The Role of Intramolecular Bifunctional Catalysis of Ester Hydrolysis in Water

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Abstract: In a search for intramolecular concerted general acid, general base and general acid, nucleophilic catalysis, the hydrolyses of four systems of esters were examined: (1) 6- and 8-quinolyl hydrogen glutarates and succinates; (2) o- and p-carboxyphenyl succinates; (3) methyl γ- and β-resorcylates; and (4) phenyl esters of 4- and 6-substituted salicylates. Bell-shaped pH-κabord profiles interpretable as resulting from bifunctional catalysis were obtained for compounds in each series. In all cases, however, only one functional group was found to participate directly in the hydrolytic reaction; the descending leg of the bell (at high pH) in all cases was shown to result from electrostatic and electronic inhibition resulting from the ionization of the nonparticipating functional group. There is, thus, no existing evidence for concerted intramolecular general acid, general base or general acid, nucleophilic catalysis for hydrolysis of esters in water.

In the enzymatic catalysis of the conversion of substrate into product, the first step involves the reversible formation of an enzyme-substrate complex; the reaction then proceeds via intracomplex participation of (generally) two or more functional groups of the enzyme, collectively known as the active site.3 The similarity between reactions within the active site and intramolecular reactions is obvious, and has prompted a great many studies in which both a reactive (ester, amide) and a catalytic (amine, carboxyl, etc.) species are combined in the same molecule.4 There are, however, only a very limited number of “models” in which two catalytic species are incorporated in the same molecule as the reactive groups. The present study was initiated in order to ascertain the validity of proposals of bifunctional (nucleophilic, general acid, or general base, general acid) catalysis of ester hydrolysis in water.

Perhaps the first system in which intramolecular bifunctional catalysis was implicated was reported by Morawetz and Oreskes,5 who observed a bell-shaped pH-log kobsd profile for the hydrolysis of o-carboxyphenyl hydrogen succinate (1). At pH 4, the observed hydrolytic rate constant for 1 was found to be about 104-fold greater than that for the hydrolysis of acetyl salicylate. Two kinetically indistinguishable pathways of nucleophilic, general acid catalysis were considered (i.e., 1a and 1b). The authors pointed out that if the reaction proceeds via 1a, the rate of solvolysis would be 24,000 times as great as that for ionized aspirin, but if via 1b, only 66 times as fast as that of o-carbomethoxyphenyl hydrogen succinate. The authors thus favored the path through 1b.

Fersht and Kirby6 have obtained a similar bell-shaped profile for the hydrolysis of 3-acetoxypthalate (2), for which there is no possibility of general catalysis of the initial nucleophilic attack. The presence of the second carboxyl group causes the observed hydrolytic rate constant for 2 at pH 3.8 to be 6.3 × 106-fold greater than that for ionized aspirin. Moreover, the authors were able to demonstrate the intermediacy of 3-hydroxyphthalic anhydride (2c) in the reaction pathway, indicating that the reaction proceeds by what they have aptly termed “series nucleophilic catalysis.”

Bender and Killian7 obtained a bell-shaped pH-log rate profile for the hydrolysis of methyl γ-resorcylate (3), indicating that the rate of hydrolysis is dependent upon the state of ionization of both o-hydroxyl groups. Two kinetically indistinguishable mechanisms involving participation by both hydroxyl groups may be written for this reaction also (3a and 3b). For case 3b, the derived rate constants indicate that the reaction is 5.4-fold faster than a similar mechanism for methyl salicylate (4b); for 3a, the calculated rate constant is 1.6 times slower than that for 4a. The near equivalence of the rate constants for hydrolysis of 3 and 4 was deemed important by the authors because of the general extreme...
lack of reactivity of ortho-disubstituted benzoate esters in comparison to mono- or unsubstituted esters.\textsuperscript{5-9}

The quinoline nitrogen has previously been shown to participate as a general base in the hydrolysis (and aminolysis) of 8-acetoxyquinoline.\textsuperscript{10} (6), giving a 500-fold rate increase over the electronically similar 6-acetoxyquinoline (5), and in the hydrolysis of 8-quinolyl phosphate,\textsuperscript{11} which presumably also proceeds via intramolecular general base catalysis. The hydrolyses of the 8-quinolyl half esters of glutaric (7) and succinic (8) acids exhibit "bell-shaped" pH–k\textsubscript{obsd} profiles which, on the basis of the work cited above, could be interpreted as bifunctional catalysis of hydrolysis. In order to evaluate the role, if any, of bifunctional catalysis in the hydrolysis of 1, 3, 7, and 8 the kinetics of hydrolysis of esters 5–18 have been determined.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure}
\caption{Figure Caption}
\end{figure}

**Experimental Section**

**Apparatus.** All spectrophotometric kinetic measurements were made on either a Gilford Model 2000 spectrophotometer equipped with four thermostaps through which water at 30 ± 0.1° was circulated, a Zeiss PMQ II spectrophotometer equipped with a brass cuvette holder through which water at 30 ± 0.1° was circulated, a Durrum-Gibson Model 13001 stopped-flow spectrophotometer equipped with a Kel-F cell and valve block through which water was circulated at 30 ± 0.2°, or a spectrophotometric titration apparatus designed around the Cary 15 spectrophotometer and Radiometer autotitrator (described elsewhere by Bruce and Maley)\textsuperscript{12} through which water was circulated at 30 ± 0.1°. Spectrophotometric titrations and titrimetric rates were carried out with the latter apparatus. Ultraviolet spectra were recorded on the Cary 15 at 30° or on a Perkin-Elmer 350 recording spectrophotometer at ambient temperature. pH measurements at 30 ± 0.1° were taken with a Radiometer Model 22 pH meter equipped with a Model 630 scale expander and a Radiometer GK2021C coated glass calomel electrode.

The hydrolysis of 10 was followed using a Radiometer TTT-1b autotitrator equipped with a PHA-630Ta scale expander and a water-jacketed 100-ml cell equipped with ℜ openings to accommodate a salt bridge leading to a calomel electrode, an EA-115 Metrom glass electrode, an inlet capillary for titrant base addition, and a thermistor. A port in the side of the cell was sealed with a serum cap allowed withdrawal of aliquots by means of a hypodermic syringe and needle. Water at 60 ± 0.2° was circulated through the cell jacket with a Haake bath. The same electrodes and the Haake bath were used with the Cary 15 for spectrophotometric titrations at 60 ± 0.2°.

Infrared spectra were recorded in potassium bromide disks using a Perkin-Elmer 137 sodium chloride spectrophotometer. Pmr spectra were recorded on a Varian A-60 spectrometer. Pmr spectra were recorded on a Radiometer A-60 spectrometer, using TMS or DSS (in water) as an internal standard. Melting points were measured on a Nalge hot stage and are uncorrected.

**Materials.** Potassium chloride, tri(hydroxymethyl)aminomethane (Sigma 7-9), potassium phosphate monobasic, sodium borate, potassium acetate, acetic acid, and formic acid were reagent grade and were used without further purification. Deionized, freshly double-distilled water was employed to prepare all solutions. Standard buffers at 30° were purchased from Fisher Scientific (7 and 10) and Beckmann (12.3). Those used at 60° were prepared by the method of Bates.\textsuperscript{13}

**8-Quinolyl Hydrogen Glutarate (7).** In a dry apparatus fitted with a silica gel drying tube were combined 10 g (0.069 mol) of 8-hydroxyquinoline (Matheson) and 7.86 g (0.069 mol) of glutaric anhydride (Matheson). The reactants were dissolved in about 80 ml of sodium-dried ether and refluxed overnight yielding a white precipitate. The solution was cooled and filtered, and the filtrate was crystallized several times from 100% ethanol, yielding 3.5 g (20%) of fine, cottony, white needles: mp 115–117°; strong ν\textsubscript{max} 1750 (ester), 1700 (acid), 1280, 1120, 830, 760 cm\textsuperscript{-1}; 1530, and 1380 cm\textsuperscript{-1}; no anhydride bands were seen. The material was then dried at 80°, under a vacuum over P\textsubscript{2}O\textsubscript{5}. Dioxane (Baker) was calculated, a Durrum-Gibson Model 13001 stopped-flow spectrophotometer equipped with a Kel-F cell and valve block through which water was circulated at 30 ± 0.2°, or a spectrophotometric titration apparatus designed around the Cary 15 spectrophotometer and Radiometer autotitrator (described elsewhere by Bruce and Maley)\textsuperscript{12} through which water was circulated at 30 ± 0.1°. Spectrophotometric titrations and titrimetric rates were carried out with the latter apparatus. Ultraviolet spectra were recorded on the Cary 15 at 30° or on a Perkin-Elmer 350 recording spectrophotometer at ambient temperature. pH measurements at 30 ± 0.1° were taken with a Radiometer Model 22 pH meter equipped with a Model 630 scale expander and a Radiometer GK2021C coated glass calomel electrode.

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**8-Quinolyl Sodium Succinate (8).** 8-Hydroxyquinoline was recrystallized three times from 100% ethanol and thoroughly dried under vacuum over P\textsubscript{2}O\textsubscript{5}. Succinic anhydride (Matheson) was refluxed for 3 hr in acetic anhydride, crystallized, and recrystallized twice more from the same solvent.\textsuperscript{12} The material was then dried at 80°, under a vacuum over P\textsubscript{2}O\textsubscript{5}. Dioxane (Baker) was scrupulously dried by the method of Fieser,\textsuperscript{16} and was distilled directly into the reaction vessel as needed. 8-Hydroxyquinoline (1 g, 0.0069 mol) was placed in a dry flask equipped with a drying tube containing silica gel and Ascarite, and dissolved in about 25 ml of dioxane. After all the sodium had reacted (about 3 hr), the succinic anhydride solution was added with a dry pipet. Heating was discontinued, and the solution was stirred at room temperature overnight. The golden precipitate was collected by filtration, thoroughly washed with sodium-dried ether, and stored under vacuum over P\textsubscript{2}O\textsubscript{5}. The powder showed strong ν\textsubscript{max} at 3450, 1750 (ester), 1660 (sodium salt of acid), 1530, and 1380 cm\textsuperscript{-1}; no anhydride bands were present. Analytical measurements were made on both the quinolyl group (multiplets at r 1–3) and the succinate methylene (multiplets at r 5–8).
The material was initially water soluble at higher concentrations, but a precipitate rapidly formed as the ester hydrolyzed. By hydrolyzing the material in acetate buffer at pH 4.6, the material was shown to contain about 76% ester sodium concentrations, but a precipitate rapidly formed as the ester hydrolyzed. Addition of the material to water containing 1.5% acetonitrile. For those esters whose disappearances were followed by monitoring loss of absorbance, the wavelengths (nm) used were: 7, 288; 8, 330 or 355; 9, 320; 10, 335; 11, 355; 12, 175; 13, 170, 1700 cm⁻¹, corresponding to the ester and the acid, respectively, and no bands corresponding to anhydride. No further purification could be obtained. Analysis of the material by the same procedure as used for 8 indicated purities of from 50 to 90% for several preparations. Rate constants obtained using materials of different purities yielded the same pH profile, indicating that the presence of impurities did not observably affect the rate of hydrolysis.

Kinetics. All kinetic measurements were performed in water at a calculated ionic strength of 1.0 (KCl) and at a temperature of 30°C, except for compound 10, which was hydrolyzed at 60°C in water containing 1.5% acetonitrile. For those esters whose disappearances were followed by monitoring loss of absorbance, the wavelengths (nm) used were: 7, 288; 8, 330 or 355; 9, 320; 10, 335; 11, 355; 12, 175; 13, 170, 1700 cm⁻¹, corresponding to the ester and the acid, respectively, and no bands corresponding to anhydride. No further purification could be obtained. Analysis of the material by the same procedure as used for 8 indicated purities of from 50 to 90% for several preparations. Rate constants obtained using materials of different purities yielded the same pH profile, indicating that the presence of impurities did not observably affect the rate of hydrolysis.

Methyl 6-Resorcylic Acid (15). 4-Methoxysalicylic acid, mp 156-161°C (lit.22 157°C) was prepared from β-resorcylic acid and dimethyl sulfate by the method of Gomberg and Johnson.18 The phenyl ester was prepared from the acid, phenol, and POCl₃ by the method of Gaylord and Kamath,19 and recrystallized from 75% ethanol to yield white needles: mp 44-45°C. Anal. Calcd for C₁₄H₁₀O₃: C, 68.84; H, 4.95. Found: C, 68.85; H, 5.00.

Phenyl 6-Methylsalicylate (16). Phenyl 6-methylsalicylate, bp 104°C (3.75 mm), was prepared from ethyl acetoacetate and crotonaldehyde by the method of Bohlmann and Prezewowski,23 Ethyl 6-methylsalicylate was then prepared from phenyl acetoacetate and 2-M NaOH to give 6-methylsalicylic acid as white needles, mp 172-174°C (lit.18 mp 169°C).

The phenyl ester was prepared from the acid and phenol by the method of Gaylord and Kamath,21 and recrystallized several times from 75% ethanol to yield white needles: mp 50-51°C. Anal. Calcd for C₁₅H₁₁O₃: C, 77.86; H, 4.48. Found: C, 77.86; H, 4.48.

Phenyl 6-Methoxysalicylate (17). 4-Methylsalicylic acid, mp 170-176°C (lit.22 mp 176°C), was prepared from acetone, ethyl formate, and ethyl acetate by the method of Prelog, et al.24 The ester was prepared by the method of Gaylord and Kamath,21 and zone sublimed to yield a waxy solid: mp 44-45°C. Anal. Calcd for C₁₄H₁₁O₃: C, 73.67; H, 5.30. Found: C, 73.84; H, 5.33.

p-Carboxyphenyl Succinate (18). In a variation of the method of Bischoff, et al.25 hydroxybenzoic acid (5.0 0.0362 mol), methanol, and potassium hydroxide (4.78, 0.0724 mol) were combined in 75 ml of water and cooled to 0°C in an ice bath. Succinic anhydride (3.8 g, 0.0362 mol) was added and the solution stirred at 0°C for 15 min. The solution was filtered to remove undissolved anhydride, and acidified with concentrated HCl, yielding a white solid. The material had a broad carbonyl absorption at 1750 and 1700 cm⁻¹, corresponding to the ester and the acid, respectively, and no bands corresponding to anhydride. No further purification could be obtained. Analysis of the material by the same procedure as used for 8 indicated purities of from 50 to 90% for several preparations. Rate constants obtained using materials of different purities yielded the same pH profile, indicating that the presence of impurities did not observably affect the rate of hydrolysis.
only practicable method.

Quinolyl Esters. In Figure 1 is plotted log \( k_{\text{obsd}} \) vs. the constant pH at which the rate constants were determined for the quinolyl esters 5, 6, 7, and 8. In Figure 2 is plotted log \( k_{\text{obsd}} \) vs. pH (pD) for esters 7 and 9 in water and ester 7 in deuterium oxide. The plots for 5 and 6 are from a previous work at 55°, and have been extrapolated to 30° by determining \( k_{\text{obsd}} \) at 30° for several pH's. The points of Figures 1 and 2 are experimental and the lines are theoretical; the lines for 5 and 6 are derived from eq 1 and those for 7 (in \( H_2O \) and \( D_2O \)), 8, and 9 from eq 2. \( K_a \) and \( K_a \) for the ester, and the free acid in the same manner were found to be linear, so that the observed absorbance could be plotted directly against time and the rate constant calculated.

All rates were followed for at least four half-lives and the values of the pseudo-first-order rate constants \( (k_{\text{obsd}}) \) were calculated by least-squares analysis of plots of \( \ln(OD_{in} - OD_{out}/OD_{in} - OD_{out}) \) vs. \( t \) for the stopped-flow measurements, or by the method of Guggenheim. Experimental \( K_a \) values for the bell-shaped profiles were calculated by the method of Alberty and Massey. All actual computations were carried out on an Olivetti-Underwood Programma 101 employing least-squares programs written in this laboratory.

Deuterium Solvent Isotope Effects. Deuterium solvent isotope effects were determined for 7 at 30° in 99.8% deuterium oxide (Stohler Isotope Chemicals), using acetic and formate buffers to maintain pH. The pD values were taken as the pH meter readings plus the proper correction at 30°.

**pKs Determinations.** Spectrophotometric titration of the resorcylates indicated that in each case, removal of the first proton was associated with one set of isosbestics, and removal of the second proton with a different set of isosbestics. Thus, in each case, \( pK_s \) could be determined via spectrophotometric titration at an isosbestic wavelength associated with \( pK_{sa} \), and *vice versa.* The wavelengths used were: 10, \( pK_{sa} \) at 315 \( nm \), \( pK_{sa} \) at 243 \( nm \); 12 \( pK_{sa} \) at 281 \( nm \), \( pK_{sa} \) at 265 \( nm \); 13, \( pK_{sa} \) at 290 \( nm \), \( pK_{sa} \) at 245 \( nm \).

The phenyl salicylates were titrated spectrophotometrically in the normal manner. The wavelengths used were: 14, 230 \( nm \); 15, 330 \( nm \); 16, 345 \( nm \); and 17, 335 \( nm \). All titrations were performed at the same temperature, ionic strength, and concentration of ester as the rate determinations.

**Results**

At constant pH, and in the presence of a great excess of buffer over ester, all spectrophotometrically determined rate constants \( (k_{\text{obsd}}) \) were found to be pseudo first order. Extrapolation of plots of \( k_{\text{obsd}} \) vs. buffer concentration to zero buffer provides as intercepts the values of the pseudo-first-order rate constants for the nonbuffer-catalyzed reactions. With the exception of Tris buffers, there was only a small buffer effect. For the stopped-flow buffer dilutions, an average value for each dilution was obtained, since the slopes of the dilutions were small, and there was a larger amount of scatter. For pH values below 2, above 11.5, or when the Cary 15 pH-stat cell apparatus was used, no external buffer was necessary.

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Table I. Hydrolytic Rate Constants for Quinolyl Esters

<table>
<thead>
<tr>
<th>Ester</th>
<th>5a</th>
<th>6a</th>
<th>7</th>
<th>7b</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{oh}(30^\circ), \text{M}^{-1}\text{min}^{-1}$</td>
<td>304</td>
<td>90.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{oh}(30^\circ), \text{M}^{-1}\text{min}^{-1}$</td>
<td>0.00022</td>
<td>0.0262</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_i(55^\circ), \text{min}^{-1}$</td>
<td>1.16</td>
<td>1.11</td>
<td>223</td>
<td>0.320</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_i(55^\circ), \text{min}^{-1}$</td>
<td>0.0058</td>
<td>0.201</td>
<td>0.0126</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_i(30^\circ), \text{min}^{-1}$</td>
<td>0.00145</td>
<td>0.00265</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$pK_{ac}(30^\circ)$</td>
<td>4.44</td>
<td>3.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$pK_{ac}(30^\circ)$</td>
<td>4.57</td>
<td>5.05</td>
<td>4.43</td>
<td>4.54</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Reference 10. b In deuterium oxide. c Kinetically determined. d Determined by spectrophotometric titration.

Although the spectrophotometrically determined rates were rigorously first order, those determined titrimetrically indicated the presence of a slowly hydrolyzable intermediate. The identity of this intermediate is easily established in the case of 8, at pH 4, since the spectrometrically observable reaction is over in seconds. A second, titrimetric rate constant is then obtained; as discussed in the Experimental Section, this rate constant is, within experimental error, identical with that obtained for the hydrolysis of succinic anhydride under the same conditions. Since succinic acid does not form the anhydride under these conditions, any mechanism written for the hydrolysis of the esters must include the intermediacy of succinic anhydride (and, therefore, presumably glutaric anhydride).

**Carboxyphenyl Succinates.** In Figure 3 is plotted $k_{obsd}$ vs. the constant pH at which the hydrolytic rate constants were determined for succinate esters 1 and 11. The data for 1 are taken from ref 5, while the points for 11 are from this study; the lines for both are theoretical, being derived from eq 3. The constants in

$$k_{obsd} = \frac{K_a(k_{ar} + k_2K_a)}{K_a(K_a + a_H) + a_H^2}$$

(3)

this equation are as previously defined. The values of the various rate and equilibrium constants are given in Table II. Both $K_a$ and $K_c$ are required to fit the data to a theoretical curve, even if one ignores the small "hump" in the pH-$k_{obsd}$ profile.

**Salicylate Esters.** The methyl esters 3, 4, and 10 were hydrolyzed at 60° in water containing 1.5% acetonitrile. In Figure 4 is plotted log $k_{obsd}$ vs. the constant pH at which the rate constants were determined. The data for 3 and 4 are taken from ref 7; the points for 10

Maugh, Bruice | Bifunctional Catalysis of Ester Hydrolysis
are from this study. The lines of Figure 4 were generated from eq 4 for 4, eq 5 for 3, and eq 3 (k_{gb} = k_{gb}' = k_{g}) for 10. The values of the various rate and equilibrium constants are given in Table III.

Table III. Hydrolytic Rate Constants for Methyl Resorcylates and Methyl Salicylatea

<table>
<thead>
<tr>
<th>Ester</th>
<th>4b</th>
<th>3b</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>pK_{a}</td>
<td>9.22</td>
<td>8.3</td>
<td>7.24</td>
</tr>
<tr>
<td>pK_{a'}</td>
<td>10.5</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>pK_{eb}</td>
<td>7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>k_{obed}, min^{-1}</td>
<td>0.149</td>
<td>0.095</td>
<td>0.0005</td>
</tr>
<tr>
<td>k_{eb}, min^{-1}</td>
<td>0.0315</td>
<td></td>
<td></td>
</tr>
<tr>
<td>k_{eb}', M^{-1} min^{-1}</td>
<td>894</td>
<td>4800</td>
<td>298</td>
</tr>
<tr>
<td>k_{eb}', M^{-1} min^{-1}</td>
<td>3.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* T = 60°, μ = 1.0 (KCl), 1.5% acetonitrile in water. b Data from ref 7. c Kinetically determined. d Determined by spectrophotometric titration. e See Discussion for explanation.

The phenyl esters 12–17 were hydrolyzed at 30°. In Figure 5 is plotted k_{obed} vs. the constant pH at which the rate constants were determined for these esters. Complete profiles were obtained only for esters 12 and 17. The points of Figure 5 are experimental and the lines are generated from eq 5 for 12, eq 3 for 13, and eq 6 for 14–17. The values for the various rate and equilibrium constants are provided in Table IV.

\[
k_{obed} = \frac{K_{a}(k_{eb} + k_{O,H} k_{eb}/a_{H})}{K_{a} + a_{H}}
\]

favored over that through path b primarily on the basis that (a) any reasonable value for the pK_{a} of the quinolyl hydroxyl group would require an exceptionally facile intramolecular catalyzed hydrolysis by the oxyanion species at the low pH end of the plateau; and (b) in analogy with the general base catalysis mechanisms for the hydrolysis of substituted aspirins,\(^\text{32,33}\) path a should

\[\text{Scheme I}\]

**Discussion**

**Quinolyl Esters.** The mechanism of the pH-independent hydrolysis of 6 has been discussed extensively in ref 10. Briefly, the deuterium solvent isotope effect (k_{H_{2}O}/k_{D_{2}O} = 2.35), the entropy of activation (\(T \Delta S^\ddagger = -8.7 \text{ kcal mol}^{-1}\)), and the different mode of reaction with H_{2}O, primary and secondary amines compared to HO-, and tertiary amines dictates an intramolecular general base catalyzed attack of H_{2}O. Two kinetically indistinguishable mechanisms were considered (Scheme I). The mechanism proceeding through path a was

\[\text{Scheme I}\]


\(\text{(33) A. R. Fersht and A. J. Kirby, ibid., 90, 5818 (1968).}\)
Table IV. Hydrolytic Rate Constants for Phenyl Salicylates

<table>
<thead>
<tr>
<th>Ester</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>$pK_{a},^b$</td>
<td>7.95</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$pK_{a},^2$</td>
<td>11.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$pK_{a},^3$</td>
<td>8.08</td>
<td>7.55</td>
<td>8.46</td>
<td>9.1</td>
<td>8.63</td>
<td>9.44</td>
</tr>
<tr>
<td>$pK_{a},^4$</td>
<td>10.58</td>
<td>10.76</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{b1}, \text{min}^{-1}$</td>
<td>0.023</td>
<td></td>
<td></td>
<td>0.0080</td>
<td>0.0038</td>
<td>0.00935</td>
</tr>
<tr>
<td>$k_{b1}, \text{min}^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td>0.00372</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{b1}, M^{-1} \text{min}^{-1}$</td>
<td>17,700</td>
<td>1890</td>
<td>206</td>
<td>1490</td>
<td>37.8</td>
<td></td>
</tr>
<tr>
<td>$k_{b1}, M^{-1} \text{min}^{-1}$</td>
<td>4.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{b1}, M^{-1} \text{min}^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td>0.0323</td>
<td>0.0025</td>
<td>0.058</td>
</tr>
</tbody>
</table>

* $T = 30^\circ, \mu = 1.0 (\text{KCl}).$  
  $^b$ Kinetically determined.  
  $^c$ Determined by spectrophotometric titration.  
  $^d$ See Discussion for explanation.

Scheme II

be favored over path $b$ (Scheme I) due to the magnitude of the $\Delta pK_a$ difference between the conjugate acids of the quinolyl nitrogen and the 8-hydroxy groups. The favoring of path $a$ over that of path $b$ was based, therefore, to a considerable extent on an anticipated analogy between systems involving a neighboring nitrogen base and a neighboring oxygen base. The reasonableness of this assumption is of course open to some criticism, and the choice between paths $a$ and $b$ (Scheme I) must be considered tentative, particularly in light of the fact that the types of differential experiments amenable to the acyl salicylate studies ($^{18}$O exchange and methanolysis) are not applicable to 4.

The hydrolysis of 8 has been shown to yield, as the immediate products of hydrolysis, both 8-hydroxyquinoline and succinic anhydride (see Experimental Section). In the case of esters 7 and 9, the intermediacy of the anhydride was not definitely established because of the similar magnitudes of the rate constants for ester disappearance and anhydride hydrolysis. In the light of the titrimetric results with 8, however, it is reasonable to assume that the three esters hydrolyze by similar mechanisms, and that cyclic anhydrides are formed on hydrolysis of the ester. To obtain anhydride intermediates, it is necessary that the terminal carboxylates of 7, 8, and 9 participate as anionic nucleophiles in an intramolecular displacement reaction. The soundness of this mechanism is demonstrated by a considerable literature dealing with intramolecular nucleophilic displacement of substituted phenols from dicarboxylic acid monophenyl esters by the carboxyl anion.34-38

In Scheme II there are presented all reasonable reaction pathways that could yield anhydride on the hydrolysis of esters 7 and 8. Paths iv and v are appropriate for the hydrolysis of ester 9 also. The relationships of the observed first-order rate constants to the hydrogen ion concentration for each pathway are given in eq 7-11.

$$k_{\text{obsd}_1} = \frac{k_iK_a}{K_a(K_i + a_H) + a_H^2}$$  \hspace{1cm} (7)

$$k_{\text{obsd}_i} = \frac{k_iK_a}{K_a[K_a(K_i + 1) + (K_i + 1)a_H] + a_H^2}$$  \hspace{1cm} (8)

$$k_{\text{obsd}_{ii}} = \frac{k_iK_a}{K_a[K_a(K_i + 1) + (K_i + 1)a_H] + a_H^2}$$  \hspace{1cm} (9)

$$k_{\text{obsd}_{iii}} = \frac{k_iK_a}{K_a(K_i a_H) + a_H^2}$$  \hspace{1cm} (10)

$$k_{\text{obsd}_{iv}} = \frac{k_iK_a}{K_a(K_i + a_H) + a_H^2}$$  \hspace{1cm} (11)

Inspection of these equations shows that eq 7, 8, and 11 have the same mathematical form as the $k_1$ term (describing a bell-shaped profile) of eq 2; eq 9 and 10 have the same form as the $k_2$ term (describing a plateau region) of eq 2. The paths are thus kinetically equivalent to the appropriate terms of the experimentally derived rate law.

In the simplest case, the hydrolysis of ester 9 produces a profile which has a bell-shaped curve centered around pH 4 and a pH-independent region at alkaline pH's (Figure 1). Steric considerations prevent direct participation by the quinoline nitrogen as either a general acid or a general base. This profile is described by eq 10 and 11 for $k_{\text{obsd}_o}$ and $k_{\text{obsd}_e}$. The unique shape of the profile, then, results simply from acid–base equilibria.

(38) T. C. Bruice and W. C. Bradbury, ibid., 90, 3808 (1968).
The hydrolysis of substituted phenyl glutarates and succinates proceeds through an anhydride intermediate resulting from intramolecular nucleophilic attack of carboxyl anion. A plot of the rate constants for intramolecular carboxyl attack in meta- and para-substituted phenyl succinates and glutarates vs. the pK_a of the phenolic leaving group is provided in Figure 6. Inspection of Figure 6 reveals two straight lines, with slopes of -1.0 for the glutarate esters and -1.14 for the succinate esters. If we then plot log k_2 for esters 7, 8, and 9 vs. the pK_a of the “normal” hydroxyl group ionization (19a, pK_a = 9.88; 19b, pK_a = 8.87), the points fall very close to the appropriate lines. Thus, in all probability, the leaving group for the formation of cyclic anhydride is the phenolic oxygen (rather than the nitrogen), the quinolinols behave like phenols of similar pK_a, and path iv represents the mechanism for the pH independent hydrolysis of these esters.

The microscopic constants for hydroxyl ionization of the protonated quinolinols are also known (20a, pK_a = 6.6; 20b, pK_a = 7.03); if we then plot log k_2 for

![Diagram](image_url)

7, 8, and 9 vs. these pK_a's, we see that these also fall nicely on the appropriate lines. The conclusion must be reached, therefore, that the pH-kobsd profiles for the esters 7, 8, and 9 arise from the INCEI effect.

**Carboxyphenyl Succinates.** The interpretation of the pH-kobsd profile for ester 11 (Figure 3) is straightforward. The pH independent rate constant (k_2) at alkaline pH occurs when the p-carboxyl group is ionized (11a); proceeding to a more acid pH, the “ascending limb”

![Diagram](image_url)

where the small k's represent microscopic ionization constants. K_a and K_b are the macroscopic ionization constants. We then have the relationships: 44

\[ K_a = k_a K_b \]

Following well-established procedures, 44-46 we may assume that K_a for 6 is a good model for k_2 for 7, and that K_a for 5 is a good model for k_2 for 9. Using these values and solving, we find that K_a = 1.1 for 7 and 0.9 for 9.

A priori, because of the similarities of 7, 8, and 9, one would expect their hydrolyses all to proceed by the same pathways, which must, therefore, be iv and v. This expectation is reinforced by the following evidence.

of the bell ($k_1$) results from protonation of the $p$-carboxyl group, which increases the leaving tendency of the phenoxide (11b). The descending limb observed at the most acid pH's then results from protonation of the succinate carboxyl group, preventing nucleophilic attack. If we make the reasonable assumption\(^{(46)}\) that the $pK_a$ for the ionization represented by 21 is similar to that for $p$-carboxmethoxyphenol ($pK_a = 8.47$\(^{(47)}\)), we may plot (Figure 6) log $k_1$ and log $k_2$ vs. the $pK_a$ of the leaving groups in 11b and 11a, respectively. These points are found to lie very close to the line for the substituted phenyl succinates: hence, the “hump” in the pH–rate profile not only is real, but also arises solely from the change in basicity of the phenoxide leaving group resulting from ionization of the $p$-carboxyl group. Inspection of structure 11 dictates that the two carboxyl groups could only participate via INCEI, as found.

The situation is much less obvious in the case of ester 1, since there are many complicating factors. In the first place, plots such as that of Figure 6 are not applicable, due to ortho-steric and intramolecular hydrogen bonding\(^{(48)}\) effects. Consider the conjugate acids of the leaving groups associated with the $k_1$ (22a) and $k_2$ (22b) terms in the hydrolysis of 1. Using $o$-

\[ \text{HOOC-} \text{O} \text{-OH} \xrightleftharpoons[][1]{H^+} \text{HOOC-} \text{O} \text{-O}^- \]

21

carboxmethoxyphenol as a model for the hydroxyl ionization of 22a, we obtain $pK_a = 10.2$;\(^{(49)}\) the $pK_a$ for the ionization of 22b, however, is 13.1.\(^{(50)}\) The high $pK_a$ of 22b is attributed to intramolecular hydrogen bonding.

The ratio of 10\(^3\) obtained for the ionization constants of the conjugate acids of the leaving groups for the $k_1$ and $k_2$ terms is observed to be very similar to the ratio of the rate constants for intramolecular carboxylate attack on the esters containing these leaving groups. $k_3/k_2 = 1380$ for ester 1. This is, however, probably just a fortuitous agreement since there is no possibility of intramolecular hydrogen bonding in the transition state (1c) associated with $k_3$ or in the leaving phenoxy. There will certainly be a difference in the basicity of the leaving phenoxy group in the reaction paths associated with $k_1$ and $k_3$, but the magnitude of this difference should be close to that observed for the similar ionization states of 11.

A perhaps more enlightening approach is to compare the $k_1$ term for 1 with the rate constant for hydrolysis of $o$-carboxmethoxyphenyl succinate (23).\(^{(51)}\) The ratio of $k_1$ for 1 to $k_1$ for 23 is found to be 8.8, rather than the 66 quoted in the text of ref 5. This small difference in rates could easily result from the differing steric requirements of the carboxyl and carboxmethoxy groups and from the small difference in the $pK_a$'s of the leaving groups. Hence, the proton of the carboxyl need not be catalytic at all.

We must then explain the 1000-fold difference in the $k_1$ and $k_2$ terms for ester 1. If the undissociated carboxyl group is not acting as a catalyst, then the ratio of $k_1$ to $k_2$ may be best ascribed to a depression in rate brought about by ionization of the $o$-carboxyl group. As stated above, the inductive change in the $pK_a$ of the leaving group due to electronic effects associated with the ionization of the carboxyl group should be comparable to that noted with 11; this should result in at least a tenfold decrease in the rate. Moreover, the presence of the $o$-carboxyl anion should have a very large electrostatic effect: its presence should tend both to destabilize formation of the transition state (1c) and the incipient hydroxyl anion. This kind of destabilization by adjacent charged moieties has previously been shown to occur,\(^{(52)}\) and should easily account for a 10- to 100-fold decrease in the rate. These effects, then, are probably sufficient to explain the observed differences in the rates for intramolecular carboxylate attack on the neutral and ionized esters. In any event, we must conclude from comparison of $k_1$ for 1 and 23 that there is no significant general acid catalysis occurring in this reaction, and that the bell-shaped profile for 1 results from a combination of intramolecular nucleophilic catalysis and electrostatic and electronic inhibition (i.e., INCEI).

The possibility remains, of course, that the hydrolysis of 1 proceeds via series nucleophilic catalysis (1a) in a manner similar to that observed for 2. The reactive intermediate would then be 1d. The basicity of the

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\((49)\) The data for ester 1 listed in Table II were obtained by enlarging the figure shown in ref 5 and, using the $pK_a$'s given in the text, fitting the curve to eq 3. The pH–log $k_{1\text{act}}$ profile for 23 is described by eq 4, and the value of the rate constant for its hydrolysis may be obtained either in the same manner from the figure of ref 5 or from the data of ref 34.

leaving group for this species (pK_a = 3) is sufficient, however, to explain the high reactivity of ester 1 as compared to the acetate, so the o-hydroxyl would appear to have no function in this mechanism either.

Salicylate Esters. The o-hydroxyl group has previously been shown to participate in the hydrolysis of various salicylate esters and amides\(^5\). In all cases, the pH-log \(k_{\text{obsd}}\) profile obtained was similar to that shown for 4 in Figure 4. The profile is generally interpreted as indicating the participation of the hydroxyl group either as a general base catalyzing the attack of water (4a) or as a general acid catalyzing the attack of hydroxide (4b). The magnitudes of the observed rate constants for hydrolysis of the salicylates in this study compared to other mono- and di-ortho-substituted benzoate esters\(^5\) indicate that in all cases at least one o-hydroxyl group must actively participate in the hydrolysis reaction.

Phenyl \(\gamma\)-resorcylate (12) was found to exhibit the same type of bell-shaped pH-log \(k_{\text{obsd}}\) profile as that previously observed for the methyl ester 3. For convenience then, a more complete study of the resorcylate system was made with the hydrolytically more labile phenyl esters 12-17 (Figure 5), whose hydrolyses may be conveniently followed spectrophotometrically. A complete profile was obtained only for the hydrolytically most susceptible ester 17. For compounds 14-16, which by all logic should have the same type profile, only the kinetically important plateau rates (from which \(k_1\) or \(k_i\) may be calculated) were obtained. Similarly, for ester 13, which should have the same profile as 10, only the upper plateau region for hydrolysis with the o-hydroxyl ionized was examined.

Two kinetically indistinguishable mechanisms may be written for esters 14-17 (and for 13 in the pH range 10-14); they are the general base catalyzed attack of water \([k_{\text{gb}}(4a)]\) and the general acid catalyzed attack of hydroxide \([k_{\text{ga}}(4b)]\), and are described by eq 4 and 12,

\[
k_{\text{obsd}} = \frac{k_{\text{gb}}K_w}{k_{\text{ga}} + a_H} \tag{12}
\]

respectively. The value of \(k_{\text{gb}}\) is obtained directly from the experimental plots, while \(k_{\text{ga}}\) is calculated from the relationship \(k_{\text{ga}}K_w = k_{\text{gb}}k_{\text{a}}\).

The most striking feature of Figure 5 or Table IV is the similarity of the observed plateau or of the calculated first-order rate constants \(k_{\text{gb}}\) for intramolecular general base catalyzed hydrolysis of the various phenyl 4- and 6-substituted salicylates: the largest and the smallest rate constants vary only by a factor of 6. The immediate conclusion is that there is no special increase or decrease of reactivity in the intramolecular general base catalyzed attack of water associated with 6-substitution of salicylate ester anions (although substituents larger than methoxyl were not examined). However, a pronounced steric decrease in the rate of uncatalyzed attack of hydroxide upon ionized salicylate esters accompanies 6-substitution (6-OCH\(_3\), 6-CH\(_3\)). This effect is not seen with substituents in the 4 position (except for 4-O\(^-\)). For the 4-O\(^-\) and 6-O\(^-\) esters, it would appear that the two oxyanions deactivate the carbonyl electronically to the extent that it is inert to unassisted attack of hydroxide.

The hydrolysis of ester 12 produces a simple bell-shaped pH-\(k_{\text{obsd}}\) profile; the two kinetically equivalent mechanisms, corresponding to 3a and 3b, are illustrated in Scheme III. The observed rate constants are related to the hydrogen ion concentration for the \(k_{\text{gb}}\) term by eq 5 and for the \(k_{\text{ga}}\) term by eq 13.

\[
k_{\text{obsd}} = \frac{k_{\text{ga}}K_wa_H}{k_{\text{gb}}(K_a + a_H) + a_H^2} \tag{13}
\]

In Figure 7 are plotted the values of \(\log k_{\text{gb}}\) and \(\log k_{\text{ga}}\) for esters 12-17 vs. the pK_a for ionization of the 2-hydroxyl. The values of \(k_{\text{gb}}\), \(k_{\text{ga}}\), and \(K_{\text{a}}\) for 12 have each been divided by two to correct for the fact that there are two o-hydroxyl groups which may participate.\(^4\) Considering first the \(k_{\text{gb}}\) points for a general base mechanism we find that all the points lie very close to a line with slope \(\beta = 0\). The \(k_{\text{ga}}\) points for a general acid mechanism are seen to lie very close to a line with slope \(\alpha = -1\). In the transition state for either mechanism, then, the proton is almost completely transferred and the observed first-order rate constant is consequently insensitive to changes in the basicity of the intramolecular catalyzing acid or base. The most im-


Figure 7. Bronsted plot of \(\log k_1(O)\) or \(\log k_1(\alpha)\) vs. the pH of the 2-hydroxyl group for phenyl salicylates.
The important feature of Figure 7 is that the points representing the hydrolysis of 12 fall on the same line as those for other 4- and 6-substituted salicylates: hence, the participation of only one hydroxyl group is sufficient to explain the observed maximum rate of hydrolysis. Again, then, we must explain the descending leg of the bell at alkaline pH's; the most obvious explanation is found in the IGCEI effect since the ortho oxyanion should strongly deactivate the ester carbonyl group to nucleophilic attack via inductive, resonance, and electrostatic interaction.

The IGCEI effect also serves to explain the pH dependence of the pseudo-first-order rate constants for hydrolysis of the methyl esters 3 and 10. The pH-log \( k_{\text{obs}} \) profile for the hydrolysis of 10 (Figure 4) is observed to have two pH-independent plateaus and two regions of slope +1. In Scheme IV are presented all reasonable pathways which might produce this type of profile. In this scheme, the observed rate constants will result from a combination of either paths \( k_{ga} \) and \( k_{ga}' \) (general acid, 1) or paths \( k_{gb} \) and \( k_{gb}' \) (general base, 2). The relationships of the observed rate constants to the hydrogen ion concentration for these pathways are given by eq 14 and 15, respectively. Inspection reveals that both equations are identical in form with the empirical equation (3). Pathways \( k_{ga} \) and \( k_{ga}' \) are analogous to the mechanism of 3b, while \( k_{gb} \) and \( k_{gb}' \) are analogous to that of 3a.

There is little doubt that the first observed ionization constant for 10 is associated with the 4-hydroxyl group, because of both the shape of the curve and the fact that hydrogen-bonded hydroxyls invariably have a higher \( pK_a \) than nonhydrogen-bonded. Considering either possible mechanism then, the initial line of slope +1 corresponds to intramolecular general base catalyzed hydrolysis with the nonparticipating (4-) hydroxyl group protonated. Ionization of this group creates a strong electron source which donates electrons to the ester carbonyl thereby deactivating it to nucleophilic attack. This inhibition is manifested in the first plateau area. The second line of slope +1 then represents catalyzed hydrolysis with the 4-hydroxyl group ionized; the perpendicular distance between the two lines of slope +1 indicates the amount of inhibition resulting from ionization of the nonparticipating hydroxyl group. The final plateau is associated with ionization of the 2-hydroxyl group in the "normal" manner. The unique shape of the profile for 10, then, as compared to that for 3, results from the fact that the nonparticipating (inhibiting) hydroxyl group has a lower \( pK_a \) than does the participating hydroxyl group.

The hydrolysis of ester 3 produces a simple bell-shaped pH-log \( k_{\text{obs}} \) profile; the two kinetically equivalent mechanisms are those of 3a and 3b, as in Scheme III. In Figure 8 are plotted the values of log \( k_{gb} \) and log \( k_{ga} \) for esters 3, 4, and 10 vs. the \( pK_a \) for ionization of the 2-hydroxyl group. Again, the values of \( k_{gb} \), \( k_{ga} \), and \( K_a \) for 3 have been divided by two as a statistical correction. The value of \( K_a' \) for the ionization represented by 10a was obtained from the relationship

\[
K_a' = K_a K_s \quad \text{(Scheme IV)}
\]

\( K_a' \) was estimated in the same manner as before, using \( pK_a = 9.80 \) (30°) for methyl 4-methoxysalicylate as a model for \( pK_a \) and \( pK_a = 7.8 \) at 30°. The values thus obtained are \( K_s = 10^{-2} \) and \( pK_a' = 9.24 \). The observed value of \( k_{gb} \) for 10 must also be divided by \( K_a' \) for correct interpretation.

Inspection of Figure 8 shows it to be virtually identical with Figure 7, so that anything said about hydrolysis of the phenyl esters applies equally to the methyl esters. Again, the points for the 6-hydroxy compounds lie very close to the lines for the other compounds. There is thus absolutely no evidence for catalytic participation by the second hydroxyl group in either of the 2,6-dihydroxy esters.

We are thus forced to the conclusion that there are no evident examples in the literature for intramolecular


\( \text{(56)} \) This work.
general acid, general base or nucleophilic, general acid catalysis of the hydrolysis of esters in water. (This statement applies to models employing two separate potential catalytic functional groups.) This result is in accord with previous searches for intramolecular bifunctional catalysis: thus, Koshland could find no evidence for concerted catalysis by imidazolyl and carboxyl groups in the hydrolysis of ester 24,57 and Coward and Bruce58 could find no evidence for intramolecular concerted general acid, general base catalysis by tertiary amino groups in the elimination of ketones. Thus, the many postulated mechanisms for enzyme mediated reactions embodying a push–pull mechanism await experimental confirmation that such a mechanism is actually possible.

**Acknowledgment.** This work was supported by grants from the National Institutes of Health and the National Science Foundation.

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**α Effects. III. The Reaction of Malachite Green with Primary Amines, Methoxylamine, and Hydrazines**

**J. Edward Dixon** and Thomas C. Bruice*

*Contribution from the Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106. Received May 20, 1970

Abstract: The rate and equilibrium constants associated with the addition of hydrazine, methylhydrazine, and methoxylamine (all possessing a pair of unshared α electrons) to malachite green are larger than are corresponding rate and equilibrium constants for primary amines of similar pKₐ. It may be concluded for this reaction that the α effect results in part from a product of greater stability than predicted from the pKₐ for amines. For the "more stable product" to transmit its stability to the transition state (kinetic α effect) it is essential that a large amount of bond formation has occurred in the critical transition state. Therefore, reactions exhibiting large Bronsted β values exhibit the α effect, whereas those with small β values, where the transition state resembles reactants rather than products, generally do not. The Bronsted β values for the reaction of amines and hydrazines with malachite green are identical at 0.4. Plots of ΔF⁺ vs. ΔF⁰ afford the linear free energy relationship, ΔΔF⁺/ΔΔF⁰ = 1.0. Thus all changes in ΔF⁰ are reflected quantitatively in a concomitant change in ΔF⁺. However, since ΔF⁰ values are close to zero and the Bronsted β ≈ 0.4, so that the transition state must reside ca. midway between reactants and products, it would appear as though factors in addition to product stability account for a portion of the kinetic α effect. The possibility that solvent effects or intramolecular general base catalysis are the source of the α effect is ruled out by consideration of activation parameters. Polarizability seems to be of little or no importance in accounting for the hyperreactivity of hydrazines in nucleophilic substitution on dinitrohalobenzenes.

The majority of previous investigations deals solely with the determination of rate constants. In the present study we sought a system that did not involve metastable intermediates and would provide: (a) rate constants, (b) equilibrium constants, and (c) Bronsted β constants. Our objective has been to determine the relationship of the decreased ΔF⁺ associated with the α effect to ground state free energies and to the position of the transition state along the reaction coordinate. Such a system has been found in the reaction of malachite green (A) with a number of amines and hydrazines (i.e., eq 1). The choice of amine addition to 24.

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## References

(1) A portion of the material to be submitted by J. E. D. in fulfillment of the requirement for the Ph.D. in Chemistry, University of California at Santa Barbara.

(2) To whom inquiries should be addressed.


