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Aminolysis of Oxalate Esters in Toluene

A Model for a Carboxylate Buried in a Hydrophobic Environment at an Active Site

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We have examined the aminolysis of the mono-*o*-nitrophenyl ester of oxalic acid by piperidine in toluene with the purpose of determining how a carboxylate "buried" at an active site might affect an enzyme-catalyzed reaction. The oxalate ester and piperidine form an ion pair ($R_2NH_2^+{}^-O_2CCO_2Ar$) even in extremely dilute toluene solutions. This conclusion is supported by the kinetic effects of acidic and basic additives and by a "concentration inversion" experiment. The oxalate ester was found to react more than three orders of magnitude faster than *o*-nitrophenyl acetate. The neighboring carboxylate in the ion pair apparently accelerates the decomposition of a tetrahedral intermediate by accepting a proton from the amine nitrogen. The implications of this anchimeric assistance to enzymatic systems is discussed briefly.

INTRODUCTION

Desolvation is an integral part of most bimolecular reactions in solution. For example, an anion must lose a significant fraction of its surrounding solvation molecules before it can form a covalent bond with carbon. When an anion is poorly solvated to begin with, it often displays unusual reactivity. Thus, chloride ion reacts with methyl iodide 2×10^6 times faster in acetonitrile than in methanol (*1*). Rate enhancements of this size have led to the proposal that enzymes activate their ionic catalytic groups in hydrophobic portions of the active sites (*2, 3*). One of the best examples is the Asp-102 carboxylate of chymotrypsin which is buried in a nonpolar region near the critical imidazole ring (*4*). Desolvation mechanisms for enzyme action, along with other mechanisms such as induced strain (*5*), vibrational activation (*6, 7*) etc., are attractive because the speed of most enzymatic reactions cannot be explained solely by the proximity of substrate to catalytic groups.

The purpose of this study was to determine how the rate and mechanism of the aminolysis of oxalic acid mono-*o*-nitrophenyl ester in toluene is affected by the neighboring carboxylate. Although work from here and elsewhere (*8, 9*) has shown that intermolecular catalysis of ester aminolyses takes place in nonpolar solvents, no intramolecular

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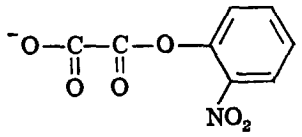
system has yet been examined. Obviously oxalate aminolysis in toluene is a highly artificial "model" in the sense that it bears little resemblance to any actual enzymatic reaction. This is true of most models. The point is that we must first understand the chemistry of simple systems before there is any hope of understanding related reactions catalyzed by enzymes. The oxalate carboxyl anion in toluene simulates, albeit crudely,



an aspartate carboxylate held in a hydrophobic environment within an enzyme. Information about the oxalate can therefore tell something about the likely behavior of a buried aspartate.

EXPERIMENTAL SECTION

Materials

Spectrograde toluene was refluxed over calcium hydride and distilled through a 30 cm Vigreux column. Triethylamine (Eastman) was refluxed and distilled over phthalic anhydride and again over potassium hydroxide, after which it was stored in an amber bottle under nitrogen. Piperidine (Aldrich) was distilled over calcium hydride and stored under nitrogen. Trifluoroacetic acid (Eastman) was used as received, whereas *o*-nitrophenyl acetate (Eastman) was recrystallized twice from benzene/hexane.

o-Nitrophenyl oxalate diester. This compound was prepared by the method of Bruice and Holmquist (*10*). In order to secure acceptable yields, it is necessary to stir the pyridine vigorously while adding the oxalyl chloride. Recrystallization of the product from benzene/hexane afforded pale yellow needles, mp, 185–186°C (Ref. (*10*), 185–187°C) in 55% yield.

o-Nitrophenyl hydrogen oxalate. The monoester was prepared by partial hydrolysis of the corresponding diester following a literature method (*10*). We found that yields could be improved considerably by using 50 rather than 300 ml of water. Crystallization of the product from chloroform/petroleum ether gave 32% of the desired product, mp, 122.5–123.5°C (Ref. (*10*), 123–124°C).

Methyl o-nitrophenyl oxalate. Methyl oxalyl chloride (0.8 ml) (Aldrich) was added dropwise with stirring to pyridine at 0°C. A solution of 1.4 g of *o*-nitrophenol in 7 ml of pyridine was then added slowly to the resulting suspension still at 0°C. Upon complete addition of the phenol, the mixture was stirred for 90 min and allowed to warm to room temperature. The reaction mixture was stirred another 30 min and added to a suspension of 300 g of ice and 150 ml of concentrated HCl. The aqueous slurry was extracted three times with ether, and the ether layers were combined and dried over anhydrous $MgSO_4$. Removal of the ether gave a yellow oil comprised of three major components, as indicated by glc analysis at 110°C using a 3-foot glass column packed with 10% SE-30 on 80/100-mesh Chromosorb W AW-DMCS.

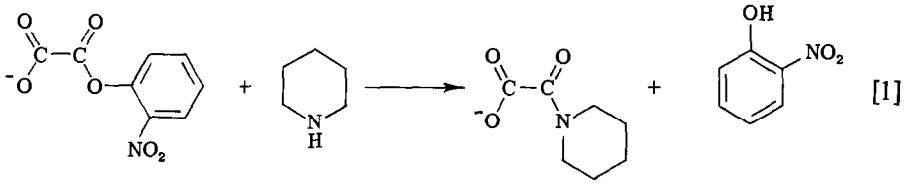
The last peak, shown by ir to be the desired product, was collected from the analytical column as a clear oil. Spectrophotometric measurement of the *o*-nitrophenoxide production upon basic hydrolysis indicated the sample to be 99% pure. *Anal.* Calcd for $C_9H_7NO_6$: C, 48.01; H, 3.14. Found: C, 48.08, H, 3.24.

Kinetic Methods

A typical procedure for a kinetic run follows. A stoppered cuvette containing 3.00 ml of a freshly prepared solution of piperidine in toluene was equilibrated at 25.0°C within the thermostated cell compartment of a Cary 14 or Acta II spectrophotometer. A small amount (20 μl) of *o*-nitrophenyl hydrogen oxalate in toluene was then added to the solution in the cuvette (with the aid of a stirring rod flattened at one end) such that the substrate concentration was 2×10^{-5} *M*. The amine concentration was always high enough so that pseudo-first-order conditions prevailed. The appearance of *o*-nitrophenol was monitored as a function of time at 352 nm (0.1 slidewire) until the reaction was over (greater than eight half-lives). Absorbance–time data were processed with the aid of an RCA Spectra 70/55 computer to secure the first-order rate constants. Experimental error in the rate constants was large (±15%) owing to the rapidity of the reactions. However, this uncertainty is small relative to the observed rate enhancements. Repeat runs performed on the two different spectrophotometers differed by less than 15%.

RESULTS AND DISCUSSION

Observed rate constants for aminolysis of the mono-*o*-nitrophenyl ester of oxalic acid by excess piperidine in toluene at 25.0° (Eq. [1]) are given in Table 1. A plot of the k_{obsd} vs [piperidine] is linear with a zero intercept (Fig. 1)[3] pointing to a simple second-order reaction. The second-order rate constant k_2, obtained from the slope of the plot, was found to equal $5.2 \pm 1.0 \times 10^2\ M^{-1}\ \text{sec}^{-1}$.



[1]

In evaluating the magnitude of a neighboring-group effect one must estimate reactivities in the absence of such an effect (*11*). Sometimes conclusions depend markedly on the choice of the model. The difficulty lies in the impossibility of removing neighboring-group participation without changing steric and electronic factors as well. We selected two other *o*-nitrophenyl esters, drawn below, as our basis of comparison. Aminolysis of methyl *o*-nitrophenyl oxalate by piperidine in toluene at 25.0°C was found to be first order in amine and to have a $k_2 = 1.7 \pm 0.5 \times 10^3\ M^{-1}\ \text{sec}^{-1}$. This is

[3] As will be shown below, the substrate protonates piperidine in toluene. Hence, the piperidine concentrations in Table 1 were reduced by an amount corresponding to the initial substrate concentration (2.0×10^{-5} *M*) prior to plotting Fig. 1. This correction changes k_2 by only 4%.

3.3 times *larger* than the corresponding k_2 for the mono-*o*-nitrophenyl ester of oxalic acid. On the other hand, the mono-*o*-nitrophenyl ester of oxalic acid reacts 1400 times faster than *o*-nitrophenyl acetate ($k_2 = 3.6 \pm 0.2 \times 10^{-1}\ M^{-1}\ \text{sec}^{-1}$). The significance of these comparisons will be clarified shortly, but first we must consider another important point.

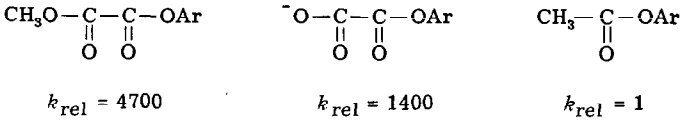


TABLE 1
OBSERVED RATE CONSTANTS FOR THE AMINOLYSIS OF *o*-NITROPHENYL HYDROGEN OXALATE BY PIPERIDINE IN TOLUENE AT 25.0°C.

Piperidine concentration (M)	k_{obsd} (sec^{-1})
4.19×10^{-4}	2.04×10^{-1}
3.14×10^{-4}	1.54×10^{-1}
2.42×10^{-4}	1.23×10^{-1}
2.42×10^{-4}	1.12×10^{-1}
2.10×10^{-4}	1.01×10^{-1}
2.10×10^{-4}	9.52×19^{-2}
1.73×10^{-4}	6.60×10^{-2}
1.39×10^{-4}	6.59×10^{-2}

One of the cardinal rules in any mechanistic study is to know the forms of the reactants precisely (*12*). Clearly, it was necessary to establish whether mono-*o*-nitrophenyl oxalate in toluene plus piperidine exists as the free acid (o-NPHO) or as the carboxylate

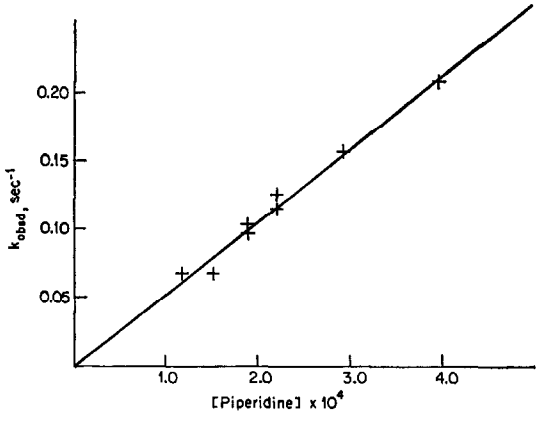


FIG. 1. Observed rate constant for aminolysis of the mono-*o*-nitrophenyl ester of oxalic acid in toluene at 25°C as a function of piperidine concentration.

(o-NPO$^-$). Unfortunately, attempts to determine the ionization state directly by ultraviolet and infrared spectroscopy were unsuccessful because of solvent absorption and solubility problems, respectively. However, the following literature data leave little doubt that o-NPHO should transfer its acidic proton to piperidine: (a) Hibbert and Satchell (*13*) have shown that mixing acetic acid with an excess amount of *n*- butylamine in ether completely destroys the acid carbonyl band at ca. 1740 cm^{-1} and creates a new band at 1560 cm^{-1}. This is best explained in terms of an ion pair ($RCO_2^-H_3N^+R$). The formation for the ion pair composed of *n*-butylamine and formic acid in ether was found to be approximately 3000 M^{-1}. Since o-NPHO is a much stronger acid than formic acid, o-NPHO would be even more favorably disposed toward transferring its proton to aliphatic amines. (b) Bruckenstein and Saito (*14*) believe that amines and organic acids in benzene form ion pairs and that the ion pairs are stabilized by long-range electrostatic forces rather than by hydrogen bonding. These authors also provide evidence that the ion pairs aggregate at concentrations near 0.01 *M*. Our own experiments were carried out at concentrations orders of magnitude more dilute than 0.01 *M*, and thus aggregation is much less likely. (c) DeTar and Novak (*15*) state that ". . . ion pair formation is the expected behavior and appears to be general for primary and secondary amines. . . ."

It seemed desirable to secure at least indirect evidence for ion pair formation in the highly dilute solutions used in the kinetic runs. One experiment designed for this purpose involved determining the kinetic effect of trifluoroacetic acid (TFA) in concentrations ranging from 24–74% of the piperidine concentration. We found that TFA reduced the observed rate constants to those which would have occurred if the amine concentration had been diminished by an amount equal to the quantity of added acid. These results indicate that TFA quantitatively converts nucleophilic amine into nonnucleophilic amine salt. We presume that o-NPHO, a strong acid with a pK_a similar to that of TFA (*16*), does likewise.

Another observation also relates to the question of the ionic state of the substrate: A *125-fold* excess of triethylamine (5.23×10^{-2} *M*) over piperidine (4.19×10^{-4} *M*) decreases the observed rate a mere 57%. This small rate perturbation by huge quantities of nonnucleophilic tertiary amine suggests that the substrate is completely ionized in the presence of 4.19×10^{-4} *M* piperidine *without* triethylamine. Additional inert base has, therefore, little effect.

Perhaps the most convincing evidence for a piperidine/o-NPHO ion pair derives from a "concentration inversion" experiment. Most kinetic runs were carried out with the piperidine in *pseudo*-first-order excess (Table 1). Under these circumstances free amine is always available to attack the ester carbonyl. However, if the o-NPHO does in fact protonate the piperidine, then one would expect a diminished or obliterated aminolysis when the *oxalate* is in excess. This was found to be the case. At 2.2×10^{-4} *M* o-NPHO and 3.0×10^{-5} *M* piperidine the reaction rate is extremely slow (roughly 10^4 slower than when the reactant concentrations are reversed).[4] The severely retarded rate caused by the concentration inversion strongly supports amine protonation under kinetic conditions.

We alluded earlier to the observation that the relative k_2's for aminolysis of O_2NC_6-$OOCR$ are 4700, 1400, and 1 for $R = -COOCH_3$, $-COO^-$, and $-CH_3$. Interpretation

[4] The slow reaction is probably an anhydride formation, but this point was not explored.

of these data depends critically upon the validity of our conclusion that the mono-*o*-nitrophenyl ester of oxalic acid loses a proton under the kinetic conditions. If the rate ratios reflected the reactivity of substrate in the conjugate acid form then the kinetic results would be totally uninteresting. Thus, similar rates for $R = -COOCH_3$ and $-COOH$ would be *expected* because the two substituents possess nearly the same size and electronegativity ($\sigma_I = 0.34$ and 0.39, respectively) (*17*). Moreover, *n*-butylaminolysis of phenyl esters C_6H_5OOCR in dioxane possesses a $\rho^* = +2.14$ (*18*). Hence, inductive withdrawal in our oxalate system by $-COOCH_3$ and $-COOH$ (with σ^*'s near 2) should both enhance k_2 by three to four orders of magnitude relative to *o*-nitrophenyl acetate. However, the mono-*o*-nitrophenyl ester of oxalic acid in piperidine/toluene does *not* bear a $-COOH$; rather it bears a carboxyl anion, and the carboxyl anion is not a highly electron-withdrawing substituent. This is seen from its Hammett σ constant (which is identical to that of hydrogen (*19*)) and from its inductive substituent constant σ_I (which is either -0.17 or $+0.06$ depending on the method of determination) (*17*). We see, therefore, that *o*-NPO^- reacts 1400 times faster than *o*-nitrophenyl acetate despite negligible or even deleterious polar and steric effects by the carboxylate. The nature of the carboxylate catalysis is the subject of the remainder of the paper.

Carboxylate catalysis in o-NPO^- is undoubtedly intramolecular in nature because the observed rates are independent of the initial substrate concentration in the region of 2×10^{-5} *M* used in the kinetics. Intramolecular nucleophilic catalysis can be discounted because it would result in a highly energetic three-membered ring. The catalytic effect is best explained in terms of the mechanism generally accepted for ester aminolysis in nonpolar solvents (Eq. [2]) (*20*). Amine adds reversibly to the ester, thereby forming

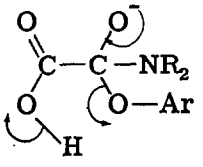
$$\mathrm{RCOOAr} + \mathrm{R_2NH} \underset{}{\overset{K}{\rightleftharpoons}} \mathrm{R{-}C(O^-)(HNR_2^+){-}OAr} \xrightarrow{k} \mathrm{RCONR_2} + \mathrm{HOAr} \qquad [2]$$

a tetrahedral intermediate that collapses to product in the rate-determining step. The observed rate acceleration by carboxylate could arise either from a more favorable preequilibrium (K) or from a faster rate of intermediate decomposition (k). Of the two possibilities, the latter seems more likely. Since the intermediate is probably zwitterionic, a neighboring carboxyl anion should not, from an electrostatic point of view, greatly enhance the intermediate stability. On the other hand, the carboxylate could appreciably increase the rate of intermediate collapse by accepting reversibly a proton from the cationic nitrogen (Eq. [3]). This would generate a second intermediate which,

$$\mathrm{{^-O{-}C(=O)(HNR_2^+){-}C(O^-){-}OAr}} \rightleftharpoons \mathrm{O{=}C(OH){-}C(NR_2){-}OAr} \qquad [3]$$

unlike the first, can eject ArO^- to form an *unprotonated* amide functionality. Conceivably, the carboxyl group also assists the departure of ArO^- as is shown in Eq. [4]. As is usually the case with complex mechanisms, the exact timing of the proton transfers is unknown and may never be known with certainty.

It must be borne in mind that the carboxylate of o-NPO$^-$ is ion-paired with piperidinium ion in toluene. Close association between the carboxylate and its counterion no doubt mitigates the carboxylate reactivity considerably. This conclusion derives in part from the fact that tetra-*n*-hexylammonium benzoate is a potent *inter*molecular catalyst for ester aminolysis in toluene, whereas octadecylammonium benzoate is much less effective (*8*). The difference lies in the fact that the benzoate of the quaternary



[4]

ammonium salt cannot be stabilized by hydrogen bonding. Moreover, the four hexyl groups in tetra-*n*-hexylammonium benzoate sterically impede electrostatic stabilization of the benzoate by the cationic nitrogen. We estimate from previous data (*8*) that intramolecular catalysis in o-NPO$^-$ would have increased rates by more than 10^6 had the counterion been a quaternary ammonium ion rather than piperidinium ion.

Recently, Rogers and Bruice studied intramolecular carboxylate catalysis in 94% CH_3CN/6% H_2O and 96% dioxane/4% H_2O in an attempt to evaluate the "charge-relay" mechanism for serine esterases (*21*). We quote from this paper two sentences concerning work by us and others (*8, 9*) on intermolecular carboxylate catalysis in non-polar solvents. "Large rate enhancements have been reported for reaction of imidazole with a phenyl ester on addition of a carboxylic acid anion when nearly anhydrous acetonitrile or toluene is employed. These results have been offered in support of the charge-relay hypothesis." Contrary to this statement, we have never claimed that model studies provide evidence for any enzymatic process, nor do we make such a claim now. We do conclude from our data that anionic groups in hydrophobic environments can accelerate a biologically relevant reaction. Of course, immersing a carboxylate in a hydrophobic portion of an active site need not necessarily lead to the impressive rate enhancements observed in the model systems. This is because such a buried carboxylate is not only more reactive than normal, it is more basic. If a buried carboxylate at an active site is not completely insulated from the external solvent, then the gain in catalytic reactivity could be negated by a corresponding decrease in carboxylate concentration. Only when the anionic charge is protected from hydrophilic regions at the active site (and this may require the presence of a bound substrate) is a large catalysis possible. It is hardly surprising that Rogers and Bruice (*21*) observed only a trivial carboxylate catalysis in their model studies of the charge-relay mechanism carried out in solvents containing greater than 2 *M* water.

ACKNOWLEDGMENTS

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