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Intramolecular General Base Catalyzed Transesterification

The Cyclization of Ethyl 2-Hydroxymethylbenzoate and Ethyl 2-Hydroxymethyl 4-Nitrobenzoate to Phthalide and 5-Nitrophthalide[1]

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The rates of cyclization of ethyl 2-hydroxymethylbenzoate to phthalide have been measured in H_2O at 30°C with $\mu = 0.5$. There is pronounced general base catalysis in the reaction with $\beta = 0.87$. The second-order rate constant for imidazole general base catalysis is decreased in D_2O as compared with H_2O by a factor of 3.46. The pH–rate constant profile obtained by extrapolation to zero buffer concentration shows hydronium ion and apparent hydroxide ion catalysis. The value of the second-order rate constant k_{OH} is 10^5 greater than k_{OH} for hydrolysis of ethyl benzoate. Ethyl 2-hydroxymethyl 4-nitrobenzoate cyclizes to 5-nitrophthalide in a similar manner. The Brönsted coefficient β for general base catalysis is 0.97, within error of unity. Thus, it is probable that general base catalysis involves rate-determining proton transfer.

INTRODUCTION

The α-chymotrypsin-catalyzed hydrolysis of esters and amides involves acylation of serine-195, with release of the alcohol or amine portion of the substrate, followed by deacylation to regenerate active enzyme. (*1–3*). In the generally accepted mechanism for the acylation step, histidine-57 functions as a general base, partially abstracting a proton from the serine hydroxyl as it attacks the carbonyl group of the substrate (*1–3*). Chemical intramolecular reactions bear a striking resemblance to corresponding enzymatic reactions proceeding through an enzyme-substrate complex (*1–3*). Consequently, the study of intramolecular catalysis has been of great importance in attempts to understand the mechanism of α-chymotrypsin and of enzyme action in general.

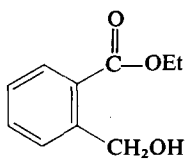
Phenoxide and alkoxide ions have been studied as intramolecular nucleophiles in reactions of substituted phenyl and ethyl carbamate esters (*4, 5*). These reactions are very efficient, and effective molarities for the neighboring group of 10^6–10^8 *M* can be calculated in comparison with analogous bimolecular reactions. Thus, a neighboring oxide ion is a powerful intramolecular nucleophile in reactions at the ester carbonyl. Capon et al. (*6*) have recently reported that aryl esters of 4-hydroxybutyric acid 5-hydroxyvaleric acid, 2-hydroxyphenylacetic acid, and 3-(2-hydroxyphenyl) propionic acid lactonize with rate constants proportional to 10^{pH-pK_w}. The second-order rate con-

[1] A portion of this work has been published as a preliminary communication in (*24*).

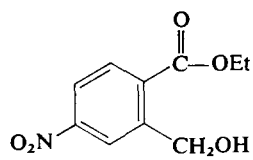
[2] National Institutes of Health Predoctoral Fellow. This work represents a portion of the Ph.D. thesis of Bruce M. Benjamin, University of Southern California, 1974.

stant at 30° C for lactonization of phenyl 4-hydroxybutyrate is ca. 3000 times greater than k_{OH} for hydrolysis of phenyl acetate at 25° C.

A neighboring hydroxymethyl group has been found to act as a nucleophile towards amides (*7–9*). In hydrolysis of γ-hydroxybutyramide an accelerated rate is found in the alkaline and neutral pH regions in comparison with acetamide and butyramide (*7*). These reactions probably involve intramolecular attack of the oxyanion on the neutral and protonated amide, respectively. Buffer catalysis is observed in the cyclization of 2-hydroxymethylbenzamide (*8*). Both general base and general acid catalysis by imidazole were reported. There have not previously been any studies of general base catalyzed transesterification reactions of alkyl esters analogous to the chymotrypsin acylation step. In the present work, we have found such catalysis in the cyclization of ethyl 2-hydroxymethylbenzoate (I) to phthalide and ethyl 2-hydroxymethyl 4-nitrobenzoate (II) to 5-nitrophthalide.



I



II

Experimental Section

Materials. All compounds and reagents obtained commercially were of reagent grade. Buffers were prepared using analytical grade materials and deionized water. Amine buffers were prepared from freshly distilled or recrystallized amines. Phthalide (mp 73–74° C) was obtained from Matheson, Coleman and Bell. 5-Nitrophthalide was prepared from 4-nitro-*o*-toluic acid by photochemical bromination in carbon tetrachloride using an adaptation of the method of Eliel and Rivard, (*10*) removal of the solvent on a rotary evaporator, and hydrolysis in excess sodium hydroxide solution to replace the α-bromo group with a hydroxyl. Addition of acid catalyzes lactonization to the phthalide which precipitated. Recrystallization from ether/hexane yielded a pure sample of 5-nitrophthalide (mp 149–151° C; lit. (*11*) 151° C).

Triethyloxonium fluoroborate was prepared by dropwise addition of epichlorohydrin in ether to gently refluxing boron trifluoride etherate in ether. The precipitate was filtered and was dissolved in acetonitrile. 2-Hydroxymethylbenzoic acid was prepared by hydrolysis of phthalide in aqueous base followed by neutralization with HCl to pH 5.5. While holding the pH constant with molar sodium carbonate, a threefold excess of the triethyloxonium fluoroborate solution in acetonitrile was rapidly added dropwise to the aqueous solution. Immediately after the pH had stabilized, the solution was extracted with ether. The ether extract was then dried over magnesium sulfate and the ether evaporated. Infrared analysis of the ester product showed a strong hydroxyl absorption peak at 3.0 μ and an intense carbonyl absorption at 5.85 μ (the infrared spectrum of phthalide has an intense carbonyl peak at 5.65 μ). Attempted distillation of the liquid product at low pressure resulted in lactonization to a pure sample of phthalide. A tetrahydropyranyl derivative of ethyl 2-hydroxymethyl benzoate was prepared by

adding the ester to dihydropyran with a few crystals of *p*-toluenesulfonic acid as a catalyst. The product was distilled, boiling at 127° C (0.5 mm). *Anal.* Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 67.91; H, 7.48.

The identical synthetic procedure was used for the preparation of ethyl 2-hydroxymethyl-4-nitrobenzoate from 5-nitrophthalide. The solid product was recrystallized from ether/hexane, melting at 63–64° C. Infrared and elemental analysis confirmed its identity and purity. *Anal.* Calcd. for $C_{10}H_{11}NO_5$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.05; H, 4.94; N, 6.19.

Kinetic methods. Ultraviolet spectra of the ester substrates and their corresponding phthalides were obtained using a Cary 15 spectrophotometer and 1 cm quartz cells containing the appropriate buffer in both sample and reference compartments. Compounds were introduced as solutions in 10 to 30 μl of acetonitrile or methanol, and spectra were taken immediately after mixing. With both ester substrates, as the reaction proceeds, the ultraviolet spectrum approaches that of the corresponding phthalide until the spectra are identical at complete reaction. In Fig. 1, initial spectra are shown for ethyl 2-hydroxymethyl 4-nitrobenzoate and the 5-nitrophthalide product.

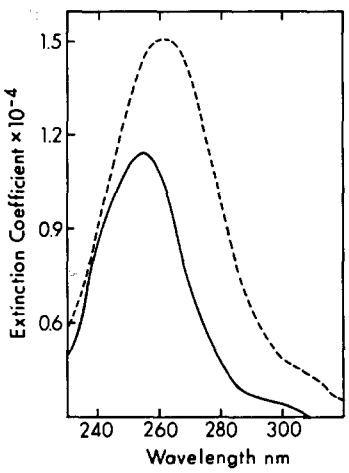


FIG. 1. Ultraviolet spectra for ethyl 2-hydroxymethyl-4-nitrobenzoate and 5-nitrophthalide. The phthalide spectra is drawn in the solid line.

The cyclization reactions were monitored by following changes in optical density due to disappearance of the ester at 275 or 254 nm. Stock solutions of substrate (1×10^{-2} *M*) were made up in anhydrous acetonitrile, 50 μl of the substrate stock solution was injected into the reaction cuvette containing 3 ml of buffer, and the reaction was monitored at the appropriate wavelength after mixing. Temperature was controlled at 30 ± 0.1° C, and the ionic strength of the buffers was kept constant at 0.5 *M* with KCl. Final concentrations were 1.64×10^{-4} *M* substrate and 1.64% acetonitrile.

Reactions too rapid for the conventional recording spectrophotometer were followed using a Durrum–Gibson stopped-flow spectrophotometer (Model D 110). The substrate was dissolved at the desired concentration in a pH 3.0 solution where it is reasonably stable. This solution was introduced into one of two identical drive syringes. The other

syringe contained a high pH buffer, such that on rapid mixing of equal volumes from the two syringes a reaction solution at the required pH was obtained. The drive syringes, mixing chamber, and cuvette were suspended in a water trough whose temperature was maintained at 30 ± 0.1° C. Optical density changes after mixing were recorded on a Hewlett–Packard storage oscilloscope (Model 1207B). With each buffer, four to six pairs of reactions with overlapping oscilloscope traces were tabulated. Reaction solution pH values were measured with a Radiometer pH meter Model 22 and GK 2303C combined electrode standardized withMallinckrodt standard buffer solutions. Pseudo-first-order rate constants were calculated with an IBM 360 computer using a program designed for rigorous least squares adjustment of nonlinear data. An Olivetti–Underwood Programma 101 was employed to calculate the least squares slopes and intercepts of linear data.

Results

Imidazole is an excellent catalyst for the cyclization of ethyl 2-hydroxymethylbenzoate (I) to phthalide, and ethyl 2-hydroxymethyl-4-nitrobenzoate (II) to 5-nitrophthalide at 30° C and $\mu = 0.5$ *M* (held constant with KCl). The plots of k_{obsd} *versus*

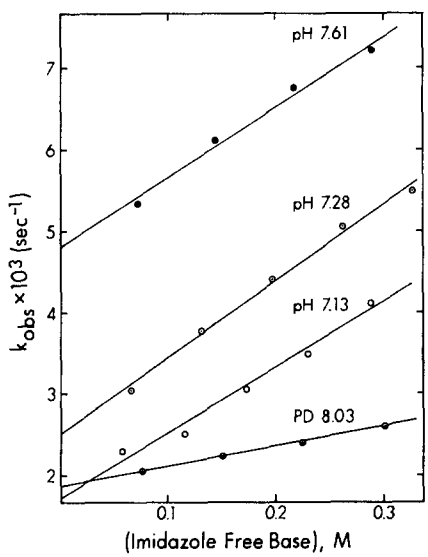


FIG. 2. Plot of k_{obsd} versus the imidazole free base concentration for cyclization of ethyl 2-hydroxymethylbenzoate to phthalide at 30°C and $\mu = 0.5$ at pH 7.13 (○), pH 7.28 (⊙), pH 7.61 (●), and pD 8.03 (◒).

imidazole free base concentration at 3 pH values in Fig. 2 for cyclization of I are linear and parallel. Thus, there is a first-order dependence on imidazole free base concentration in the reaction and k_{obsd} is given by Eq. (1). The value of k_{Im}, the second-order

$$k_{obsd} = k_0 + k_B(B) \quad (1)$$

rate constant for general base catalysis by imidazole, in the case of I, is $8.75 \times 10^{-3}\ M^{-1}$ sec^{-1}. Rate constants were also obtained in D_2O as the solvent, the ratio of rate constants ($k_{Im}^{H_2O}/k_{Im}^{D_2O}$) being 3.46.

A variety of other bases also act as intermolecular general base catalysts of the cyclization reactions of I and II. The values of k_B, the second-order rate constants, are given in Tables 1 and 2. Figures 3 and 4 show linear plots of the logarithms of the second-

TABLE 1

RATE CONSTANTS FOR SPONTANEOUS AND GENERAL BASE CATALYSIS[a]

Buffer	pH	k_0 (sec^{-1})	pK_a	k_B (M^- sec^{-1})
Acetate	5.60	5.00×10^{-5}	4.76	4.71×10^{-5}
Cacodylate	6.22	2.21×10^{-4}	6.33	6.05×10^{-4}
Imidazole	7.13	1.72×10^{-3}	7.00	8.75×10^{-3}[b]
	7.28	2.50×10^{-3}		
	7.61	4.80×10^{-3}		
	pD[c] = 8.03	1.87×10^{-3}	7.54	2.52×10^{-3}
Tris	8.13	2.08×10^{-2}	8.10	1.71×10^{-2}
Borate	9.38	1.79×10^{-1}	9.14	2.83×10^{-1}
N,*N*-Dimethylethanolamine	9.72	4.42×10^{-1}	9.13	2.53×10^{-1}
Diethylamine	10.98	6.16	10.89	10.00
HCl[d]	0.00	3.36×10^{-3}		
	0.38	1.59×10^{-3}		
	0.58	9.29×10^{-4}		
	1.03	3.33×10^{-4}		
	2.54	1.00×10^{-5}		

[a] For cyclization of ethyl 2-hydroxymethylbenzoate. Cyclization was monitored at 254 nm at 30°C and $\mu = 0.5\ M$ with KCl; 1.64% acetonitrile.

[b] Second-order rate constants for buffer catalysis were averaged when more than one pH series was run with the same buffer.

[c] Reaction run in D_2O as the solvent; pD = pH + 0.41.

[d] Acid catalysis was measured in HCl solutions with μ kept constant at 0.5 M with KCl except at 1.0 M acid.

order rate constants for general base catalysis versus pK_a of the catalyzing base. The slope of Fig. 3, β, for general base catalyzed cyclization of ethyl 2-hydroxymethylbenzoate is 0.87 ($r = 0.994$). From Fig. 4, the Brönsted coefficient for general base catalyzed cyclization of ethyl 2-hydroxymethyl-4-nitrobenzoate is 0.97 ($r = 0.995$). Of interest in Figs. 3 and 4 is the fact that buffers of different type and charge (i.e., amines, aliphatic acids, borate) give a linear relationship.

The rate constants k_0 for spontaneous cyclization at 30° C were obtained by extrapolation to zero buffer concentration. Spontaneous cyclization of I and II is subject to both hydroxide and hydronium ion catalysis. The values of k_0 are given in Tables 1 and 2. Figure 5 shows the log k_0 versus pH profiles. The limbs of the profiles have slopes of +1.0 and −1.0 for hydroxide and hydronium ion catalysis, respectively. Second-order rate constants for I are $k_{OH} = 10^4\ M^{-1}\ sec^{-1}$ and $k_H = 3.35 \times 10^{-3}\ M^{-1}\ sec^{-1}$.

TABLE 2

RATE CONSTANTS FOR SPONTANEOUS AND GENERAL BASE CATALYSIS[a]

Buffer	pH	k_0 (sec^{-1})	pK_a	k_B(M^{-1} sec^{-1})
Acetate	5.54	2.46×10^{-4}	4.76	1.57×10^{-4}
Cacodylate	6.20	8.22×10^{-4}	6.33	2.42×10^{-3}
Imidazole	7.14	5.47×10^{-3}	7.00	2.29×10^{-2}[b]
	7.41	1.10×10^{-2}		
	7.76	2.07×10^{-2}		
	pD[c] = 8.02	9.09×10^{-3}	7.54	7.25×10^{-3}
Tris	8.26	7.57×10^{-2}	8.10	1.20×10^{-1}
N,*N*-Dimethylethanolamine	9.80	2.51	9.13	1.45
Diethylamine	10.92	2.91×10^{1}	10.89	1.22×10^{2}
HCl[d]	−0.32	2.60×10^{-3}		
	0.00	7.95×10^{-4}		
	0.38	3.40×10^{-4}		
	0.58	2.21×10^{-4}		
	1.03	8.37×10^{-5}		

[a] For cyclization of ethyl 2-hydroxymethyl-4-nitrobenzoate. Cyclization was monitored at 275 nm at 30°C at $\mu = 0.5$ *M* with KCl; 1.64% acetonitrile.

[b] Second-order rate constants for buffer catalysis were averaged when more than one pH series was run with the same buffer.

[c] Reaction run in D_2O as solvent; pD = pH + 0.41.

[d] Acid catalysis was measured in HCl solutions with μ kept constant at 0.5 *M* with KCl except at 1.0 *M* acid.

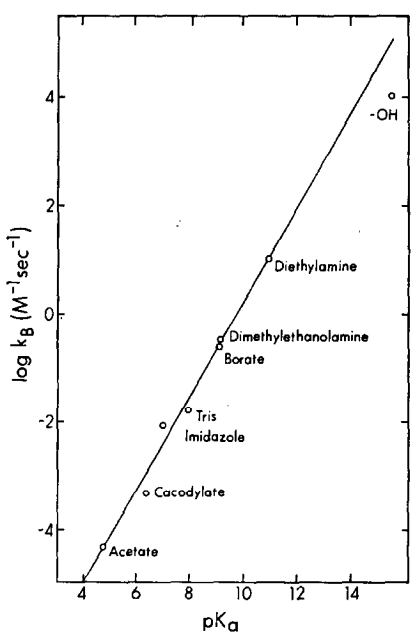


FIG. 3. Brönsted plot of log k_B versus the pK_a of the catalyzing base in the cyclization of ethyl 2-hydroxymethylbenzoate to phthalide at 30°C in H_2O ($\mu = 0.5$).

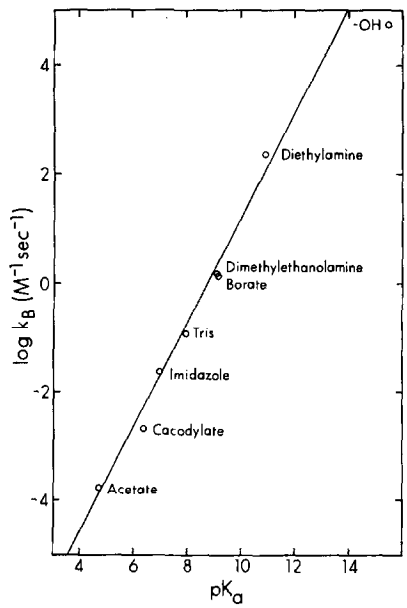


FIG. 4. Brönsted plot of log k_B versus the pK_a of the catalyzing base in the cyclization of ethyl 2-hydroxymethyl-4-nitrobenzoate to 5-nitrophthalide at 30°C in H_2O ($\mu = 0.5$).

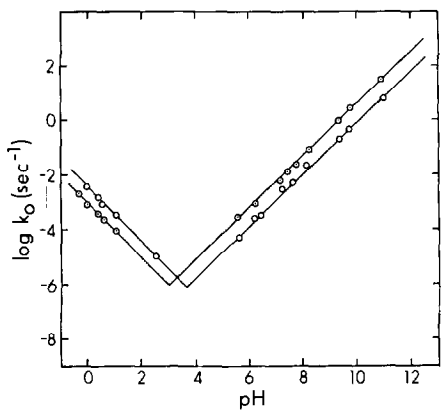


FIG. 5. Plot of log k_0 versus pH for cyclization of ethyl 2-hydroxymethylbenzoate (○) and ethyl 2-hydroxymethyl-4-nitrobenzoate (⊙) to phthalide and 5-nitrophthalide at 30°C in H_2O with $\mu = 0.5$. The points were obtained by extrapolation to zero buffer concentration.

The minimum equation for k_{obsd} is thus given by Eq. (2). The observed rate constants for cyclization catalyzed by hydroxide ion are less in

$$k_{obsd} = k_H(H^+) + k_{OH}(OH^-) + k_B(B) \quad (2)$$

D_2O than H_2O ($k_{OH}^{H_2O}/k_{OH}^{D_2O} = 1.14$). In calculating second-order rate constants the ion-product of water, K_w, and K_{D_2O} at 30° C were taken to be 1.47×10^{-14} and $0.2 \times$

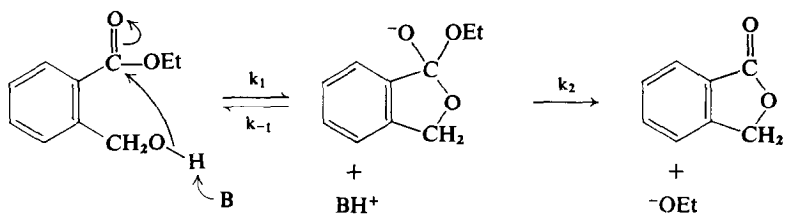
10^{-14}, respectively. The point for hydroxide ion catalyzed cyclization on the Brönsted plot of Fig. 3 is roughly a log unit below the line established with general base catalysts.

Figure 5, which gives the log k_0 versus pH profiles, shows that the rate of reaction of ethyl 2-hydroxymethyl-4-nitrobenzoate is similar to that of the unsubstituted ester. The rate constant for apparent hydroxide ion catalysis is increased only fivefold ($k_{OH} = 5.0 \times 10^4\ M^{-1}\ sec^{-1}$) by substitution of a nitro group in the 4-position while the rate constant for acid catalysis is decreased by a like amount ($k_H = 7.95 \times 10^{-4}\ M^{-1}\ sec^{-1}$). The second-order rate constant k_{Im} for imidazole-catalyzed cyclization of II is decreased by a factor of 3.15 in D_2O compared with H_2O, and the rate constant for the apparent hydroxide ion catalyzed reaction, k_{OH}, is decreased by a factor of 1.15 in D_2O. The D_2O solvent isotope effect on k_{OH} for I and II is possibly due in part to an increase in the pK_a of the hydroxymethyl group is D_2O.

Discussion

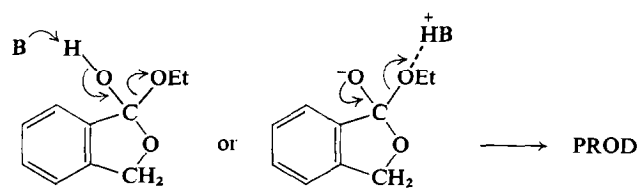
Buffer catalysis is observed in the intramolecular transesterification of ethyl 2-hydroxymethylbenzoate to phthalide. There is general base catalysis by imidazole and a variety of other bases. The mechanism could, therefore, be closely analogous to the mechanism for acylation of α-chymotrypsin by ethyl esters (Eq. (3) or a kinetic equivalent). A Brönsted plot of log k_B for general base catalysis of the cyclization of I versus the pK_a of the catalyzing base is linear (Fig. 3) with a slope (β) of 0.87, showing that proton transfer is appreciable in the transition state. There are several kinetically equivalent mechanisms for the general base catalysis observed in the cyclization of I and II. Equations (3)–(6) illustrate these mechanisms.

(a) General base catalysis of formation of a tetrahedral intermediate.



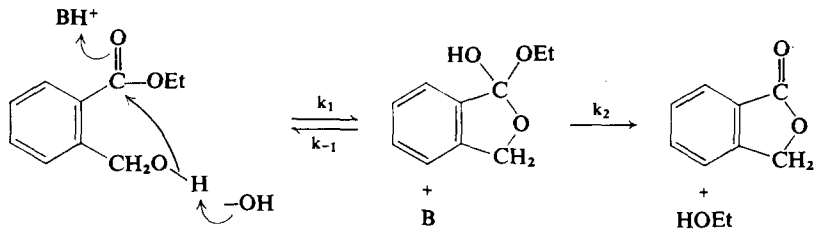
(3)

(b) General base or general acid catalysis of breakdown of a tetrahedral intermediate.



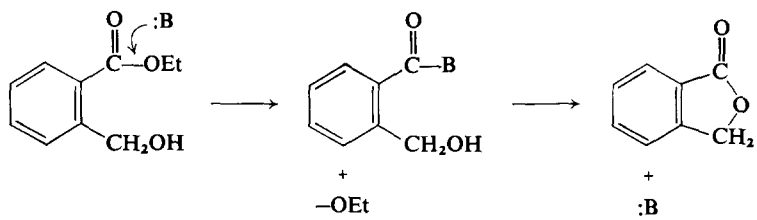
(4)

(c) General acid-specific base catalysis.



(5)

(d) Nucleophilic catalysis by the base.

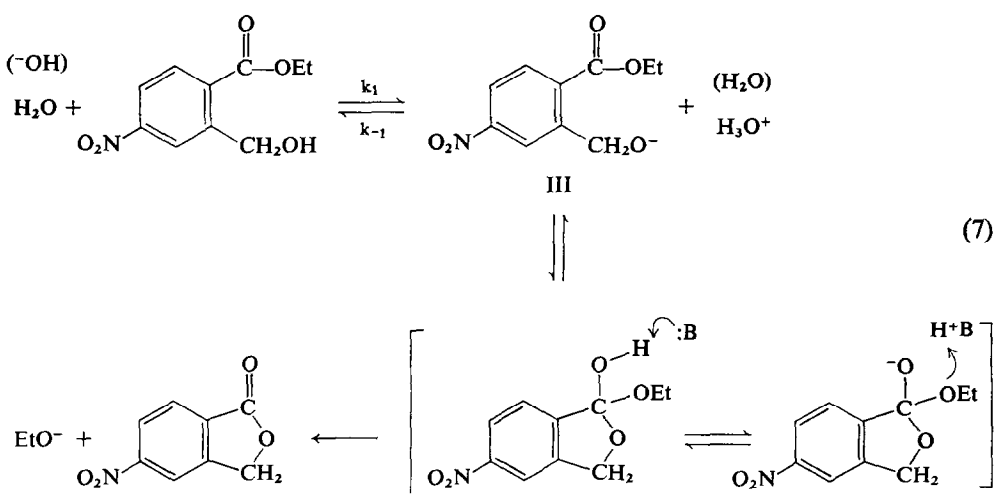


(6)

Intermolecular nucleophilic catalysis (Eq. (6)) can be eliminated as a possible mechanism on the basis of several arguments. Consideration of the relative pK_a values of the leaving group and nucleophiles strongly suggests that should nucleophilic attack occur the tetrahedral intermediate would undergo preferential breakdown to starting materials. Imidazole is a much better leaving group than ethoxide (pK_a 16). For this reason, nucleophilic catalysis by imidazole has not been observed in hydrolysis of aliphatic esters (*1–3*). In addition, the Brönsted plots are linear with the rate constants for catalysis by bases of differing types fitting on the same line. The Brönsted plot for nucleophilic displacements at the ester carbonyl of *para*-nitrophenyl acetate is characterized by separate lines for different types of nucleophiles (for example, primary amines, imidazoles, or oxy anion nucleophiles) (*12*). In Brönsted plots for general base catalyzed reactions, however, bases of widely varying type have been found to give rate constants lying on the same line (*13*, *14*). Nucleophilic reactions are not subject to major D_2O solvent isotope effects (*15*). Rate constants obtained in D_2O as the solvent give $k_{1m}^{H_2O}/k_{1m}^{D_2O} = 3.46$ for imidazole-catalyzed cyclization of ethyl 2-hydroxymethylbenzoate, showing that proton transfer is occurring in the critical transition state. Thus, a nucleophilic mechanism (Eq. (6)) for general base catalysis in this system can be conclusively eliminated. The D_2O solvent isotope effect, however, cannot be used further to distinguish between specific and general catalyzed processes whose rate equations are kinetically indistinguishable.

The kinetically equivalent general base mechanisms of Eqs. (3), (4), and (5) differ not only in the site of catalysis but in that the rate-determining steps are different. If a

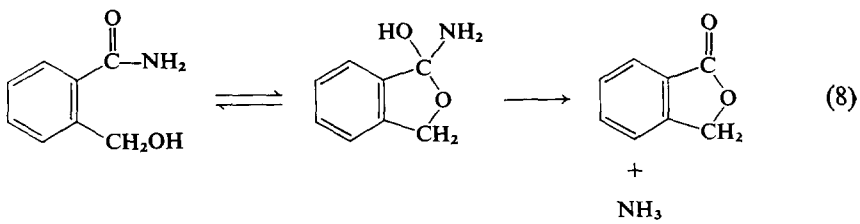
tetrahedral intermediate is present at steady state concentrations then $k_{obsd} = k_1k_2/(k_{-1} + k_2)$. If $k_{-1} \ll k_2$ then k_1 (nucleophilic attack) will be rate limiting. On the other hand, if $k_2 \ll k_{-1}$ then breakdown of the tetrahedral intermediate would be rate-determining. The rate-determining step for intramolecular carboxylate ion attack on phenyl esters is breakdown of a tetrahedral intermediate (*16*), whereas in bimolecular hydroxide ion catalysis formation of a tetrahedral intermediate is rate-determining (*16*, *17*). In alcoholysis reactions of alkyl esters it might be expected that the tetrahedral intermediate would partition equally between products and starting materials since the leaving groups in the two cases would be of nearly the same basicity. In bimolecular reactions this is probably the case, but in the intramolecular reactions of I and II, ethoxide ion expulsion from a tetrahedral intermediate would be accompanied by a favorable translational ΔS^* in comparison with breakdown to starting material. However, rotational ΔS^* differences should favor breaking the five-membered ring. This, coupled with the fact that the pK_a of the hydroxymethyl group of I may be somewhat less than that of ethanol, prohibits an unequivocal choice in regard to rate-determining step. It is possible that in the case of I both steps may be kinetically important. The nitro substituent of II should reduce the pK_a of the hydroxymethyl group. At the same time, expulsion of ethoxide ion would be more difficult. Therefore, in the case of II, the observed general base catalysis may be catalysis of the breakdown of a tetrahedral intermediate (Eq. (4)). General base catalysis of the cyclization of ethyl 2-hydroxymethyl 4-nitrobenzoate is characterized by a Brönsted coefficient of 0.97, within error of unity, which is characteristic of a diffusion-controlled proton transfer in the thermodynamically unfavorable direction (*18*,). General base catalyzed formation of a tetrahedral intermediate (Eq. (3)) can be ruled out if proton transfer is rate-determining. The second-order rate constant (k_1) for imidazole-catalyzed ionization of the hydroxymethyl group to alkoxide ion (III in Eq. (7)) would be given by $k_1 = k_{-1}\ K_a/K_{Im}$, where K_a and K_{Im} are the dissociation constants of the hydroxymethyl group and imidazolium ion, respectively, The magnitude of the rate constant for the reverse reaction k_{-1} should be that of a diffusion-controlled reaction



(10^{10} M^{-1} sec^{-1}). Assuming a reasonable pK_a for II of 14–16, a value of k_1 of 10–10^3 M^{-1} sec^{-1} can be calculated, which is several orders of magnitude greater than that observed.

A value of 2×10^{-2} M^{-1} sec^{-1} for the rate constant for imidazole-catalyzed cyclization is also much less than that required for general acid catalyzed formation of a tetrahedral intermediate (Eq. (5)) if proton transfer is a stepwise process (assuming a reasonable value for the pK_a of the hydroxymethyl group). It is apparent that rate-determining proton transfer must be preceded by a preequilibrium step. Thus, catalysis of the breakdown of a tetrahedral intermediate (Eq. (7)) is the preferred mechanism.

Belke et al. (*8*) reported both general base and general acid catalysis in the lactonization of 2-hydroxymethylbenzamide (Eq. (8)). The Brönsted coefficient for general base catalysis is 1.0, again indicating that proton transfer is rate-determining. A Brönsted plot of log k_B versus pK_a of the catalyzing base shows apparent curvature,



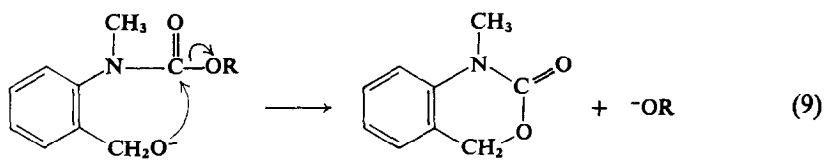
(8)

the slope changing to 0.2 at high pK_a, indicating a change in rate-determining step at about pH 8. In the hydrolysis of 1-benzylimino-1, 3-dihydroisobenzofuran, a tetrahedral intermediate similar to that of Eq. (8) is formed (*19*). 2-Hydroxymethyl benzamide is the major product at high pH whereas phthalide is the product at low pH showing preferential amine expulsion from the tetrahedral intermediate at low pH. It was concluded by Belke et al. (*8*) that imidazole-catalyzed cyclization of 2-hydroxymethylbenzamide to phthalide is a good model for acylation of α-chymotrypsin by amide substrates. The present findings with the corresponding ethyl esters I and II make derivatives of 2-hydroxymethylbenzoic acid reasonable models for reaction of α-chymotrypsin with both ester and amide substrates.

Lactonization of phenyl 4-hydroxybutyrate is catalyzed by acetate and phosphate buffers in what was considered to possibly be general base catalyzed reactions although no further evidence was presented (*6*). Imidazole catalysis of phenoxide ion release proceeds with nucleophilic attack by imidazole at the carbonyl of the ester. Consequently, in this nonrigid system, where the leaving group is a phenol, general base catalyzed intramolecular alcoholysis is not sufficiently advantageous to compete with nucleophilic attack by imidazole. Rearrangement of 2-hydroxymethyl-4-nitrophenyl trimethylacetate to its benzyl ester counterpart has, however, recently been reported (*20*) to be catalyzed by imidazole with a D_2O solvent isotope effect of 2.4. A general base mechanism was preferred, and therefore, it was concluded that the enzymatic reactions of *p*-nitrophenyl esters probably occur through a general base catalyzed pathway. Thus, it is chemically reasonable that acylation of α-chymotrypsin by both alkyl and aryl esters utilizes general base catalysis by histidine-57.

Extrapolation of the plots of k_{obsd} for cyclization of I and II versus buffer concentration to zero buffer concentration gives values of the rate constants for spontaneous cyclization. This reaction is subject to hydroxide and hydronium ion catalysis with $k_{OH} = 10^4\ M^{-1}\ sec^{-1}$ and $k_H = 3.55 \times 10^{-3}\ M^{-1}\ sec^{-1}$ in the case of I and $k_{OH} = 5 \times 10^4\ M^{-1}\ sec^{-1}$ and $k_H = 7.95 \times 10^{-4}\ M^{-1}\ sec^{-1}$ with II. Thus, the spontaneous reaction is a relatively facile process. In comparison, ethyl esters normally require high pH and elevated temperatures for rapid hydrolysis. In the case of ethyl benzoate, k_{OH} is $3.0 \times 10^{-2}\ M^{-1}\ sec^{-1}$ at 25° C in H_2O (*21*). Therefore, k_{OH} for I is approximately 10^5 greater, showing that a neighboring hydroxymethyl group is a powerful intramolecular nucleophile in transesterification reactions.

Neighboring phenoxide and alkoxide ions have previously been found to be excellent intramolecular nucleophiles toward carbamate esters (*4*, *5*). The pH-rate constant profile for cyclization of phenyl *N*-(2-hydroxyphenyl) *N*-methylcarbamate is sigmoidal with pK_{app} of 9.0. Preequilibrium ionization of the phenolic hydroxyl is followed by intramolecular nucleophilic attack by the oxygen anion on the ester carbonyl. In comparison with intermolecular nucleophilic attack by a phenoxide ion of the same pK_a on the unsubstituted ester phenyl *N*-methyl *N*-phenylcarbamate, the effective molarity of the neighboring phenoxide ion is $3 \times 10^8\ M$. Intramolecular alkoxide ion attack takes place in cyclization of *p*-nitrophenyl and ethyl esters of 2-hydroxymethyl *N*-methylcarbanilic acid (*5*) as in Eq. (9). Plots of log k_{obsd} versus pH are linear with



(9)

slopes of 1.0, indicating apparent hydroxide ion catalysis. The value of k_{OH} for the nitrophenyl ester is 3×10^5 greater than k_{OH} for hydrolysis of *p*-nitrophenyl *N*-methylcarbanilate and 10^5 greater than the second-order rate constant for bimolecular transesterification of *p*-nitrophenyl *N*-methylcarbanilate by pentaerythritol. Ring closure also occurs rapidly with the corresponding ethyl ester, the k_{OH} value being 10^6 greater than k_{OH} for hydrolysis of ethyl *N*-methylcarbanilate. Thus, the rate enhancements provided by a neighboring hydroxymethyl group are comparable for carbamate and carboxylate esters. The reactions differ in that buffer catalysis is not detected in cyclization of the carbamates. It is possible that the deactivated carbonyl of carbamate esters necessitates nucleophilic attack by a fully developed negative charge.

Bender et al (*22*) attempted to analyze α-chymotrypsin catalysis in terms of individual mechanistic factors. It is now possible to discuss the enzyme in terms of more rigorous chemical analogies than could be employed at the time of Bender's analysis. Bender (*22*) estimated that the alcoholysis of a carboxylic derivative would proceed at a rate approximately 100-fold faster than hydrolysis of the corresponding compound. It is now clear that the presence of a neighboring hydroxymethyl group will allow intramolecular alcoholysis to proceed at least 10^5 more rapidly than hydroxide ion-catalyzed hydrolysis.

Thus, a factor of 10^3 in the rate of α-chymotrypsin reactions, which had to be attributed to other factors, can now be accounted for satisfactorily in terms of the chemistry of the reaction. The most important single piece of information that is still missing for a complete analysis of the rates of α-chymotrypsin reactions is the effective molarity of an intramolecular general base in intramolecular alcoholysis reactions of esters and amides (see, however, (*9*)). With amide substrates, it appears that α-chymotrypsin catalysis will be explainable (*8*, *9*, *23*) in terms of: (1) the intracomplex character of the alcoholysis reaction involving serine-195; (2) general base catalysis by histidine-57; and (3) the intramolecular nature of the general base catalysis, without the necessity of invoking features dependent upon unknown chemistry (charge–relay, etc.)

From the second-order rate constant for imidazole-catalyzed cyclization of the ethyl ester I (9×10^{-3} M^{-1} sec^{-1}) and the rate constant for acylation of α-chymotrypsin by *N*-acetyl L-tyrosine ethyl ester (*22*) (4000 sec^{-1}), it can be calculated that in order to attain a rate constant of the magnitude seen in the α-chymotrypsin reaction, a neighboring imidazole would have to possess an effective molarity of 400,000 *M*. This is not necessarily an unreasonable value, but in view of the lack of chemical information on this point it should be considered that other factors may be important in the enzymatic reaction with ester substrates. Intramolecular alcoholysis is of comparable efficiency with esters and amides of 2-hydroxymethylbenzoic acid where the steric situation is similar. It appears, however, that this is not the case with α-chymotrypsin catalysis. It can be concluded that either steric factors are not optimal in the active site for attack of serine-195 on amide substrates, or that enzymatic acylation with ester substrates involves relatively advantageous mechanistic features.

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