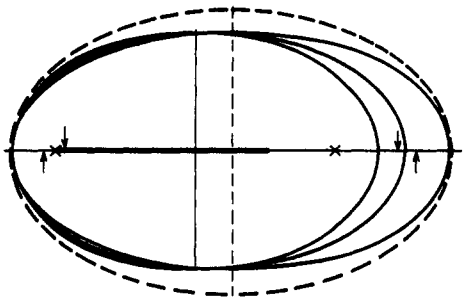

Figure 2. Illustration of how the ellipsoidal cavity changes with change in d_+ from 4.2 to 5.2 to 6.9 Å (see Table II) and no change in the lateral dimension (solid figures). Broken line is for proportional change in a and b. Movement of the focuses (originally X) for the 2.7 Å change in d_+ is indicated by arrows, pointing up for the solid, down for the broken-line figure.

For the more unlikely extreme where the lateral (b) and directly incremented (a) cavity dimensions are assumed to expand proportionally with bulk, effects approaching the size of those for the small spherical cavities are noted. This correspondence does not seem unreasonable from reconsideration of average molecule to cavity boundary distances in Figure 1, even though the ellipsoids of concern are rather eccentric (e from 0.77–0.84).

Much more likely upon end-group substitutions is that b expands only fractionally compared to a with bulk, i.e., $a\Delta b/b\Delta a = f$, and that f is $^1/_3$–$^1/_2$. In such a case, since it may be shown that ΔpK_a varies essentially linearly with this fraction, bulk effects of only 0.1–0.15 ΔpK_a unit are to be expected in all solvents, certainly now of a size small enough to be obscured by neglected effects and experimental uncertainties.

Further examination of Table II reveals that whereas a reasonable substituent effect for p-$CH_2NMe_3^+$ relative to p-CH_3 can only be obtained in the spherical model calculations by employing very shallow imbedding distances, here this effect is satisfactorily reproduced by employing the physically based distances discussed above. Obviously this effect is rather insensitive to d_+ variation, as the bulk effect results imply.[12]

Only in the matter of solvent-effect range does this body of results appear at all at variance with experiment. Here the change in ΔpK_a accompanying change from H_2O to 75% ethanol (ϵ_s/ϵ_i from 39 to 20) amounts to ~25–35% of the H_2O value, where the comparable experimental variation is ~95%. These predicted results are similar to those obtained from the spherical cavity models. It may be significant that results obtained upon reduced ionizable-proton to cavity-wall distance specification and with solvent boundary smoothing (~45%, see respectively footnote 12 and parenthesized entries of Table II) are in somewhat better agreement, but it seems inappropriate to attempt to refine this argument further at this time.

In short, it appears that bulk effects are only to be expected if interacting site distances to solvent are increased in all dimensions, i.e., are truly buried deeper within their cavities. Movement inward (relatively) in a spherical cavity, at least along a diameter, implies such burial, probably inappropriately for the present substrates. Structural considerations and various reactivity cross-comparisons suggest ellipsoidal cavities provide better physical representations of such acids in continuum solvents.[13] These generalizations, it should be recognized, may not hold in the event of strong specific interactions, which may have alternative directional characteristics, or if the shape of cavities become grossly distorted from ellipsoidal.

Registry No. $HO_2CC_6H_4$-p-$CH_2NEt_3^+$, 85371-51-3; $HO_2CC_6H_4$-p-$CH_2NMe_3^+$, 85371-50-2; $HO_2CC_6H_4$-p-$CH_2N(Bu\text{-}t)_3^+$, 85371-52-4.

(12) Somewhat greater sensitivity to the imbedding distance of the ionizable proton is noted, decreasing to 0.72 and 1.03, respectively, for ϵ_s/ϵ_i of 39 and 20, when d_H = 1 Å and d_+ = 3.2 or 5.9 Å, (again with b unchanged).

(13) It may be useful, as a final detail, to dissect on a mathematical level how constancy of ΔpK_a, through constancy of ϵ_{eff}, may be maintained upon bulk variation. Increased bulk strongly decreases ξ_2 (=$\rho_+/0.5R$) by severely shortening ρ_+ while lengthening R (see arrows of Figure 2 and note that $\rho = \rho_+ + \rho_H$, $\rho_i = a - d_i$). ξ_1 (=$\rho_H/0.5R$), on the other hand, only changes slightly (decreases for b unchanged or increases for proportionally varied b). The overall effect of ξ decreasing therefore should be one of decreasing ϵ_{eff} in harmony with intuition (see footnote 11 and, e.g., Figures 1–4 of ref 8). Where b is unchanged or varies slowly with increase in bulk, however, λ is also found to decrease (e.g., from 1.30 to 1.18 for d_+ increased from 4.2 to 6.9 with b = 4.4). Decreasing λ is accompanied by increasing ϵ_{eff} (sharply in this region, as the figures of ref 8 again reveal). Apparently, the two effects are almost completely compensatory for the geometries of interest. This suggests (at least) two physically realizable tests of the theory. In the first, invariant frameworks characterized by larger λ values (more spherical, e.g., 2,3,5,6-substituted p-$CH_2NR_3^+$ benzoic acids) could be similarly examined to see if greater (observable) bulk effects pertain. Simultaneous increases in lateral and terminal bulk should provide even larger differences.

Inhibition of Ester Hydrolysis by a Lipid Vesicle Membrane

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The second-order rate constants for the imidazole- or hydroxide-mediated hydrolysis of [3-nitro-4-(palmitoyloxy)phenyl]trimethylammonium iodide (1), [11-(4-nitrocarbophenoxy)undecyl]trimethylammonium bromide (2), and [5-nitro-2-(palmitoyloxy)benzyl]trimethylammonium halide (3) were obtained in the absence and presence of phospholipid vesicles. The rate data were obtained under conditions where only bound ester contributed to the kinetics. In general, small retardations of the hydrolysis rates were effected by binding of the substrates to lipid bilayers with choline phosphate headgroups. The imidazole rates appear to be more sensitive to the depth within the bilayer of the reaction center. The relative rates also can be interpreted in terms of an intramolecular electrostatic acceleration of the hydroxide rate for 3 in aqueous solution, which is inhibited upon binding of this amphiphilic ester to the lipid bilayer membrane.

Lipid bimolecular layers are the backbone of biological membranes[2] and, as such, must organize a wide variety of biological reactions. Vesicles, formed by sonication of aqueous phospholipid dispersions, provide a convenient

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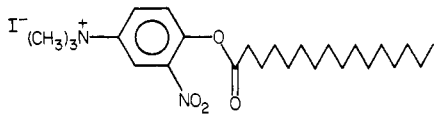
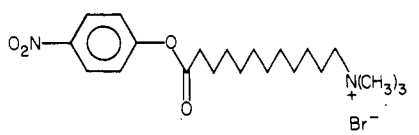
controlled model for biological membranes in that they possess this bilayer structure and are capable of mimicing the transport, electrical, and binding properties of authentic cell surfaces.[3] We are employing this model in a program to study the effect of a membrane environment on chemical reactivity. The aim of this effort will be to elucidate the subtle perturbations effected by this environment on simple reactions with relatively well-understood behavior in homogeneous solution. This approach contrasts with the many successful searches for large catalytic effects induced by micelles[4] and surfactant bilayers.[5] We report here the first results to emerge from this effort, which show that the interfacial region can reorganize a reaction center to alter the reactivity patterns normally observed in homogeneous solution.

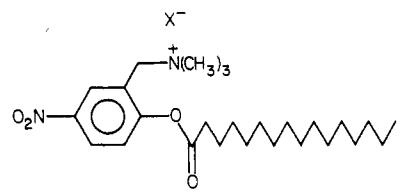
The esters 1, 2, and 3 were prepared via straightforward



1



2



3

syntheses. They are sufficiently polar to provide homogeneous solutions, and their amphiphilic structures assure binding to lipid bilayers. Each of these esters is subject to hydrolysis at convenient rates by either saponification or imidazole-mediated nucleophilic catalysis. Thus the effect of a lipid bilayer environment can be assessed for both ionic and neutral rate-determining transition states.

Experimental Section

Melting points were obtained with an open capillary tube on a Laboratory Devices apparatus (Model Meltemp) and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined at 60 MHz on a Varian Associates Model A-60 or Model EM-360A spectrometer. Infrared (IR) spectra were obtained by using a Perkin-Elmer Model 457 spectrometer. Elemental analyses were performed by Galbraith Lab, Inc., Knoxville, TN, or Atlantic Microlab, Inc., Atlanta, GA.

4-Nitrophenol, 4-amino-2-nitrophenol, 2-hydroxy-5-nitrobenzyl bromide, trifluoroacetic anhydride, 4-(dimethylamino)pyridine, and *N,N*-dicyclohexylcarbodiimide were obtained from Aldrich Chemical Co. and used without further purification. Palmitoyl chloride was obtained from Aldrich and redistilled in vacuo. Palmitic acid and trimethylamine were obtained from Eastman Kodak; the former was recrystallized twice from ethanol before use.

[3-Nitro-4-(palmitoyloxy)phenyl]trimethylammonium Iodide (1). (4-Hydroxy-3-nitrophenyl)trimethylammonium iodide was prepared according to the procedure of Bruice et al.[6] The ester was prepared according to the method of Hassner et al.[7] with some modification in the workup.

To 2.00 g of (4-hydroxy-3-nitrophenyl)trimethylammonium iodide (phenol) (6.17 mmol) in 40 mL of methylene chloride were added 1.58 g palmitic acid (6.17 mmol), 1.275 g of *N,N*-dicyclohexylcarbodiimide (DCC) (6.17 mmol), and 0.1 g of 4-(dimethylamino)pyridine (DMAP). The reaction mixture was stirred at ambient temperature for 2 h and then refluxed for 12 h. The progress of the reaction was followed by the gradual disappearance of the orange crystals of the phenol and the appearance of a yellow precipitate. The reaction was stopped when all the phenol disappeared.

The yellow precipitate, which contains both the ester and *N,N*-dicyclohexylurea, was suction filtered, washed with methylene chloride (3 × 50 mL), and transferred to a beaker where it was digested with a 300-mL mixture of acetonitrile, acetone, and water (4:2:1). Then it was suction filtered, washed with 3 × 50 mL of acetonitrile, 3 × 50 mL of water, and 5 × 50 mL of acetone. The solid residue was dried in vacuo overnight. The product was recrystallized three times from isopropyl alcohol and dried again in vacuo over P_2O_5. The ester was obtained as a yellow powder in 30% yield: mp 156–156.5 °C; IR (Nujol mull) 1776 cm^{-1}; NMR (Me_2SO-d_6–$CDCl_3$) δ 0.9 (t, 3 H), 1.3 (br s, 28 H), 3.75 (s, 9 H), 7.65–8.90 (ABX, 3 H) ppm. Anal. Calcd for ($C_{25}H_{43}IN_2O_4$): C, 53.39; H, 7.65; I, 22.58; N, 4.98; Found: C, 53.62; H, 7.86; I, 22.34; N, 5.02.

[11-(4-Nitrocarbophenoxy)undecyl]trimethylammonium Bromide (2). A mixture of 2.00 g of 12-bromododecanoic acid (7.17 mmol) and 1.51 g of trifluoroacetic anhydride (7.17 mmol) was allowed to react at 25 °C for 30 min, until a pasty liquid was formed and all the acid dissolved. To this was added 1.00 g of *p*-nitrophenol (7.88 mmol), and the mixture was heated at 80 °C, under nitrogen atmosphere, for 12 h. The mixture was cooled, taken up in diethyl ether, and washed with 3 × 50 mL of ice-cold 5% sodium bicarbonate. The ether phase was dried over anhydrous magnesium sulfate, and the ether evaporated under reduced pressure. Infrared spectrum of the viscous liquid residue indicated complete ester formation. This product was then dissolved in 50 mL of benzene and cooled to −5 °C, and 1.2 equiv (0.75 mL) of anhydrous trimethylamine was added. The mixture was magnetically stirred at −5 °C for 1 h, gradually warmed up to room temperature, and allowed to stir for an additional 10 h. The precipitate that formed was suction filtered, washed with benzene, and recrystallized twice from diethyl ether/isopropyl alcohol and once from a mixture of ethyl acetate and isopropyl alcohol. The product was dried in vacuo over P_2O_5. The ester was obtained in 62% yield: mp 84–86 °C: IR (Nujol mull) 1760 cm^{-1}; NMR (Me_2SO-d_6) δ 1.4 (br s, 20 H), 3.3 (s, 9 H), 3.3–3.7 (m, 2 H), 7.4–8.6 (AB, 4 H). Anal. Calcd for ($C_{21}H_{35}BrN_2O_4$): C, 54.91; H, 7.63; Br, 17.40; N, 6.10; Found: C, 55.02; H, 7.30; Br, 17.44; N, 6.08.

[5-Nitro-2-(palmitoyloxy)benzyl]trimethylammonium Bromide (3). To 5.00 g of 2-hydroxy-5-nitrobenzyl bromide (20.1 mmol) in 150 mL of methylene chloride were added 2.28 g of 2,6-lutidine (20.1 mmol) and 5.92 g of palmitoyl chloride (20.1 mmol). The solvent was refluxed for 2 h. The precipitated 2,6-lutidine hydrochloride was filtered off. An IR spectrum of the filtrate indicated presence of ester. Anhydrous trimethylamine was cooled to −78 °C; a 2.3-mL aliquot (26 mmol) was rapidly pipetted and added to the cooled (−78 °C) filtrate. The mixture was allowed to warm up to room temperature while it was magnetically stirred and then refluxed for 1 h. The resulting precipitate was suction filtered and washed thoroughly with methylene chloride until a white powder was obtained. The powder was recrystallized twice from diethyl ether–isopropyl alcohol. The

(1) NIH Research Career Development Awardee, 1980–1985 (CA-677).
(2) Singer, S. J.; Nicolson, G. L. *Science (Washington, D.C.)* **1972**, *175*, 720–731.
(3) (a) Bangham, A. D.; Hill, M. W.; Miller, N. G. A. In "Methods in Membrane Biology"; Korn, E. D., Ed.; Plenum Press: New York, 1974; Vol. 1, p 1. (b) Huang, C. *Biochemistry* **1969**, *8*, 344–352.
(4) (a) Bunton, C. A. *Pure Appl. Chem.* **1977**, *49*, 969. (b) Fendler, J. H.; Fendler, E. J. In "Catalysis in Micellar and Macromolecular Systems"; Academic Press: New York, 1975.
(5) (a) Fendler, J. H. *Acc. Chem. Res.* **1980**, *13*, 7. (b) Kunitake, T.; Shinkai, S. *Adv. Phys. Org. Chem.* **1980**, *17*, 435–487.
(6) Bruice, T. C.; Katzhendler, J.; Fedor, L. B. *J. Am. Chem. Soc.* **1968**, *90*, 1333–1348.
(7) Hassner, A.; Alexanian, V. *Tetrahedron Lett.* **1978**, 4475–4478.

ester was obtained in 54% yield after drying it in vacuo over P_2O_5 overnight: mp 158–160 °C; IR (Nujol mull) 1783 cm^{-1}; NMR (Me_2SO-d_6) δ 0.8 (t, 3 H), 1.3 (br s, 28 H), 3.3 (s, 9 H), 4.9 (s, 2 H), 7.4–8.8 (ABX, 3 H). The elemental analysis showed the counterion to be a mixture of chlorine and bromine in the approximate ratio Cl^-:Br^- = 1.1:1. Anal. Calcd for ($C_{26}H_{45}Br_{0.47}Cl_{0.53}N_2O_4$): C, 61.79; H, 8.91; Br, 7.73; Cl, 3.83; N, 5.55; Found: C, 61.81; H, 8.99; Br, 8.23; Cl, 4.07; N, 5.59.

Preparation of Vesicles. Egg lecithin was purchased from Sigma Chemical Co. and is the only phospholipid used in this study. The appropriate amount of lipid in chloroform–methanol (9:1, v/v) was transferred to a 10-mL glass vial, and the solvent was evaporated with a stream of nitrogen. The lipid was left behind as a thin film in the bottom of the vial. It was then put in a desiccator in vacuo to remove the last traces of solvent. The necessary volume of the desired buffer was then added and the suspension sonified at 50% power with a Branson sonifier (Model 185) equipped with a titantium probe for 15 min at 0 °C under a stream of N_2. For lipid concentrations higher than 1 mg/mL, sonication times of up to 1 h were used. Esters were usually added as a concentrated ethanol solution from a syringe or a pipet to the buffer contained in a 3-mL cuvette of 1-cm path length that had already been thermally equilibrated at the desired temperature; in no case did the final ethanol concentration in the cuvette exceed 3% (v/v). The reaction solution was mixed by inverting the stoppered cuvette. In some instances, the ester was cosonicated with the lipid in distilled water and the buffer with the appropriate pH added later when the kinetic run was initiated. Vesicles were always used the same day they were prepared.

Negatively stained samples are examined with a Philips EM201 transmission electron microscope. Grids are prepared by placing a drop of a 1:1 mixture of the vesicle suspension and 4% ammonium molybdate, pH 7, on a parlodion/carbon grid and blotting off the excess after 1 min of settling.

Kinetic Studies. The following buffers were obtained from Fisher Scientific Co.: pH 7 (0.05 M KH_2PO_4–NaOH), pH 8 (0.05 M K_2HPO_4–NaOH), pH 9 (0.1 M H_3BO_3–KCl–NaOH), and pH 10 (0.05 M K_2CO_3–KH_2BO_3–NaOH). Appropriate quantities of palmitoyllysophosphatidylcholine (Sigma Chemical Co.) or cetyltrimethylammonium bromide (Fluka) were dissolved in the buffers for the comparison studies in micelles as discussed under Results. Kinetic measurements in homogeneous solution were either carried out in the pure or appropriately diluted buffer of the desired pH. The desired ionic strength was maintained by addition of potassium chloride. All solvents were deaerated by boiling and cooling under N_2. Doubly glass distilled water was used throughout.

The rates of hydrolysis were followed spectrophotometrically via the appearance of phenolate anion. For homogeneous solutions, a Beckman Model 25 UV–vis spectrometer sufficed. For kinetics of turbid vesicle preparations, a dual wavelength photometer, which has been previously described,[8] was employed. The output of this photometer is the difference in optical density between the wavelength of the phenolate and a reference wavelength at which no change in chromophore absorbance is expected; this technique substantially cancels any artifactual contributions to the kinetics from changes in sample turbidity. Sample and reference wavelengths employed for the three esters were, respectively, 402 and 438 nm for 1, 405 and 440 nm for 2, and 400 and 440 nm for 3.

Reactions were generally followed to four half-lives. The pH of the solution was checked before and after the reaction to assure constancy. Pseudo-first-order rate constants, k_1', were obtained by fitting the data to a single exponential. Second-order rate constants for hydroxide were obtained from the slope of a plot of k_1' vs. [OH] between pH 9 and 10 at an ionic strength of 0.05 M. Buffer catalysis was insignificant as determined by diluting the buffer at constant ionic strength. Similarly, second-order rate constants for imidazole-catalyzed hydrolysis were obtained from the dependence of k_1' on [imidazole]. The rates were all determined in Fisher pH 9 buffer, and at least three rate determinations were obtained at each of at least five different imidazole concentrations.

(8) Fatah, A. A.; Loew, L. M. *J. Org. Chem.* **1980**, *45*, 4333–4336.

Table I. Second-Order Rate Constants (M^{-1} min^{-1}) at 35.0 °C for the Hydrolysis of Esters 1, 2, and 3

conditions[a]	1	2	3
k^{-OH}	2.7×10^2	2.1×10^3	4.6×10^3
k^{-OH}_{ves}	1.3×10^2	5.3×10^2	4.6×10^2
k^{IM}	1.2×10^2	44	1.9×10^2
k^{IM}_{ves}	80	1.3	76

[a] Subscripts indicate whether or not lipid vesicles were present in the aqueous medium. Superscripts indicate whether rates refer to slopes of pseudo-first-order rates vs. [hydroxide] or vs. [imidazole].

Table II. Lipid Concentration Dependence of Ester Kinetics at pH 9.0, 35.0 °C

egg lecithin concentration, mg/mL	$k_1' \times 10^2$ (min^{-1})[a] Im = 5.90×10^{-4} M 1	Im = 1.66×10^{-2} M 2	Im = 3.75×10^{-4} M 3
0	7.6	72.7	19.7
0.5	4.5	3.3	3.4
1	4.7	3.4	3.9
2	4.3	3.3	3.3
4	4.6	3.8	3.5

[a] Imidazole was included at the indicated concentrations to accelerate the reactions to conveniently measured rates. The values for 0 lipid concentrations were taken as the intercept of plots of k_1' vs. [imidazole].

It was found that for 1 and 3 the rates above ester concentration of 10^{-4} M became severely concentration dependent, presumably as a result of the onset of self-aggregation.[8] Therefore, all of the experiments reported here were with ester concentrations at or below 6×10^{-5} M.

Results

The hydrolysis rate constants for esters 1, 2, and 3 are presented in Table I. It is not the purpose of this paper to try to rationalize the differences between these compounds that can arise presumably from a combination of variables such as steric, solvation, electrostatic, or conjugative effects. It might be more instructive, however, to take the rate constants in rows 1 and 3 as measures of the intrinsic reactivity of these substrates and try to rationalize the changes induced in each case by a lipid bilayer environment.

The most obvious observation is that relatively small rate retardations are effected by binding to the lipid vesicles. The structures of the three esters are such as to allow the reaction centers to remain close to the bilayer surface either because this is the most stable orientation (1 and 3) or because the appropriate conformation is populated on a time-averaged basis. The dipolar nature of the choline phosphate headgroup provides a highly ionic environment, but slightly deeper within the membrane a nonpolar environment may be presented to the reaction centers. A more detailed attempt to rationalize the individual vesicle-induced rate changes will be presented in the Discussion. First, however, we will describe a series of experiments designed to test whether the esters are completely bound, if the vesicles are sufficiently stable during the reactions, and if the reactivity in micelles is significantly different from that in vesicles.

As a test for the degree of binding of the esters, pseudo-first-order rate constants were obtained at 6×10^{-5} M ester with lipid concentrations varying between 0.5 and 5 mg/mL (Table II). For all three esters, rates were constant over this range of lipid concentrations but were significantly retarded relative to the rates obtained in the

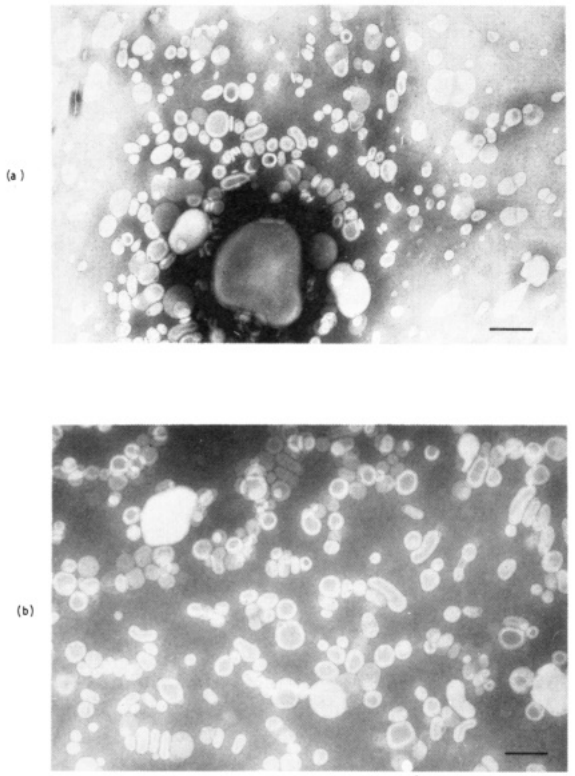


Figure 1. Electron micrographs of negatively stained vesicle preparations before (a) and after (b) hydrolysis of ester **3**. Bar = 1000 Å.

absence of lipid vesicles. The vesicle data presented in Table I were obtained at 1 or 2 mg/mL of lipid and 4 or 6×10^{-5} M ester. The absence of any lipid concentration dependence in this range assures that the data in lines 2 and 4 of Table I represent the kinetics of only vesicle-bound esters, the low concentration of any unbound ester rendering it kinetically insignificant. Paranthetically, as already noted in the Experimental Section, there was no self-micellization of the esters at these concentrations.

Also of concern was the effect of any hydrolysis of the lipid itself under the reaction conditions. While this would not be expected to be extensive on the time scale of the hydrolysis of the activated aryl esters, even a small amount of hydrolysis could conceivably disrupt the vesicles. Also, the aryl ester hydrolysis products could themselves disrupt the vesicles. We obtained electron micrographs of negatively stained preparations before and after typical kinetic runs and observed an increase in the average vesicle size from ca. 250 to 500 Å. These are shown in Figures 1a and 1b, respectively. This is consistent with the known tendency of even small concentrations of fatty acids to induce vesicle fusion.[9] The turbidity change resulting from this increase in particle size does not distort the kinetic traces obtained with the dual wavelength photometer;[8] indeed, standard log plots were satisfactorily linear over the four half-lives for which data were typically taken (actual rate constants were obtained from linear regression analyses).

Pseudo-first-order rates for the saponification of the esters in the presence of micelles are compiled in Table III. As can be seen from a comparison of rows 1 and 4, the effects of the membrane environment on k_1' are identical with the effect on the actual second-order rate constants discussed above (cf. Table I). The cationic micelles composed of cetyltrimethylammonium bromide clearly display the typical large rate accelerations expected

(9) Kanton, H. L.; Prestegard, J. H. *Biochemistry* **1975**, *14*, 1790–1794.

Table III. Pseudo-First-Order Rates of Ester Hydrolysis at 35.0 °C, pH 9.0

conditions	k_1' [SD] × 10^2 (min^{-1})[a] 1	2	3
no added solute	1.17 [0.02]	2.87 [0.06]	11.3 [0.3]
2×10^{-3} M cetyltrimethylammonium bromide	42.9 [1.3]	4.58 [0.06]	67.2 [4.5]
1 or 2×10^{-3} M[b] lysolecithin	1.20 [0.03]	0.83 [0.04]	2.91 [0.02]
1 mg/mL of egg lecithin	0.66 [0.05]	0.89 [0.10]	1.22 [0.08]

[a] SD = standard deviation. [b] As for egg lecithin, the rates were not dependent on lysolecithin concentration above its cmc of ca. 0.5×10^{-3} M.

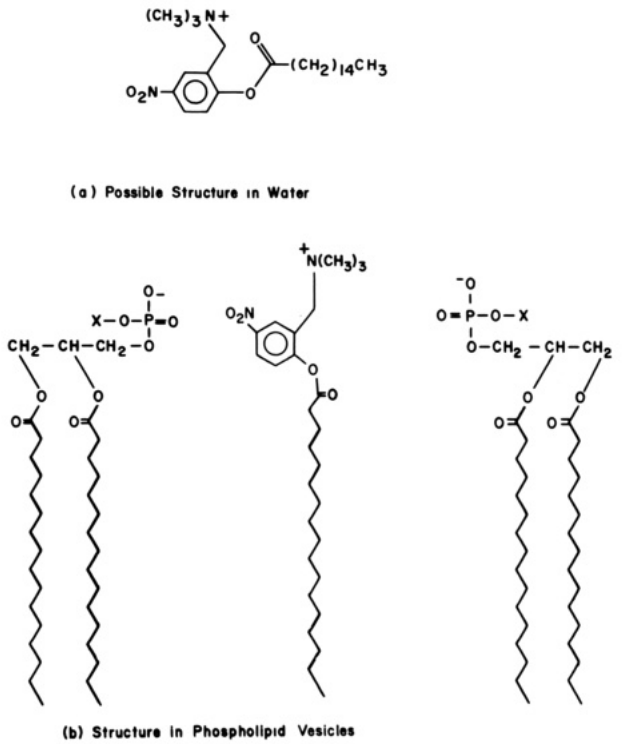


Figure 2. Postulated electrostatic interactions in solution (a) and at the surface of a phospholipid bilayer (b) of the positively charged ammonium group with the reaction center in **3**.

for an anionic reactant. More relevant to the question of any special effects of a bilayer vs. a micellar environment are the data obtained with palmitoyl lysophosphatidylcholine. This material has the same zwitterionic headgroup as lecithin but forms micelles instead of bilayers.[10] The hydrolysis of ester **1** is not retarded at all by these micelles, ester **3** is retarded somewhat less effectively, and hydrolysis of ester **2** is retarded equally effectively by the lysolecithin micelles and the lecithin vesicles.

Discussion

The hydroxide-mediated hydrolysis rates for **1**, **2**, and **3** are retarded by factors of ~2, ~4, and ~10, respectively, by the lipid vesicles. The retardation for **1** and **2** can be simply rationalized on the basis of having isolated the reaction centers (i.e., the carbonyl carbon) from the aqueous medium upon binding of the esters to the membrane; since the reaction center in **2** is much further removed from the hydrophilic trimethylammonium group,

(10) Hamori, E.; Michaels, A. M. *Biochim. Biophys. Acta* **1971**, *231*, 496–504.

one would expect it to be more deeply buried in the hydrophobic region of the lipid bilayer, on average, and hence display a somewhat more retarded rate than does 1. This cannot explain the still greater retardation displayed by 3, which should have its reaction center at a depth in the membrane quite close to that of 1.

We offer the rationale illustrated in Figure 2 to explain this excessive rate retardation displayed by 3. It is reasonable to expect that the positive charge on the trimethylammonium can provide an electrostatic stabilization of the anionic transition state in the saponification and thus accelerate the reaction in aqueous solution (Figure 2a). We propose that the highly ionic atmosphere at the surface of the vesicle membrane can screen out this interaction; the opposing hydrophobic and hydrophilic forces on the appropriate segments of 3 may, in fact, lead to a preferred conformation in which the trimethylammonium group is kept away from the incipient negative charge on the carbonyl oxygen (Figure 2b).

The imidazole rates in Table I can be used to provide additional support for this argument. Again, first examining 1 and 2, retardations of ~2 and ~34-fold, respectively, are experienced for the vesicle bound relative to the free esters. thus, the imidazole rates are considerably more sensitive to the environment of the reaction center than the hydroxide rates; this is to be expected since two neutral reactants are combining to form a charge-separated transition state. Ester 3 displays a rate retardation similar to that of 1, as might be expected on the basis of depth considerations alone: the larger rate retardation found for hydroxide is not observed with imidazole catalysis! The absence of the electrostatic effect is expected for a neutral nucleophile. Electrostatic effects in the hydrolysis of aspirin monoanion[11] and 1-acetoxy-4-methoxypyridinium cation,[12] as examples, are present for anionic nucleophiles but absent for neutral amine nucleophiles. Thus, the imidazole results actually provide a control for the behavior of 3 in the absence of electrostatic effects and support the premise that the larger hydroxide rate retardation is not simply due to an unexpected deep placement of the reaction center in the bilayer.

Naturally, these ideas are tentative and would be strongly bolstered by independent data on the orientations and conformations of the substrates within the bilayer. The results with positively charged micelles indicate that electrostatic influences may dominate purely orientational effects. The results presented in this paper do suggest, however, that whatever their precise molecular origin, the lipid bilayer structure can produce some subtle perturbations to reactivity beyond those normally observed within micelles.

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Registry No. 1, 85405-76-1; 2, 85405-77-2; 3, 85405-78-3; (4-hydroxy-3-nitrophenyl)trimethylammonium iodide, 18618-47-8; palmitic acid, 57-10-3; 12-bromodecanoic acid, 73367-80-3; *p*-nitrophenol, 100-02-7; trimethylamine, 75-50-3; 2-hydroxy-5-nitrobenzyl bromide, 772-33-8; palmitoyl chloride, 112-67-4; cetyltrimethylammonium bromide, 57-09-0; palmitoyllysophosphatidylcholine, 14863-27-5.

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Synthesis and Stereochemistry of Tetra-*o*-tolylethene and 1,1,2,2-Tetra-*o*-tolylethane

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The synthesis of tetra-*o*-tolylethene and 1,1,2,2-tetra-*o*-tolylethane through reductive coupling of di-*o*-tolyl ketone by $TiCl_3/LiAlH_4$ in THF is described. The ^1H NMR spectra of tetra-*o*-tolylethene display several coalescences between −90 and 60 °C. Evidence is presented for a propeller conformation undergoing a four-ring flip as the threshold mode. At 60 °C, the three-ring flip is rapid on the NMR time scale. Internal rotations in 1,1,2,2-tetra-*o*-tolylethane are rapid over the whole temperature range studied so that little stereochemical information could be obtained for this compound.

From a stereochemical point of view, the polyaryl systems[1-12] studied up to now belong to two types. In compounds of type I at least two aryl rings are bound to each atom of a central unit. The whole structure displays a propeller conformation observable on the NMR time scale, with the aryl rings acting as propeller blades.[3] The internal

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