NaCl solution and dried. After filtering off the desiccant, the solvent was removed under vacuum, resulting in a slightly yellow oil. This oil was distilled under vacuum, affording a colorless liquid (2.57 g, 17% yield): bp 130–133 °C (5.5 mmHg) (lit.[15] bp 122 °C (5 mmHg)); ^{1}H NMR ($CDCl_3$) δ 1.40 (6 H, s, 3,3-$(CH_3)_2$), 2.60 (2 H, s, CH_2), 7.05 (4 H, m, ArH); MS (EI) m/e 176 (M) 161 (M – CH_3). Anal. Calcd for $C_{11}H_{12}O_2$: C, 74.97; H, 9.16. Found: C, 75.10; H, 8.86.

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3-(2'-Hydroxyphenyl)-3,3-dimethylpropanol (7). Compound **6** (0.5 g, 2.84 mmol) dissolved in 30 mL of dry tetrahydrofuran was added dropwise to a suspension of lithium aluminum hydride (0.16 g, 4.2 mmol) in 50 mL of anhydrous tetrahydrofuran, and the mixture was stirred for 1 h. TLC analysis (20% ethyl acetate–hexane) showed the reaction to be complete. The excess lithium aluminum hydride was quenched by dropwise addition of a 10% HCl solution. The aqueous mixture was then extracted twice with 50 mL of ether, and the ethereal layers were combined and dried. After filtering off the desiccant, evaporation of the ether resulted in a white solid. Recrystallization of this solid in acetone and hexane afforded a white crystalline solid (0.4 g, 78% yield): mp 107–110 °C (lit.[14] mp 110–111 °C); ^{1}H NMR ($CDCl_3$) δ 1.51 (6 H, s, 3,3-$(CH_3)_2$), 2.82 (2 H, t, 2-CH_2), 3.61 (2 H, t, 1-CH_2), 4.16 (1 H, t, 1-OH), 5.86 (1 H, s, 2'-OH), 6.64–6.99 (4 H, m, ArH); MS (EI) *m/e* 180 (M) 135 (M – CH_2CH_2OH). Anal. Calcd for $C_{11}H_{16}O_2$: C, 73.30; H, 8.95. Found: C, 72.98; H, 9.18.

3-(2'-(Benzyloxy)phenyl)-3,3-dimethylpropanol (8). Compound **7** (5 g, 27.7 mmol) was combined with benzyl chloride (4.8 mL, 1.5 equiv), KI (6.9 g, 1.5 equiv), and K_2CO_3 (7.66 g, 2 equiv) in 150 mL of acetone and heated to reflux under N_2 for 12 h. After TLC analysis (20:80, ethyl acetate–hexane) showed the reaction to be complete, the reaction mixture was stirred for several hours with 150 mL of a 20% aqueous ammonium hydroxide solution in order to convert the excess benzyl chloride into benzylamine. The product and benzylamine were then extracted from this solution with 200 mL of ether. The benzylamine was then removed from this ethereal layer by stirring with 200 mL of 0.1 N HCL for 3 h. After evaporation of the ether, the resulting compound was further purified on a silica gel column (20:80, ethyl acetate–hexane), yielding 5.43 g of a slightly yellow oil for a 72% yield: bp 134–136 °C (0.2 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.49 (6 H, s, 3,3-$(CH_3)_2$), 2.78 (2 H, t, 2-CH_2), 3.56 (2 H, t, 1-CH_2), 4.19 (1 H, t, 1-OH), 5.04 (2 H, s, benzyl CH_2), 6.70–7.07 (4 H, m, ArH), 7.38 (5 H, s, phenyl); MS (EI) *m/e* 270 (M), 225 (M – CH_2CH_2OH), 162 (M – benzyloxy), 91 (benzyl). Anal. Calcd for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20. Found: C, 79.60; H, 8.00.

3-(2'-(Benzyloxy)phenyl)-3,3-dimethylpropionaldehyde (9). Compound **8** (2.46 g, 9.1 mmol) was dissolved in methylene chloride and added dropwise to a stirring suspension of pyridinium chlorochromate (3.9 g, 2 equiv) also in methylene chloride. The reaction was complete in 2 h, and the black solution was then poured onto a silica gel column and eluted with methylene chloride to remove the chromium salts. The black residue was washed several times with methylene chloride, and this solvent was run through the same column. The compound eluted from this column was then further purified on another silica gel column (20:80, ethyl acetate–hexane). Evaporation of the eluate resulted in a slightly yellow oil (2 g, 82% yield): bp 143–146 °C (0.1 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.52 (6 H, s, 3,3-$(CH_3)_2$), 2.92 (2 H, s, CH_2), 5.01 (2 H, s, benzyl CH_2), 6.62–6.97 (4, H, m, ArH), 7.37 (5 H, s, phenyl) 9.23 (1 H, t, ald); MS (EI) *m/e* 268 (M), 161 (M – benzyloxy), 91 (benzyl). Anal. Calcd for $C_{18}H_{20}O_2$: C, 80.56; H, 7.51. Found: C, 80.21; H, 7.67.

3-(2'-(Benzyloxy)phenyl)-3,3-dimethylpropionic Acid (10). Compound **9** (1.23 g, 4.58 mmol) was dissolved in 50 mL of absolute ethanol and combined with $AgNO_3$ (3.11 g, 4 equiv) dissolved in 5 mL of water. To this solution was added dropwise 25 mL of a 2.67 M KOH solution (5 equiv) with vigorous stirring. The reaction was complete in 2.5 h, at which time the silver salts were filtered off and the ethanol was removed under vacuum. The resulting aqueous solution was extracted two times with 30 mL of ether, and these washings were discarded. The aqueous layer was then reacidified with a 0.1 N HCl solution, resulting in a cloudy solution which was then reextracted three times with 50 mL of ether. The combined ether fractions were dried, and removal of the desiccant and ether afforded a white solid (0.94 g, 72% yield): mp 114 °C; ^{1}H NMR ($CDCl_3$) δ 1.50 (6 H, s, 3,3-$(CH_3)_2$), 2.90 (2 H, s, CH_2), 5.00 (2 H, s, benzyl CH_2), 7.00 (4 H, m, ArH), 7.35 (5 H, s, phenyl), 8.89 (1 H, s, COOH); MS (EI) *m/e* 284 (M), 176 (M – benzyloxy), 161 (M – benzyloxy and CH_3), 91 (benzyl). Anal. Calcd for $C_{18}H_{20}O_3$: C, 76.03; H, 7.09. Found: C, 75.89; H, 7.06.

4-Methoxyaniline 3-(2'-(Benzyloxy)phenyl)-3,3-dimethylpropionic Acid Amide (11). Compound **10** (0.1 g, 0.35 mmol) was dissolved in 15 mL of freshly distilled methylene chloride along with anisidine (0.065 g, 1.5 equiv), dicyclohexylcarbodiimide (0.108 g, 1.5 equiv), and 4-(dimethylamino)pyridine (0.01 g). The reaction mixture was stirred under an inert atmosphere for 12 h. TLC (20:80, ethyl acetate–hexane) of an aliquot from the reaction mixture showed three major spots. The resulting mixture was purified on a silica gel column (20:80, ethyl acetate–hexane). The spot with an R_f value of 0.85 was believed to be the desired product and was the first material to elute from the column. This first fraction was recrystallized with acetone and hexane, resulting in a white solid (60 mg, 44% yield): mp 95–97 °C; ^{1}H NMR ($CDCl_3$) δ 1.60 (6 H, s, 3,3-$(CH_3)_2$), 2.96 (2 H, s, CH_2), 3.78 (3 H, s, OCH_3), 5.21 (2 H, s, benzyl CH_2), 6.45 (1 H, s, NH), 6.67–7.19 (4 H, m, ArH), 6.79–6.89 (4 H, d, amine-ArH), 7.47 (5 H, s, phenyl); UV λ_{max} (acetonitrile) 250 (ϵ = 9930); MS (EI) *m/e* 389 (M), 298 (M benzyl), 282 (M – benzyloxy), 213 (M – CH_2 – amide), 161 (M – benzyloxy and anisidine), 122 (anisidine), 91 (benzyl). Anal. Calcd for $C_{25}H_{27}NO_3$: C, 77.09; H, 6.99; N, 3.60; Found C, 76.88; H, 6.84; N, 3.43.

4-Methoxyaniline 3-(2'-Hydroxyphenyl)-3,3-dimethylpropionic Acid Amide (2b). Compound **11** (0.01 g, 0.026 mmol) was treated according to the same conditions employed for the conversion of **5** to **1b**. The resulting hydroxy amide was too unstable to characterize in detail and was treated according to the conditions described in the Experimental Section entitled Hydroxy Amide Stock Solutions.

4,4,5,7-Tetramethyl-3,4-dihydrocoumarin (12). Methyl 3,3-dimethylacrylate (28 g, 0.246 mol, 1.5 equiv), 3,5-dimethylphenol (20 g, 0.164 mol), and 9 mL of concentrated sulfuric acid were treated according to the procedure for the formation of compound **6**. The reaction afforded a white solid (19.97 g, 60% yield): mp 89–90 °C (lit.[12] mp 90–92 °C); ^{1}H NMR ($CDCl_3$) δ 1.45 (6 H, s, 4,4-$(CH_3)_2$), 2.25 (3 H, s, 5-(CH_3)), 2.45 (3 H, s, 7-(CH_3)), 2.60 (2 H, s, CH_2), 6.70 (2 H, s, Ar-H); MS (EI) *m/e* 204 (M), 189 (M – CH_3), 145 (M – 4CH_3), 91 (phenol – 3H). Anal. Calcd for $C_{13}H_{16}O_2$: C, 76.43; H, 7.90. Found: C, 76.50; H, 7.81.

3-(2'-Hydroxy-4',6'-dimethylphenyl)-3,3-dimethylpropanol (13). Compound **12** (10 g, 48.9 mmol) and lithium aluminum hydride (1.8 g, 1 equiv) in anhydrous tetrahydrofuran were treated according to the procedure for the formation of compound **7**. Recrystallization from acetone and hexane gave a white crystalline material (8.46 g, 83% yield): mp 112–114 °C; ^{1}H NMR ($CDCl_3$) δ 1.50 (6 H, s, 3,3-$(CH_3)_2$), 2.20 (3 H, s, 6'-(CH_3)), 2.25 (2 H, t, 1-CH_2), 2.45 (3 H, s, 4'-(CH_3)), 3.60 (2 H, t, 2-CH_2), 4.39 (1 H, t, 1-OH), 5.58 (1 H, s, 2'-OH), 6.20 (1 H, s, ArH), 6.35 (1 H, s, ArH); MS (EI) *m/e* 163 (M – CH_2CH_2 – OH), 135 (M – CH_2CH_2OH and 3,3-$(CH_3)_2$), 123 (M – propyl side chain). Anal. Calcd for $C_{13}H_{20}O_2$: C, 74.95; H, 9.68. Found: C, 74.60; H, 9.90.

3-(2'-(Benzyloxy)-4',6'-dimethylphenyl)-3,3-dimethylpropanol (14). Compound **13** (4.5 g, 21.6 mmol), benzyl chloride (4.1 g, 1.5 equiv), potassium iodide (5.38 g, 1.5 equiv), and potassium carbonate (5.97 g, 2 equiv) were dissolved or suspended in acetone, and the mixture was treated according to the procedure for the formation of compound **8**. The resulting mixture was purified on a silica gel column (20:80, ethyl acetate–hexane) yielding a slightly yellow oil (4.4 g, 69% yield): bp 145–150 °C (0.05 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.50 (6 H, s, 3,3-$(CH_3)_2$), 2.10 (2 H, t, 2-CH_2), 2.20 (3 H, s, 6'-(CH_3)), 2.45 (3 H, s, 4'-(CH_3)), 3.45 (2 H, t, 1-CH_2), 4.36 (1 H, t, 1-OH), 5.00 (2 H, s, benzyl CH_2), 6.45 (1 H, s, ArH), 6.55 (1 H, s, ArH), 7.30 (5 H, s, phenyl); MS (EI) *m/e* 298 (M), 253 (M – CH_2CH_2OH), 207 (M – benzyl), 91 (benzyl). Anal. Calcd for $C_{20}H_{26}O_2$: C, 80.50; H, 8.78. Found: C, 80.15; H, 8.80.

3-(2'-(Benzyloxy)-4',6'-dimethylphenyl)-3,3-dimethylpropionaldehyde (15). Compound **14** (0.54 g, 1.8 mmol) was added to a suspension of pyridinium chlorochromate (0.776 g, 2 equiv) in methylene chloride, and the mixture was treated according to the procedure for the formation of compound **9**. The reaction resulted in a slightly yellow oil (0.4 g, 75% yield): bp 160–163 °C (0.1 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.50 (6 H, s, 3,3-$(CH_3)_2$), 2.20 (3 H, s, 6'-CH_3), 2.50 (3 H, s, 4'-CH_3), 2.90 (2 H, d, CH_2), 5.00 (2 H, s, benzyl CH_2), 6.55 (1 H, s, ArH), 6.65 (1 H, s, ArH), 7.35 (5 H, s, phenyl) 9.45 (1 H, t, ald); MS (EI) *m/e* 296 (M), 205 (M – benzyl), 189 (M – benzyloxy), 91 (benzyl). Anal. Calcd for $C_{20}H_{24}O_2$: C, 81.04; H, 8.16. Found: C, 80.91; H, 8.02.

3-(2'-(Benzyloxy)-4',6'-dimethylphenyl)-3,3-dimethylpropionic Acid (16). Compound **15** (0.63 g, 2.1 mmol) dissolved

in 50 mL of ethanol was combined with 5 mL of water containing silver nitrate (0.9 g, 2.5 equiv). The mixture was treated according to the procedure for the formation of compound **10**. Recrystallization from acetone and hexane gave a white solid (0.163 g, 43% yield): mp 105–108 °C; ^{1}H NMR ($CDCl_3$) δ 1.53 (6 H, s, 3,3-$(CH_3)_2$), 2.19 (3 H, s, 6′-CH_3), 2.47 (3 H, s, 4′-CH_3), 2.85 (2 H, d, CH_2), 4.96 (2 H, s, benzyl CH_2), 6.50 (1 H, s, ArH), 6.58 (1 H, s, ArH), 7.38 (5 H, s, phenyl), 11.13 (1 H, s, COOH); MS (EI) *m/e* 312 (M), 204 (M – benzyloxy), 189 (M – benzyloxy and CH_3), 91 (benzyl). Anal. Calcd for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74. Found: C, 76.70; H, 7.68.

4-Methoxyaniline 3-(2′-(Benzyloxy)-4′,6′-dimethylphenyl)-3,3-dimethylpropionic Acid Amide (17). Compound **16** (0.05 g, 0.16 mmol), anisidine (0.03 g, 1.5 equiv), dicyclohexylcarbodiimide (0.05 g, 1.5 equiv), and (dimethylamino)-pyridine (0.01 g) were treated according to the procedure for the formation of compound **11**. The reaction resulted in the production of a white solid (0.035 g, 52% yield): mp 129–130 °C; ^{1}H NMR ($CDCl_3$) δ 1.60 (6 H, s, 3,3-$(CH_3)_2$), 2.25 (3 H, s, 6′-CH_3), 2.45 (3 H, s, 4′-CH_3), 2.90 (2 H, s, CH_2), 3.75 (3 H, s, OCH_3), 5.00 (2 H, s, benzyl CH_2), 6.50 (1 H, s, NH), 6.75 (6 H, m, ArH), 7.35 (5 H, s, phenyl); MS (EI) *m/e* 417 (M), 310 (M – benzyloxy), 91 (benzyl). Anal. Calcd for $C_{27}H_{31}NO_3$: C, 78.07; H, 6.99; N, 3.37. Found: C, 77.89; H, 7.11; N, 3.41.

4-Methoxyaniline 3-(2′-Hydroxy-4′,6′-dimethylphenyl)-3,3-dimethylpropionic Acid Amide (3b). Compound **17** (0.01 g, 0.024 mmol) was treated according to the same conditions employed for the conversion of **5** to **1b**. The resulting hydroxy amide was too unstable to characterize in detail and was treated according to the conditions described in the Experimental Section entitled Hydroxy Amide Stock Solutions.

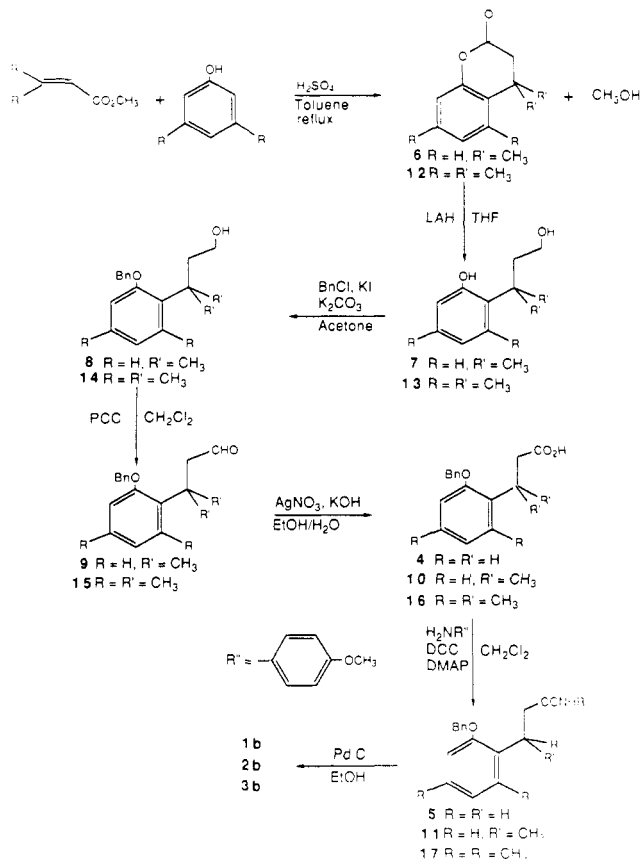
3-(2′-Hydroxyphenyl)propanol (18). 3,4-Dihydroucoumarin (10 g, 67 mmol) and lithium aluminum hydride (2.56 g, 1 equiv) were subjected to the same conditions as used in the formation of **7**. Purification afforded a colorless oil (9.32 g, 91% yield): bp 135–137 °C (0.5 mmHg) (lit.[14] bp 124–125 °C (0.4 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.84 (2 H, q, 2-CH_2), 2.76 (2 H, t, 3-CH_2), 3.56 (2 H, t, 1-CH_2), 4.27 (1 H, t, 1-OH), 6.05 (1 H, s, 2, -OH), 6.89–7.18 (4 H, m, ArH); MS (EI) *m/e* 152 (M), 107 (M – CH_2CH_2OH). Anal. Calcd for $C_9H_{12}O_2$: C, 71.05; H, 7.89. Found: C, 71.44; H, 7.96.

Hydroxy Amide Stock Solutions. The benzyl-protected hydroxy amides (**5**, **11**, and **17**) were stored in the solid state until needed for kinetics. The benzyl groups were then removed by catalytic hydrogenation, and the purity of the resultant solution was verified by the HPLC assay described below. It was found that by using cold temperatures and short reaction times it was possible to generate even the most reactive hydroxy amides without significant appearance of the product lactones. The hydroxy amides were then diluted to a concentration of 5×10^{-4} M with acetonitrile and stored as frozen stock solutions at −78 °C. All hydroxy amide stock solutions were stable at this temperature over the course of the study.

Kinetic Measurements. The previously described stock solutions were diluted five times with the appropriate aqueous buffer, resulting in a final concentration of hydroxy amide of 1×10^{-4} M and a final solvent milieu of 20‰ acetonitrile in water. Buffers were prepared from commercial reagent grade materials, using deionized, distilled water. The buffers employed in these studies were phosphate in the pH regions of 2.0 to 3.0 and 6.0 to 8.0, formate in region of 3.3 to 4.3, acetate in the region of 4.0 to 5.5, and carbonate at pH 10.0. pH measurements were made with an Orion digital Ionalyzer model 701A. The majority of the reactions were conducted at a temperature of 30 ± 0.5 °C maintained by a Precision shaking water bath. The accelerated stability studies were carried out in sealed ampules which were maintained at temperatures of 50, 70, and 90 °C by Stabil-Therm Constant Temperature ovens. The ionic strength was fixed at 0.3 with NaCl. Aliquots were removed from the reaction mixture at various times and frozen in a dry ice/acetone bath which stopped the reaction instantaneously. These samples were later analyzed by the HPLC assay described below.

HPLC Assay Conditions. The loss of hydroxy amide, as well as the appearance of lactone in the samples, was monitored through the use of a Shimadzu HPLC. 4-Methoxyaniline, which possessed a low extinction coefficient and exhibited poor peak shape under the current HPLC conditions, was not analyzed. At the concentrations employed, it did not appear in the chromatography. The compounds were quantified by measuring their peak areas in relation to those of standards chromatographed under the same conditions. The isocratic assay was conducted on an ODS Hypersil C-18 column along with a mobile phase consisting of 30–50% acetonitrile in 0.01 M phosphate buffer pH 3.0, resulting in retention times of less than 15 min for all compounds. A detection wavelength of 250 nm was used. The acetonitrile was HPLC grade obtained from Fisher, and the water was doubly distilled and filtered by a Milli Q Water System.

Scheme I. Synthetic Route to the Model Hydroxy Amides



pK_a Measurements. All pK_a measurements were carried out under conditions of solvent, temperature, and ionic strength identical with those used in the kinetic studies. It was not possible to determine the pK_a values for the phenolic groups of most of the hydroxy amides due to their rapid lactonization in the requisite pH range. Therefore, pK_a values for the stable diol intermediates (**7**, **13**, and **18**) of each series were determined as well as the pK_a value for the hydroxy amide **1b**. The contribution to pK_a value exerted by the side chain propyl amide (taken to be the difference between the pK_a values of **18** and **1b**) was then assumed to be constant for the three series, allowing an estimation of the pK_a values for the more reactive hydroxy amides. The pK_a values which were estimated are in parentheses. Using this method, the following pK_a values were obtained for **18** and **1b**, 11.62 ± 0.05 and 11.91 ± 0.05, respectively; for **7** and **2b**, 12.14 ± 0.07 and (12.43), respectively; and for **13** and **3b**, 12.60 ± 0.04 and (12.89), respectively. All the pK_a values were determined spectrophotometrically using a Shimadzu UV-260 spectrophotometer. In the case of compound **13** it was necessary to use the method of spectral extrapolation.[16]

Results

The model hydroxy amides (**1b**, **2b**, and **3b**) were prepared according to the synthetic steps outlined in Scheme I. During the synthesis of these compounds, the very

(16) Cohen, L. A.; Jones, W. M. *J. Am. Chem. Soc.* **1963**, *85*, 3397.

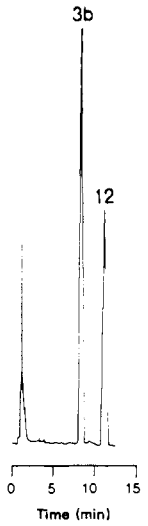


Figure 3. A representative chromatogram showing the separation of hydroxy amide **3b** and lactone **12** by the HPLC assay described in the Experimental Section.

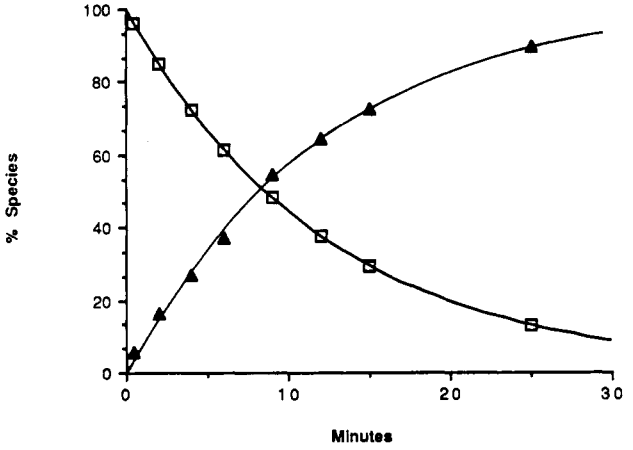


Figure 4. A representative plot showing the pseudo-first-order loss of hyroxy amide **3b** (□) and formation of lactone **12** (▲) at pH 6.0, 50 mM phosphate, μ = 0.3 and 30 °C. Under all conditions, the products of the lactonization reaction were lactone **12** and *p*-methoxyaniline with conservation of mass.

quality it was hoped to exploit—an equilibrium strongly favoring the lactone over the hydroxy acid in compounds containing a total or partial "trimethyl lock"—was found to create some difficulties. Attempts to open the lactone rings of compounds **6** and **12** and protect the phenolic hydroxy in a single step were unsuccessful. It was, therefore, necessary to reductively open the lactones **6** and **12** to the diol forms **7** and **13**, respectively, which are incapable of lactonization; then in a second synthetic step, protect the phenolic hydroxyl with a benzyl group. Standard oxidants were then used to oxidize the side chain primary alcohol back to the carboxylic acid. Because problems were encountered in generating acid chlorides, the desired amides **11** and **17** were formed via coupling of the acids **10** and **16**, respectively, with *p*-methoxyaniline using dicyclohexylcarbodiimide. A simpler scheme could be employed in the synthesis of **1b**. Compound **4**, which was prepared with commonly employed reactions and from commercially available starting materials, was converted to the corresponding acid chloride which was then reacted with *p*-methoxyaniline to yield **5**. Presumably the inability to form acid chlorides with the side chain acids of compounds **10** and **16** was due to steric hindrance to access of the carbonyl carbon by the geminal dimethyl groups at position 3. In the final step, the benzyl protecting groups from **5**, **11**, and **19** were removed by catalytic hydrogenation to yield the desired hydroxy amides **1b**, **2b**, and **3b**, respectively.

As described in the Experimental Section, the lactonization kinetics were determined by HPLC analysis. This assay measured the disappearance of the hydroxy amides as well as the appearance of the lactone. A representative HPLC chromatogram showing the separation of the hydroxy amide **3b** from its product lactone **12** is shown in Figure 3. Kinetic experiments were conducted with control of temperature, pH, buffer concentration and ionic strength. In all cases, the reactions afforded only the lactone and *p*-methoxyaniline with conservation of mass (Figure 4). Figure 5, parts a and b, shows the time-dependency of disappearance of **2b** and **3b**, respectively, at various pH values between 1 and 8. The plots demonstrate that the loss of both hydroxy amides followed pseudo-first-order kinetics to completion at all pH values. Furthermore, a comparison of the time axis of the two plots demonstrates the greater reactivity of **3b**, which contains the complete trimethyl lock. Consistent with previous results,[10] the order of reactivity of the three compounds was found to be **3b** ≫ **2b** > **1b**. The lactonization reactions of **2b** and **3b** were studied in detail over the pH range of 1–8 with thorough investigation of the buffer catalysis. The slowness of the lactonization reaction for compound **1b** at 30 °C made it impossible to study this reaction in detail. Therefore, the lactonization of **1b** was investigated at elevated temperatures as described below.

Figure 6, parts a–d, illustrates that at each pH the observed pseudo-first-order rate constants for the lactoni-

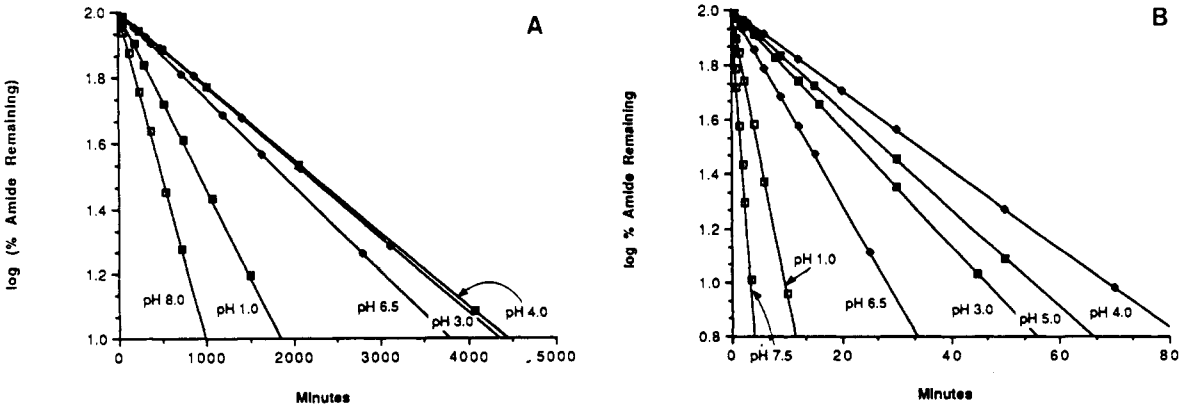


Figure 5. These figures provide an overview of the kinetic data for the pseudo-first-order loss of **2b** (Figure 5a) and **3b** (Figure 5b) at 30 °C and μ = 0.3. Each line corresponds to a single experiment selected from the set of experiments conducted at the indicated pH. The rate constants depicted here are not buffer-independent.

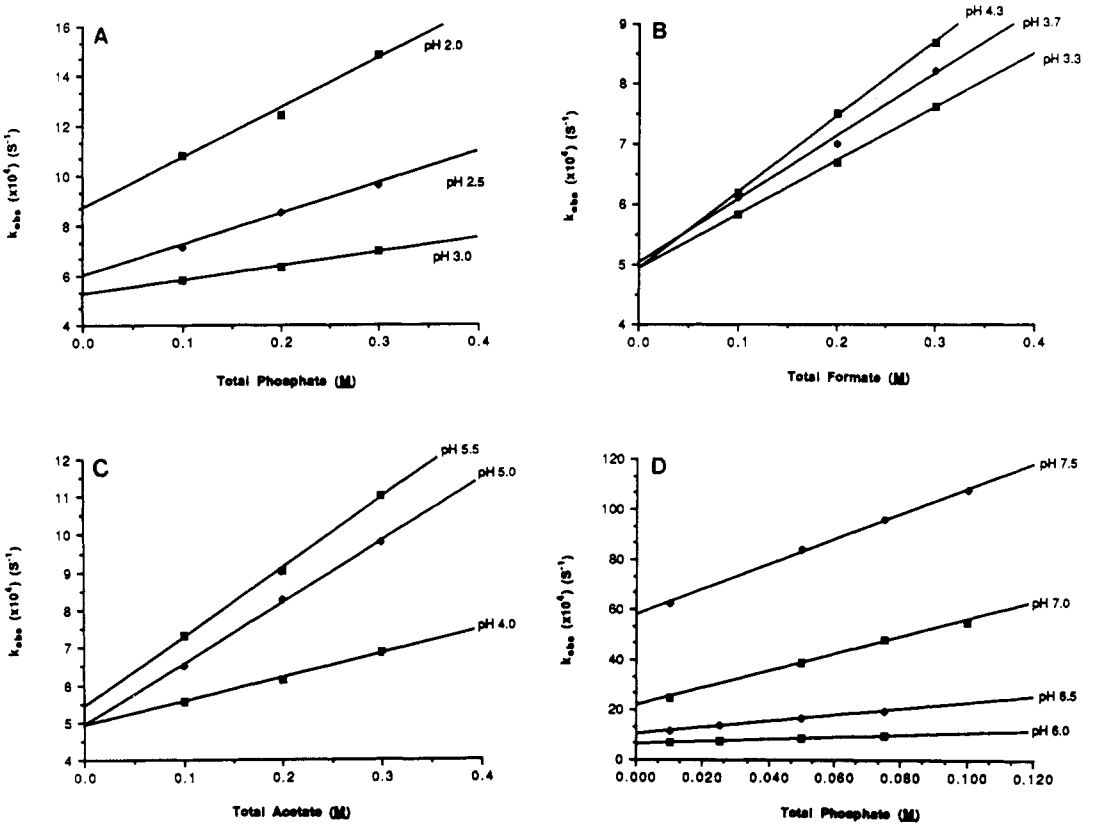


Figure 6. Buffer dilution plots showing the dependence of the observed rates of lactonization of **3b** as a function of the total buffer concentration. Each data point corresponds to the average of three determinations. The lines in these plots obey eq 2, which indicates that the slopes correspond to values of k_{cat} (Table II) and the intercepts correspond to values of k_o (Table I). The experiments were conducted over the following pH ranges utilizing the buffers indicated in the plots: Figure 6a, pH 2.0–3.0; Figure 6b, pH 3.3–4.3; Figure 6c, pH 4.0–5.5; Figure 6d, pH 6.0–7.5.

Table I. Apparent First-Order, Buffer-Independent Rate Constants (k_o) for the Loss of Hydroxy Amides 2b and 3b[a]

	k_o, s^{-1} ($\times 10^4$)	
pH	**2b**	**3b**
1.0	0.205 ± 0.010	39.9 ± 1.1
2.0	(9.28 ± 0.15) × 10^{-2}	8.67 ± 0.50
2.5		5.98 ± 0.19
3.0	(7.82 ± 0.04) × 10^{-2}	5.26 ± 0.11
3.3		4.92 ± 0.06
3.7		5.01 ± 0.21
4.0	(7.97 ± 0.04) × 10^{-2}	4.90 ± 0.13
4.3		4.94 ± 0.07
5.0	(7.87 ± 0.50) × 10^{-2}	4.92 ± 0.11
5.5		5.41 ± 0.16
6.0		6.48 ± 0.10
6.5	(8.22 ± 0.22) × 10^{-2}	10.2 ± 0.2
7.0		21.6 ± 0.9
7.5	0.110 ± 0.002	57.7 ± 0.7
8.0	0.181 ± 0.002	

[a] The values of k_o were determined by plotting the observed pseudo-first-order rate constants for the loss of **2b** and **3b** versus the total buffer concentration according to eq 2. On the buffer dilution plots (Figures 6a–d for **3b**, data not shown for **2b**), the intercepts correspond to values of k_o.

Table II. Second-Order, Buffer-Dependent Rate Constants (k_{cat}) for the Loss of Hydroxy Amides 2b and 3b[a]

	k_{cat}, M^{-1} s^{-1} ($\times 10^4$)	
pH	**2b**	**3b**
2.0	(6.20 ± 0.69) × 10^{-2}	20.0 ± 2.3
2.5		12.2 ± 0.9
3.0	(3.15 ± 0.20) × 10^{-2}	5.60 ± 0.52
3.3		8.95 ± 0.26
3.7		10.5 ± 0.3
4.0	(6.20 ± 0.17) × 10^{-2}	6.45 ± 0.60
4.3		12.6 ± 0.3
5.0	0.230 ± 0.023	16.3 ± 0.5
5.5		18.5 ± 0.8
6.0		39.9 ± 2.1
6.5	0.410 ± 0.033	124 ± 3
7.0		341 ± 14
7.5	1.59 ± 0.03	508 ± 11
8.0	2.05 ± 0.03	

[a] The values of k_{cat} were determined by plotting the observed pseudo-first-order rate constants for the loss of **2b** and **3b** versus the total buffer concentration according to eq 2. On the buffer dilution plots (Figures 6a–d for **3b**, data not shown for **2b**), the slopes correspond to values of k_{cat}.

zation of hydroxy amide **3b** showed a linear dependence on the total buffer concentration. The series of nonparallel lines in these figures were found to obey eq 2.

$$k_{obs} = k_o + k_{cat}[B_T] \quad (2)$$

A similar relationship (data not shown) was observed with the lactonization data for compound **2b**. This relationship indicates that the loss of hydroxy amide is catalyzed by buffer as well as by nonbuffer species. The intercepts of the lines correspond to values of k_o (the apparent first-order, buffer-independent rate constant) shown in Table I, while the slopes correspond to values of k_{cat} (the second-order, buffer-dependent rate constant) which are shown in Table II. The slopes and intercepts of the lines were obtained by linear least-squares analysis with the correlation coefficients generally exceeding 0.995. The rate constant k_{cat} can in theory (eq 3) be composed of contributions from both the acidic and basic forms of the buffer. Secondary plots of k_{cat} versus the mole fraction of the basic buffer species for the lactonization of **3b** are shown in Figure 7, parts a–d. The relationships between k_{cat} and

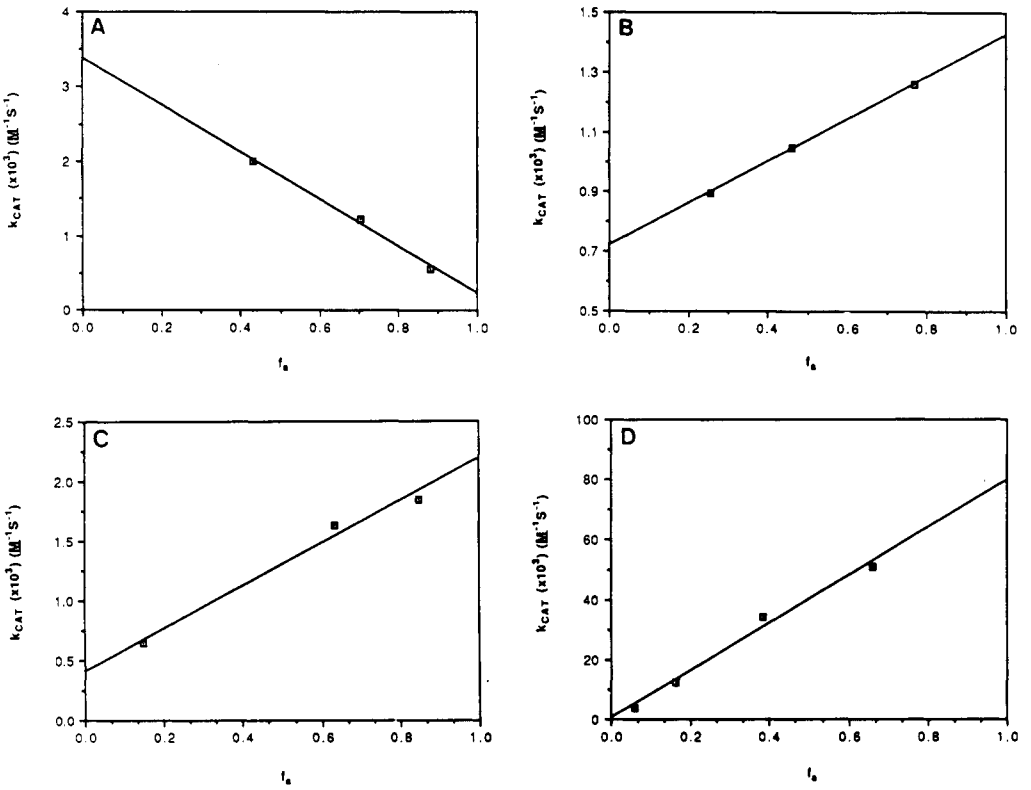


Figure 7. Plots of k_{cat}, the slopes of Figures 6a–d, versus f_{a^-}, the fraction of basic buffer species present at various pH values. Figure 7a shows the dependence of k_{cat} on the fraction of monobasic phosphate present in the pH range of 2.0–3.0. Figure 7b shows the dependence of k_{cat} on the fraction of formate anion present in the pH range of 3.3–4.3. Figure 7c shows the dependence of k_{cat} on the fraction of acetate anion present in the pH range of 4.0–5.5. Figure 7d shows the dependence of k_{cat} on the fraction of dibasic phosphate anion present in the pH range of 6.0–7.5.

Table III. Rate Constants for Buffer Catalysis in the Lactonization of Hydroxy Amides 2b and 3b

	rate constants[a] M^{-1} s^{-1} ($\times 10^4$)	
	2b	**3b**
$k_{H_3PO_4}$	9.11×10^{-2}	34.2 ± 1.5
$k_{H_2PO_4^-}$	2.36×10^{-2}	2.22 ± 2.65
k_{HCOOH}		7.21 ± 0.11
k_{HCOO^-}		14.2 ± 0.2
k_{HOAc}	1.09×10^{-2}	4.09 ± 1.40
k_{OAc^-}	0.356	21.9 ± 2.6
$k_{H_2PO_4^-}$	2.74×10^{-2}	3.83 ± 25.4
$k_{HPO_4^{2-}}$	2.38	794 ± 69

[a] The rate constants for catalysis by the acidic and basic forms of the buffer were determined by plotting the second-order, buffer-dependent rate constant k_{cat} versus the fraction of basic buffer species present (f_{a^-}) at a given pH. The values of k_{cat} show a dependence on f_{a^-} which is described by eq 3. The y intercept at f_{a^-} = 0 on these plots (Figures 7a–d for **3b**, data not shown for **2b**) corresponds to the rate constant for catalysis by the acidic component. The y intercept at f_{a^-} = 1 on these plots corresponds to the rate constant for catalysis by the basic component.

the mole fraction of basic buffer species were found to obey eq 3 in which f_{A^-} is the mole fraction of the buffer in its

$$k_{cat} = k_{GA} + (k_{GB} - k_{GA})f_{A^-} \quad (3)$$

basic form at a given pH. The y intercept of these lines (f_{A^-} = 0) provides k_{GA} (the second-order rate constant for general acid catalysis) and that at f_{A^-} = 1 provides k_{GB} (the second-order rate constant for general base catalysis). The specific rate constants for catalysis of the lactonization of **2b** and **3b** by buffer components are given in Table III.

The lack of coincidence of the y intercepts in Figure 6, parts a–d, indicates that in addition to catalysis by the solvent water there are also contributions to the lactonization rate by hydronium and hydroxide ions. The pH dependency of these intercepts (k_o) is shown in Figure 8,

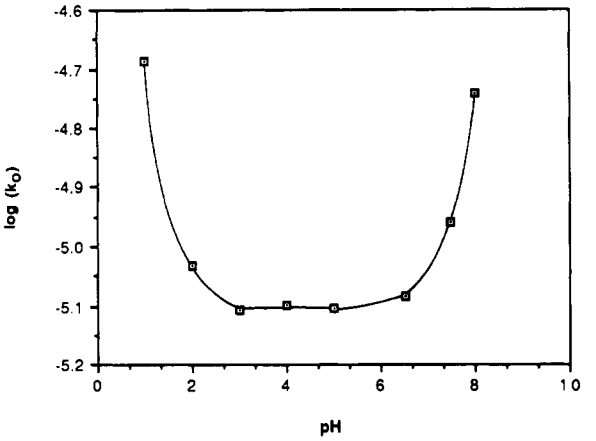


Figure 8. Buffer-independent pH–rate profile for the lactonization of **2b** over the pH range 1–8. Values shown as squares represent the intercepts of the buffer dilution plots (data not shown), while the solid line is the theoretical curve calculated from eq 4 and the data of Table I.

which contains the buffer-independent pH–rate profile for the lactonization of **3b**. Similar buffer dilution plots (data not shown) were generated for compound **2b**, and the pH dependency of those k_o values is shown in Figure 9. The pH–rate profiles for **2b** and **3b** are similar and reflect the situation where k_o shows a dependence on hydronium and hydroxide ions as described in eq 4. The curves shown

$$k_o = k_{H^+}[H_3O^+] + k_{H_2O} + k_{OH^-}[OH^-] \quad (4)$$

in Figures 8 and 9 are theoretical curves determined by a fit of the data to eq 4 by means of a nonlinear least-squares curve-fitting program. The values of the calculated specific rate constants are summarized in Table IV.

Table IV. Rate Constants for Specific Catalysis[a]

compd	k_{H^+}, M^{-1} s^{-1}	k_{H_2O}, s^{-1}	k_{OH^-}, M^{-1} s^{-1}
2b	$(1.26 \pm 0.01) \times 10^{-4}$	$(7.91 \pm 0.05) \times 10^{-6}$	10.1 ± 0.13
3b	$(3.51 \pm 0.01) \times 10^{-2}$	$(4.88 \pm 0.03) \times 10^{-4}$	$(1.67 \pm 0.04) \times 10^{4}$

[a] The rate constants for specific catalysis were determined by a fit of the experimentally determined values of k_o to eq 4 by means of the nonlinear least-squares curve fitting program RS 1.

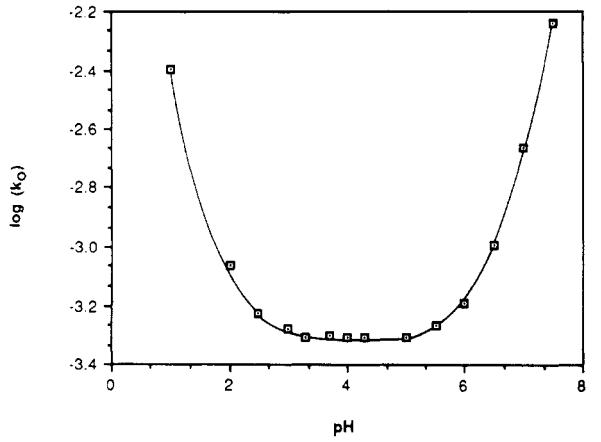


Figure 9. Buffer-independent pH–rate profile for the lactonization of **3b** over the pH range 1–7.5. Values shown as squares represent the intercepts of the buffer dilution plots (Figures 6a–d), while the solid line is the theoretical curve calculated from eq 4 and the data of Table I.

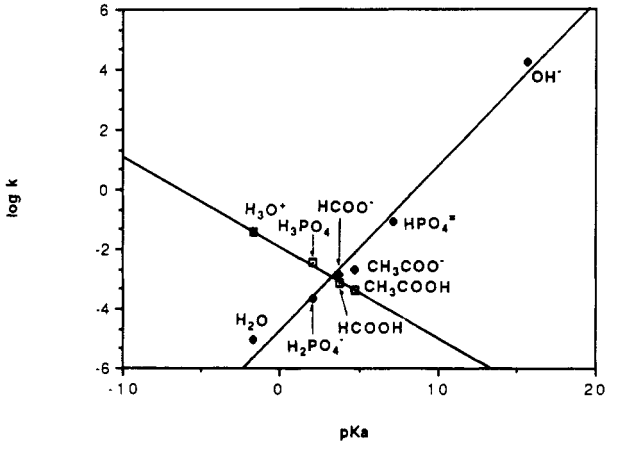


Figure 10. Brønsted plots for the acid and base catalysis of lactonization of **3b**.

Figure 10 shows the Brønsted plots for the lactonization of **3b**. These plots relate the log of the second-order catalytic constants for the lactonization reaction and the pK_a of the species responsible for the catalysis. The β line, which relates the log of the catalysis constants for species acting as bases to the pK_a of those species, was found to have a slope or β value equivalent to 0.54 ± 0.04. The α line, which relates the log of the catalysis constants for species acting as acids to their pK_a values, exhibited a slope or α value equivalent to 0.30 ± 0.02. The sum of α and β for this reaction is 0.84 ± 0.04. Correlation coefficients of 0.996 and 0.991 for the α and β lines, respectively, indicate that the Brønsted relationships are linear.

The lactonization of **1b** was determined at elevated temperatures because of the slowness of the reaction at 30 °C. The rate constant for the hydroxide ion catalyzed reaction was determined at pH 10, a pH shown by the previous pH–rate profiles to be well into the specific base-catalyzed region of this reaction. The reaction was measured at 90, 70, and 50 °C. Under these conditions of temperature and pH, the product lactone was hydrolyzed

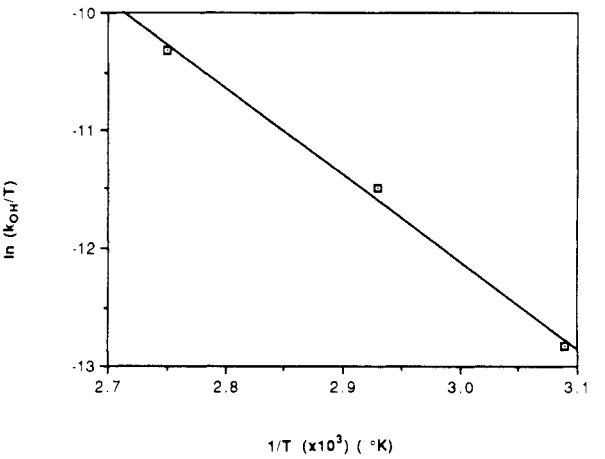


Figure 11. Eyring plot of ln (k_{OH}/T) versus $1/T$ (K) for the lactonization of **1b**. The values of k_{OH} were determined at pH 10.0 and 50, 70, and 90 °C.

to the corresponding hydroxy acid **1a**. The temperature dependence of rate constants as described by transition-state theory is shown in eq 5. Rearranging and linearizing eq 5 results in eq 6, which describes the temperature dependency of the rate constants shown in Figure 11. In eqs 5 and 6, R represents the universal gas constant, h represents Planck's constant, and K represents Boltzmann's constant. A value of 14.7 ± 0.8 kcal/mol for $\Delta H^{\ddagger}$, the enthalpy of activation, was calculated from the slope of this line, while the intercept was used to calculate a value of -9.5 ± 2.3 eu for $\Delta S^{\ddagger}$, the entropy of activation. There was some concern that at elevated temperatures amide hydrolysis might become a competing reaction with lactonization. To investigate this possibility the stability of compound **5**, an intermediate in the synthesis of **1b**, was also studied at 90 °C. Compound **5**, which still contained its benzyl protecting group, could not undergo lactonization but its amide bond could be hydrolyzed. No hydrolysis of compound **5** was observed within 50 h at pH 10 and 90 °C. Under these same conditions, **1b** was found to lactonize completely.

$$k = KT/h \exp(-\Delta H^{\ddagger}/RT) \exp(\Delta S^{\ddagger}/R) \quad (5)$$

$$\ln (k/T) = \Delta S^{\ddagger}/R + \ln (K/h) - \Delta H^{\ddagger}/RT \quad (6)$$

The magnitude of steric rate enhancement within the set of hydroxy amides was evaluated at pH 7.5 with 0.1 M phosphate. The comparison was made at this pH because of its closeness to physiological pH. The buffer concentration of 0.1 M was chosen because it was the highest concentration at which the lactonization of **3b** could be measured at pH 7.5. Rate enhancement was evaluated by dividing the values of k_{obs} for each compound by the value determined for compound **1b**. As expected, the order of reactivity of the set was **3b** $\gg$ **2b** > **1b**. The rate constants as well as the extent of rate enhancement in the set are shown in Table V.

Discussion

The first formal investigation into the lactonization of *o*-hydroxyhydrocinnamic acid derivatives[17] provided a clear

Table V. Rate Enhancement of Lactonization

compd	k_{obs},[a] s^{-1}	$t_{1/2}$, s	k_{rel}[b]
1b	4.13×10^{-7}	1.68×10^{6} (19.4 days)	1
2b	1.83×10^{-5}	3.79×10^{4} (10.5 h)	44.3
3b	1.06×10^{-2}	65.4	2.57×10^{4}

[a] The observed pseudo-first-order rate constants (k_{obs}) for the loss of **1b**, **2b**, and **3b** were measured at pH 7.5, 0.1 M phosphate buffer, μ = 0.3 and 30 °C. [b] The values of k_{rel} were determined by taking the ratio of k_{obs} for the three hydroxy amides to the value determined for **1b**.

demonstration of the advantage in terms of rate enhancement that intramolecular reactions have over their intermolecular counterparts. Since this initial study, the reaction has been more extensively investigated. The first study into the lactonization of *o*-hydroxyhydrocinnamic acids conducted by Milstien and Cohen utilized a series of analogues of compound **2a** (Figure 2) containing various electron donating and withdrawing groups placed at the 5'-position.[18] The lactonization of these compounds was investigated in weakly acidic media and the reaction was observed to be catalyzed both concurrently and independently by the acidic and basic buffer species. There was no evidence of concerted catalysis by both buffer species. The results of a linear free energy relationship between lactonization rates and variations in the 5'-substituents led the authors to conclude that the rate-limiting step in the lactonization of these compounds is the breakdown of the tetrahedral intermediate. Later work involving many structurally diverse *o*-hydroxyhydrocinnamic acids also accepted breakdown of the tetrahedral intermediate as the rate-determining step in the reaction.[12,13]

In a subsequent study by the same authors,[12] it was observed that the addition and substraction of methyl groups at positions 3, 3', 4', and 6' had a tremendous effect on the rate of lactonization and that lactonization rates varying by over 10 orders of magnitude could be observed within the set of methyl-substituted hydroxy acids. The lactonization of these compounds, which were investigated over the pH range of 6.0–8.0, was observed to be catalyzed concurrently, but not concertedly, by both the acidic and basic forms of the buffer. The most reactive compound in the series—**3a** with an additional methyl group at position 3'—was found to possess a half-life of only 6 s at pH 7 and 30 °C. Rate enhancement of this magnitude within a set of structural analogues was unprecedented. These results generated much interest, and many explanations were presented to account for the enhancement.[19,20] The dispute was settled when Caswell and Schmir[13] determined that the lactonization rates for the most reactive compounds, although substantial, were over-estimated. The revised rate enhancement in the case of compound **3a** compared to **1a** is approximately 10^5, which is in better agreement with the enhancement observed in other systems containing the trimethyl lock.[14] These data were later reevaluated by DeTar,[21] and the rate enhancement was estimated to be closer to a factor of 4000. The author states that in all reactions involving reversibly formed tetrahedral intermediates the experimental rate constants are actually the product of the mechanistic constant and a product distribution f_p. If f_p is approximately equal to one, then the experimental constant is a good measure of the mechanistic constant. DeTar[21] contends that f_p is actually 0.001 for **1a**, resulting in a 100-fold increase in the acid-catalyzed lactonization rate for this compound. This reduces the rate enhancement factor with **3a** to 4000. Further studies into lactonization of *o*-hydroxyhydrocinnamic acids have demonstrated that the size of the substituent at the 6'-position has an effect on the lactonization rate. With a series of halogens placed at this position, it was observed that the larger the van der Waals radius of the substituent in the 6'-position the faster the rate of lactonization of the hydroxy acid.[22]

The trimethyl lock has been found to generate similar rate enhancements for other types of reactions and in a few cases has promoted reactions which were considered to be unlikely or unusual. The effects of this system on nucleophilic displacement of various side chain propanol monoesters by either phenol or phenolate anion was investigated.[14] Within a series containing the same leaving group and the same nucleophile, the appropriate alkyl substitution produced a rate enhancement factor of almost 10^6. The presence of the trimethyl lock in the benzoquinonepropionic acid [3-(3',6'-dioxo-2',4',5'-trimethylcyclohexa-1',4'-dienyl)-3,3-dimethylpropionic acid] caused the side chain carboxyl group to undergo conjugate addition with the double bond of the quinone system.[23] The addition of a carboxyl group to an activated double bond, a reaction which is rarely observed, occurred in this system only with those compounds containing the complete trialkyl lock. A less facile example of intramolecular conjugate addition was observed with similar quinonoid compounds containing a side chain hydroxyl group in place of the carboxyl group.[24] The anion of the hydroxyl group was required for this addition and, therefore, the reaction occurred only in highly basic solution. The chemistry of the quinonoid compounds containing a side chain functional group of intermediate oxidation state—the aldehyde—has also been investigated.[25] Although it was expected that this side chain would be unreactive, it was found that in aqueous solution the hemiacetal form of the aldehyde could undergo conjugate addition in a fashion analogous to that of the hydroxyl group. A further demonstration of the power of the trimethyl lock was shown by an investigation into the formation of homophthalic anhydrides. In this study, compounds containing the trimethyl lock exhibited rate enhancements of 10^5 for cyclization.[26]

During the earlier investigations of *o*-hydroxyhydrocinnamic acid lactonization, no carboxylic acid derivatives were studied. The lactonization of such derivatives would result in the release of the compound to which the acid was coupled. Presumably these release rates could be controlled by the extent of methyl substitution in a manner analogous to the results observed with the free acids. The results of this study support this conclusion. We have observed the same order of reactivity with the amides (**3b** $\gg$ **2b** > **1b**) as was seen with their analogous acids (**3a** $\gg$ **2a** > **1b**), as well as a similar extent of rate enhancement.[12,13] In general, the buffer catalysis observed in the present study is in agreement with the results determined in the lactonization of the hydroxy acids. The lactonization of hydroxy amine **3b** was catalyzed concurrently and independently by both the acidic and basic form of the buffer in the pH regions of 2.0–3.0, 3.3–4.3, and 4.0–5.5 by the

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phosphate, formate, and acetate buffers, respectively. In the region of 6.0–7.5, only the basic form of the phosphate buffer was observed to catalyze the reaction and the contribution by the acidic form was determined to be statistically indistinguishable from zero (see Table III). Evidence is presented below which indicates that when both the acidic and basic buffer forms catalyze the reaction, they act independently.

The pH–rate profiles determined in this study (Figures 8 and 9) exhibit none of the complexity which can often appear in such profiles. Both are broad U-shaped curves described perfectly by the relationship of eq 4. Complexity in pH–rate profiles is usually the result of either ionization occurring in the reacting molecule or a change in the reaction mechanism. The phenolic hydroxyl group is the only potentially ionizable group in these model compounds. The pK_a values of the hydroxyl groups of **1b**, **2b**, and **3b** range from 11.9 and 12.9. As the reaction was investigated only up to pH 8.0, there would be virtually none of the ionized form present in the pH range studied. Therefore, it comes as no surprise that curvature due to ionization fails to appear in the pH–rate profile.

The second potential source of pH–rate profile complexity is the possibility of a change in the rate-determining step of the reaction over the pH range investigated. With most coumarinic acids, the rate-determining step for lactonization in the acidic region changes from formation of the tetrahedral intermediate at low pH to its breakdown at a somewhat higher pH.[27-31] In contrast, the lactonization of hydrocoumarinic acids in acidic medium shows no evidence for a change in rate-determining step with increasing pH.[13] The pH–rate profiles for hydroxy amides **2b** and **3b** in the acidic region also remain linear down to pH 1 with no negative deviations from a slope of −1. Such deviations usually signal the approach of a change in the rate-determining step. In many examples of hydroxy amide lactonization, breaks in the pH–rate profile are observed at pH 7–8. These breaks are indicative of a change in the rate-determining step from the formation of the tetrahedral intermediate, which is rate-determining at pH values below neutrality, to rate-limiting breakdown of the tetrahedral intermediate in the basic region.[10,31,32] No such breaks in the pH region of 7–8 were observed in the pH profiles for compounds **2b** and **3b**. Because of the dramatic extent of specific base catalysis, it was impossible to measure the rate of this reaction at pH values greater than 7.5. Therefore, the possibility cannot be ruled out that a change of rate-determining step would have been observed at higher pH values.

The plots shown in Figure 10 demonstrate that a Brønsted type relationship holds for the general acid and base catalysis in the lactonization of **3b**. These plots were used to determine values for α and β. Empirically one finds that the values of α and β generally fall between 0 and 1. When $\alpha = 1$ the structural features of the acid catalyst which determine its acidity are fully expressed during proton transfer in the transition state. This is most easily visualized if proton transfer is essentially complete in the transition state. By contrast, the situation where $\alpha = 0$ is consistent with a transition state in which essentially no proton transfer has occurred. The inverse is true for values of β. It is a common interpretation to suggest that intermediate values of α or β reflect intermediate degress of proton transfer. The values of α and β determined for the lactonization of **3b** are intermediate between 0 and 1, which is consistent with the observation of general catalysis in the reaction. One should note that the log of the catalysis constants versus pK_a is a linear relationship for both sets of constants, indicating that the general acids and bases are acting as proton donors and acceptors in the reaction. The monobasic phosphate ion ($H_2PO_4^-$) has the potential to act as a bifunctional catalyst in the pH range investigated; however, this does not appear to be the case because of the position of this value relative to the β line. The value for monobasic phosphate acting as a base falls near the β line—actually slightly below it. If this species were acting as a bifunctional catalyst, one would expect it to be displaced positively from the β line. In contrast to our results, evidence of bifunctional catalysis with the $H_2PO_4^-$ ion in the lactonization of various *o*-hydroxyhydrocinnamic acids was observed.[12]

One possible mechanism for catalysis of the lactonization reaction by buffer components would involve nucleophilic catalysis by buffer anions. In this mechanism the basic form of a undefined buffer species attacks the carbonyl carbon directly, resulting in a tetrahedral intermediate which breaks down with loss of amine. The resulting mixed anhydride intermediate is then attacked by the phenolic hydroxyl group, resulting in lactone production. This mechanism dictates that the basic buffer species act as a nucleophile rather than as a proton donor; it can be eliminated on the basis of the linearity of the β line of the Brønsted plot, which indicates that the bases are acting as proton acceptors. If nucleophilic catalysis were in effect, a linear relationship for the β line would not be expected.

Another possible mechanism to explain buffer catalysis is one in which a general acid and general base act in concert on the tetrahedral intermediate. In this mechanism, if β expresses the extent to which the proton is removed by the general base from the oxygen atom, then the extent to which the oxygen atom still possesses its proton is expressed as $1 - \beta$. This degree of proton transfer is passed alternatively as β and then $1 - \beta$ through each bond of the transition state, resulting in a value of $1 - \beta$ as indicative of the extent to which the general acid still possesses its proton. However, this value is by definition α indicating that for this mechanism to hold the sum of α and β must equal one within experimental error. The experimentally determined sum of α and β was 0.84 ± 0.05, which would seem to disqualify this mechanism as well.

Accepting that breakdown of the tetrahedral intermediate is rate-determining for this reaction over the pH range studied, it is necessary to describe a mechanism for the reaction which is consistent with the observations. First of all, general acid and base catalysis was clearly observed for the reaction. This requires that proton transfer occur in the transition state of the rate-determining step, which in this case is breakdown of the tetrahedral intermediate. Furthermore, the Brønsted data indicate that it is unlikely that acids and bases act in concert on the transition state. The mechanisms shown in Scheme II, a–c, are consistent with the hypothesis that the buffer species are acting singly as proton donors or acceptors in the transition state. The mechanism shown in Scheme IIa is an overall general acid mechanism in which the basic form of the buffer triggers the breakdown of the protonated form of the tetrahedral intermediate by abstracting the phenolic proton. A potential overall general base mechanism is described in Scheme IIb where

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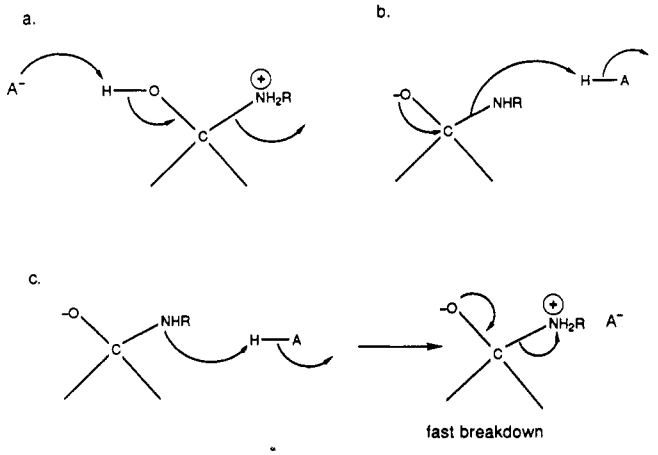
Scheme II.[a] Potential Mechanisms for Overall Acid- and Base-Catalyzed Breakdown of Tetrahedral Intermediate in Which the Acids and Bases Act Singly on the Transition State



[a] Part a is an overall acid-catalyzed mechanism, and parts b and c are both overall base-catalyzed mechanisms.

breakdown of the anionic form of the tetrahedral intermediate is simultaneous with proton abstraction by the departing amine from a general acid. A second potential overall general base mechanism which involves two steps is shown in Scheme II. In the first step, the nonbonded electrons on the amine nitrogen abstract a proton from a general acid resulting in a zwitterionic tetrahedral intermediate which breaks down very rapidly in the second step.

This initial study has been limited to amide prodrug forms for two reasons. First, amides are less reactive than esters or thioesters; thus, the complication of direct hydrolysis is minimized. The direct hydrolysis mechanism seems highly unlikely given the lack of amide hydrolysis in the accelerated stability study of compound **5** and the very slow rate of amide hydrolysis reported for structurally similar amides.[33] The second reason amides were investigated is that amines have not been as successfully handled by existing prodrug approaches as have alcohols and acids.[34,35] It should be noted, however, that the side chain carboxylic acid is a versatile synthetic handle which should allow the application of this promoiety to a variety of amines, thiols and alcohols with the only requirement being the ability to form a stable condensation product with the carboxylic acid.

This study has employed only one amine, which could raise the issue of what would happen if amine structure were varied. In subsequent work[36] with a related system containing the full trimethyl lock, we found that amine structure exerted no effect on lactonization rates. Evidently the steric driving force overrides any electronic effects provided by changes in the amine structure.

The data shown in Table V indicate that the lactonization of amides of the various methyl-substituted *o*-hydroxyhydrocinnamic acids follows the same methyl-dependent hierarchy as previously established with the free acids. Clearly amine release rates from such promoieties could be strictly controlled through synthetic manipulation. In fact, it should be possible to achieve release rates varying by many orders of magnitude in a biological system. Given that compound **3b** exhibited a half-life of 65 s at pH 7.5 and 30 °C, one would expect promoieties containing this methyl substitution to exhibit very short reconversion half-lives at physiological pH. Certainly the rate of this reaction will differ in a biological milieu from that determined in acetonitrile/water mixtures; however, it should still exceed the lactonization rate of any previously studied hydroxy amides and therefore meet one of the requirements of the prodrug system shown in Figure 1. It is also impossible to predict how the reaction rate will be affected by catalysis by physiological components or by possible plasma protein binding. It has been reported that the lactonization of 2-(hydroxymethyl)benzamide—an example of hydroxy amide lactonization—is not subject to catalysis by plasma components.[5] However, general catalysis has frequently been observed in the lactonization of hydroxy amides, which would lead one to expect catalysis by plasma components, the in vivo sources of general acids and bases.

This manuscript has dealt only with the hydroxy amide intermediate referred to in Figure 1 as the "prodrug". Future publications from this laboratory will deal with the derivatized hydroxy amide form referred to as the "pro-prodrug". It is essential for the success of the delivery system that the pro-prodrug exhibit good in vitro stability; that is, the pro-prodrug must contain some structural feature capable of controlling the reactivity of the transient prodrug form. During the preparation of this manuscript, Carpino et al.[37] described a satisfactory pro-prodrug form when they reported the reductive lactonization of quinone propionic acid amide analogues of the hydroxy amides utilized in this study. The reduction of the quinone portion of these pro-prodrugs resulted in the release of amines and alcohols at rates approaching those observed in this study. A well-documented method of blocking the lactonization of hydroxy amides has been esterification of the hydroxyl group.[4-6] Because of the susceptibility of esters to enzymatic cleavage, this synthetic modification results in pro-prodrug forms which, while preventing the chemical conversion of the prodrug, still exhibit good in vivo reconversion rates. Esterification with a variety of ester acyl groups could impart dramatic changes to the physiochemical properties of the pro-prodrug. As evidenced by the high percentage of acetonitrile utilized in these studies, these hydroxy amides have limited water solubility—especially compound **3b**. More water soluble acyl groups could provide a solution to this solubility problem. Furthermore, the highly lipid soluble promoieties described here could find their greatest utility in imparting greater lipid solubility to those compounds which already possess high water solubility but have difficulty crossing biological membranes. Structural modifications to the promoieties described in this report with the intent of providing novel control mechanisms for hydroxy amide lactonization as well as altering its solubility are issues which will be addressed in future publications.

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