Interfacial Physical Organic Chemistry. Imidazole-Catalyzed Ester Hydrolysis at a Water–Heptane Boundary

F. M. Menger

Contribution from the Department of Chemistry, Emory University, Atlanta, Georgia 30322. *Received February 17, 1970*

Abstract: A methodology of interfacial bioorganic chemistry is developed which allows detailed examination of a catalyzed ester hydrolysis occurring at a liquid–liquid boundary. When aqueous imidazole solutions are stirred rapidly under carefully controlled conditions with heptane solutions of *p*-nitrophenyl laurate, ester hydrolysis takes place at the hydrocarbon–water interface of the dispersed heptane droplets. Hydrolysis rates are determined as a function of the following reaction variables: stirring speed, concentration of reactants, temperature, viscosity of the hydrocarbon phase, volume of the heptane and water solutions, deuterium and salt content of the water, lauroylimidazole content of the heptane, presence of an amphiphile, and structure of the catalyst. Interesting differences are found between the heterogeneous hydrolysis and the corresponding homogeneous reaction. The mechanism of the interfacial hydrolysis, including the mode of imidazole catalysis and the nature of the rate-determining step, is tentatively established.

Despite the fact that the majority of biological reactions are interfacial in nature,[1] studies of bioorganic mechanisms have been performed, for the most part, under homogeneous conditions where experimental problems are not as severe. There is, therefore, an obvious need to investigate mechanisms of organic reactions occurring at interfaces between two liquid phases. Such interfacial organic reactions are interesting and potentially useful aside from any biological relevance. In the present paper we report preliminary results with a heterogeneous imidazole-catalyzed ester hydrolysis. This particular reaction was selected because the corresponding homogeneous process is relatively simple and thoroughly understood. Kinetic runs were carried out by stirring aqueous imidazole solutions with heptane solutions of *p*-nitrophenyl laurate, a water-insoluble ester. Catalyzed hydrolysis of the ester occurred at the hydrocarbon–water interface of the dispersed heptane droplets. The rate of hydrolysis (determined by spectrophotometric assay of the *p*-nitrophenolate in the water phase) was studied as a function of stirring rate, temperature, concentration of reactants, deuterium content of the water, viscosity of the hydrocarbon, etc.

Several decades ago, Bell[4] examined the surface reaction between benzoyl-*o*-toluidine in benzene and potassium permanganate in water to produce benzoylanthranilic acid. His experimental procedure differed from ours in that the hydrocarbon and water were allowed to remain in two layers each of which was stirred separately. We, on the other hand, dispersed the hydrocarbon by rapid mixing in order to secure a large interfacial area. The main conclusion of Bell[4] was that the oxidation is caused by permanganate ions striking an adsorbed layer of benzoyl-*o*-toluidine. More recently, Pollard and Westwood[5,6] successfully analyzed the kinetics of metal exchange between a stirred metallic mercury surface and diphenylmercury in solution. Exchange in the liquid–liquid system takes place *via* an S_Ei mechanism.

Catalysis and reaction kinetics at liquid interfaces have been investigated mainly by means of monomolecular films adsorbed onto water surfaces. The subject has been reviewed elsewhere,[7,8] and only one example will be mentioned here. Molecules of an uncompressed oleic acid film on water lie flat along the water surface. Consequently, the double bonds of such a film are readily oxidized to glycol by a water layer containing potassium permanganate.[9] If the film is compressed, the rate of oxidation decreases markedly because the oleic acid chains are forced to stand vertically out of the water, thereby placing the double bonds several carbon atoms away from available oxidizing agent. These findings illustrate an important difference between interfacial reactions and reactions occurring in the bulk phase. Orientation of reactant groups in the bulk phase is usually random. Molecules at an interface, on the other hand, have well-defined orientations. If a multifunctional molecule positions itself at a liquid–liquid boundary so that only one of the labile groups is near the interface, then there is a possibility of a high degree of reaction specificity. We are currently attempting to devise synthetic methods based on this idea. The present paper, however, is devoted exclusively to mechanistic aspects of interfacial organic chemistry.

Experimental Section

Materials. Imidazole (Eastman) was crystallized from dry benzene, dried under reduced pressure, and stored in a desiccator. Spectroquality heptane (197 mμ uv cut-off) was used as obtained from Matheson Coleman and Bell. Pierce Chemical Co. and Nutritional Biochemical Corp. supplied the *p*-nitrophenyl laurate and *p*-nitrophenyl palmitate. Lauroylimidazole was prepared

(1) Even many enzymatic reactions which normally take place in the free solution, such as chymotrypsin-catalyzed hydrolyses, can be considered interfacial processes. The binding site of chymotrypsin is almost certainly hydrophobic.[2] There is evidence that at least part of the catalytic site is aqueous.[3] This means that a substrate at the active site traverses a boundary between a polar and a nonpolar region.

(2) T. M. Spotswood, J. M. Evans, and J. H. Richards, *J. Amer. Chem. Soc.*, **89**, 5052 (1967).

(3) M. B. Hille and D. E. Koshland, *ibid.*, **89**, 5945 (1967).

(4) R. P. Bell, *J. Phys. Chem.*, **32**, 882 (1928).

(5) D. R. Pollard and J. V. Westwood, *J. Amer. Chem. Soc.*, 87, 2809 (1965).

(6) D. R. Pollard and J. V. Westwood, *ibid.*, **88**, 1404 (1966).

(7) J. T. Davies, *Advan. Catal.*, **6**, 1 (1954).

(8) G. L. Gaines, "Insoluble Monolayers at Liquid-Gas Interfaces," Interscience, New York, N. Y., 1966, Chapter 7.

(9) A. H. Hughes and E. K. Rideal, *Proc. Roy. Soc., Ser. A*, **140**, 253 (1933).

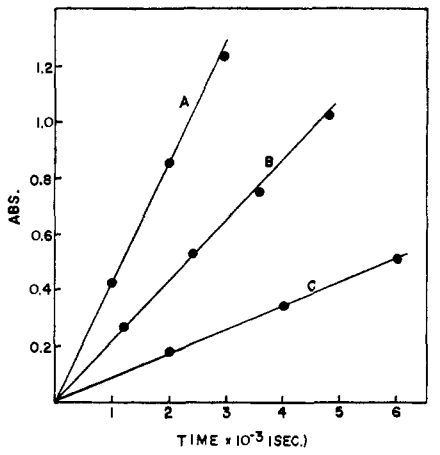

Figure 1. Plots of absorbance of the water phase (400 mμ) *vs.* time for the interfacial reaction between *p*-nitrophenyl laurate in heptane (10.0 ml, 2.49×10^{-3} *M*) and aqueous imidazole (20.0 ml, pH 8.03) at 25.0°, 1020 rpm. Imidazole concentrations are 0.298 *M* (A), 0.149 *M* (B), and 0.0596 *M* (C).

according to the procedure of Staab.[10] 1-Methylimidazole (Aldrich) was purified by distillation through a vacuum-jacketed Vigreux column.

Reaction Procedure and Kinetics. The reaction vessel and stirrer used in this work were similar to those described by Pollard and Westwood.[5] The cylindrical vessel was 43 mm high and 43 mm in diameter and had a capacity of about 50 ml. Since the rate parameters depend on the stirring efficiency and thus on the geometry of the apparatus, it was necessary to guard against possible breakage by making several identical vessels and stirrers at the outset of the project. Most of the kinetic runs were performed with a single vessel; when it eventually broke, it was replaced by a spare with no significant effect on the rate constants. The stirring rod was attached to a Fisher Stedi-Speed stirring motor connected to a constant-voltage transformer. The stirring rate, measured by a calibrated Cenco neon stroboscope, could be maintained at better than ±1%. The reaction flask was immersed in a constant-temperature bath with a stability of ±0.1°.

A typical kinetic run was carried out as follows. An aqueous solution of imidazole (20.0 ml, 0.147 *M*, pH 8.03) and a heptane solution of *p*-nitrophenyl laurate (10.0 ml, 2.49×10^{-3} *M*) were added to a reaction vessel thermostated at 25.0°, and the stirring rod and vessel cover were set into place. The use of stops ensured that the stirring rod was always in the same position relative to the reaction vessel; this was important because reaction rates were not reproducible if the height of the stirring rod above the bottom of the vessel changed from run to run. Stirring of the reaction mixture was then initiated at 1020 rpm. After a measured amount of time, the stirring was stopped, the phases were allowed to separate, a 3-ml aliquot was removed from the water layer, and the aliquot was analyzed spectrophotometrically for *p*-nitrophenolate anion (400 mμ). The apparatus was dismantled and cleaned, and the above procedure was repeated in order to secure an absorbance reading at another time value. In this manner we obtained absorbance *vs.* time plots such as are shown in Figure 1.

In no run was more than 6% of the initial *p*-nitrophenyl laurate content of the heptane layer hydrolyzed. Except when indicated otherwise, plots of the absorbance of the water layer *vs.* time were found to be linear, as is expected for the initial portion of a simple reaction. The initial reaction velocities (in moles per liter per minute) were calculated from the slopes of these linear plots divided by the extinction coefficient of *p*-nitrophenol at pH 8.03 (*i.e.*, 16,700). In some cases, especially with repeat experiments, the velocities were calculated from only one run using eq 1; A is the absorbance reading at 400 mμ using a 10-mm cuvette, ϵ is the extinction coefficient of *p*-nitrophenol, T is the time in minutes.

$$V = A/\epsilon T \qquad (1)$$

The stock imidazole buffers had to be refrigerated or else the observed rates decreased substantially from day to day. Apparently, bacterial growth releases trace amounts of surface active materials which perturb the reaction.[11] As will be shown later, the velocity is extremely sensitive to the presence of surfactants.

The small percentage of observed reaction made it necessary to demonstrate that the increase in absorbance at 400 mμ was not caused by extraction of *p*-nitrophenol (a possible impurity in the ester) from the heptane phase into the water layer. Since stirring the ester solution with phosphate buffer (pH 8.03) led to no absorbance increase, the appearance of *p*-nitrophenolate in the water layer during the kinetic runs must be the result of a catalyzed ester hydrolysis.

One reason that *p*-nitrophenol laurate was selected as the substrate for the present work was that the compound is very water insoluble. This lessened the danger of the ester being extracted into the water phase where hydrolysis would occur homogeneously. *p*-Nitrophenyl laurate turned out to be a good choice in this respect; we present strong evidence in the Discussion that hydrolysis does indeed take place at the hydrocarbon–water interface. The substrate had the disadvantage of possibly hydrolyzing to products which are surface active. This was one of the reasons we determined only initial reaction rates. The reaction velocity was found to decrease sixfold upon addition of 3×10^{-4} *M* lauric acid to the water; the amount of ester hydrolyzed at the completion of the kinetic runs was kept well below this level. If there had been substantial product inhibition, the absorbance *vs.* time plots (Figure 1) would curve downward. The possibility of undetectable trace amounts of impurities in the ester was also a concern. In most of the reported experiments the ester concentration in the heptane was maintained at 2.49×10^{-3} *M*, so that impurities, if any, remained constant from run to run. Analysis of the *p*-nitrophenyl laurate by vapor phase chromatography showed no lauric acid (*i.e.*, the lauric acid content was less than 1%).

Interfacial reaction rates depend on the size and shape of the vessel and stirring rod. Values of our rates will, therefore, be difficult to duplicate in other laboratories, but this is not really a serious problem. *Absolute* values of reaction rates mean little. Only *differences* between rate constants are important. Our conclusions regarding the mechanism of the interfacial ester hydrolysis are based on how reaction rates *change* with changes in reaction variables. For example, we find that the rate does not depend on the temperature, and we conclude from this that the ester hydrolysis is diffusion controlled.

Kinetic runs were performed in duplicate or triplicate. The reproducibility of the data, neglecting an occasional erratic run, was ±5%. This is considered satisfactory in view of the heterogeneous nature of the system. Runs involving surfactant (in which suspended droplets increased the uncertainty in the absorbance measurements) had slightly more error. There were, however, severe reproducibility problems with the hydroxide ion experiments, especially at high concentrations of base, and only two runs using dilute solutions are reported here. No conclusion in this paper is based on a small change in reaction velocity. The "standard run" (described in detail in the second paragraph of this section) was performed before, during, and after each set of experiments. The constancy of the standard runs ensured that no extraneous factor was suddenly appearing and influencing the reaction rates.

Results

When a heptane solution of *p*-nitrophenyl laurate was stirred rapidly with an aqueous imidazole solution (pH 8.03), the ester hydrolyzed and *p*-nitrophenolate appeared in the water. Since stirring the heptane solution with a phosphate buffer (pH 8.03) resulted in no *p*-nitrophenolate formation, the ester hydrolysis must be imidazole catalyzed. In a typical run, 20.0 ml of 0.149 *M* aqueous imidazole was stirred with 10.0 ml of 2.49×10^{-3} *M* ester in heptane at 1020 rpm. A plot of the absorbance of the water phase as a function of time (Figure 1B) is linear. The slope of the line divided by the extinction coefficient of *p*-nitrophenol at pH 8.03 is defined as the initial reaction velocity (see discussion in the Experimental Section). The velocity was determined as a function of several reaction variables, and the results of these studies are listed

(10) H. A. Staab, *Angew. Chem., Int. Ed. Engl.*, 1, 351 (1962).

(11) I am indebted to Dr. E. Reiner of the National Communicable Disease Center for suggesting this possibility.

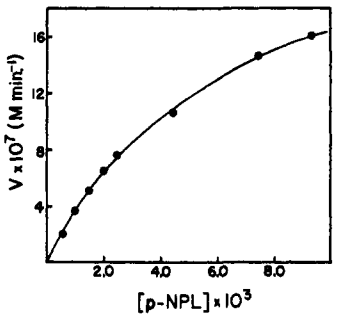

Figure 2. Plot of reaction velocity *vs.* concentration of *p*-nitrophenyl laurate in the heptane phase. Runs were performed with 20.0 ml of 0.149 *M* aqueous imidazole (pH 8.03) and 10.0 ml of ester in heptane at 25.0°, 1020 rpm.

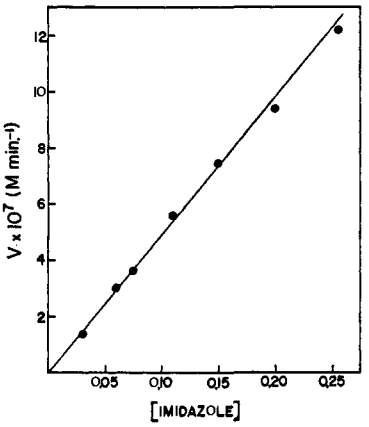

Figure 3. Plot of reaction velocity *vs.* concentration of imidazole in the aqueous phase. Runs were performed with 20.0 ml of aqueous imidazole (pH 8.03) and 10.0 ml of 2.49 × 10^{-3} *M p*-nitrophenyl laurate in heptane at 25.0°, 1020 rpm.

below (initially without any accompanying discussion or interpretation in order that the entire set of experiments may be referred to easily).

1. The velocity is proportional to the stirring rate from 600 to 1700 rpm.[12]

2. A plot of the reaction rate *vs.* concentration of ester in the heptane shows a pronounced saturation effect (Figure 2).

3. The reaction is first order in imidazole. A plot of rate *vs.* concentration of imidazole in the water is linear from 0.03 to 0.26 *M* (Figure 3).

4. The initial velocity is proportional to the volume of the heptane solution (Table I). The reaction rate is

Table I. The Effect of the Volume of Heptane Solution on the Velocity of the Interfacial Reaction between *p*-Nitrophenyl Laurate and Imidazole[a]

Volume, ml	$V \times 10^7$, M min^{-1}
2.0	2.0
4.0	3.5
6.0	4.8
8.0	6.2
10.0	7.5

[a] Runs were performed with aqueous imidazole (20.0 ml, 0.149 *M*, pH 8.03) and *p*-nitrophenyl laurate in heptane (2.49 × 10^{-3} *M*) at 25.0°, 1020 rpm.

(12) Runs were performed with 20.0 ml of 0.149 *M* aqueous imidazole (pH 8.03) and 10.0 ml of 2.49 × 10^{-3} *M p*-nitrophenyl laurate in heptane at 25°, 1020 rpm unless indicated otherwise.

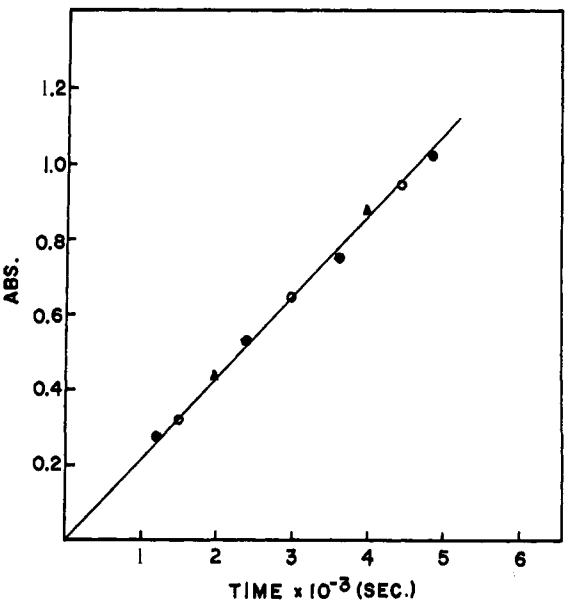

Figure 4. Plot of absorbance of the water phase (400 mμ) *vs.* time for the interfacial reaction between *p*-nitrophenyl laurate in heptane (10.0 ml, 2.49 × 10^{-3} *M*) and aqueous imidazole (20.0 ml, 0.149 *M*, pH 8.03) at 1020 rpm. Temperatures are 15.0° (○), 25.0° (●), and 30.0° (▲).

less sensitive to the volume of aqueous imidazole. When the volume of aqueous imidazole is decreased stepwise from 20.0 to 16.0 ml, the rate increases by 30%. Further reduction in the volume of water (2.0-ml increments) leads to small decreases in the reaction velocity until the rates at 10.0 and 20.0 ml are nearly the same.[13]

5. Addition of sodium chloride to the water phase (0.02–1.10 *M*) causes a small rate increase (Table II).

Table II. The Effect of Sodium Chloride on the Velocity of the Interfacial Reaction between *p*-Nitrophenyl Laurate and Imidazole[a]

[Sodium chloride], M	$V \times 10^7$, M min^{-1}
0.00	7.5
0.02	7.7
0.06	8.0
0.12	8.1
0.33	8.6
0.75	9.0
1.10	9.7

[a] Runs were performed with aqueous imidazole (20.0 ml, 0.149 *M*, pH 8.03) and *p*-nitrophenyl laurate in heptane (10.0 ml, 2.49 × 10^{-3} *M*) at 25.0°, 1020 rpm.

6. *p*-Nitrophenyl palmitate and *p*-nitrophenyl laurate react heterogeneously at nearly the same rate.

7. The hydrolysis is only slightly affected by a change in hydrocarbon viscosity of two orders of magnitude. When light paraffin oil is used for the nonaqueous phase instead of heptane, the observed reaction rate increases 1.3-fold.

8. The rate of the heterogeneous ester hydrolysis is independent of the temperature. Absorbance *vs.* time plots at 15, 25, and 30° are superimposable (Figure 4).

(13) In these runs the calculated velocities were multiplied by $v/20$ where v is the volume of aqueous imidazole.

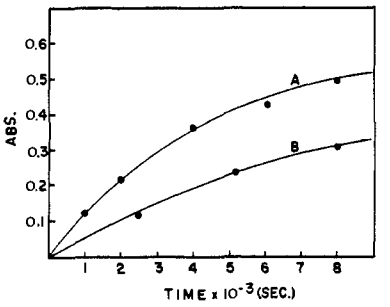

Figure 5. Plots of absorbance of the water phase (400 mμ) *vs.* time for the interfacial reaction between *p*-nitrophenyl laurate in heptane (10.0 ml, 2.49 × 10^{-3} *M*) and aqueous potassium hydroxide (20.0 ml) at 25.0°, 1020 rpm. Hydroxide concentrations are 0.05 *M* (A) and 0.025 *M* (B).

9. Absorbance *vs.* time plots for hydroxide ion catalyzed interfacial hydrolyses of *p*-nitrophenyl laurate[14] are markedly curved (Figure 5), unlike the corresponding plots for the imidazole-catalyzed reaction (Figure 1). As will be shown later, this curvature is the result of product inhibition. The catalytic efficiency of the hydroxide ion can be estimated from the slopes of the curves (Figure 5) at zero time. It is found that the base-catalyzed interfacial hydrolysis is first order in hydroxide ion and that hydroxide ion is roughly twice as effective a catalyst as imidazole. Hydroxide ion is a 31-fold better catalyst than imidazole in the homogeneous hydrolysis of *p*-nitrophenyl acetate.[15]

10. The N-methylimidazole-catalyzed hydrolysis is first order in amine and eight times slower than the imidazole-catalyzed reaction. N-Methylimidazole is 75% as good a catalyst as imidazole in the homogeneous hydrolysis of *p*-nitrophenyl acetate.[16,17]

11. The initial reaction velocity is decidedly slower with a polar phase composed of deuterium oxide rather than water (V_{H_2O}/V_{D_2O} = 1.7). Homogeneous imidazole-catalyzed hydrolysis of *p*-nitrophenyl acetate displays no solvent isotope effect.[18]

12. The interfacial hydrolysis is inhibited by remarkably small amounts of laurate anion added to the aqueous phase (Figures 6 and 7). Thus, 3 × 10^{-4} *M* laurate anion decreases the observed reaction velocity sixfold. The homogeneous hydrolysis of *p*-nitrophenyl acetate is totally unaffected by laurate anion at this low concentration.[19] We will show later that the data in Figure 6 can be described by a Freundlich adsorption isotherm.

13. Addition of lauroylimidazole (a possible reaction intermediate) to the heptane phase (5 × 10^{-4} *M*) has only a small effect on the hydrolysis rate of *p*-nitrophenyl laurate (Figure 7B). The lauroylimidazole hydrolyzes slowly under the heterogeneous conditions, thereby releasing laurate anion which inhibits the reaction as described above.

(14) These runs were performed with 20.0 ml of aqueous potassium hydroxide and 10.0 ml of 2.49 × 10^{-3} *M* *p*-nitrophenyl laurate in heptane at 25.0°, 1020 rpm.
(15) M. L. Bender, *Chem. Rev.*, **60**, 53 (1960).
(16) T. C. Bruice and G. L. Schmir *J. Amer. Chem. Soc.*, **79**, 1663 (1957).
(17) M. L. Bender and B. W. Turnquest, *ibid.*, **79**. 1656 (1957).
(18) M. L. Bender, E. J. Pollock, and M. C. Neveu, *ibid.*, **84**, 595 (1962).
(19) F. M. Menger and C. E. Portnoy, *ibid.*, **89**, 4698 (1967).

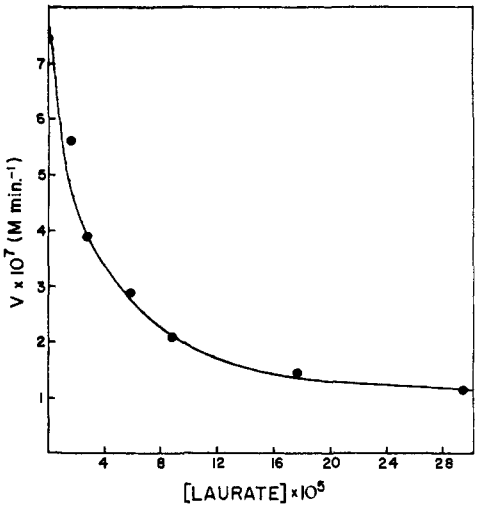

Figure 6. Plot of the reaction velocity *vs.* concentration of lauric acid added to the aqueous phase. Runs were performed with 20.0 ml of 0.149 *M* aqueous imidazole (pH 8.03) and 10.0 ml of 2.49 × 10^{-3} *M* *p*-nitrophenyl laurate in heptane at 25.0°, 1020 rpm.

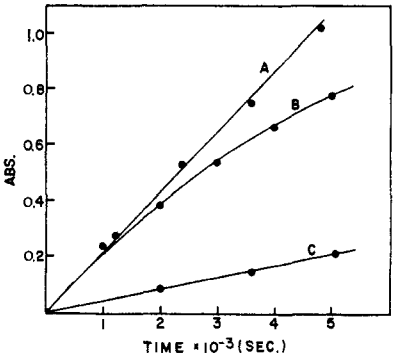

Figure 7. Plots of absorbance of the water phase (400 mμ) *vs.* time for the interfacial reaction between *p*-nitrophenyl laurate in heptane (10.0 ml, 2.49 × 10^{-3} *M*) and aqueous imidazole (20.0 ml, 0.149 *M*, pH 8.03) at 25.0°, 1020 rpm. Plot A, no additive; plot B, 5.1 × 10^{-4} *M* lauroylimidazole added to heptane phase; plot C, 2.5 × 10^{-4} *M* laurate anion added to the aqueous phase.

These then are the observations upon which we base our analysis. Obviously, any initial entry into an undeveloped and complicated area leaves behind many tentative conclusions and unanswered questions. Nevertheless, reasonable interpretations of the experimental results are possible, and these are delineated in the next section.

Discussion

We must first consider whether the observed ester hydrolysis is indeed an interfacial reaction. Alternatively, the hydrolysis could be a homogeneous reaction which proceeds either in the bulk water phase or in the bulk heptane phase. The sum total of the experimental results points strongly to a true interfacial process.

Ester hydrolysis in the free aqueous solution (eq 2)

$$\text{ester}_{(\text{heptane})} \rightleftharpoons \text{ester}_{(\text{water})}$$
$$\text{ester}_{(\text{water})} + \text{imidazole}_{(\text{water})} \longrightarrow \text{products} \qquad (2)$$

is unlikely on the basis of the following observations. (A) A solution of *p*-nitrophenyl laurate in heptane was

stirred with water for 60 min. The aqueous layer was removed, made strongly alkaline in order to hydrolyze any dissolved ester, and analyzed for *p*-nitrophenolate. None was found. Partitioning of ester from heptane into water under the conditions of the kinetic runs is, therefore, undetectably small. (B) *p*-Nitrophenyl laurate and *p*-nitrophenyl palmitate react heterogeneously at nearly the same rate. If tiny amounts of ester were dissolving and hydrolyzing in the water phase, then one would have expected the palmitate (with a carbon chain four atoms longer than that of laurate) to hydrolyze more slowly because it is presumably less soluble in water. (C) During our work on *p*-nitrophenyl laurate hydrolysis[20] it was found that colloidal or subcolloidal aggregates of ester molecules are extremely sensitive to the presence of salts. Addition of sodium chloride (0.2–1.0 *M*) causes precipitation from optically clear solutions of ester aggregates (8×10^{-6} *M*). If ester hydrolysis occurs only in the bulk water phase of the heptane–water mixture, then addition of sodium chloride to the water should drastically reduce the reaction velocity by "salting out" the ester. Table II shows that, on the contrary, the rate is slightly enhanced by large amounts of sodium chloride. (D) The behavior of the heterogeneous hydrolysis does not parallel that of homogeneous hydrolyses of *p*-nitrophenyl esters. For example, the heterogeneous hydrolysis is extremely sensitive to the presence of trace amounts of laurate anion (Figure 6), whereas homogeneous hydrolyses are not.[19] Homogeneous imidazole-catalyzed hydrolysis of *p*-nitrophenyl acetate displays no solvent isotope effect,[18] whereas the corresponding heterogeneous reaction of *p*-nitrophenyl laurate has a "solvent" isotope effect of 1.7. Finally, the reactivities of hydroxide ion and N-methylimidazole in the heptane–water system are smaller, relative to that of imidazole, than would be expected on the basis of the known reactivities of these species in the bulk water phase—see (9) and (10) of the previous section.

A homogeneous chemical reaction occurring solely in the dispersed hydrocarbon phase is also unlikely. When *p*-nitrophenyl laurate was added to heptane, which had been saturated with both water and imidazole, no *p*-nitrophenol could be detected after 75 min. This is not surprising since aminolyses of esters by weak amines in nonpolar solvents are very slow reactions[21] and since heptane (equilibrated with water using an electric shaker) does not dissolve imidazole even to the extent of 5×10^{-3} *M* (25.0°). The lack of an observable reaction after 75 min does not preclude the possibility that imidazole and ester are in an unfavorable equilibrium with acylimidazole and *p*-nitrophenol within the heptane phase (eq 3). If this is the

$$\text{imidazole} + \text{ester} \rightleftharpoons \text{acylimidazole} + p\text{-nitrophenol} \quad (3)$$

case, then the function of the water phase might be simply to remove one or both of the products, thereby driving the equilibrium constantly to the right. The following points can be made with regard to such a mechanism.

In the first place, eq 3 is certainly not applicable to the heterogeneous hydroxide ion catalyzed hydrolysis (Figure 5) since the reaction of an ester with hydroxide ion is *irreversible*. Second, the mechanism predicts a linear plot of velocity *vs.* [*p*-nitrophenyl laurate], whereas Figure 2 is in fact markedly curved.[22] Moreover, if the equilibrium (eq 3) is established rapidly, then removal of product into the aqueous phase must be rate determining. This is unlikely because extraction of *p*-nitrophenol from a heptane solution (1.3×10^{-4} *M*) into an aqueous imidazole buffer under the usual reaction conditions[12] was found to occur instantaneously (*i.e.*, in less than 10 sec).

Equation 3 is also inconsistent with Figure 3, a linear plot of velocity *vs.* [imidazole]. If the heptane becomes saturated with imidazole during the rapid stirring, then addition of *more* imidazole to the water should not increase the velocity. In other words, the reaction should not be first order in imidazole as is observed. The following experiment bears on this point. Heptane was saturated with imidazole by boiling the two reagents together under reflux for several hours. The heptane was cooled, filtered, and used to prepare a solution of *p*-nitrophenyl laurate. When a kinetic run was performed with this solution, it was found that saturating the heptane phase with imidazole beforehand has no effect on the reaction velocity. This result disproves the presence of a homogeneous reaction in heptane which is first order in imidazole by virtue of the fact that saturation of the heptane with respect to imidazole is never achieved.

N-Methylimidazole was found to be only eightfold less effective a catalyst as imidazole. Since N-methylimidazole and ester react to form *ionic* products (eq 4), one would have expected a much larger difference between the two catalysts if both equilibria (eq 3 and eq 4) were proceeding in the bulk heptane phase.[23]

$$\text{N-methylimidazole} + \text{ester} \rightleftharpoons \text{acyl-N-imidazolium ion}^+ + p\text{-nitrophenolate}^- \quad (4)$$

The above arguments point strongly to an interfacial reaction, but they do not preclude the possibility that part of the hydrolysis is noninterfacial in nature. Indeed, the distinction between an interfacial and homogeneous process is vague. We visualize the hydrocarbon–water interface as a three-dimensional region containing both water and heptane. There is no sharp demarcation between interfacial and noninterfacial solvent. The susceptibility of an ester molecule to catalyzed hydrolysis undoubtedly depends on its particular location within the continuum. We have shown above that the properties of the heterogeneous hydrolysis are qualitatively those which should be expected in a solvent that is neither completely hydrocarbon nor completely aqueous.

The reaction velocity increases linearly with both the stirring rate and the volume of heptane (Table I). Faster stirring and greater volumes of heptane lead to larger interfacial areas and, therefore, to larger reaction rates. The finding that the interfacial hydrolysis is only slightly affected by a change in hydrocarbon viscosity of two orders of magnitude is surprising.

(20) F. M. Menger and C. E. Portnoy, *J. Amer. Chem. Soc.*, **90**, 1875 (1968).

(21) Unpublished observations.

(22) Another possible explanation for the saturation effect, which cannot be excluded at present, is that an undetectable trace impurity in the ester (such as lauric acid) adsorbs at the interface and inhibits the reaction.

(23) *p*-Nitrophenol is a weaker acid in acetonitrile than in water by 10.7 pK_a units: J. F. Coetzee, *Progr. Phys. Org. Chem.*, **4**, 45 (1967). The equilibrium constant for eq 4 in heptane would therefore be expected to be many orders of magnitude less favorable than that for eq 3.

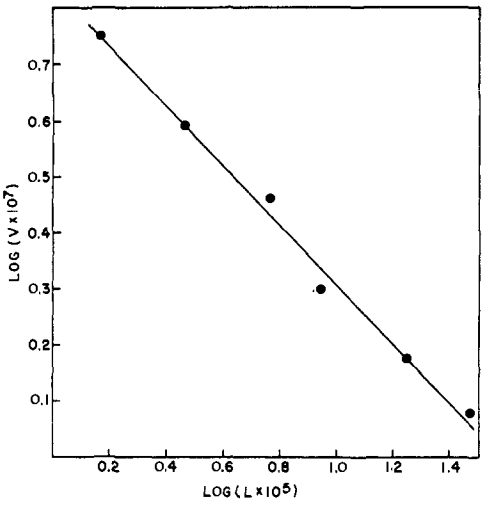

Figure 8. Freundlich isotherm plot of log (reaction velocity) *vs.* log (laurate anion concentration). Data are taken from Figure 6.

The droplet size may depend on both the ease of dispersal of the hydrocarbon and the resistance of the droplets to coagulation. Perhaps these factors compensate for each other in the case of a viscous hydrocarbon solvent such as paraffin oil. Attempts to photograph the oil-in-water dispersion at several stirring speeds, and thereby determine the distribution of droplet sizes, have not yet been successful. Consequently it is impossible to attach significance to small rate differences which might reflect changes in the contact area between the two phases. For example, at the present time we do not know whether the deuterium oxide isotope effect of 1.7 is the result of a change in interfacial area or a change in the nature of the interface arising from structural differences between deuterium oxide and water. With most of the experiments (such as the temperature studies from 15 to 30°) we make the reasonable assumption that the interfacial area remains fairly constant.

It was important to establish whether imidazole participates as a nucleophile or as a general base during the interfacial-catalyzed hydrolysis of *p*-nitrophenyl laurate. Both modes of catalysis are known in homogeneous imidazole-catalyzed ester hydrolyses.[24,25] Although *p*-nitrophenyl esters are normally subject to nucleophilic attack by imidazole, a change to a general base mechanism under the heterogeneous conditions was a distinct possibility. Water within the interfacial region would be expected to be highly structured.[26,27] Since proton transfers are more facile in ice than in liquid water,[28] an interfacial general base mechanism might be favored over a nucleophilic process. The evidence, as we shall now see, is quite to the contrary.

Small amounts of laurate anion added to the water phase markedly inhibit the interfacial hydrolysis (Figure 6). Thus, 3×10^{-4} *M* laurate decreases the observed reaction velocity sixfold. This rate inhibition cannot be ascribed to a decrease in surface area because the soap would, if anything, enhance the dispersion of the hydrocarbon droplets. The simplest explanation for the inhibition is that the laurate anion competes with the reactants for adsorption sites at the interface.[29] Alternatively, the inhibition may be an electrokinetic phenomenon (*i.e.*, perturbation of the interfacial structure by adsorbed soap may adversely affect the rate-determining step of the reaction). Experiments with different surfactants and with systems of known surface area will shed more light on the subject. In any event, the data in Figure 6 are important because they demonstrate that interfacial hydrolysis occurs by nucleophilic catalysis rather than by a general base mechanism. If a general base mechanism were operative, then laurate anion would be formed directly as one of the two reaction products, and the rate would continually decrease as the reaction proceeded. Yet Figure 1A shows no product inhibition even after 3000 sec when the laurate anion concentration in the water is 7×10^{-5} *M* (corresponding to a threefold rate decrease in Figure 6). Thus, laurate anion is not an initial reaction product. Instead, lauroylimidazole must form *via* nucleophilic attack by imidazole on the carbonyl carbon of the ester. If this conclusion is correct, then lauroylimidazole should hydrolyze to laurate anion and imidazole relatively slowly under the standard reaction conditions,[12] and this is exactly what is found. Figure 7B shows the effect of lauroylimidazole in the heptane phase (5.1×10^{-4} *M*) on the hydrolysis of *p*-nitrophenyl laurate. The rate of ester hydrolysis is seen to decrease slowly as the lauroylimidazole hydrolyzes to laurate anion. If *all* the lauroylimidazole had hydrolyzed instantaneously, the reaction rate would have been diminished by one order of magnitude (Figure 6).[30]

The sensitivity of the reaction velocity to the presence of an amphiphile (Figure 6) is noteworthy because regulation of reaction rates by small quantities of chemically inert substances is prevalent in biological systems.[31] The laurate inhibition (Figure 6) can be represented by a Freundlich adsorption isotherm (Figure 8).[32] The Freundlich equation (eq 5), which is empirical in nature, relates the amount of material adsorbed per unit surface area (X) to the concentration of material in solution (C). K and N are constants.

$$X = KC^N \qquad (5)$$

The linearity of Figure 8 suggests that the interfacial area is not greatly affected by addition of trace amounts of surfactant.

The interfacial hydroxide ion catalyzed hydrolysis of *p*-nitrophenyl laurate produces *p*-nitrophenolate and laurate anion. Absorbance *vs.* time plots for the reaction would therefore be expected to display product inhibition and, indeed, these plots (Figure 5) are markedly curved.

Equations 6–9 summarize the pathway for interfacial hydrolysis as we have thus far described it. IM, E, L,

(24) W. P. Jencks and J. Carriuolo, *J. Amer. Chem. Soc.*, **83**, 1743 (1961).
(25) M. L. Bender and W. A. Glasson, *ibid.*, **81**, 1590 (1959).
(26) J. L. Kavanau, "Water and Solute-Water Interactions," Holden-Day, San Francisco, Calif., 1964, p 78.
(27) G. Némethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).
(28) M. Eigen, *Angew. Chem., Int. Ed. Engl.*, **3**, 1 (1964).
(29) A. K. Chatterjee and D. K. Chattoraj, *J. Colloid Interface Sci.*, **26**, 1 (1968).
(30) For a discussion of hydrolysis of acylimidazoles under homogeneous conditions, see W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1272, 1280 (1959).
(31) R. L. Letsinger and T. E. Wagner, *J. Amer. Chem. Soc.*, **88**, 2062 (1966).
(32) D. J. Shaw, "Introduction to Colloid and Surface Chemistry," Butterworths, London, 1966, p 112.

and P are imidazole, ester, laurate anion, and product, respectively; subscripts W, H, and I refer to water, heptane, and interface. No attempt will be made to analyze the system quantitatively such as we have previously done for less complicated micellar reactions.[19] The interfacial mechanism is consistent with the parabolic laurate inhibition curve (Figure 6) and with the saturation effect observed in the velocity *vs.* [*p*-nitrophenyl laurate] plot (Figure 2). Equation 9 represents nucleophilic attack by interfacial imidazole on the carbonyl group of interfacial ester.

$$IM_W \rightleftarrows IM_I \quad (6)$$

$$E_H \rightleftarrows E_I \quad (7)$$

$$L_W \rightleftarrows L_I \quad (8)$$

$$IM_I + E_I \longrightarrow P \quad (9)$$

An interfacial reaction may be viewed as a five-step process:[5] (a) transport of reactants to the interface, (b) adsorption of reactants onto the interface, (c) chemical reaction at the interface, (d) desorption of products from the interface, and (e) transport of products from the interface. The insensitivity of the initial reaction velocity to a 15° temperature change (Figure 4) suggests that the chemical reaction at the interface (eq 9) is not entirely rate determining. Interfacial reactions, of course, need not have the same activation parameters as the corresponding bulk phase reaction.[33] In micellar systems, for example, activation energies often differ from those for the same reaction in the water phase.[34,35] Yet there is no known case of a micellar reaction being independent of the temperature. Since small temperature coefficients are characteristic of diffusion-controlled reactions,[36] the migration of reactants into the interfacial region must be at least partially rate determining. If this conclusion is correct, then the laurate anion inhibition (Figure 6) may be the result of retarded transport of one or both of the reactants to the reaction site. Adsorbed gelatin is known to affect adversely the movement of diethyl phthalate across a hexadecane–water interface.[37]

In summary, we have determined the dependence of interfacial hydrolysis rates on stirring speed, concentration of reactants, temperature, viscosity of the hydrocarbon, volume of the heptane and water solutions, deuterium and salt content of the water, lauroylimidazole content of the heptane, presence of an amphiphile, and nature of the catalyst. Interesting differences were found between heterogeneous and homogeneous hydrolyses. The mode of imidazole catalysis and the nature of the rate-determining step were discussed. Most importantly perhaps, a methodology of interfacial bioorganic chemistry was developed.

Acknowledgment. I thank the Research Corporation and the National Science Foundation for financial support. I am also very grateful for a National Institutes of Health Career Development Award.

(33) Activation parameters for the homogeneous reaction of imidazole with *p*-nitrophenyl acetate in water are $\Delta H^{\ddagger} = 7.0$ kcal/mol and $\Delta S^{\ddagger} = -10.7$ eu: T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. 1, W. A. Benjamin, New York, N. Y., 1966, p 56.

(34) C. A. Bunton, E. J. Fendler, and J. H. Fendler, *J. Amer. Chem. Soc.*, **89**, 1221 (1967).

(35) C. A. Bunton and L. Robinson, *ibid.*, **90**, 5972 (1968).

(36) E. A. Moelwyn-Hughes, "The Kinetics of Reactions in Solution," Oxford University Press, London, 1933, p 267.

(37) A. Ghanem, W. I. Higuchi, and A. P. Simonelli, *J. Pharm. Sci.*, **58**, 165 (1969).

The Thermal Disproportionation of Aryl Arenethiolsulfinates. Kinetics and Mechanism[1a]

Paolo Koch, Ennio Ciuffarin, and Antonino Fava[1b]

Contribution from the Istituto di Chimica Generale, Universita di Pisa, 56100 Pisa, Italy. *Received January 26, 1970*

Abstract: Aryl arenethiolsulfinates decompose thermally in inert solvents to give mainly the products of disproportionation, disulfide and thiolsulfonate. The rate of decomposition displays first-order kinetics within a run. However, massive changes of the initial concentration show that the first-order coefficient increases with increasing concentration. The rate law is $R = k_1[ArS(O)SAr] + k_2[ArS(O)SAr]^{1.5}$. Experiments in the presence of the stable radical DPPH show that DPPH disappears with zero-order kinetics within a run. In the presence of olefins or in the solvent acetonitrile the rate is independent of the initial concentration of thiolsulfinate. The overall effect of substituents on the phenyl rings is rather small. The above evidence and that which comes from tracer experiments is interpreted in terms of a radical process: a unimolecular decomposition along with an induced decomposition. The unimolecular initiation process is believed to be the homolytic fission of the S(O)–S bond, which appears to involve 34.5 kcal/mol. The induced decomposition is characterized by $\Delta H^{\ddagger} = 22.6$ kcal/mol. Various mechanistic paths are suggested which may be either radical displacement at sulfur or oxygen atom transfer reactions.

The recent discovery of an easy route to optically active thiolsulfinates[2,3] has revived the interest in the chemistry of this class of substances. Two papers already appeared dealing with their stereochemistry,[4,5]

(1) (a) The financial support of C.N.R., Rome, is gratefully acknowledged. (b) Address correspondence to this author at the Department of Chemistry, Louisiana State University, Baton Rouge, La. 70803.

(2) W. E. Savige and A. Fava, *Chem. Commun.*, 417 (1965).

(3) J. L. Kice and G. B. Large, *Tetrahedron Lett.*, 3537 (1965).

(4) P. Koch and A. Fava, *J. Amer. Chem. Soc.*, 90, 3867 (1968).