then vacuum distilled to give 2.1 g (30%) of the desired product, bp 92–95° (0.5 mm).

After preparative gas chromatography on an 8-ft column of 20% SE-52 on Chromosorb W the sample showed the following: nmr $\delta_{\mathrm{TMS}}^{\mathrm{CCl_4}}$ 7.1 (m, 5 H), 3.05 (s, 3 H), 2.5 to 1.0 (m, 8 H), 0.6 and 0.7 (2 s, integrating to 2 H).

Anal. Calcd for $C_{14}H_{18}O$: C, 83.16; H, 8.91; O, 7.92. Found: C, 82.99; H, 8.71; O, 8.13.

The mass spectrum showed a parent peak at m^+/e 202; no higher peaks were present.[42]

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(42) Preparative gas chromatography was unable to separate the desired product from those produced by addition to the nonconjugated olefin. These compounds, however, would not interfere with the charge-transfer spectrum of the desired 6-methoxybicyclo[4.1.0]-1-phenylheptane.

Lactonization of Coumarinic Acids. Kinetic Evidence for Three Species of the Tetrahedral Intermediate[1a]

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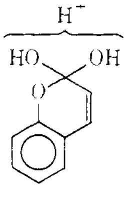
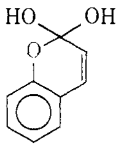
Received August 10, 1973

Abstract: The lactonization of four coumarinic acids substituted on the ring and side chain with methyl groups has been studied at 30° and at pH 0–7. The variation of the rate of lactonization with pH is strongly dependent on the position of the methyl substituents. With 4,6,8-trimethylcoumarinic acid, the pH–rate profile is consistent with the participation of three kinetically significant intermediates, and provides kinetic evidence for the existence of an anionic tetrahedral intermediate in the formation of a phenyl ester. The nonlinear dependence of the rate of lactonization of coumarinic acids on formate and acetate buffer concentration offers additional support for the postulation of intermediates in these reactions.

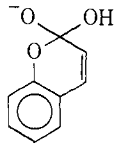
Studies of the kinetics of lactonization of coumarinic acids have led to the proposal that the reaction involves cationic and neutral tetrahedral addition intermediates.[2,3] The nature of the rate-limiting step depends on pH; for most of the compounds studied, the rate-limiting step is believed to be the formation of intermediates at low pH, and their decomposition at higher pH. This change in rate-determining step occurs because the two intermediates partition differently between reactants and products. The cationic intermediate (T^+) breaks down mainly with expulsion of water to yield the coumarin product, while the neutral intermediate (T^0) usually reverts to the reactant coumarinic acid.[4] No evidence was obtained for the existence of an anionic intermediate (T^-). If such a species is formed, it would be expected to break down generally with expulsion of phenoxide ion. The observation[3] that electron-donating substituents in the aromatic ring enhanced the departure of water relative to that of phenol from T^0 led us to consider the possibility that a suitably substituted coumarinic acid might form a neutral intermediate from which water would leave predominantly. If this coumarinic acid formed an anionic intermediate which preferred to expel phenoxide ion, the kinetics of the lactonization reaction should indicate a new change in rate-determining step, and, incidentally, provide hitherto lacking evidence for the existence of the anionic intermediate.



T^+



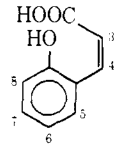
T^0



T^-

Results

The lactonization of four methyl-substituted coumarinic acids (**1–4**) was studied by spectrophotometric means in aqueous solution, μ = 1.0 (LiCl), at 30°. To avoid the necessity of extrapolating rate constants to zero buffer concentration, rate constants at pH >2.5 were determined in unbuffered solution and pH was kept constant by automatic titration with the pH-stat.[5] With **1**, some measurements were made in buffered solution; the values extrapolated to zero buffer con-



1, 4-CH_3

2, 5,8-$(CH_3)_2$

3, 4,6,8-$(CH_3)_3$

4, 4,5,7-$(CH_3)_3$

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(1) (a) This research was supported by a grant from the National Science Foundation. (b) Postdoctoral Research Fellow of the National Institutes of Health, 1971–1973.

(2) (a) E. R. Garrett, B. C. Lippold, and J. C. Mielck, *J. Pharm. Sci.*, 60, 396 (1971); (b) B. C. Lippold and E. R. Garrett, *ibid.*, 60, 1019 (1971).

(3) R. Hershfield and G. L. Schmir, *J. Amer. Chem. Soc.*, **95**, 7359 (1973).

(4) The position of the proton in T^+ is not specified. Similarly, the structure here given for T^0 does not exclude other structures of zero net charge.

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

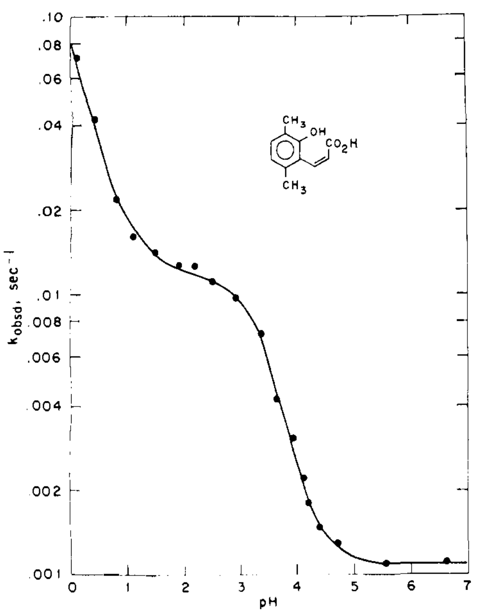

Figure 1. pH-rate profile for the lactonization of **2** at 30°, μ = 1.0. The solid line is calculated from eq 3 and 4, using constants of Table II.

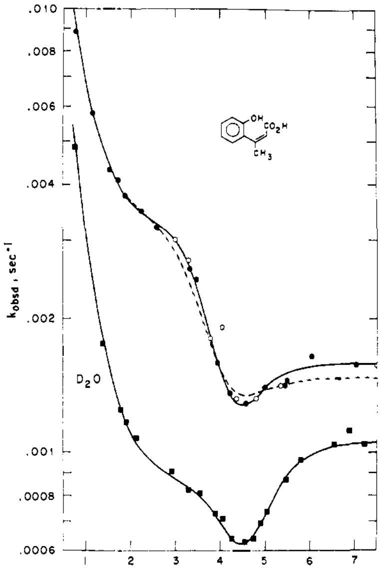

Figure 2. pH-rate profile for the lactonization of **1** at 30°, μ = 1.0. The open circles are rate constants extrapolated to zero buffer concentration and were not used in the calculation of the least-squares curve. For the reaction in H_2O, the dashed line is calculated from eq 3 and 6 (two intermediates) and the solid line from eq 3 and 4 (three intermediates). The solid line for the reaction in D_2O is calculated from eq 6. All constants used are listed in Table II. For calculation of pD, see Experimental Section.

centration agreed well with those determined by the pH-stat method. The rate data are listed in Table I.[6]

The overall appearance of the pH-rate profiles for lactonization varies appreciably with the structure of the coumarinic acid. With the 5,8-dimethyl compound (Figure 1), there is seen a gradual, though nonlinear, decrease in rate with increasing pH, culminating in a constant rate of reaction at pH >5. This behavior is characteristic of most ring[3] and side chain[2] substituted coumarinic acids which have been studied. In the case

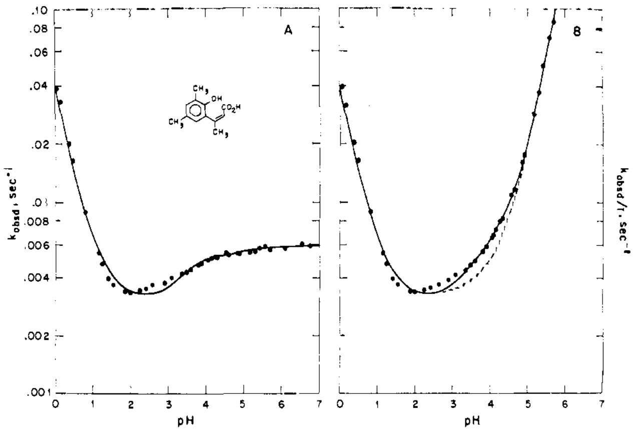

Figure 3. pH-rate profile for the lactonization of **3** at 30°, μ = 1.0: (A) observed first-order rate constants; (B) observed first-order rate constants divided by mole fraction of neutral substrate. The solid lines are calculated from: (A) eq 3 and 4; (B) eq 4, using constants of Table II. The dashed line is calculated from the expression $k_{obsd}/f = (3.3 \times 10^{-3} + 1.142 \times 10^7 K_w/[H^+])$ sec^{-1}.

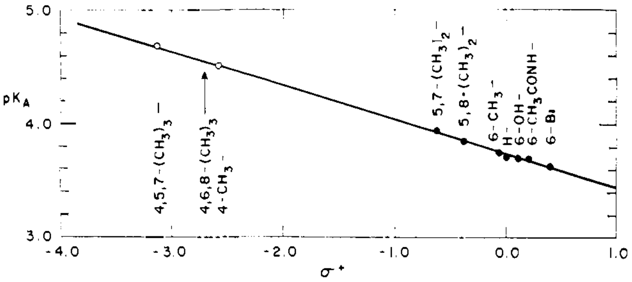

Figure 4. The dependence of the pK_a of substituted coumarinic acids on σ^+. The solid circles were used to calculate the least-squares line. The arrow indicates the calculated pK_a of **3**.

of 4-methylcoumarinic acid (Figure 2), a shallow but unmistakable minimum in rate occurs at pH 4.5. Limited data have been previously reported for this compound at 25°.[2b] A rate minimum (*ca.* 3.3×10^{-3} sec^{-1}) at pH 2-2.5 is the predominant feature of the pH-rate profile for 4,6,8-trimethylcoumarinic acid (Figure 3A). At higher pH, there occurs a gradual increase in rate over several pH units, leveling off at about 6×10^{-3} sec^{-1}. The isomeric 4,5,7-trimethylcoumarinic acid is extraordinarily stable to lactonization in acid solution, reacting about 10^3 times more slowly than **1**-**3**, and obeying a simple rate law (eq 1) over the narrow pH

$$k_{obsd} = k_H[H^+] + k_{H_2O} \qquad (1)$$

range (−0.2 to 1.09) where rate measurements could conveniently be made ($k_H = 2.67 \times 10^{-5}$ M^{-1} sec^{-1}; $k_{H_2O} = 4.4 \times 10^{-6}$ sec^{-1}). It has long been known that 4,5,7-trimethylcoumarin could be converted to a coumarinic acid stable enough to be isolated.[7]

pK_a values (Table II) for the carboxyl group of coumarinic acids **1**, **2**, and **4** were determined by spectrophotometric titration. This method could not be used with 4,6,8-trimethylcoumarinic acid, owing to the small differences in molar extinction coefficient between the coumarinic acid and its anion, and to the rapid rate of lactonization. The acid dissociation constant of **3** was estimated as follows. The pK_a values[3] of five 6-substituted coumarinic acids and of 5,6- and 5,8-dimethyl-

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(6) See paragraph at end of paper regarding supplementary material.

(7) L. A. Jordan and J. F. Thorpe, *J. Chem. Soc.*, 107, 387 (1915).

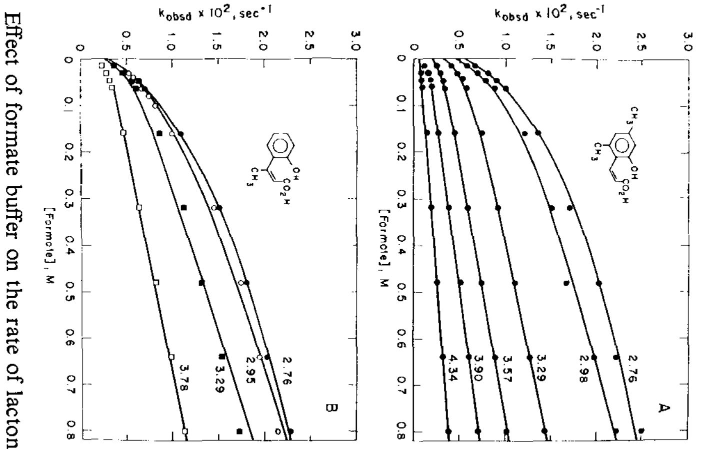

Figure 5. Effect of formate buffer on the rate of lactonization of coumarinic acids. The pH of each series of reactions is shown. For reactions at pH ≤ 3.57, the lines are calculated from eq 10, with $k_1^{BH} = 0$, using the constants in Table III. For **1**, the constants k_1, k_1', K', P^0, and P^+ are from Table II. For 5,7-dimethylcoumarinic acid, these constants are from ref 3. For data at higher pH, the straight line is the final slope reached at high buffer concentration.

coumarinic acids correlate well with σ^+ constants[8] ($\rho^+ = -0.30$, $r = 0.99$; Figure 4). An empirical "σ^+" value of -2.57 was calculated for the 4-methyl substituent from the regression line (eq 2) thus obtained.

$$\text{p}K_a = -0.30\sigma^+ + 3.73 \qquad (2)$$

The reliability of eq 2 for the prediction of the pK_a values of 4-methyl-substituted coumarinic acids was tested with 4,5,7-trimethylcoumarinic acid, for which the calculated value of 4.69 may be compared with the measured value of 4.68. Use of eq 2 leads to a calculated value of pK_a = 4.54 for 4,6,8-trimethylcoumarinic acid. The predominant substituent effect in **3** is that of the 4-methyl group, whose influence is much larger than that of the two meta oriented ring methyl functions. Consequently, **3** is expected to be only slightly more basic than **1**. In any event, it will be shown in the Discussion that the uncertainty in the exact value of the pK_a of **3** can have at most a minor effect on the interpretation of the kinetics of lactonization of **3**.

Second-order rate constants (k_{OH}; Table II) for the alkaline hydrolysis of the coumarins corresponding to **1**–**4** were calculated from measurements made at [OH⁻] = 0.1–0.5 *M*. The sensitivity of 4,5,7-trimethylcoumarin to base-catalyzed hydrolysis is normal, in contrast to the much reduced rate of lactonization of the coumarinic acid.

Buffer Catalysis. As with other phenolic acids,[9] the lactonization of coumarinic acids is subject to general

Table II. Rate and Equilibrium Constants for the Lactonization of Coumarinic Acids and the Hydrolysis of Coumarins[a,b]

Compd	pK_a	pK'	pK''	P^+	P^0	P^-	10^2k_1, M^{-1} sec⁻¹	$10^3k_1'$, sec⁻¹	$10^{-7}k_1''$, M^{-1} sec⁻¹	$10^{-6}B_{OH}$,[c,d] M^{-1} sec⁻¹	k_{OH},[d,e] M^{-1} sec⁻¹	$10^{-6}K_{eq}$[f]
4-CH₃	4.50	3.48 ± 0.1	3.78 ± 0.2	0.9970 ± 0.0007	0.183 ± 0.014	0.08 ± 0.04	3.75	3.35 ± 0.04	4.19		0.352	9.57
		3.45 ± 0.06	g	0.9975 ± 0.0003	0.299 ± 0.013	g	3.68	3.96	g	3.11	0.352	8.84
4-CH₃ (D₂O)[h]	4.79	4.41 ± 0.1	g	0.9989 ± 0.0002	0.0125 ± 0.06	g	2.41	1.055	g	1.145	i	i
5,8-(CH₃)₂	3.84	3.40 ± 0.05	3.60 ± 0.11	0.9980 ± 0.0003	0.152 ± 0.013	0.047 ± 0.015	7.03	11.7 ± 0.2	22.6		0.760	14.0
		3.60 ± 0.05	g	0.9988 ± 0.0001	0.143 ± 0.014	g	6.97	12.5	g	10.75	0.760	14.17
4,6,8-(CH₃)₃	4.54[j]	3.95	3.95	0.9995	0.664	0.215	3.73	3.00	5.31		0.152	75.1
6-OH[k]	3.69	3.18 ± 0.03	3.22 ± 0.1	0.9894 ± 0.0009	0.0539 ± 0.013	0.0191 ± 0.0047	8.27	4.86 ± 0.11	20.7			
		3.24 ± 0.02	g	0.992 ± 0.0005	0.067 ± 0.005	g	8.02	5.36	g	3.81		
4,5,7-(CH₃)₃	4.68						0.00267	0.0044		i	0.157	

[a] 30°, μ = 1.0. [b] Parameters are defined in Table V and Scheme I. For P^+, P^0, P^-, pK', pK'', and k_1', standard deviations are given. For compounds for which two sets of constants are given, the upper line refers to eq 4 (three intermediates) and the lower line to eq 6 (two intermediates). [c] Base-catalyzed lactonization (see eq 6). [d] Based on activity of hydroxide ion and pK_w = 13.83. [e] Base-catalyzed hydrolysis of coumarins. [f] For conversion of neutral coumarinic acid to lactone. [g] Not defined. [h] Activity scale; see Experimental Section. [i] Not measured. [j] Estimated value. [k] Data from ref 3.

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(8) J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," Wiley, New York, N. Y., 1963, p 204. With the 5,7- and 5,8-dimethyl derivatives, σ^+ for the 5 substituent was taken to be equal to that of the 7-methyl group, and additivity was assumed.

(9) (a) S. Milstien and L. A. Cohen, *J. Amer. Chem. Soc.*, **92**, 4377 (1970); (b) *ibid.*, **94**, 9158 (1972).

Table III. Buffer Catalysis of the Lactonization of Coumarinic Acids[a]

Coumarinic acid	Buffer	pH[b]	% free base	No. of points	Concn, M	Obsd slope $\times 10^2$, M^{-1} sec^{-1} [c]	Corr. slope $\times 10^2$, M^{-1} sec^{-1} [d]	k_1^{B}, M^{-1} sec^{-1}	P^{B}
4-CH_3	Formate	2.76	0.25	8	0.032–0.8			0.41	0.097
		2.95	0.33	11	0.016–0.8			0.41	0.112
		3.29	0.50	9	0.016–0.8			0.57	0.056
		3.78	0.75	9	0.016–0.8	1.08	1.71		
	Acetate	4.03	0.33	8	0.016–0.64	1.01	4.05	0.57	0.075
		4.36	0.50	8	0.016–0.64	1.11	3.82	0.49	0.078
		4.81	0.75	8	0.016–0.64	0.89	3.60		
		5.35	0.90	8	0.016–0.64	0.45	3.98		
5,7-$(CH_3)_2$	Formate	2.76	0.25	9	0.016–0.8			0.57	0.055
		2.98	0.33	9	0.016–0.8			0.65	0.065
		3.29	0.50	8	0.032–0.8			0.60	0.042
		3.57	0.67	9	0.016–0.8			0.48	0.044
		3.78	0.75	8	0.016–0.8	1.37	1.82		
		3.90	0.80	9	0.016–0.8	0.70	1.70		
		4.34	0.90	8	0.032–0.8	0.39	1.97		
	Acetate	4.01	0.33	3	0.06–0.1	0.72	4.74		
		4.26	0.50	3	0.06–0.1	0.75	4.68		
		4.80	0.75	4	0.04–0.1	0.39	4.42		
		5.23	0.90	4	0.04–0.1	0.17	4.24		

[a] 30°, $\mu = 1.0$. [b] ± 0.04. [c] Slope of linear plots of k_{obsd} *vs.* total buffer concentration. [d] Observed slope divided by f (eq 3) and by mole fraction of buffer in conjugate base form.

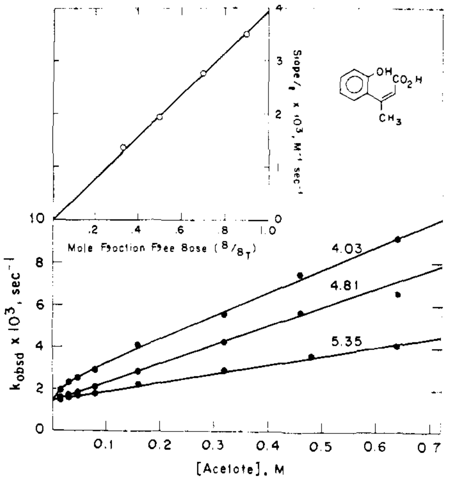


Figure 6. Effect of acetate buffer on the rate of lactonization of **1**. For pH 4.03, the solid line was calculated from eq 10 ($k_1^{BH} = 0$), using the constants in Tables II and III. For data at higher pH, the straight line is the final slope reached at high buffer concentration. Insert: dependence of the final slopes of acetate-catalyzed reactions (divided by mole fraction of neutral substrate) on the mole fraction of the buffer in conjugate base form.

acid–base catalysis by buffer components. The effects of formate and acetate buffers on the rates of lactonization of 4-methyl- and 5,7-dimethylcoumarinic acids were examined over a wide range of concentrations and at several pH values (Table III). Plots of k_{obsd} *vs.* total formate buffer concentration exhibit pronounced curvature at low pH (Figure 5) and approach lines of constant, nonzero slope. Curvature disappears at higher pH, and is nearly absent in acetate buffer (Figure 6). With 6-nitrocoumarinic acid, k_{obsd} increases linearly with formate buffer (pH 3.0, 33% free base), although considerable curvature is seen with other coumarinic acids under the same conditions (Figures 5 and 7).

Isotope Effects. The solvent deuterium isotope effect was determined for the lactonization of coumarinic acid and of 6-bromo-, 6-methyl-, and 5,8-dimethylcoumarinic acids in the pH region where the rate-limiting step is the water- and acid-catalyzed formation of a tetrahedral intermediate.[3] For the limited range of acid concentrations used, the rate law is accurately given by eq 1. With coumarinic acids bearing more electron-withdrawing substituents (6-nitro, 6-cyano, 6-carboxamido), the isotope effect was measured in the pH range corresponding to rate-limiting, acid-catalyzed breakdown of the tetrahedral intermediate,[3] where $k_{obsd} = k_H[H^+]$. In the case of the relatively reactive 4-methylcoumarinic acid, lactonization in D_2O was measured over a wide range of acidities (Table I and Figure 2). The isotope effects (k_H/k_D or k_{H_2O}/k_{D_2O}) are summarized in Table IV.

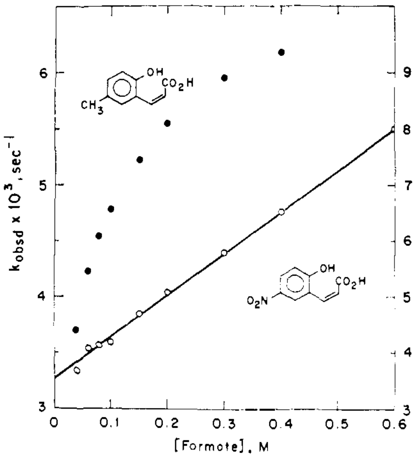


Figure 7. Effect of formate buffer (pH 3.0, 33% free base) on rate of lactonization of 6-methyl- (left ordinate) and 6-nitrocoumarinic (right ordinate) acids.

The sizable isotope effect associated with the water-catalyzed formation of T^0 is in accord with the previously[3] proposed mechanism for that step, involving proton transfer by water or hydronium ion in the transition state. With the ring-substituted compounds, there seems to be a trend to a smaller isotope effect with in-

Table IV. Isotope Effects in the Lactonization of Substituted Coumarinic Acids[a,b]

Substituent	[DCl], *M*	k_{H_2O}/k_{D_2O}[c]	k_H/k_D[d,e]	k_H/k_D[d,f]
5,8-$(CH_3)_2$	0.01–0.2	5.1	1.13	
4-CH_3	*g*	3.2	1.55	2.77
6-CH_3	0.1–1.0	(3.0)[h]	1.56	
H	0.01–0.2	3.8	1.60	
6-Br	0.1–1.0	2.3	1.68	
6-$CONH_2$	0.005–0.025			0.96
6-CN	0.005–0.025			0.86
6-NO_2	0.005–0.025			0.75

[a] 30°, $\mu = 1.0$. Four concentrations of DCl were used for each compound except for 4-CH_3. [b] Rate data for H_2O are from ref 3 except for 5,8-dimethyl and 4-CH_3. [c] k_{H_2O} corresponds to $k_1'P^+$ of general scheme. [d] Concentration scale. [e] Rate-limiting formation of intermediate; k_H corresponds to k_1P^+ of general scheme. [f] Rate-limiting breakdown of intermediate; k_H corresponds to $k_1'P^+/K'$ of general scheme. [g] See Tables I and II, and Figure 2. Activities of D^+ converted to concentrations using expression pD = −log [DCl] − 0.22. [h] Approximate value.

creasing electron withdrawal from the phenolic group. Acid-catalyzed formation of the intermediate exhibits a small normal isotope effect, while acid-catalyzed breakdown shows an inverse isotope effect, except for **1**. Interpretation of the isotope effect on the breakdown step is complicated by the combination of the effects on the equilibrium for formation of the intermediate and on the rate of its breakdown.

Comparison of the pH-rate profiles for the lactonization of **1** in H_2O and D_2O allows calculation of a solvent isotope effect of about 3 for k_3/k_2. No estimate of the isotope effect on k_3'/k_2' could be made; least-squares analysis of the scattered data in the pH region from which this constant is calculated showed that the standard deviation of P^0 exceeded its absolute value.

Discussion

The existence of two regions of acid catalysis in the pH–rate profile for the lactonization of coumarinic acids[2,3] and of a mercapto acid[10] (when the rate is expressed in terms of neutral substrate, eq 3) has provided

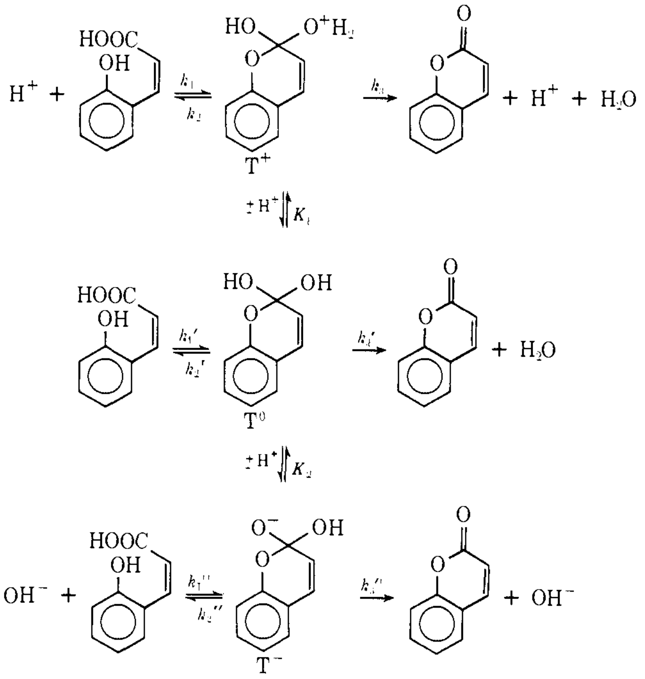
$$\frac{k_{\text{obsd}}}{f} = \frac{k_{\text{obsd}}}{[H^+]/([H^+] + K_a)} \tag{3}$$

evidence for a mechanism involving cationic and neutral intermediates, as well as for a change in rate-determining step with pH.[2,3] Detailed analysis of the pH–rate profile allowed calculation of the partitioning ratios of the intermediates, *i.e.*, of the relative rates of the conversion of each intermediate to reactants and products.[3] The hydrolysis of methyl thiolformate[11] at acid pH proceeds *via* the same type of mechanism, but with rate constants such that the two regions of acid catalysis overlap severely. In that case, the kinetic evidence for the presence of intermediates consists of small but systematic positive deviations of the observed rate constants from a simple rate law such as eq 1.

Analogously, the occurrence in several acyl transfer reactions of two regions of base catalysis separated by a region where the rate is less dependent on or independent of pH has been interpreted in terms of the participation of neutral and anionic intermediates on the reaction pathways.[12–16]

In what follows, we present a summary of the important kinetic properties of an acyl transfer reaction proceeding through cationic, neutral, and anionic intermediates in acid–base equilibrium (Scheme I). The steady-state rate equation (eq 4) for the pH dependence

Scheme I



$$\frac{k_{\text{obsd}}}{f} = \frac{\begin{array}{r} k_1'[P^+[H^+]^2 + P^0K'[H^+] + P^-K'K''] \times \\ [1 + ((1 - P^-)K''/(1 - P^0)[H^+]) + \\ ((1 - P^+)[H^+]/(1 - P^0)K')] \end{array}}{[H^+]^2 + K'[H^+] + K'K''} \tag{4}$$

of the rate of lactonization may be expressed in a number of alternate, equivalent ways, each containing six independent kinetic parameters. We have found it useful to collect the various rate and equilibrium constants of Scheme I into three partitioning ratios, P^+, P^0, and P^-, for the cationic (T^+), neutral (T^0), and anionic (T^-) intermediates, and two constants K' and K'' which indicate the pH values were partial or complete changes in rate-determining steps occur with varying pH if $P^+ \neq P^0 \neq P^-$ (see Table V for the definition of these terms).[17a] In the extreme case, the complex rate equation (eq 4) will generate a pH–rate profile (Figure 8) exhibiting two

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[10] R. Hershfield and G. L. Schmir, *J. Amer. Chem. Soc.*, **94**, 6788 (1972).

[11] R. Hershfield and G. L. Schmir, *ibid.*, **94**, 1263 (1972).

[12] L. R. Fedor and T. C. Bruice, *ibid.*, **87**, 4138 (1965).

[13] B. A. Cunningham and G. L. Schmir, *ibid.*, **89**, 917 (1967).

[14] G. M. Blackburn and W. P. Jencks, *ibid.*, **90**, 2638 (1968).

[15] G. L. Schmir, *ibid.*, **90**, 3478 (1968).

[16] R. Hershfield and G. L. Schmir, *ibid.*, **95**, 3994 (1973).

[17] (a) The difference between eq **4** and that previously given[18] for the three-intermediate system results from the different definition of P^+, P^0, and P^-. (b) The limiting kinetic expressions for regions A–D and for the separations between these regions (Figure **8**) are obtained from the overall rate equation for the T^+–T^0 system[3,10] and the assumption that $1 \simeq P^+ \gg P^0 \simeq 0$. The expressions for the regions D–G are similarly obtained from the overall rate equation for the T^0–T^- system and the assumption that $1 \simeq P^0 \gg P^- \simeq 0$. Clearly, both sets of assumptions cannot hold simultaneously in any one reaction involving T^+, T^0, and T^-. Put another way, if well-defined separations are seen in the range of pH corresponding to A–D, they are not expected to be seen in the range D–G because P^0 and P^- will probably both be near zero. In the unlikely event that $1 \simeq P^+ \gg P^0 \ll P^- \simeq 1$, the overall profile would be that of Figure 8, except that the vertical separation between regions F and D is now given by P^-/P^0 and the horizontal separation between regions E and G is given by $(1 - P^0)/(1 - P^-)$.

[18] R. K. Chaturvedi and G. L. Schmir, *J. Amer. Chem. Soc.*, **91**, 737 (1969).

Table V. Kinetic Expressions for the pH–Rate Profile for the Lactonization of Coumarinic Acids *via* Three Intermediates[a]

pH range[b]	k_{obsd}/f
A	$k_1P^+[H^+] = k_1'P^+(1 - P^+)[H^+]/(1 - P^0)K'$
B	$k_1'P^+$
C	$k_1[(1 - P^0)/(1 - P^+)]P^+[H^+] = k_1'P^+[H^+]/K'$
D	$k_1'P^0$
E	$k_1'(1 - P^-)P^0K''/(1 - P^0)[H^+] = k_1''P^0K_w/[H^+]$
F	$k_1'[(1 - P^-)/(1 - P^0)]P^0$
G	$k_1'P^-(1 - P^-)K''/(1 - P^0)[H^+] = k_1''P^-K_w/[H^+]$

Definitions of parameters	Equilibrium relationships
$K' = (k_2' + k_3')K_1/(k_2 + k_3)$	
	$k_1 = k_1'(1 - P^+)/(1 - P^0)K'$
$K'' = (k_2'' + k_3'')K_2/(k_2' + k_3')$	
$P^+ = k_3/(k_2 + k_3)$	
$P^0 = k_3'/(k_2' + k_3')$	$k_1' = k_1''(1 - P^0)K_w/(1 - P^-)K''$
$P^- = k_3''/(k_2'' + k_3'')$	

[a] Rate and equilibrium constants refer to Scheme I and eq 4. K_w = ion product of water = 1.47×10^{-14}. [b] See Figure 8 and ref 17b.

regions of acid catalysis and two regions of base catalysis. The complete separation of these regions, presented to facilitate visualization of the interrelationships between the several segments of the profile, is not likely to be frequently observed, since it requires that $P^+ \gg P^0 \ll P^-$, and $pK'' \gg pK'$. In favorable cases, some or all of the three partitioning ratios and the constants K' and K'' may be estimated by simple inspection of the pH-rate profile corrected for ionization of the reactant (eq 3). The relevant relationships are indicated in Figure 8, and the kinetic expressions for the limiting rate regions A–G are listed in Table V.[17b] The expressions which relate the constants k_1, k_1' and k_1'' are based on the assumption of acid–base equilibrium between the species T^+, T^0, and T^-.

The general pH profile is reduced to more familiar profiles in some special cases. For example, if $P^+ = P^0 = P^-$, eq 4 becomes identical in form with that for the summation of acid, base, and uncatalyzed reactions (eq 5), and no change in rate-limiting step occurs with

$$\frac{k_{obsd}}{f} = k_1'P^+\left(1 + \frac{K''}{[H^+]} + \frac{[H^+]}{K'}\right) \tag{5}$$

changing pH. If $P^0 = P^-$, regions E and F vanish and the pH-independent region D gives way to a simple base-catalyzed reaction at higher pH. This profile has been observed in the lactonization of coumarinic acids[3] and is consistent with a mechanism involving a change in rate-determining step in acid solution ($P^+ \neq P^0$) but no further change in rate-limiting step at higher pH. As has been pointed out earlier,[3,10] the nature of the rate-limiting step in various pH ranges cannot be assigned on the basis of the kinetics alone, and additional information is needed. Put another way, identical curves may be generated by the use of the set of parameters (P^+, P^0, P^- or $1 - P^+$, $1 - P^0$, $1 - P^-$), with the consequent reversal of the assignment of the rate-limiting step.

The Mechanism of Lactonization. The simplest reaction mechanism which appears to be in accord with the kinetics of the lactonization of 5,8-dimethyl- and 4-methylcoumarinic acids is that previously employed for the lactonization of 13 coumarinic acids.[3] The main features of this proposal (Scheme I, upper two lines) are the inclusion of cationic (T^+) and neutral (T^0) intermediates, which partition differently between reactants and products (*i.e.*, $P^+ \neq P^0$). Consequently, there occurs a change in rate-limiting step as a funtion of pH (at pH = pK'). At pH >4, there begins a base-catalyzed reaction (in terms of neutral substrate), possibly, but not necessarily, involving an intermediate. The steady-state rate equation based on this mechanism (eq 6) may be used to generate pH-rate profiles which

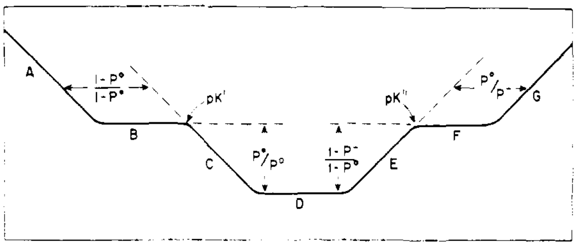

Figure 8. Schematic representation of the pH dependence of the rate of lactonization according to Scheme I and eq 4. The limiting regions of the pH profile have zero or unit slopes. The kinetic parameters are defined in Table V.

$$\frac{k_{obsd}}{f} = \frac{k_1([H^+]P^+ + K'P^0)([H^+] + K'(1 - P^0)/(1 - P^+))}{H^+ + K'} + B_{OH}[OH^-] \tag{6}$$

are in reasonable agreement with observation both for **2** (curve not shown) and **1** (Figure 2, dashed line). With the 5,8-dimethyl derivative, the parameters obtained from a least-squares curve fitting to eq 6 (Tables II) are slightly different from those previously[3] reported for this compound, owing to the more precise data now obtained at pH 3–5 using the pH-stat method.

4-Methylcoumarinic acid differs from all previously studied coumarinic acids in that its pH profile exhibits a *minimum* at pH 4–5. This unusual behavior does not reflect a change in mechanism but is simply a consequence of the high pK_a of the carboxyl group of **1**. Minima in the pH-rate profile will be observed whenever a base-catalyzed term in the rate law becomes important prior to extensive deprotonation of the substrate carboxyl group. When conversion of the substrate to its unreactive conjugate base form takes place, the opposing effects of increasing pH on the concentration of RCOOH and of hydroxide ion result finally in a constant rate of lactonization.

The rate law of eq 6 does not account for the kinetics of lactonization of 4,6,8-trimethylcoumarinic acid. Regardless of what values of the parameters k_1, P^+, P^0, and K' are chosen to account for the kinetics of lactonization at pH <2.5, the dependence of k_{obsd}/f on pH should follow the dashed line in Figure 3 if the previous mechanism were applicable to this compound. The positive deviations of the data in the pH range 2.5–4.5 are qualitatively similar to, though less obviously interpretable than, those seen in other reactions involving neutral and anionic intermediates *and* a change in rate-limiting step involving these intermediates. Whatever uncertainty exists in the pK_a of **3** does not affect these conclusions. The pK_a of **3** is certainly greater than 4.50, and most of the deviations shown in Figure

3B take place at pH <4.5, *i.e.*, in a pH range where little or no error in the calculation of k_{obsd}/f would be introduced by a small error in the pK_a of **3**.

The kinetics of lactonization of **3** were analyzed in terms of the complete mechanism of Scheme I and eq 4, in which it is assumed that three kinetically significant tetrahedral intermediates exist. This assumption is equivalent to the statement that $P^+ \neq P^0 \neq P^-$, and furthermore that significant differences occur between these constants. If P^0 and P^- were both near zero, the pH–rate profile would be dominated by region A–D (Figure 8), and the incursion of a species T^- in the reaction would be essentially imperceptible. Similarly, if $P^+ \simeq P^0$, regions D–G may be well defined, but the existence of a species T^+ probably could not be proven.

When eq 4 is used with the constants summarized in Table II, reasonable agreement is found between the experimental rate data and the calculated pH dependence of k_{obsd} (Figure 3A) or k_{obsd}/f (Figure 3B). In this case only, the use of a least-squares curve-fitting procedure did not lead to convergence, for reasons which are not clear. The calculated curve was achieved by trial and error variation of the six constants, and standard deviations were not calculated. The limits of error estimated for the various constants are not large, and are probably no more than ± 0.1 in pK' and pK'', ± 0.05 in P^0 and P^-, while P^- must be >0.995.

In summary, the principal evidence for the existence of an anionic intermediate in the lactonization of **3** consists of the positive deviations of the rate data from any rate law not including an anionic species which partitions differently from the neutral intermediate. The participation of cationic and neutral intermediates has been convincingly demonstrated with 17 other coumarinic acids.[2,3] Although the various portions of the pH–rate profile of **3** are not well separated, and the assignment of values to the rate and equilibrium constants cannot be made simply by inspection, the values required to give reasonable agreement between observed and calculated curves provide further support for the favored mechanism. For example, the ratio k_3/k_2 is large (*ca.* 2000), as expected[3] for coumarinic acids with electron-donating groups in the ring. The value of pK' (3.95) may be compared to those found for other methylated coumarinic acids (3–3.6) where this constant was unambiguously defined by the rate data. The relatively high value of P^0 is consistent with the trend in P^0 observed with a series of ring-substituted coumarinic acids.[3]

Reasoning as earlier,[3] *i.e.*, that an anionic intermediate is more likely to expel phenoxide than hydroxide ion, it follows that at pH <3, the rate-limiting step for the lactonization of **3** is the formation of an intermediate through neutral and cationic pathways. At pH >5, breakdown of T^- is mainly rate limiting (though not completely, since expulsion of phenoxide ion from P^- is only four times faster than expulsion of hydroxide ion). In the narrow pH range of 3–5, there is a transition from T^+ to T^0 to T^- as the predominant intermediate, although the neutral species T^0 never plays an important kinetic role in the reaction.

The participation of an anionic intermediate with **3** suggested that the pH–rate profile of other basic coumarinic acids (*i.e.*, those exhibiting relatively high values of P^0 when analyzed in terms of cationic and neutral intermediates only, using eq 6) might show kinetic evidence for T^- also. Least-squares curve fitting of the data for 4-methyl-, 5,8-dimethyl-, and 6-hydroxycoumarinic[3] acid to eq 4 yielded the constants summarized in Table II. The values of the parameters P^+, P^0, and k_1, deduced from the simpler two-intermediate analysis, do not change very much. Application of the three-intermediate scheme provides values of P^- which seem to be significant. In the case of 4-methylcoumarinic acid, the fit of the rate data to eq 4 (Figure 2, solid line) seems distinctly better than that to eq 6. Little improvement in fit was seen with the other two substrates. In all cases, pK'' is near pK', and the values of P^- are small but not negligible. Clearly, the pH–rate profiles of these three compounds do not offer unequivocal evidence for the existence of T^-, but in view of the probable participation of this species in the case of **3**, it is likely that Scheme I is generally valid for coumarinic acids. Reliable individual values of K'', P^-, and k_1'' can be obtained only when P^0 is sufficiently greater than P^- that curve fitting can distinguish between eq 4 and eq 6.

The formation of T^- from **3** may represent the first instance where kinetic evidence has been obtained for the existence of an anionic tetrahedral intermediate derived from a phenyl ester. It has long been known that ^{18}O exchange does not occur in the alkaline hydrolysis of phenyl benzoate.[19] This observation has been taken to mean that if an intermediate is formed, it breaks down predominantly (>99%) with expulsion of phenoxide ion, and isotope exchange does not occur. The low value of 3.7 for k_2''/k_3'' in the case of **3** is unexpected. Possibly, the driving force for the facile expulsion of hydroxide ion resides in the stability of the coumarin product. It would be of interest to determine if detectable ^{18}O exchange occurs in the alkaline hydrolysis of 4,6,8-trimethylcoumarin, as would be expected from the present results.

It is uncertain whether the slow acid- and water-catalyzed lactonizations of 4,5,7-trimethylcoumarinic acid (Table II) correspond to rate-limiting formation or decomposition of the intermediate. Space-filling models indicate unfavorable steric interactions between the 4- and 5-methyl groups in the coumarin product and in the tetrahedral intermediate. No evidence for a base-catalyzed reaction is seen at pH 1.8 (the highest pH studied), although base-catalyzed terms are already detectable at pH 2.5–3.0 with **3**. This suggests that the base-catalyzed lactonization of **4** is retarded by at least 20-fold (and possibly much more). Since the rate of alkaline hydrolysis of the corresponding coumarin is normal, it is probable that the equilibrium constant for the overall reaction is much lower with **4** than with **3**, and that steric factors cause destabilization of the tetrahedral intermediate (and of the transition state leading to it) as well as appreciable destabilization of the product.

Buffer Catalysis. The nonlinear dependence of rate on buffer concentration offers additional support for the existence of intermediates in the lactonization of coumarinic acids. Changes in rate-limiting step with increasing buffer concentration have been reported for many reactions involving intermediates at steady-state concentrations.[10,13–15,20] A transition from a step

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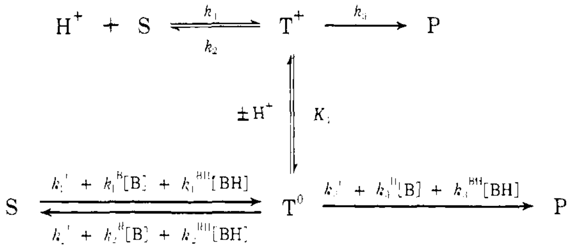
[19] C. A. Bunton and D. A. Spatcher, *J. Chem. Soc.*, 1079 (1956).

[20] (a) E. H. Cordes and W. P. Jencks, *J. Amer. Chem. Soc.*, **84**, 4319 (1962); (b) R. B. Martin, A. Parcell, and R. I. Hedrick, *ibid.*, **86**,

susceptible to buffer catalysis to one which is not catalyzed by buffer leads to a hyperbolic dependence of rate on buffer concentration. Frequently, both steps are catalyzed by buffer components, but to different extents, so that plots of k_{obsd} *vs.* [buffer] exhibit curvature and reach limiting lines of nonzero slope.

Reactions which undergo changes in rate-limiting step with pH and are catalyzed by buffers are generally expected to show curvature in the plots of k_{obsd} *vs.* [buffer].[20k] Consider a lactonization reaction proceeding *via* cationic (T^+) and neutral (T^0) intermediates, and susceptible to general acid–base catalysis (Scheme II).

Scheme II



At low pH, curvature will be observed if $k_3/(k_2 + k_3) \neq k_3^{\mathrm{BH}}/(k_2^{\mathrm{BH}} + k_3^{\mathrm{BH}})$ or $P^+ \neq P^{\mathrm{BH}}$, *i.e.*, if the nature of the rate-limiting step at zero buffer concentration is not identical with that of the step at high buffer. Similarly, curvature will be seen at high pH if $k_3'/(k_2' + k_3') \neq k_3^{\mathrm{B}}/(k_2^{\mathrm{B}} + k_3^{\mathrm{B}})$, *i.e.*, $P^0 \pm P^{\mathrm{B}}$. Note that if $P^+ = P^{\mathrm{BH}}$, and $P^0 = P^{\mathrm{B}}$, the rate-limiting step does not change with increasing buffer concentration at the extremes of pH, and no curvature is expected there. However, curvature will be seen at intermediate pH values, except for the fortuitous and unlikely situation where the change in rate-limiting step at high buffer occurs precisely at the same pH as that of the change in rate-limiting step at zero buffer. In favorable cases, analysis of data such as those of Figures 5 and 6 will lead to values for the partitioning ratios (P^{B} and P^{BH}) of the intermediate in the presence of general acid–base catalysts, in much the same way as the analysis of the pH–rate profiles at zero buffer led to values for the partitioning ratios P^+ and P^0 (see Appendix).

In experiments where the plot of k_{obsd} *vs.* [buffer] showed little or no curvature, straight lines were drawn through the data at high buffer concentration, and their slopes are given in Table III, column 7. Division of these slopes by the mole fraction of substrate in neutral form (f) and by the mole fraction of buffer in conjugate base form (α) yields essentially constant values for acetate catalysis with both coumarinic acids and for formate catalysis with the 5,7-dimethyl derivative (Table III, column 8; see also Figure 6, inset). Since significant curvature is not observed at these pH values, the rate-limiting step is the same at zero buffer concentration and in the presence of buffer, *i.e.*, breakdown of the tetrahedral intermediate is rate limiting at all buffer concentrations. The constancy of the corrected final slopes indicates that this step is subject solely to general base catalysis. In the lactonization of *o*-hydroxyphenylpropionic acids, the corresponding step was found to be subject to both general acid and base catalysis, although base catalysis was the more important of the two processes.[9a]

If, at all pH values, the buffer-catalyzed reaction consists mainly of general base catalysis which occurs with rate-limiting breakdown of the intermediate, curvature in the plot of k_{obsd} *vs.* [buffer] should increase with decreasing pH, as the rate-limiting step at zero buffer changes from breakdown to formation of the intermediate. The experiments summarized in Figures 5 and 6 are, at least qualitatively, consistent with this expectation. For these curved plots, the data at each pH were fitted by least squares to eq 11, using previously calculated values of the parameters describing the reaction at zero buffer concentration. Values of k_1^{B} and P^{B} (Table III) were calculated for each pH from the pairs of L and N constants, using eq 12 and 14 and the assumption that both formation and breakdown of intermediates are susceptible only to general base catalysis (*i.e.*, $k_1^{\mathrm{BH}} = 0$).

Satisfactory agreement is found between the curves calculated from eq 10 and the experimental observations (Figures 5 and 6). In all cases, P^{B} is small, *i.e.*, $k_2^{\mathrm{B}} > k_3^{\mathrm{B}}$, and the rate-limiting step in the buffer-catalyzed lactonization is the general base catalyzed breakdown of T^0 (or a kinetic equivalent). The reasonably constant values of k_1^{B} support the assumption that general acid catalysis of the formation step is not significant, although a minor contribution cannot be ruled out.

The term $k_1^{\mathrm{B}}P^{\mathrm{B}}$ [$= k_1^{\mathrm{B}}k_3^{\mathrm{B}}/(k_2^{\mathrm{B}} + k_3^{\mathrm{B}})$] may be evaluated either from the limiting slope of the buffer plots after correction for substrate and buffer ionization, or from the separate values of k_1^{B} and P^{B} calculated from the least-squares fit to eq 11. For reasons which are not understood, poor agreement was found in the formate catalysis experiments between the values of $k_1^{\mathrm{B}}P^{\mathrm{B}}$ calculated by the curve-fitting procedure at low pH and those obtained directly from the linear buffer plots at high pH. Possibly, the plots of k_{obsd} *vs.* [buffer] at low pH have not yet reached a limiting slope, suggesting that the deduced values of k_1^{B} and P^{B} should be considered only approximate. The general conclusions concerning the nature of the rate-limiting step are not expected to be significantly affected by this uncertainty.

Nonlinear increases in rate with increasing formate buffer concentration (pH 3.0, 33% free base) were seen with three different methyl-substituted coumarinic acids, but not with 6-nitrocoumarinic acid (Figures 5 and 7). With the latter compound, lactonization proceeds with rate-limiting breakdown of intermediates throughout the entire pH range studied,[3] so that a change in rate-determining step with increasing formate buffer is not expected, and none is seen.

In view of the linear dependence of rate on buffer concentration observed with the 6-nitro compound, it is probable that the nonlinear rate increases observed[10] under nearly identical experimental conditions for thiolactonization and the hydrolysis of a ketene-*O,S*-acetal were the result of a change in rate-determining step, and not of buffer aggregation. Others[21] have suggested

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2406 (1964); (c) W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, **86**, 5616 (1964); (d) R. B. Martin, R. I. Hedrick, and A. Parcell, *J. Org. Chem.*, **29**, 3197 (1964); (e) G. E. Lienhard and W. P. Jencks, *J. Amer. Chem. Soc.*, **87**, 3855 (1965); (f) S. O. Eriksson and C. Holst, *Acta Chem. Scand.*, **20**, 1892 (1966); (g) D. R. Robinson and W. P. Jencks, *J. Amer. Chem. Soc.*, **89**, 7098 (1967); (h) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, p 477; (i) W. P. Jencks and K. Salvesen, *J. Amer. Chem. Soc.*, **93**, 6294 (1971); (k) T. Okuyama and G. L. Schmir, *ibid.*, **94**, 8805 (1972); (l) D. J. Hupe, M. C. R. Kendall, G. T. Sinner, and T. A. Spencer, *ibid.*, **95**, 2260 (1973).

(21) (a) M. M. Kreevoy and D. E. Konasewich, *J. Phys. Chem.*, **74**, 4464 (1970); (b) W. J. Albery, J. S. Curran, and A. D. Campbell-Crawford, *J. Chem. Soc., Perkin Trans. 2*, 2185 (1972).

that the nonlinear effects of acetate buffer on the hydrolysis and methanolysis of ketone-*O*,*O*-acetals, ascribed[22] to buffer aggregation, may also represent changes in rate-determining steps.

Experimental Section

4-Methylcoumarin was prepared by the method of von Pechmann and von Krafft,[23] using 70% sulfuric acid, and was recrystallized from benzene–petroleum ether; mp 78–80° (lit.[23] mp 81–82°).

4,6,8-Trimethylcoumarin,[24] prepared from 2,4-xylenol and ethyl acetoacetate, and recrystallized from ethanol, had mp 115.5–116.5° (lit.[24] mp 116–117°); mass spectrum molecular ion at *m/e* 188. **4,5,7-Trimethylcoumarin,**[24] similarly obtained from 3,5-xylenol, had mp 174–175° (lit.[24] mp 175–176°).

Other coumarins were samples used in the previous study.[3]

Deuterium chloride (38% in D_2O, >99% D) and deuterium oxide (99.7% D) were obtained from Merck, Sharp and Dohme of Canada, Ltd.

Kinetic Measurements. The kinetic methods were as previously described.[3] Owing to their low solubility and large equilibrium constant for lactone formation, stock solutions of **1** and **3** were prepared in 50% ethanol and contained 0.1 *M* NaOH. The addition of 0.015 ml of stock solution was used to initiate the lactonization reaction. The final concentration of substrate was 1–2 × 10^{-4} *M*.

All reaction rates were measured spectrophotometrically (at 310 nm for compounds not previously described) with a total absorbance change of 0.4–0.8 unit. For experiments in which the pH was kept constant by means of a Radiometer TTT 1a pH-stat,[5] 0.015 ml of stock solution was added to 20 ml of aqueous 1.0 *M* LiCl and 0.01 *M* HCl (μ = 1.0, LiCl) was used as titrant. Approximately 0.2 ml of titrant was consumed per reaction.

pK_a values were determined by spectrophotometric titration as previously described.[3]

For the determination of the deuterium solvent kinetic isotope effect, constant pH was maintained either with DCl or with the pH-stat. In 0.005–0.01 *M* HCl or DCl solutions (30°, μ = 1.0, LiCl) eq 7 or 8 were obeyed. k_H/k_D ratios were calculated on the basis of acid *concentrations*. Therefore, kinetic parameters previously[3] based on the *activity* of hydrogen ion were multiplied by 1.622 to convert to a concentration-based scale. When the concentration of DCl was known, it was used directly; otherwise DCl concentration was calculated from the pH meter reading and eq 8. A term of 0.40 log unit, which relates (pH meter reading) D_2O to pD has been previously determined at low ionic strength, where the activity and concentration scales for hydronium ion do not differ.[25,26] Because the activity and concentration of hydrogen ion are unequal in 1 *M* LiCl at 30° (eq 7), the expression for pD is given by eq 9.

$$(\text{pH meter reading})_{H_2O} = -\log[\text{HCl}] - 0.21 \tag{7}$$

$$(\text{pH meter reading})_{D_2O} = -\log[\text{DCl}] - 0.43 \tag{8}$$

$$\text{pD} = (\text{pH meter reading})_{D_2O} + 0.22 \tag{9}$$

Acknowledgment. We are grateful to Mr. Tad S. Stashwick for skillful assistance with the kinetic studies.

Appendix

For a lactonization reaction following Scheme II, the steady-state rate equation relating k_{obsd} to buffer concentration and pH (eq 10) contains eight independent parameters.

$$\frac{k_{\text{obsd}}}{f} = \frac{\left[k_1[\text{H}^+] + k_1' + \frac{[\text{B}_\text{T}]K_a}{([\text{H}^+] + K_a)}\left(k_1{}^\text{B} + k_1{}^\text{BH}\frac{[\text{H}^+]}{K_a}\right)\right] \times \left[[\text{H}^+]P^+ + K'P^0 + \frac{[\text{B}_\text{T}]K_a}{([\text{H}^+] + K_a)}\frac{K'(1 - P^0)}{k_1'}\left(\frac{P^\text{B}k_1{}^\text{B}}{1 - P^\text{B}} + \frac{P^\text{BH}k_1{}^\text{BH}[\text{H}^+]}{(1 - P^\text{BH})K_a}\right)\right]}{[\text{H}^+] + K' + \frac{[\text{B}_\text{T}]K_a}{([\text{H}^+] + K_a)}\left[\frac{K'(1 - P^0)}{k_1'}\left(\frac{k_1{}^\text{B}}{1 - P^\text{B}} + \frac{k_1{}^\text{BH}[\text{H}^+]}{(1 - P^\text{BH})K_a}\right)\right]} \tag{10}$$

$$\frac{k_{\text{obsd}}}{f} = \frac{[k_1[\text{H}^+] + k_1' + [\text{B}_\text{T}]L][[\text{H}^+]P^+ + K'P^0 + [\text{B}_\text{T}](K'(1 - P^0)/k_1')M]}{[\text{H}^+] + K' + [\text{B}_\text{T}][(K'(1 - P^0)/k_1')N]} \tag{11}$$

Four parameters (k_1, k_1', P^0, and P^+) describe the reaction at zero buffer concentration and are defined in Table V. K' is related to the other constants as shown in Table V. Four parameters ($k_1{}^\text{B}$, $k_1{}^\text{BH}$, P^B, and P^BH) describe the general acid and general base catalyzed reaction [$P^\text{B} = k_3{}^\text{B}/(k_2{}^\text{B} + k_3{}^\text{B})$; $P^\text{BH} = k_3{}^\text{BH}/(k_2{}^\text{BH} + k_3{}^\text{BH})$]. f is the mole fraction of coumarinic acid in the neutral form; K_a is the acid dissociation constant of the buffer; $[\text{B}_\text{T}] = [\text{B}] + [\text{BH}]$.

Equation 10 may be rewritten as eq 11 where all constants are known from the pH-rate profile at zero buffer concentration, except for L and N (see eq 12–15).

$$L = k_1{}^\text{B}\alpha + k_1{}^\text{BH}(1 - \alpha) \tag{12}$$

$$M = \frac{P^\text{B}k_1{}^\text{B}\alpha}{1 - P^\text{B}} + \frac{P^\text{BH}k_1{}^\text{BH}(1 - \alpha)}{1 - P^\text{BH}} \tag{13}$$

$$N = \frac{k_1{}^\text{B}\alpha}{1 - P^\text{B}} + \frac{k_1{}^\text{BH}(1 - \alpha)}{1 - P^\text{BH}} \tag{14}$$

$$M = N - L \tag{15}$$

$$\alpha = \frac{K_a}{[\text{H}^+] + K_a} \tag{16}$$

Each plot of k_{obsd}/f *vs.* $[\text{B}_\text{T}]$ at constant pH is determined by two constants only (L and N), assuming that the zero buffer parameters have been independently determined. The four buffer-related parameters are easily calculated from the intercepts at at $\alpha = 0$ and $\alpha = 1$ of plots of L and N *vs.* α. The limiting slope reached at high buffer concentration is given by $L(N - L)/N$, which is equal to $k_1{}^\text{B}P^\text{B}\alpha$ when general acid catalysis is absent.

Supplementary Material Available. Table I will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-73-8032.

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[22] (a) V. Gold and D. C. A. Waterman, *J. Chem. Soc. B*, 839, 849 (1968); (b) V. Gold and S. Grist, *ibid.*, 2272 (1971).

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