spectrum; see Table III). Purification was accomplished by evaporative distillation or preparative glpc, but the two isomers could not be separated.

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Stereopopulation Control. I. Rate Enhancement in the Lactonizations of *o*-Hydroxyhydrocinnamic Acids[1,2]

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Abstract: The kinetics of lactonization of a series of *o*-hydroxyhydrocinnamic acids have been studied in the pH range 7–8 (30°, 20% dioxane–imidazole buffer) and in the pH range 6–7 without buffer. Concurrent, but not concerted, catalysis by both the acidic and basic forms of the buffer is observed. The introduction of appropriate alkyl substitution on both the aromatic ring and the side chain leads to rate-enhancement factors as high as 5×10^{10}; in comparison with the bimolecular esterification of phenol with acetic acid, the rate enhancement is greater than 10^{15} *M*. In the most favorable case studied, the half-time for formation of 4,4,5,7,8-pentamethylhydrocoumarin (pH 7) is 6 sec, with 90% of the total rate being due to buffer catalysis. The effect is attributed to a unique interlocking of methyl groups, which produces a severe conformational restriction of the side chain and a ground-state geometry highly favorable to formation of the transition state. Analysis of rate data suggests that the conformational effect operates, primarily, to increase the steady-state concentration of tetrahedral intermediate, but also causes the intermediate to be more sensitive to acid than to base catalysis in the rate-determining breakdown step. This phenomenon is presented as a model for the conformational restraint imposed by an enzyme on its substrate, and for the large acceleration effects resulting therefrom.

The rate constants for many enzyme-catalyzed reactions have been estimated to exceed those for their nonenzymatic, bimolecular counterparts by factors of 10^{10} to 10^{18}.[3] In some instances, such comparison is impossible because a close nonenzymatic parallel is undemonstrable, or is immeasurably slow, under reasonable conditions of pH, temperature, and solvent. The "magical" action of an enzyme on its substrate has been attributed,[3,4] principally, to the combined effects of (a) reduction in kinetic order (formation of an enzyme–substrate complex); (b) activation of the substrate by distortion of ground-state geometry or of electron-density distribution; (c) general acid–general base catalysis (possibly concerted) by protein functional groups; (d) favorable polarity of the active-site region; and (e) restriction of conformational freedom of the substrate. Rough estimates of the rate enhancement values of these factors, individually, have been based on the kinetic characteristics of many nonenzymatic model reactions; despite the use of liberal estimates, however, a sizable portion of the total rate enhancement achieved by the enzyme has yet to be justified.

Our immediate concern is with the rate-enhancement factor associated with the severe loss of conformational freedom of a substrate, resulting from its binding to an enzyme. The enzyme has available a variety of devices for binding a substrate tightly, while manipulating its conformation: attractive forces, such as covalent-bond, hydrogen-bond, and metal-chelate; forces which may be attractive or repulsive, such as electrostatic, van der Waals, dipole, and charge-transfer. The enzyme may be considered capable of "freezing" the substrate into a single conformation,[5] presumably that most favorable for achievement of the enzyme's mission. Although the rate-enhancement value of this factor has been estimated at 10^3 to 10^4, such magnitude is based on rather limited model data, and on systems in which the degree of conformational restriction fails to approach that possible in an enzyme–substrate complex.[6] Thus, a more detailed examination of the phenomenon of conformational restriction seems warranted.

Suitable intramolecular model systems require that

(1) A preliminary account of this work has been published: S. Milstien and L. A. Cohen, *Proc. Nat. Acad. Sci. U. S.*, **67**, 1143 (1970).

(2) For prior studies related to this investigation, see (a) S. Milstien and L. A. Cohen, *J. Amer. Chem. Soc.*, **91**, 4585 (1969); (b) *ibid.*, **92**, 4377 (1970).

(3) D. E. Koshland, Jr., and K. E. Neet, *Annu. Rev. Biochem.*, **37**, 359 (1968).

(4) (a) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, Chapter 1; (b) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, New York, N. Y., 1966, Chapter 1; (c) M. L. Bender, F. J. Kezdy, and C. R. Gunter, *J. Amer. Chem. Soc.*, **86**, 3714 (1964); (d) T. C. Bruice in "The Enzymes," Vol. II, 3rd ed, P. D. Boyer, Ed., Academic Press, New York, N. Y., 1970, Chapter 4.

(5) At least that portion of the substrate in contact with the enzyme's active site.

(6) Theoretical arguments have been advanced for rate-enhancement factors as high as 10^8 due to the "freezing" process: M. I. Page and W. P. Jencks, *Proc. Nat. Acad. Sci. U. S.*, **68**, 1678 (1971).

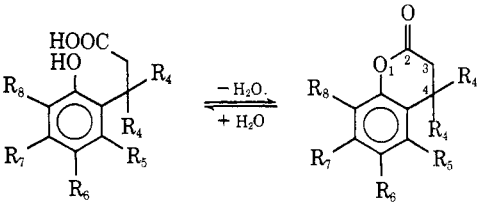
Table I. Hydrocoumarins

Compd	Mp or bp (mm), °C	Formula	Calcd, % C	Calcd, % H	Found, % C	Found, % H
2d	98–100 (0.2)[a]	$C_{12}H_{14}O_2$	75.76	7.42	75.24	7.62
2e	90–92[b]	$C_{13}H_{16}O_2$	76.44	7.90	76.69	7.82
2f	97–99[b]	$C_{13}H_{16}O_2$	76.44	7.90	76.47	7.97
2g	70–72[b]	$C_{14}H_{18}O_2$	77.03	8.31	76.93	8.10
2h	146–147[c]	$C_{14}H_{17}NO_4$[d]	63.86	6.51	63.58	6.44
2i	186–187[b]	$C_{14}H_{18}O_3$	71.77	7.74	71.77	7.53

[a] Lit. bp 165° (20 mm): J. Colonge, E. LeSech, and R. Marey, *Bull. Soc. Chim. Fr.*, 776 (1957). [b] Solid lactones were recrystallized from benzene or hexane. [c] Recrystallized from ether. [d] Calcd for N, 5.32%; found, 5.47%.

conformational freedom at a reaction site be limited by manipulation from a distance, such that, ideally, neither the electronic nor the steric properties of the reaction partners experience serious alteration. One logical approach, the Thorpe–Ingold or *gem*-dialkyl effect, makes use of the phenomenon of van der Waals repulsion.[7] The favorable influence of appropriate alkyl substitution, at a distance, on both rates and equilibria of intramolecular reactions has been known for at least 50 years; rate enhancements achieved in this way cover a wide range, extending beyond 10^5 in rare cases.[8] van der Waals repulsion, however, is only one of a number of devices available to the investigator for controlling the freedom of rotation of single bonds from a distance; others include hydrogen bonding, electrostatic attraction or repulsion, lone-pair repulsion, and resonance overlap. Because of the diversity of factors which can be used to produce the same experimental result (rate enhancement), we have found it desirable to introduce the more general term *stereopopulation control* to include all such devices. In this paper, and in subsequent papers of the series, we shall describe a variety of model systems in which severe narrowing of the distribution of conformer populations leads to rate enhancements of magnitude sufficient to complete the accounting of enzymic catalysis, or at least to reduce the deficit considerably.

In 1969, Thanassi and Cohen[9] proposed a scheme for mitochondrial oxidative phosphorylation which required, in part, the esterification of ubihydroquinone with a nonactivated protein carboxyl group. Support for the proposal was sought by simulation in models. As a bimolecular process, esterification in a phenol–carboxylic acid system is extremely unfavorable both in rate and in equilibrium. The intramolecular parallel, *o*-hydroxyhydrocinnamic acid (**1a**), although lactonizing extensively in strong mineral acid,[10] shows no significant tendency to do so under the mild conditions of a biological system; accordingly, alkyl-substituted analogs were investigated.[2] The introduction of 4,4-dimethyl groups (numbered as in **2**)[11] promoted lactonization to a degree which prompted a study of even more extensively alkylated systems. The rates and equilibria for lactonization of the phenolic acids **1a–h** have been examined in detail and provide the basis for this report.



1 **2**

a, $R_4 = R_5 = R_6 = R_7 = R_8 = H$
b, $R_4 = R_6 = H$; $R_5 = R_7 = R_8 = CH_3$
c, $R_5 = R_6 = R_7 = R_8 = H$; $R_4 = CH_3$
d, $R_5 = R_6 = R_7 = H$; $R_4 = R_8 = CH_3$
e, $R_6 = R_8 = H$; $R_4 = R_5 = R_7 = CH_3$
f, $R_6 = R_7 = H$; $R_4 = R_5 = R_8 = CH_3$
g, $R_6 = H$; $R_4 = R_5 = R_7 = R_8 = CH_3$
h, $R_4 = R_5 = R_7 = R_8 = CH_3$; $R_6 = NO_2$
i, $R_4 = R_5 = R_7 = R_8 = CH_3$; $R_6 = OH$

Experimental Section[12]

4,4-Dimethylhydrocoumarins (2d–g, 2i) were prepared by alkylation of the appropriate phenols with the methyl ester of 3-methylcrotonic acid, according to the method previously described.[2b] Yields of purified lactones ranged from 10 to 30%, no effort being made to effect improvements. Physical and analytical data are given in Table I. Hydrocoumarin (**2a**) was obtained commercially, and **2c** has been described previously.[2b]

5,7,8-Trimethylhydrocoumarin (2b). Although direct alkylation of certain phenols with acrylonitrile[13] or with methyl acrylate[14] has been achieved, these methods failed with 2,3,5-trimethylphenol; however, condensation of the phenol with malic acid, according to the method of Clayton,[15] provided a 50% yield of 5,7,8-trimethylcoumarin, mp 104–106° (hexane).[16]

Anal. Calcd for $C_{12}H_{12}O_2$: C, 76.57; H, 6.43. Found: C, 76.31; H, 6.26.

Since catalytic hydrogenation of the coumarin proved to be very slow, the lactone ring was opened, prior to reduction of the double bond, by heating a solution of the coumarin in 50% aqueous ethanol, to which had been added a slight excess of sodium hydroxide. Hydrogenation of the phenolic acid salt with palladium–charcoal then proceeded rapidly to completion. Following removal of catalyst and ethanol, the aqueous solution was acidified to pH 1, the mixture was heated at reflux for 2 hr, and the lactone was isolated by ether extraction. The solvent was evaporated and the product was recrystallized from hexane, mp 68–70°. Homogeneity was confirmed by tlc and mass spectroscopy.

Anal. Calcd for $C_{12}H_{14}O_2$: C, 75.76; H, 7.42. Found: C, 75.87; H, 7.24.

Nitration of 4,4,5,7,8-Pentamethylhydrocoumarin (2g). Nitration of the lactone, by use of fuming nitric acid in acetic anhydride–acetic acid solution at −5°,[2b] provided two isomeric products which were separated by silica gel chromatography (elution with ether). The faster moving product was the desired 6-nitrolactone (**2h**), obtained in 50% yield: nmr ($CDCl_3$) 1.50 (6, s, 4,4-$(CH_3)_2$),

(7) For a comprehensive bibliography, see ref 1.
(8) J. F. Bunnett and T. Okamoto, *J. Amer. Chem. Soc.*, **78**, 5363 (1956).
(9) (a) J. W. Thanassi and L. A. Cohen, *Biochim. Biophys. Acta*, **172**, 389 (1969); (b) *J. Amer. Chem. Soc.*, **89**, 5733 (1967).
(10) H. Hochstetter, *Justus Liebigs Ann. Chem.*, **226**, 355 (1884).
(11) For the sake of clarity and consistency, the chroman numbering system (as in **2**) has been retained for the phenolic acids (**1**).
(12) All analyses were performed by the Analytical Services Section of this laboratory, under the direction of Dr. W. C. Alford. Melting points and boiling points are uncorrected.
(13) K. Sato, T. Amakusu, and S. Abe, *J. Org. Chem.*, **29**, 2971 (1964).
(14) T. Amakusu and K. Sato, *ibid.*, **31**, 1433 (1966).
(15) A. Clayton, *J. Chem. Soc.*, **93**, 2016 (1908).
(16) Prepared by another route, mp 110–111°: G. Manecke and G. Bourwieg, *Chem. Ber.*, **92**, 2958 (1959).

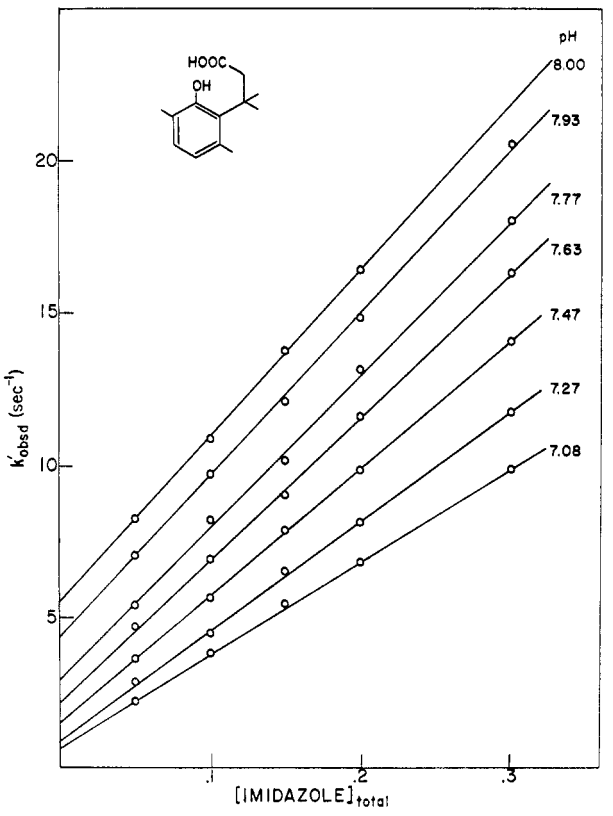


Figure 1. Typical set of buffer dilution plots for lactonization (**1f**); k'_{obsd} includes adjustment for f_{RCOOH}.

2.18 (3, s, 8-CH_3), 2.27 (3, s, 7-CH_3), 2.33 (3, s, 5-CH_3), and 2.62 ppm (2, s, 3-CH_2).

The second nitration product was obtained in 38% yield, mp 124–125° (ether): nmr ($CDCl_3$) 1.43 (6, s, 4,4-$(CH_3)_2$), 2.28 (3, s, 8-CH_3), 2.32 (3, s, 7-CH_3), 2.60 (2, s, 3-CH_2), 5.65 (2, s, 5-CH_2NO), and 7.00 ppm (1, s, 6-H). The spectrum is indicative of nitration of one of the aromatic methyl groups, probably that at C-5 (**4**; see Discussion).

Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.86; H, 6.51; N, 5.32. Found: C, 63.69; H, 6.41; N, 5.47.

Phenolic Acid Salts. The very rapid lactonization and favorable equilibria made it impossible to isolate salts of the phenolic acids, except in the cases of **1a–1d**. Stock solutions for kinetic runs were prepared by heating, in sealed tubes, solutions of the lactones in 50% aqueous ethanol, to which had been added 2 equiv of sodium hydroxide. These mixtures were heated at 100° for 10–15 hr, at which point uv spectra showed ring opening to be complete. In a large excess of 1 *N* sodium hydroxide (aqueous ethanol), ring opening proceeded readily at 25°. Solutions stored at strongly alkaline pH, at −5°, showed only a slight tendency to relactonize over a period of several weeks.

The sodium salt of **1d** was isolated by the procedure previously described.[2b]

Anal. Calcd for $C_{12}H_{15}O_3Na$: C, 62.60; H, 6.53. Found: C, 62.35; H, 6.71.

Despite the use of anaerobic conditions, a stock solution of the salt of the hydroquinone acid (**1i**) could not be obtained free of the corresponding quinone. Attempts were made to generate **1i**, *in situ* in a sealed uv cell, by reduction of the pure quinone[17] with sodium borohydride at high pH. Immediately following reduction, however, the uv spectrum was identical with that of the lactone.

Kinetic Measurements. A description of the apparatus, kinetic methods, and calculations has been published.[2b] Because of the low solubility of the lactones used in this study, all buffers were prepared with 20% (by volume) of purified dioxane.[18] Several comparative runs showed that this quantity of dioxane had no significant effect on rate data. All measurements were made at 30° and at a total ionic strength of 0.3 *M* (NaCl or LiCl).[19] Buffer solutions were prepared with imidazole which had been twice recrystallized from benzene. Rates of lactonization were measured spectrophotometrically by following the increase in absorption at 240–260 nm (lactone formation), or the decrease in absorption at 280 nm (phenol consumption). Some kinetic measurements were also made, in the absence of buffer, by use of pH-Stat–spectrophotometer combination.[20]

pK_a Measurements. The pK_a of imidazole, in 20% dioxane–water (μ = 0.3 *M*), was found to be 7.22. It was not possible to determine pK_a values for the carboxyl groups of most of the phenolic acids owing to their rapid lactonization in the requisite pH range.[21] The pK_a's of **1c** and **1d** in 20% dioxane–water (μ = 0.3 *M*, 30°) were found to be 5.00 ± 0.05. This value was taken for the remainder of the compounds. In another investigation,[22] the pK_w in 40% dioxane (μ = 0.3 *M*, 30°) was found, from spectroscopic data, to be 14.81. By combination of this value and the data of Harned and Fallon,[23] an extrapolated value of 14.40 was estimated for 20% dioxane (μ = 0.3 *M*, 30°).

Results

Rates of lactonization were followed spectrophotometrically with control of temperature, pH, buffer concentration, and ionic strength. In our earlier study of this reaction with **1c** and its para-substituted derivatives, specific rate constants had been obtained for the spontaneous (or water-catalyzed) reaction (k'_{H_2O}),[24] for catalysis by hydronium ion ($k'_{H_3O^+}$), and for catalysis by the components of formate and acetate buffers. Lactonization had been studied up to pH 6, the reaction becoming quite slow at higher pH values. On the other hand, *o*-hydroxyhydrocinnamic acids containing the 4,4,5-trimethyl system (**1e–h**) lactonize so rapidly that rate data could not be obtained conveniently *below* pH 7. Lactonization of the latter group of acids was followed in imidazole buffer in the pH range 7–8 and, as in the case of **1c**, concurrent and independent catalysis by both buffer species was observed. For the pH range 6–7, a few kinetic runs were performed in buffer-free media, pH being maintained constant by use of a pH-Stat.[20]

The cyclizations of **1c–h** were found to follow pseudo-first-order kinetics essentially to completion. Infinity spectra for **1e–h** were identical with those of the lactones, recorded under the same conditions. Infinity spectra for **1a–d** reached equilibrium values; kinetic data were adjusted accordingly (see below). Values of k_{obsd} were divided by f_{RCOOH} to provide k'_{obsd}.[24] The latter values were plotted against total buffer concentration for each pH value, providing a series of nonparallel slopes (k'_{Im_T}), which are illustrated in Figure 1 and which obey eq 1. Values of k'_{Im_T} and of k'_0

$$k'_{obsd} = k'_{Im_T}[Im_T] + k'_0 \quad (1)$$

(the buffer-independent rate constant) were obtained by least squares analysis, correlation coefficients generally exceeding 0.995. Secondary plots of k'_{Im_T} *vs.*

(17) See paper III: R. T. Borchardt and L. A. Cohen, *J. Amer. Chem. Soc.*, **94**, 9175 (1972).

(18) W. Dasler and C. D. Bauer, *Ind. Eng. Chem., Anal. Ed.*, **18**, 52 (1946).

(19) Essentially identical rates and equilibria were obtained with either salt.

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

(21) pK_a Values for the phenolic groups are reported in paper II of this series: R. T. Borchardt and L. A. Cohen, *J. Amer. Chem. Soc.*, **94**, 9166 (1972).

(22) See following paper, part II (ref 21).

(23) H. S. Harned and L. D. Fallon, *ibid.*, **61**, 2374 (1939); see also E. M. Wooley, D. G. Hurkot, and L. G. Hepler, *J. Phys. Chem.*, **74**, 3908 (1970).

(24) Throughout this report, k' refers to specific rate constants which have been adjusted to the fraction of undissociated carboxyl species (f_{RCOOH}) present in a given medium.

Table II. Summary of Specific Rate Constants for Lactonization (M^{-1} sec^{-1})[a]

Compd	$k'_{H_3O^+}$	$k''_{H_3O^+}$[b]	k'_{ImH^+}	k''_{ImH^+}	k'_{Im}	k''_{Im}	k'_{OH^-}	k''_{OH^-}	k'_{H_2O}[c]	k''_{H_2O}[c]
1a	5.9×10^{-6} [d]		3.5×10^{-10} [e]							
1b	4.0×10^{-5} [d]	5.9×10^{-6}								
1c	2.62×10^{-2} [f]		1.25×10^{-6}		1.12×10^{-2}		204		3.6×10^{-5}	
1d	9.85×10^{-2}	2.63×10^{-2}	4.50×10^{-6}	1.31×10^{-6}	2.70×10^{-2}	0.98×10^{-2}	413	149	6.6×10^{-5}	2.6×10^{-5}
1e	5.0×10^{5}	2.8×10^{5}	2.70	1.60	14.4	10.9	3.0×10^{6}	2.3×10^{6}	2.0×10^{-2}	1.6×10^{-2}
1f	1.5×10^{6}	3.0×10^{5}	7.50	1.64	62.2	19.5	1.2×10^{7}	3.8×10^{6}	5.0×10^{-2}	1.8×10^{-2}
1g	2.0×10^{6}	3.0×10^{5}	13.2	2.22	64.9	17.7	1.4×10^{7}	3.8×10^{6}	6.0×10^{-2}	2.0×10^{-2}
1h	4.2×10^{5}	2.0×10^{6}	5.7	34.0	23.6	33.8	5.0×10^{6}	1.4×10^{7}	3.0×10^{-2}	6.4×10^{-2}

[a] At 30°, in 20% dioxane, $\mu = 0.3$ M; all rate constants have been adjusted to f_{RCOOH} and for equilibria, where applicable. [b] Values of k' which have been adjusted for the electronic and buttressing effects of ring substituents. [c] Sec^{-1}. [d] Calculated from rate constants for lactone hydrolysis and from equilibrium constants. [e] Extrapolated from $k'_{H_3O^+}$ and a Brønsted slope of 0.47. [f] From ref 2b.

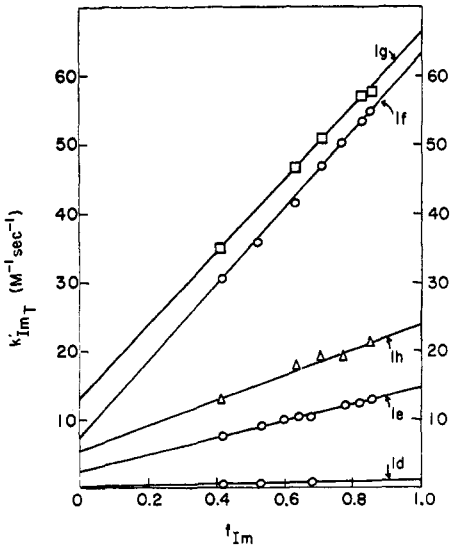


Figure 2. Plots of k'_{Im_T} (the slopes of Figure 1) *vs.* f_{Im}, the fraction of imidazole free base in the buffer at various pH values.

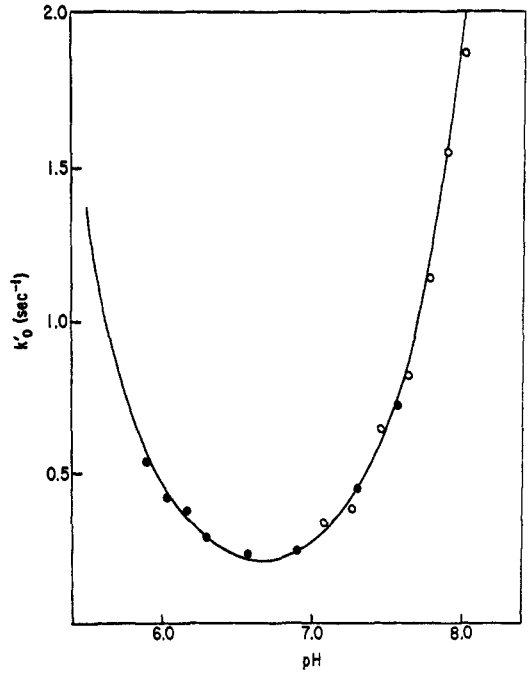


Figure 3. Typical plot of values of k'_0 *vs.* pH (for **1h**). The solid line shows the theoretical curve calculated from eq 3 and the data of Table II. Values shown as open circles are based on the intercepts of buffer dilution plots (Figure 1); those shown as solid circles are based on values of k_0 determined in buffer-free media.

mole fraction of imidazole free base in the buffer are shown in Figure 2. The lines of Figure 2 obey eq 2,

$$k'_{Im_T} = k'_{ImH^+}(f_{ImH^+}) + k'_{Im}(f_{Im}) \quad (2)$$

in which f_{Im} is the mole fraction of imidazole free base and f_{IMH^+} is the mole fraction of imidazolium ion present at a given pH. Thus, the y intercept (Figure 2) at $f_{Im} = 0$ provides k'_{ImH^+} and that at $f_{Im} = 1$ provides k'_{Im}.[25] A summary of specific rate constants (obtained by least-squares analysis) for catalysis by the imidazole buffer components is given in Table II.

The lack of coincidence of the y intercepts in Figure 1 suggests that, in addition to a spontaneous, or water-catalyzed, lactonization, possible contributions from hydronium and/or hydroxide ion catalysis must be considered. The intercepts of Figure 1 (k'_0) may be treated as summations of the several terms of eq 3.

$$k'_0 = k'_{H_2O} + k'_{H_3O^+}[H_3O^+] + k'_{OH^-}[K_w/H_3O^+] \quad (3)$$

This equation suggests that plots of k'_0 *vs.* $[H_3O^+]$ or pH should provide inverse bell-shaped curves, a portion of one such plot being shown in Figure 3. Because of the great increase in rates of lactonization at lower pH values, it was not practical to obtain values of k'_0 from buffer dilution plots below pH 7. Additional values of k'_0 (pH 6–7) were obtained from kinetic runs in buffer-free media. Results obtained by the two methods were in quite satisfactory agreement (Figure 3). The kinetics of lactonization above pH 8 was not explored because of the steepness of the slopes (Figure 3) and because of possible complications (in spectra and kinetics) resulting from phenol ionization. Approximate values for the specific rate constants of eq 3 were obtained by combination of trial and error and the use of a computer program for multiple-linear-regression analysis. In general six to eight values of k'_0 were used to provide the best values for the three unknowns of eq 3. The results are given in Table II for **1e–h**. A portion of the calculated curve (for **1h**) is shown as the solid line in Figure 3. Because of the errors involved in using small intercept values, as well as those of the curve-fitting analysis, these specific rate constants should be viewed as approximations, whose range may fall within a factor of 2–3. For the less reactive phenolic acids, **1c** and **1d**, $k'_{H_3O^+}$ and k'_{H_2O} were determined by direct measurement of rates of lactonization in dilute hydrochloric acid. For these compounds, k'_{OH^-} was calculated by difference in eq 3.

An effort was made to measure the rate of lacto-

(25) Reference 4a, p 165.

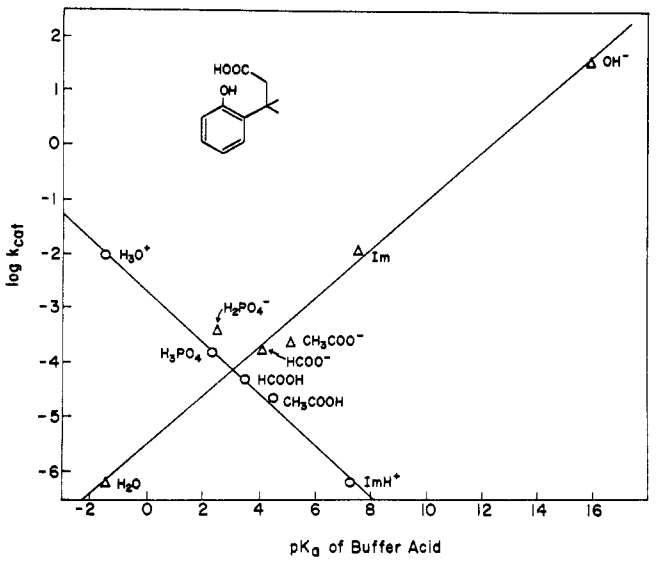


Figure 4. Brønsted plots for acid and base catalysis of lactonization of **1c**.

nization of the parent acid, **1a**. Even in 1 *N* hydrochloric acid, the process is almost immeasurably slow, the problem being compounded by an equilibrium position highly unfavorable to the lactone. The position of equilibrium in dilute hydrochloric acid was determined spectrophotometrically by approach from either direction at 5–6 acid concentrations. The amount of phenolic acid present at equilibrium (30°) was found to be 96.4 ± 1.4%. Since identical rates and equilibria were obtained in lithium chloride as in sodium chloride solution, specific cation effects appear to be absent. From the rate constant for acid hydrolysis of the lactone **2a** (1.65×10^{-4} M^{-1} sec^{-1}) and from the equilibrium constant, the rate constant for lactonization ($k'_{H_3O^+}$) of **1a** was calculated (Table II). A similar method was used in the case of **1b–2b** ($k^{hyd}_{H_3O^+} = 1.04 \times 10^{-4}$ M^{-1} sec^{-1}). Because the equilibrium constant for lactonization of **1b** is not as unfavorable as for **1a** (Table III), the rate constant for lactonization ($k'_{H_3O^+}$) could be measured directly for **1b**, providing a value of 4.2×10^{-5} M^{-1} sec^{-1}. For compounds containing the 4,4-dimethyl system (**1c**, **1d**), the lactone is strongly favored at equilibrium (*ca.* 96%); the corrections in lactonization rate due to equilibria are, therefore, relatively small. In the cases of **2e–h**, no ring opening in 1 *N* hydrochloric acid could be detected spectroscopically. The dependence of equilibrium position on the type of alkyl substitution is summarized in Table III.

Table III. Hydroxy Acid–Lactone Equilibria

Compound	Lactone or ester, %[a]	K_{eq}[b]	$\Delta F°$, cal/mol
Phenol + acetic acid[c]	3.8×10^{-4}	3.8×10^{-6}[d]	+7390
1a	3.6	0.0373	+1980
1b	38.3	0.621	+290
1c	96.2	25.67	−1950
1g[e]	>99	>99	<−2770

[a] At 30°, 20% dioxane, μ = 0.3 *M*. [b] Calculated as [lactone]/[hydroxy acid]; activity of water taken as 1.0. [c] At 25°, μ = 0; value of $\Delta F°$ taken from J. Gerstein and W. P. Jencks, *J. Amer. Chem. Soc.*, **86**, 4655 (1964). [d] M^{-1}. [e] Since no hydrolysis of the lactone could be detected in acidic media, the true values are probably larger than the minimal estimates given.

By combination of the specific rate constants for lactonization of **1c**, obtained in our earlier study,[2b] with the additional values reported here, a more extensive Brønsted plot could be constructed, as shown in Figure 4. It should be noted that the rate constant for general-base catalysis by $H_2PO_4^-$ is anomalous, being 10–20 times the predicted value. This observation suggests the occurrence of bifunctional catalysis in the breakdown of the tetrahedral intermediate,[26] a possibility which is being examined further. Brønsted slopes for the more reactive compounds, although based on the two or three points available in each case, show internal consistency and reasonable agreement with the much more reliable values for **1c** and **1d** (Table IV).

Table IV. Brønsted Slopes for Acid and Base Catalysis of Lactonization[a,b]

Compd	α	β	Compd	α	β
1c	0.47	0.48	**1f**	0.6	0.5
1d	0.48	0.48	**1g**	0.6	0.5
1e	0.6	0.5	**1h**	0.6	0.5

[a] Brønsted slopes for **1e–h** are approximate values, probably valid to ±0.1 unit. [b] Values of log k_{cat} and of pK_a are statistically corrected.

Before any comparisons could be made of specific rate constants resulting solely from conformational restriction, it was necessary to adjust the data of Table II for electronic and buttressing effects. Our previous study[2b] had shown that electron release into the phenolic ring of **1c** produces a modest increase in rate constants for lactonization. In order to extrapolate this factor to the other compounds, certain assumptions were made: (1) values of ρ for **1c** are applicable to all the compounds studied;[27] (2) the value of ρ for imidazolium ion catalysis is roughly comparable to that for acetic acid catalysis, −1.50;[2b] ρ for acetate ion (−0.80) is applicable to imidazole and hydroxide ion catalysis;[28] (3) for methyl groups at C-5 or C-7, a σ value of −0.076 is applicable throughout; for C-8 methyl, σ = −0.15; (4) the electronic effect of the propionic acid side chain on the ring is not significantly altered by alkyl substitution at C-4; (5) either by buttressing or by effecting a slight restriction of rotation of the phenolic hydroxyl, the 8-methyl group introduces a steric rate enhancement factor of 2.1. This factor is the ratio of $k'_{H_3O^+}$(**1d**)/$k'_{H_3O^+}$(**1c**), following adjustment for electronic effects in **1d**. The specific rate constants obtained by application of these adjustments are given in Table II as k'' values.

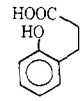
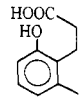
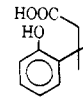
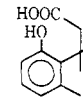
The nitro group of **1h** is undoubtedly twisted out of coplanarity with the benzene ring by the adjacent methyl

(26) For example, B. A. Cunningham and G. L. Schmir, *J. Amer. Chem. Soc.*, **88**, 551 (1966). For a comprehensive bibliography, see P. R. Rony, *ibid.*, **91**, 6090 (1969).

(27) The further assumption is made that ρ is the same for 20% dioxane–water as for water alone.

(28) Values of ρ include contributions due to electronic effects on the steady-state concentration of the tetrahedral intermediate as well as those influencing the rate-determining (buffer-catalyzed) breakdown of the intermediate.[2b]

Table V. Effect of Conformational Restriction on Lactonization Rate[a,b]

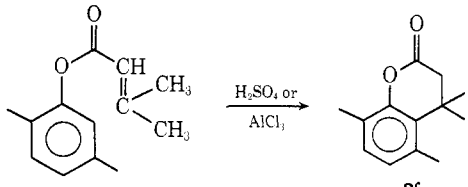
	Phenol + acetic acid	 1a	 1b	 1c	 1g
$k'_{H_3O^+}$	1×10^{-10} [c]	5.9×10^{-6}	5.9×10^{-6}	2.6×10^{-2}	3.0×10^{5}
k'_{rel}		1	1	4400	5.1×10^{10}
k'_{ImH^+}		3.5×10^{-10}		1.2×10^{-6}	2.2
k'_{rel}		1		3430	6.3×10^{9}

[a] Specific rate constants (k', M^{-1} sec^{-1}) taken from Table II; for **1b** and **1g**, values of k'' were used. [b] Since k'' includes corrections for electronic and buttressing effects, only that portion of the molecule contributing to conformational rate enhancement is shown. [c] M^{-2} sec^{-1}; for the source of this value, see Discussion.

groups. A modified σ value for this substituent of +0.90 was derived from the pK_a of 3,5-dimethyl-4-nitrophenol (8.25).[29] Even after making the various adjustments, the specific rate constants for lactonization of **1h** are somewhat greater than expected. This enhancement may be the result of a small buttressing effect of the 6-nitro group on the adjacent 5-methyl. By comparison with the exponential rate-enhancement factors obtained, all such adjustments are minor; thus, any errors in the electronic parameters or assumptions would have a negligible effect on the overall conclusions of the study.

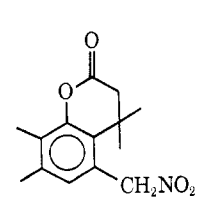
Discussion

The methods available for the synthesis of *o-tert*-butyltoluene or of its functional analogs are extremely limited in number and in scope.[30] The facile approach to this system, as represented by **2e–i**, was achieved by acid-catalyzed alkylation of the appropriate phenol with methyl 3-methylcrotonate, leading directly to the substituted hydrocoumarin. A probable intermediate is the phenolic ester (**3**) which can itself be



3 2f

cyclized to the same product in the presence of aluminum chloride. Thus, the steric factors which normally retard the introduction of methyl and *tert*-butyl groups at adjacent ring positions may be compensated and surmounted by the gain in probability of an intramolecular process. In the case of an asymmetrically substituted phenol, one ortho position must be blocked to ensure that cyclization occurs in the more hindered direction. Thiolactones have been prepared by cyclization of the analogous thiophenolic esters with aluminum chloride, but not by direct alkylation of the thiophenol.[31] Synthesis of the corresponding lactams has not yet been achieved by either method. Strong electron-withdrawing substituents on the phenolic ring retard or prevent alkylation. Thus, **2h** could not be obtained directly from the nitrophenol, but was prepared by nitration of **2g**. This nitration leads to a by-product which, on the basis of its nmr spectrum, is considered to be **4**.[31] Anomalous side-chain nitration



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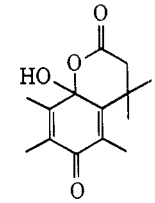


5

of polyalkylbenzenes has been observed previously.[32]

Although no difficulty was experienced in the preparation of the 6-hydroxylactone (**2i**), the corresponding hydroquinone acid (**1i**) was found to undergo air oxidation in alkaline media with extreme rapidity. Efforts to generate **1i** by reduction of the quinone, *in situ*, with sodium borohydride were thwarted, since reduction appeared to regenerate **2i** directly. The reduction may proceed by direct hydride attack on the hemiketal **5**,[33] present in a small equilibrium concentration.

Because of the wide spread in rate constants for lactonization (>10^{11}), $k'_{H_3O^+}$ is the only catalytic constant which could be evaluated for all of the compounds studied. The most reliable values are those for **1b–1d**; that for **1a** had to be calculated from the rate of lactone hydrolysis and the equilibrium constant for the lactonization reaction. Values of $k'_{H_3O^+}$ for **1e–h** were obtained by resolution of the buffer-independent rate constants (k'_0) into their components (eq 3). Despite the errors inherent in such methods of evaluation, we are confident of the exponential factors obtained, both because of their consistency before and after electronic correction and because the Brønsted slopes for these compounds (Table IV) are close to the more reliable values for **1c** and **1d**. Since cyclization of **1a** could not be observed in imidazole buffer, a value of k'_{ImH^+} for this compound was calculated by assuming its Brønsted α to be the same as for **1c**.

The rate-enhancement factors (based on k' and k'') resulting from nonbonded interaction between C-4 and C-5 are summarized in Table V, relative to *o*-hydroxy-

(29) G. W. Wheland, R. M. Brownell, and E. C. Mayo, *J. Amer. Chem. Soc.*, **70**, 2492 (1948). A modified σ value of +0.84 has previously been estimated: R. W. Taft, Jr., and H. D. Evans, *J. Chem. Phys.*, 27, 1427 (1957).

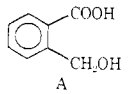
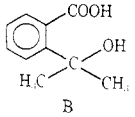
(30) For example, K. T. Serijan, H. F. Hipsher, and L. C. Gibbons, *J. Amer. Chem. Soc.*, **71**, 873 (1949); A. W. Burgstahler, D. J. Malfer, and M. O. Abdel-Rahman, *Tetrahedron Lett.*, 1625 (1965); W. A. Gibbons and H. Fischer, *ibid.*, 43 (1964).

(31) Details will be reported separately.

(32) V. Migrdichian, "Organic Synthesis," Vol. 2, Reinhold, New York, N. Y., 1957, p 1598; J. W. Baker and C. K. Ingold, *J. Chem. Soc.*, 2462 (1926).

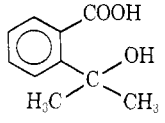
(33) For example, see M. Oxman and L. A. Cohen, *Biochim. Biophys. Acta*, **113**, 412 (1966).

Table VI. Specific Rate Constants for Lactonization at 20° (M^{-1} sec^{-1})

Compound	$k'_{H_3O^+}$	k'_{HCOOH}	k'_{HCOO^-}	k'_{CH_3COOH}	$k'_{CH_3COO^-}$	k'_{H_2O}[a]	α	β
 A	3.02×10^{-3} [b,c]	1.63×10^{-6}	7.45×10^{-6}			4.0×10^{-7}	0.57	0.55
 B	33.3[d]	3.76×10^{-2}	1.50×10^{-2}	8.70×10^{-3}	3.85×10^{-2}	1.2×10^{-3}	0.53	0.50
k'_B/k'_A	1.1×10^4	2.3×10^4	2.0×10^3			3.0×10^3		

[a] Sec^{-1}. [b] All rate constants for compound A were obtained at 60° and extrapolated to 20° by use of a value of E_a = 15.6 kcal/mol. [c] Extrapolation of the data of ref 35b to 20° provides a value of $k'_{H_3O^+} = 2.76 \times 10^{-3}$. [d] Extrapolation of the data of ref 35a to 20° provides an identical value of $k'_{H_3O^+}$.

hydrocinnamic acid (**1a**). The order of rate-enhancement factors is revealed qualitatively by inspection of space-filling models.[34] While there appears to be no barrier to free rotation in the nonbonded methyl–methylene case (**1b**), the *gem*-dimethyl–hydrogen interactions in **1c** and **1d** provide a barrier which may be compared with that of tetramethylsuccinic acid. Similar nonbonded systems have been studied previously in the cases of **6**[2a,35] and **7**,[36] as well as in our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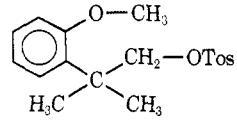
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earlier work on the lactonization of **1c**.[2b] The rate-enhancement effect of *gem*-dimethyl substitution, in the case of **6**, is summarized in Table VI.[37] The rate constant for acid-catalyzed lactonization of **6**, at 30°, is about 3000-fold that for **1c**; this difference is probably due to participation by an alcohol rather than by the less nucleophilic phenol, and to the formation of a five- rather than a six-membered lactone. As found for the various *o*-hydroxyhydrocinnamic acids, the lactonization of **6** and of its parent (*o*-hydroxymethylbenzoic acid) is subject to catalysis by both general acids and general bases (*cf.* ref 35a). Intramolecular participation by an aryl ether, in the case of **7**, was not observed in the absence of the *gem*-dimethyl substituent,[36] prohibiting numerical comparisons.

The occurrence of *gem*-dimethyl–methyl interaction (**1e–h**) produces a rate-enhancement effect of considerably greater magnitude. In fact, so effective is the restriction to rotation that two, and possibly all three, skeletal bonds of the side chain may be considered largely frozen into a conformation highly suitable for formation of the tetrahedral intermediate.[38] The specific rate constant for acid-catalyzed lactonization of **1g** is greater than that of **1a** by a factor of 5×10^{10}, corresponding to a decrease in $\Delta F^{\pm}$ of *ca.* 15 kcal/mol, or an apparent 5 kcal per skeletal bond frozen.

(34) Catalin, C. P. K., and Leybold models were used in these constructions.

(35) (a) D. P. Weeks and X. Creary, *J. Amer. Chem. Soc.*, **92**, 3418 (1970); (b) J. F. Bunnett and C. F. Hauser, *ibid.*, **87**, 2214 (1965).

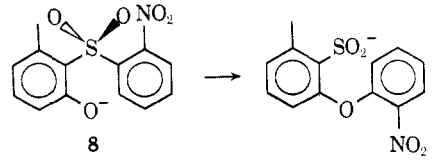
(36) R. Heck, J. Corse, E. Grunwald, and S. Winstein, *ibid.*, **79**, 3278 (1957).

(37) The rate constants in this Table are refinements and extensions of those given in ref 2a.

(38) *Cf.* Figure 1 in ref 1. For arguments against steric overcrowding contributing significantly to this rate enhancement, see paper III (ref 17).

In the more flexible system leading to succinic and glutaric anhydrides, rate-enhancement factors already indicate a decrease in $\Delta F^{\pm}$ of 3 kcal/mol per bond restricted.[4d] As emphasized at the close of this discussion, conformational freezing, *per se*, must be considered only partially responsible for the overall decrease in $\Delta F^{\pm}$.

A somewhat comparable case has been observed in studies of the Smiles rearrangement (**8** → **9**).[8] In **8**,


8 → **9**

the aryl methyl group fits between the oxygen atoms of the tetrahedral sulfone, promoting the conformation needed to produce a tetrahedral intermediate in the addition–elimination process. The locking effect of the aryl methyl group is sufficient to produce a rate-enhancement factor of almost 10^6.

The half-life of **1g** at pH 7 (0.3 *M* buffer, 30°) was found to be *ca.* 6 sec, 90% of the total rate being contributed by buffer catalysis. At pH 6, the half-life was estimated (by calculation) to be 1 sec. As the pH is increased, lactonization becomes slower: pH 8, 22 sec; pH 11, 60 sec (calcd). Even at pH 14, a storage temperature of −5° was found necessary to retard lactonization over a period of 1–2 weeks.

It is evident from the data of Table III that a gradual increase in conformational restriction serves to increase the fraction of lactone present at equilibrium. Whether the gradation in values of ΔF (through 5 kcal or more) is to be attributed to changes in enthalpy or entropy (or both) has yet to be determined. From the equilibrium constant (Table III) for phenyl acetate formation (3.8×10^{-6} M^{-1}) and $k_{H_3O^+}$ for hydrolysis of phenyl acetate (2.77×10^{-5} M^{-1} sec^{-1}),[39] the rate constant for formation of the ester in aqueous acid (1 *M*, 25°) is estimated to be 10^{-10} M^{-2} sec^{-1}. The rate constant for lactonization of **1a** is, therefore, greater than that of its bimolecular counterpart by a factor of 6×10^4 *M* and that of **1g** is greater by a factor of 3×10^{15} *M*.

In our earlier study[2b] of lactonization in the series represented by **1c**, we had concluded that rapid, revers-

(39) E. Tommila and C. N. Hinshelwood, *J. Chem. Soc.*, 1801 (1938).

ible formation of a tetrahedral intermediate is dependent on f_{RCOOH} and is followed by a rate-limiting breakdown catalyzed by hydronium ion or by either buffer component. Values of Brønsted α or β for the more conformationally restricted series are sufficiently close to those for **1c** and **1d** to suggest a similar mechanism for lactonization.[40] If the geometries of the tetrahedral intermediates are essentially identical in each case, the very large rate enhancements must represent significantly greater steady-state concentrations of tetrahedral intermediate for **1e–h**; in this event, rate-enhancement factors should be similar for all catalytic species. Examination of Table VII reveals, however,

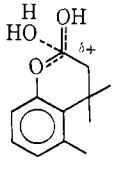
Table VII. Dependence of Rate Enhancement Factor on Catalytic Species[a]

	k''(**1g**)/k'(**1c**)		k''(**1g**)/k'(**1c**)
$k'_{H_3O^+}$	1.1×10^7	k'_{Im}	1.6×10^3
k'_{ImH^+}	1.8×10^6	k'_{OH^-}	1.8×10^4

[a] Based on the specific rate constants of Table II; for **1g**, the corrected rate constants (k'') were used.

that the overall rate-enhancement factor is *not* independent of the type of catalysis involved and that, in fact, general acid catalysis is more effective than is general base catalysis by a factor of about 10^3. A similar but more modest differentiation is also evident in the data of Table VI. It would appear that the effect of conformational modification must be *composite* in nature, influencing the breakdown of the tetrahedral intermediate as well as its formation.

At least two explanations for this phenomenon may be advanced by reference to the difference in mechanisms of the acid-and base-catalyzed breakdown of the intermediate.[2b] In the general acid-catalyzed process, breaking of a C–O bond may be considered the rate-limiting event (**10**). Since this cleavage places a partial



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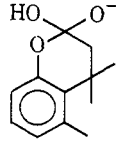

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positive charge on C-2, the more effectively the remaining oxygen atoms can stabilize the developing positive charge, the more rapid should be the breakdown. If the 4,4,5-trimethyl interaction causes the dihydropyran ring to exist in a conformation such that overlap of the ring oxygen with the C^+ species is better than with 4,4 substitution alone, resonance stabilization would be more effective for the former case.[41] In addition, inspection of models suggests that one hydroxyl group in the tetrahedral intermediate is more crowded by a C-4 methyl in the 4,4,5 system than in the 4,4; thus, relief of crowding could also account for the results.[42] Since proton removal may be significantly rate limiting in base-catalyzed breakdown (**11**), neither of these factors should assume importance. The **1g**/**1c** ratio for base catalysis may than be taken as a rough measure of the degree to which conformational restriction by the 4,4,5 system enhances the steady-state concentration of the tetrahedral intermediate. Since we are dealing with two distinct mechanisms for acid and base catalysis, the question–which is the true conformational rate enhancement factor?–becomes meaningless; both are true. These considerations suggest that conformational differences may exist between lactones **1g** and **1c**, or other cyclic systems containing the same sets of alkyl substituents.[43]

The rate-acceleration effects observed in this series may be attributed, primarily, to a considerable increase in the population of a conformer highly favorable to the formation of the tetrahedral intermediate. It is improper or inaccurate, however, to view the proximity or propinquity phenomenon, in our extreme cases, simply as the result of an enhanced probability for intramolecular collision. Severe conformational locking can, and may, lead to rate acceleration by various other means,[4d,44] all resultants of the same structural modification of a basic system, and all serving to bring the ground state closer in identity to a transition state or intermediate. Among such factors may be included improvement in the angular relation of nucleophilic and electrophilic centers,[45] partial negation of van der Waals and electrostatic repulsion in the ground state, partial orbital overlap in the ground state, and exclusion of solvent between the reaction partners (as well as their partial desolvation). Present knowledge offers no practical means of resolving an overall rate-enhancement factor into contributions from these several effects, nor does theory offer a sound basis for further speculation thereon at this time.

The stimulus for this investigation arose from the absence of a convincing test-tube precedent for a biochemical speculation. The proposal that phenol–carboxyl esterification might occur spontaneously in a protein matrix[9] has now been amply demonstrated. Furthermore, the inclusion, in theories of enzyme catalysis, of considerably larger rate-enhancement factors than previously assumed for substrate freezing is mandatory.[6] Finally, the quest for enzyme simulation in model systems by matching of reaction rate in a comparable environment, and with a total dependence on general acid–general base catalysis, is closer to realization.

Acknowledgment. We are indebted to Drs. W. P. Jencks and G. L. Schmir for valuable comments and criticisms.

(40) In the absence of experimental support, we assume tetrahedral breakdown to be rate-limiting for **1a** and **1b** as well.

(41) The argument is analogous to that invoked for distortion of hexoses by lysozyme: ref 4a, p 304.

(42) L. Eberson and H. Welinder, *J. Amer. Chem. Soc.*, **93**, 5821 (1971).

(43) In subsequent papers, conformational changes in the heterocyclic ring will be shown to be responsible for a variety of contrasting patterns of reactivity.

(44) Reference 4a, pp 15–21.

(45) D. R. Storm and D. E. Koshland, Jr., *Proc. Nat. Acad. Sci. U. S.*, **66**, 445 (1970). See, however, T. C. Bruice, A. Brown, and D. O. Harris, *ibid.*, **68**, 658 (1971), and ref 4d.