ether, dried, evaporated, and distilled. The major fraction was collected at 100–104° (0.3 mm). The colorless oil slowly crystallized. The solid was recrystallized from ether at low temperature (−50°) to give mp 51–52°; nmr (CCl_4, TMS) (concentrated solution), τ 2.62 (1 H), 2.81 (5 H, multiplet), 6.4 (6 H); (concn, 94.5 mg/ml), 2.6 (1 H), 2.79 (5 H), 6.30 (3 H), and 6.36 (3 H).

Anal. Calcd for $C_{11}H_{12}O_3$: C, 68.72; H, 6.29. Found: C, 68.74; H, 6.09.

Acknowledgment. The authors are grateful to the Petroleum Research Fund for support of this work under Grant PRF 742A-4.

The Aminolysis and Amidinolysis of *p*-Nitrophenyl Acetate in Chlorobenzene. A Facile Bifunctional Reactivity.

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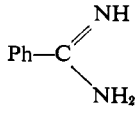
Contribution from the Department of Chemistry, Emory University, Atlanta, Georgia. *Received February 7, 1966*

Abstract: Benzamidine has been shown to react with *p*-nitrophenyl acetate in chlorobenzene with a second-order rate constant of 3.45 M^{-1} sec^{-1}. *n*-Butylamine, a nucleophile with a basicity similar to that of benzamidine, reacts with *p*-nitrophenyl acetate in chlorobenzene by means of a third-order process, the rate of which is little affected by the presence of large amounts of a tertiary amine. Benzamidine reacts at least 15,000 times faster than *n*-butylamine monomer. This reactivity of benzamidine with the ester in the aprotic solvent is attributed to the bifunctional nature of the nucleophile. The mechanism of proteolytic enzyme action is discussed in terms of these results.

The rates of nucleophilic reactions of carboxylic acid derivatives in media containing high concentrations of organic solvents are slow compared with the rates in pure water.[1] Jencks and Gilchrist[2] have recently shown, for example, that tetrahydrofuran and ethanol inhibit the reaction of methylamine with phenyl acetate in water. It is therefore tempting to assume that the reactive sites of proteolytic enzymes are regions of high water content. On the basis of this assumption, most models of these enzymes have been studied in aqueous solutions. It is possible, however, that the catalytic sites of the enzymes are nonpolar regions and that the transition states of the enzymatic reactions are neutral in character. Indeed, none of the transition states described in the recently proposed mechanism for α-chymotrypsin-catalyzed reactions[3] involves creation of charge. In order to test the idea that a nucleophilic attack on an ester may occur readily in a nonpolar medium if the process proceeds by means of a neutral transition state and tetrahedral intermediate, we have examined the reaction of benzamidine with *p*-nitrophenyl acetate (*p*-NPA) in chlorobenzene at 25°. Benzamidine is a bifunctional nucleophile which can concertedly attack the carbonyl carbon of the ester and deliver a proton to the carbonyl oxygen, thereby forming the tetrahedral intermediate without creation of charge. For comparison purposes, the reaction of *n*-butylamine with *p*-NPA in chlorobenzene was also examined. *n*-Butylamine is similar to benzamidine in basicity but does not possess its bifunctional character.



Experimental Section

Materials. Chlorobenzene was reagent grade material which had been distilled three times from P_2O_5 through an efficient column. Two kinetic runs were performed in chlorobenzene which had been shaken with several portions of H_2SO_4, washed with aqueous K_2CO_3, and distilled five times from P_2O_5. The more careful purification of the solvent did not change the rate constants. Acetonitrile, spectro quality, was distilled repeatedly from P_2O_5.

Benzamidine was prepared from its hydrochloride salt (Aldrich), sublimed three times, and handled under nitrogen in a dry box: λ_{max} (H_2O, pH 7) 268 mμ (log ε 2.91) and 229 mμ (log ε 3.96) (lit.[4] 268 mμ (log ε 2.95) and 228 mμ (log ε 4.06)).

n-Butylamine was reagent grade material distilled twice from KOH and once from zinc dust.

Kinetics. The reaction of benzamidine with *p*-NPA was followed by measuring the increase in absorbance at 320.0 mμ, due to *p*-nitrophenol formation, using a Cary 14 spectrophotometer thermostated at 25.0 ± 0.1°. The reactions were initiated by adding 50 μl of benzamidine in acetonitrile to a cuvette containing 3.00 ml of 1.07×10^{-5} *M* *p*-NPA in chlorobenzene. In all the runs the benzamidine was in greater than 20-fold excess over the ester, so that pseudo-first-order conditions prevailed. The reactions with the three highest concentrations of benzamidine were followed to completion, and the first-order plots were linear to greater than 85% reaction. Stoppered cuvettes were used in all cases.

The *n*-butylamine reactions were carried out in a similar manner except that the ester was added to the amine and that 360.0 mμ was used. The absorbance readings at completion of the reactions varied somewhat with the *n*-butylamine concentration, indicative of complexation. However, Beer's law is strictly obeyed at any constant excess *n*-butylamine concentration for a large range of *p*-nitrophenol concentrations, as would be expected from the Benesi–Hildebrand equation.[5]

Release of *p*-nitrophenol was quantitative with both the aminolysis and amidinolysis of *p*-NPA. The second product of the reaction between *p*-NPA and benzamidine, N-acetylbenzamidine, could not be detected by ultraviolet spectrophotometry under the conditions used for the kinetics because of solvent absorption. It is known that amidines and esters do indeed react to give acylamidines. For example, Titherly and Hughes[6] heated phenyl benzoate with benzamidine at 50° to prepare N-benzoylbenzamidine. When

(1) M. Gordon, J. G. Miller, and A. R. Day, *J. Am. Chem. Soc.*, 70, 1946 (1948).
(2) W. P. Jencks and M. Gilchrist, *ibid.*, 88, 104 (1966).
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(4) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).
(5) L. J. Andrews and R. M. Keefer, "Molecular Complexes in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964.
(6) A. W. Titherly and E. C. Hughes, *J. Chem. Soc.*, 99, 1493 (1911); see also D. A. Peak, *ibid.*, 215 (1952).

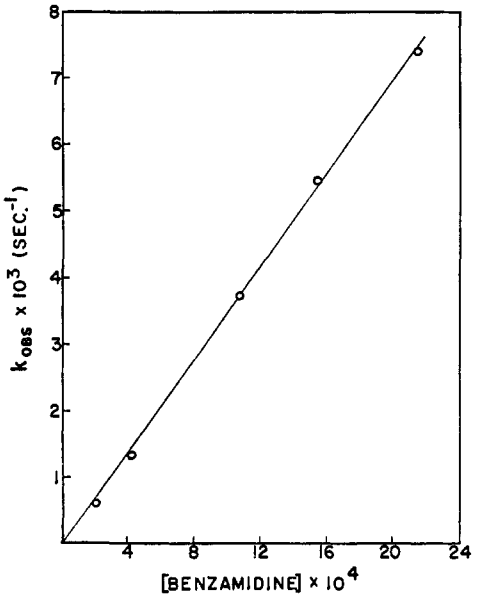


Figure 1. A plot of the observed rate constants for the reaction of benzamidine with 1.07×10^{-5} *M* *p*-nitrophenyl acetate in 1.6% acetonitrile–chlorobenzene at 25.0° *vs.* benzamidine concentration.

p-NPA is mixed with excess benzamidine in chloroform, the ester carbonyl stretching band at 5.67 μ disappears and a strong band at 6.25 μ appears. This band remains after the chloroform solution is shaken with pH 10 buffer.

Results

The observed pseudo-first-order rate constants for the reaction of *p*-NPA with excess benzamidine are presented in Table I. The rate constant does not vary with *p*-NPA concentration at a constant excess benzamidine concentration. A plot of k_{obsd} *vs.* [benzamidine] (Figure 1) is linear and has a zero intercept. These data show that the reaction is a second-order process with a rate constant of 3.45 M^{-1} sec^{-1}. This is a remarkably large rate constant for a nucleophilic reaction of an ester in a nonpolar solvent. It is only fourfold smaller than that for the reaction of *p*-NPA with hydroxide ion *in water*. As will be shown in the next section, the rate constant is very much larger than that for *n*-butylaminolysis of *p*-NPA in chlorobenzene.

Table I. The Observed Rate Constants for the Reaction of Benzamidine with *p*-Nitrophenyl Acetate[a]

[Benzamidine], *M*	[*p*-NPA] × 10^5 *M*	k_{obsd} × 10^3, sec^{-1}
2.15×10^{-3}	1.07	7.42
1.54×10^{-3}	1.07	5.46
1.08×10^{-3}	1.07	3.73
4.30×10^{-4}	1.07	1.34
2.15×10^{-4}	1.07	0.62
2.13×10^{-3}	2.12	7.51

[a] 25.0°, in 1.6% acetonitrile–chlorobenzene (v/v). The presence acetonitrile does not affect the rate constants.

The observed pseudo-first-order rate constants for the reaction of *p*-NPA with *n*-butylamine are presented in Table II. These data show that the reaction is first order in ester and second order in amine. A plot of k_{obsd} *vs.* [*n*-butylamine]^2 is linear and has a zero intercept. In this plot there is a tenfold change in total *n*-butylamine concentration and a corresponding 100-fold change in rate constants. It was also shown that *p*-NPA reacts a mere 30% faster in a mixture of 0.26 *M* N-methylpiperidine and 0.10 *M* *n*-butylamine as it does in 0.10 *M* *n*-butylamine.

Table II. The Observed Rate Constants for the Reaction of *n*-Butylamine with *p*-Nitrophenyl Acetate[a]

[Butylamine], *M*	[*p*-NPA] × 10^4 *M*	k_{obsd}, sec^{-1}
0.215	3.25	2.96×10^{-3}
0.172	3.25	1.90×10^{-3}
0.151	3.25	1.44×10^{-3}
0.108	3.25	7.10×10^{-4}
0.0862	3.25	4.59×10^{-4}
0.0689	3.25	2.88×10^{-4}
0.0221	3.25	2.91×10^{-5}
0.215	0.650	2.95×10^{-3}

[a] 25.0°, in chlorobenzene.

Discussion

The precise linear relationship between k_{obsd} and [benzamidine] (Figure 1) means that benzamidine must exist essentially all in the monomeric form or else all in the dimeric form in the concentration range used (2×10^{-4} to 2×10^{-3} *M*). This follows because the

$$2\text{Ph—C}\lessgtr^{\text{NH}}_{\text{NH}_2} \rightleftharpoons \text{Ph—C}\lessgtr^{\text{H}}_{\text{NH}_2\cdots\text{N}}\!\!\cdots\text{H}_2\text{N}\gtrless\text{C—Ph}$$

dimer is without doubt an unreactive species (the reactive atoms are engaged in complexation). If both monomer and dimer were present in appreciable amounts at the concentration range used here, then the k_{obsd} *vs.* [benzamidine] plot would be curved; at the higher benzamidine concentrations there would be a greater percentage of unreactive dimer in solution than at lower concentrations. Nevertheless, the data does not exclude a situation in which there is 1% monomer and 99% dimer. This is considered unlikely, however. It would mean that the *true* second-order rate constant for the reaction of benzamidine monomer with *p*-NPA would be 100-fold larger than that observed, 345 M^{-1} sec^{-1}! As will be shown below, mechanistic conclusions are drawn, in part, from the fact that 3.45 M^{-1} sec^{-1} is very much larger than the second-order rate constant for the reaction of *n*-butylamine monomer with *p*-NPA. Postulating dimeric benzamidine species would only increase the magnitude of this difference. It should be noted, however, that benzoic acid in benzene is largely dissociated in the concentration range used for benzamidine.[7]

Since the benzamidine and *n*-butylamine reactions are of different order, it is difficult to compare their rate constants. If the concentration of nucleophiles is specified arbitrarily to be 0.0221 *M* (the lowest *n*-butylamine concentration studied), then the k_{obsd} for benzamidinolysis would be more than 2500 times larger than k_{obsd} for butylaminolysis. A more meaningful comparison would be one between the benzamidinolysis reaction and the reaction of a *single* *n*-butylamine molecule (the monomer) with the ester, for these two reactions are of the same order in nucleophile. Such a

(7) B. C. Barton and C. A. Kraus, *J. Am. Chem. Soc.*, 73, 4561 (1951).

comparison would provide a measure of the acceleration due to the bifunctionality of the benzamidine (neglecting the relatively small pK_a difference between the two nucleophiles). To estimate the reactivity of a single amine species, it is assumed that the k_{obsd} is the sum of two terms.

$$k_{obsd} = k_1A_f + k_2A_f^2$$

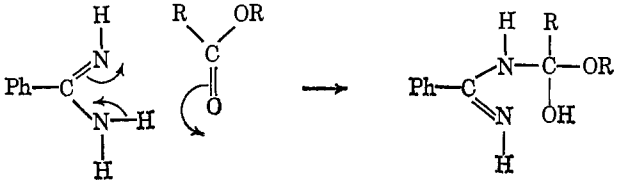
A_f represents the concentration of nonassociated *n*-butylamine. If it is a dimer that is reactive, then the second term would include an association constant, but such an expression would be kinetically indistinguishable from the one given. Since the concentration of the monomeric amine is very nearly equal to the total concentration of amine in the chlorobenzene (A_t), the k_{obsd} is given by

$$k_{obsd} = k_1A_t + k_2A_t^2$$

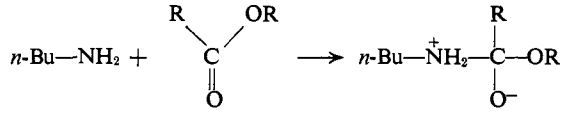
The work of Feeney and Sutcliffe[8] on the association of ethylamine in CCl_4 shows clearly that equating A_f and A_t is valid for the concentration range used in this study. Since a plot of k_{obsd}/A_t *vs.* A_t passes through the origin, it only is possible to place an upper limit on the value of k_1 by estimating the uncertainty in the zero intercept. It is found that k_1 must be less than 2×10^{-4} M^{-1} sec^{-1}. This means that benzamidine reacts with *p*-NPA in chlorobenzene at least 15,000 times faster than *n*-butylamine monomer!

Benzamidine thus shows a remarkable reactivity. It reacts with *p*-NPA in chlorobenzene about as fast as hydroxide ion reacts with *p*-NPA in water. Benzamidine (pK_a = 11.6) is 1.5×10^4 more reactive than *n*-butylamine (pK_a = 10.6) in chlorobenzene. (We have also investigated a nonaromatic amidine and estimate that it reacts 3×10^5 times faster than *n*-butylamine). It is clear that differences of such magnitude between the two types of reactants, both containing a nitrogen atom as the attacking nucleophile, are significant even if there is a disparity in the nucleophilicity–basicity relationship as described in the Brønsted law. A large disparity exists, for example, in the comparison of anilines and phenoxides of similar basicity.[9] The anilines are nearly 100-fold better nucleophiles, but this difference is particularly large because nitrogen bases and oxygen bases are being compared. It is not valid to attribute the much greater difference in reactivity between benzamidine and butylamine to Brønsted disparities alone.

We must conclude that the unusual reactivity of benzamidine is due to the bifunctional nature of the reactant.

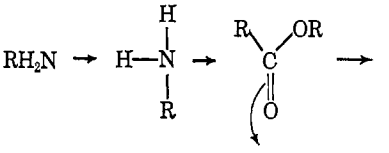


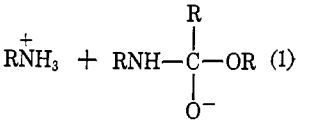
Benzamidine can react in a concerted process to form a neutral tetrahedral intermediate, which would rapidly collapse to acylamidine and *p*-nitrophenol. Such a reaction is facile because there is no charge formation in the transition state which resembles the tetrahedral intermediate.[10] Nucleophilic attack by *n*-butylamine monomer, on the other hand, involves the creation of charge which is inhibited in the nonpolar solvent.



Proton transfer may occur to some extent during nucleophilic attack, but the geometry for such a transfer is not nearly as favorable as in the benzamidine case where a six-membered cyclic transition state exists. The benzamidinolysis reaction is reminiscent of the 2-hydroxypyridine-catalyzed mutarotation of tetramethylglucose.[11] A concerted reaction similar to one proposed for the benzamidinolysis of *p*-NPA has been suggested for the reactions of several heterocycles with esters in acetonitrile.[12]

It has been shown that the butylaminolysis of *p*-NPA in chlorobenzene is second order in amine (Figure 2). Because of the nature of the solvent and the unlikelihood of a termolecular reaction, it is probable that amine dimer is the reactive species, although this cannot be proved from kinetic data. In any case, it is possible to write two mechanisms for the reaction. The first





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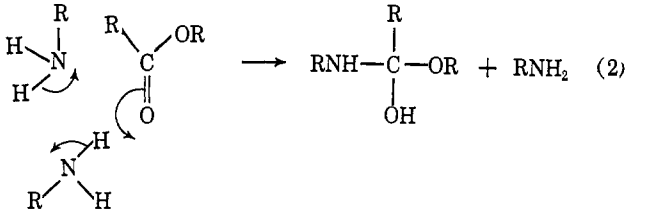

(2)

involves charge creation, and is analogous to the general base catalysis which occurs in water.[2] The second mechanism, first proposed by Bruice and Mayahi,[13] is a cyclic concerted process which involves no charge formation in the transition state. If the first mechanism is correct, then addition to the *n*-butylamine–ester system of a teritary amine should accelerate the reaction, for it too can remove a proton from the nucleophile. If the second mechanism is correct, then the tertiary amine should have little effect on the rate for it cannot participate in the concerted process—it has no labile proton. We found that *p*-NPA reacts a mere 30% faster in a mixture of 0.26 *M* N-methylpiperidine and 0.10 M *n*-butylamine as it does in 0.10 M *n*-butylamine, suggesting that the cyclic mechanism is correct. The small rate increase with the addition of large amounts of tertiary amine may well be due to a "medium effect." The effect is certainly much smaller than

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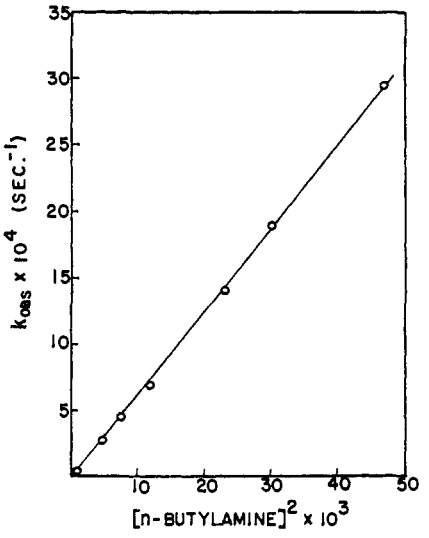

Figure 2. A plot of the observed rate constants for the reaction of *n*-butylamine with 3.25×10^{-4} *M* *p*-nitrophenyl acetate in chlorobenzene at 25.0° *vs.* the square of the *n*-butylamine concentration.

would be expected if the tertiary amine were interchangeable with *n*-butylamine as a reactive species. These results with *n*-butylamine further demonstrate the advantage of the nucleophile(s) possessing bifunctional character so that charge formation in the nonpolar medium is precluded.

The benzamidine model supports the suggestion that multifunctional catalysis by proteolytic enzymes may occur in a cyclic fashion in nonpolar regions of the active sites. Since these enzymes catalyze hydrolyses, water is obviously present in the regions of catalytic activity. This does not mean that the regions are aqueous in nature. A single water molecule within a hydrophobic environment may be bound in a position suitable for reaction with the acyl-enzyme intermediate. This water molecule could react with the carbonyl carbon, simultaneously losing a proton which would be delivered (with the aid of intervening groups such as an imidazole ring or other water molecules) to the carbonyl oxygen. In this way hydrolysis could occur without charge generation. A similar mechanism could be envisioned for the formation of the acyl enzyme, but involving the serine hydroxyl rather than a water molecule. It is just such a process that has been proposed by Bender and Kézdy.[3]

There is already substantial evidence that the binding site of α-chymotrypsin is a hydrophobic region.[14] The facility of the nucleophilic reaction of a carboxylic acid derivative in the medium of dielectric constant 5.6 raises the possibility that the *entire* active site is nonpolar in character.

Acknowledgment. The author wishes to express his gratitude to Professor Myron Bender for support of a postdoctoralship (Grant H 5726 of the National Institutes of Health) during which this project was conceived and the initial experiments were performed. We also appreciate assistance from the McCandless Fund of Emory University.

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A Comparative Study of the Alkaline Hydrolysis of *o*-Hydroxy-α-toluenesulfonic Acid Sultone and Phenyl α-Toluenesulfonate

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Contribution from the Department of Chemistry, University of Chicago, Chicago, Illinois 60637. *Received May 24, 1966*

Abstract: The five-membered cyclic sulfonate, *o*-hydroxy-α-toluenesulfonic acid sultone, undergoes alkaline hydrolysis 7×10^5 times faster than its open-chain analog, phenyl α-toluenesulfonate. This is the second observation of a very large rate enhancement for the hydrolysis of a five-membered cyclic sulfur-containing ester.

In recent years studies on the alkaline hydrolysis of five-membered cyclic esters of phosphoric, phosphonic, and sulfuric acid have been reported. An investigation on the simplest cyclic ester of phosphoric acid, ethylene phosphate, revealed that its potassium salt hydrolyzes at *ca.* 10^7 times the rate observed for the corresponding salt of the open-chain analog, dimethyl phosphate.[2] The alkaline hydrolysis of ethylene phosphate has been demonstrated to occur exclusively with P–O bond cleavage, whereas dimethyl phosphate hydrolyzes to a large extent with C–O bond cleavage.[3,4] As a result of this difference in the modes of bond cleavage, the rate enhancement for the attack at the phosphorus atom of the cyclic ester by hydroxide ion is estimated to be greater than 10^8. Further studies have shown that this large rate enhancement is not unique for five-membered cyclic esters of phosphoric acid. The five-membered cyclic ester of phosphonic acid, lithium propylphostonate, is hydrolyzed 6×10^5 times as fast as the open-chain compound, sodium ethyl

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